TEHRY UNG KIM
Nutritional and Developmental Studies in Pecan: Studies on Zinc, Nitrogen, and Seasonal Fluctuation of Carbohydrate and Nutrients
(Under the Direction of HAZEL WETZSTEIN)

Studies were conducted in pecan (*Carya illinoinensis* Wangenh. K. Koch) including 1) the effect of zinc supply on growth, development, and nutrient uptake of different seedstocks, 2) evaluation of zinc deficiency at macro- and microscopic levels, 3) the effect of different nitrogen sources on growth, development and nutrient uptake, and 4) seasonal changes in nutrients and carbohydrates.

Zinc efficiency was compared in two pecan seedstocks, Stuart and Curtis. Stuart exhibited more severe deficiency ratings than Curtis. Stuart seedstocks grown under minus zinc versus plus zinc conditions exhibited significantly higher foliar concentration of P, Ca, Mg, and Cu, while Curtis leaves contained significantly higher Mn and lower S.

A microscopic evaluations of zinc deficiency were conducted using light and transmission electron microscopy. Zinc deficiency symptoms varied with severity ranging from interveinal mottling, overall chlorosis, necrosis, to marginal curving. Changes in leaf thickness and mesophyll cell organization were associated with zinc deficiency. Cells in zinc deficient leaves had limited cytoplasmic content and accumulated phenolic compounds in vacuoles. Extensive starch accumulation was observed in chloroplasts. This work represents the first detailed microscopic evaluations of zinc deficiency in leaves.

The effects of nitrogen form on plant growth and nutrient uptake were evaluated. Plants grown with a 75:25 (ammonium:nitrate) ratio exhibited significantly lower biomass, decreased root/shoot ratio, and lower specific leaf weight than observed in lower ammonium treatments. The highest root growth was observed in the 50:50 ratio treatment. Levels of Ca, Mg, and Mn in leaves were higher in 25:75 than in 75:25. Total

nitrogen uptake on a dry weight basis was highest in the 75:25 treatment. Plants exhibited preferential uptake of ammonium nitrogen under all nitrogen regimes.

Changes in soluble carbohydrate levels were related to leaf and fruit developmental stages. Glucose level declined during cotyledon elongation and reserve deposition in fruit. Fructose levels declined during fruit storage reserve accumulation. After fruit maturation, stems exhibited a dramatic increase of sucrose along with a marked decrease of starch. Seasonal variation of essential element levels in leaves indicated developmental stage-related nutrient movement. During leaf development, all essential elements accumulated in leaves.

INDEX WORDS : Pecan, Nutrition, Zinc, Nitrogen, Carbohydrate, Season, Deficiency, Ultrastructure

NUTRITIONAL AND DEVELOPMENTAL STUDIES IN PECAN: STUDIES ON ZINC, NITROGEN, AND SEASONAL FLUCTUATION OF CARBOHYDRATE AND NUTRIENTS

by

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Dedication

Dedicated to my parents

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CHAPTER 1

Introduction

Pecan (*Carya illinoinensis* Wangenh. K. Koch) is a hardwood tree in the Juglandaceae family that produces an edible nut with high commercial value. With the successful development of accurate and consistent leaf analysis, pecan growers have been able to improve tree performance with improved fertilization regimes. However, nutritional problems frequently limit orchard productivity (Smith, 1991). Notable nutritional problems are described for nitrogen (Sparks, 1976; Smith and Cotten, 1985), phosphorus (Sparks, 1988), potassium (Diver et al., 1984), magnesium (Worley et al., 1975; Sparks, 1986), and zinc (Hu and Sparks, 1990; Hu and Sparks, 1991; Wood and Payne, 1997). There are inherent difficulties associated with monitoring nutrient uptake and utilization in field studies. Likewise, direct evaluations of nutritional effects on growth and development are difficult.

Some specific areas of investigation were conducted for this work. These include studies on 1) evaluation of zinc deficiency at macro- and microscopic levels, 2) the effect of zinc supply on growth, development, and nutrient uptake of pecan seedlings with an evaluation of different seedstocks, 3) the effect of different nitrogen sources on growth, development and nutrient uptake, and 4) seasonal changes in nutrients, carbohydrates, and plant structure. The nutritional studies evaluating nitrogen and zinc used hydroponic culture. The last study was temporal evaluations using mature trees. An advantage of hydroponics is that the uptake and utilization of subtle differences in nutrients can be followed and growth readily assessed.

Zinc deficiency is the most well-known and at one time one of the most widely

occurring deficiencies in the pecan belt (Sparks and Madden, 1977). Rosette, a disorder in pecan resulting from inhibition of shoot elongation, has been recognized as a major cultural problem since the early part of the last century and discovered to be caused by zinc deficiency (Sparks, 1993). Researchers have reported the physiological impact of zinc deficiency in pecan, which includes diminishing chlorophyll synthesis and gas exchange (Hu and Sparks, 1991), inhibition of reproductive development (Hu and Sparks, 1990), and decreased carbonic anhydrase activity (Snir, 1983). Differences in the frequency of Zn deficiency and efficiency have been observed in different pecan cultivars. A reproducible method using somatic embryogenesis has been developed which has potential as a means to produce clonal rootstocks in pecan (Mathews and Wetzstein, 1993). The identification of genotypes with enhanced zinc efficiency would be of great interest.

As a plant nutrient, nitrogen is the element in highest demand in terms of quantity and makes up about 2-3% of plant dry matter. Nitrate and ammonium are the major sources of inorganic nitrogen taken up by higher plants. Whether ammonium or nitrate as a source of nitrogen supply is better for plant growth and yield depends on the plant species (Marschner, 1997). Ammonium and nitrate comprise about 80% of the total cations and anions taken up by plants. Thus the form of nitrogen supply has a strong but inverse impact on the uptake of other cations and anions, on cellular pH regulation, and on rhizosphere pH. Ammonium assimilation demands carbon skeletons and decreases external medium pH and rhizosphere pH by excreting protons. Soil pH has been reported by Sparks (1977) to have a striking effect on pecan growth. A linear increase of shoot diameter was observed at soil pH values between 4.5-6.1. A very pronounced change in soil pH has been demonstrated from the application of nitrogen fertilizers within relatively short time periods. Of interest would be to document preference for nitrogen source and how nitrogen source affects plant growth and development in this species. Such information would be very useful in developing optimal fertilization protocols.

Higher plants can exhibit marked seasonal changes in nutrient content, carbon

metabolism and organ development. Critical developmental stages can influence both nutrient and carbohydrate allocation patterns. A number of studies have followed changes in carbohydrate (Smith et al., 1986) and nutrient content (Cresswell and Wickson, 1986) in pecan leaves. However, the objective of these studies was to assess the effect of various treatments such as fungicide application (Worley, 1973), artificial defoliation (Worley, 1978; Worley, 1979), stem cutting (Smith et al., 1975) and fruit loads (Worley, 1973; Wood and McMeans, 1981; Smith et al., 1986; Wood, 1989). An integrated study of temporal carbohydrate, nutrient and structural changes in pecan leaves and shoots would provide insight into differences in seasonal requirements for this crop.

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CHAPTER 2

Literature Review

1. Pecan

1.1. General

Pecan (*Carya illinoinensis* (Wang) K. Koch) is the most widely-cultivated, largest, longest-lived, and most valuable member of the North American hickory group native to the Mississippi River Valley (Hanna, 1987). Its native habitat ranges from southwest Texas to southeast Alabama, extending north to southern Illinois and south to the mountains of northern Mexico. Pecan is cultivated beyond its native range, from South Carolina and North Carolina across the country to the southwest into California (Madden and Hensz, 1979; Hanna, 1987). The leading states in pecan nut production are Georgia, Texas, New Mexico, Alabama, Louisiana and Oklahoma. Pecan production in other countries such as Australia, Brazil, Israel, Mexico and South Africa is limited to cultivars developed in the United States (Sparks, 1992).

The main product of economical importance of pecan is the nut, with a high nutritional value including a high oil content (73-75%), carbohydrates (12-15%), proteins (9-10%), vitamins A and E, ascorbic acid, thiamine, riboflavin and niacin (Brison, 1974). In addition, drugs, essential oils and cosmetics can be obtained from the nut s oil. Leaves are sources of lauric acid, juglone (an antihemorrhagic), and protein (Duke, 1983). Other uses of pecan include wood for furniture, flooring and veneer, smoking of meats, and as an ornamental shade tree (Harlow, 1991).

Pecan is a diploid (2n=32) deciduous tree, a member of the Juglandaceae family, which includes hickories and walnuts. About 20-25 species of large trees comprise the genus *Carya*. Pecan leaves are alternate and compound, varying in size, from the small leaves of Cheyenne to the large leaves of Mahan . Leaflets are usually 5-10 cm long and vary from 9-17 leaflets per leaf. Variations in color are observed, from the yellow-green leaves of Desirable to the extremely dark green leaves of Pawnee (Sparks, 1992).

Pecan is a monoecious tree with staminate (male) and pistillate (female) flowers on the same tree. Pistillate flowers are borne in terminal spikes on new shoot growth, while pendulous staminate inflorescence are borne on the base of the shoot and along the length of the supporting one-year-old wood (Sparks, 1992; Wetzstein and Baker, 1993). Staminate flowers differentiate during spring on current season growth, with pollen shedding the next spring, whereas pistillate flowers differentiate at the time growth resumes in spring (Wetzstein and Sparks., 1983; Wetzstein and Sparks, 1984). Pollen is produced in large quantities which enhances the chances of pollination by wind, the main type of pollen dispersal. In pecan, the times of anther dehiscence and pistil receptivity differ, a condition referred as to dichogamy. Pecan is heterodichogamous, with different genotypes exhibiting both protandrous (pollen dehiscence prior to stigma receptivity) or protogynous (stigma receptivity prior to pollen dehiscence) flower habits. Dichogamy usually promotes cross-pollination, although a short period of overlap in some cultivars may favor self-pollination. Geographic location and year-to-year variations also affect dichogamy. Therefore, a proper selection of compatible cultivars must be considered for adequate pollination (Sparks, 1992).

Pecan fruits are comprised of the kernel enclosed by the shell, referred to as the nut. The nut is enclosed by the shuck developed from the floral involucre, which dries and opens at maturity, separating from the nut, usually in four valves. Fruit maturation occurs in the fall of the same year and it is directly related to the cultivar and its region of origin (Peterson and Annable, 1990; Sparks, 1992).

1.2. Pecan Nutrition

Nutritional problems frequently limit orchard productivity. These problems may be apparent such as chlorotic leaves or abnormal growth. On the other hand, trees may have no outward appearance of nutritional problems yet production or nut quality is reduced. Diagnostic procedures to identify or avoid nutritional problems have allowed scientists and growers to improve fertility programs for pecan.

Although all the nutrients essential for other plants are likewise essential for pecan, nutritional problems in pecan basically center around N, P, K, and Zn. Nitrogen, P and K become especially critical once the tree reaches reproductive maturity. All tree nutrients are suppressed strikingly in leaf tissue by fruiting and, for this reason, N, P and K have been proposed to be contributing factors to the irregular or alternate bearing habit of pecan. That is, N, P, and K are suppressed during the on year of fruiting and replenished during the off year in the same manner as carbohydrates (Sparks and Payne, 1985). Experiments to test this hypothesis have failed because N, P, and K have not been simultaneously increased to an optimum level. Thus, the idea remains a hypothesis. The failure to increase N, P, and K simultaneously points up the number one problem in pecan nutrition; that is, an inadequate understanding of factors influencing uptake of the nutrients from the soil. In most orchards, more fertilizer is probably being applied than will be utilized by the tree, but the amounts applied are apparently needed to override the factors interfering with uptake. Preliminary data indicate that time of fertilizer application has a dominant effect on tree response (Sparks, 1989). Timing of application needs further study. Also, a better understanding of competitive effects between various nutrients on absorption and soil factors, such as pH, influencing uptake is needed. Because fertilizer is a major expense in pecan production, improving the efficiency of nutrient uptake will result in immediate savings to the grower.

1.2.1. Nitrogen

The lowest recommended N concentration sufficiency range for pecan is 2.3 - 3% in Oklahoma and the highest is 2.7 - 2.9% in Georgia and Alabama (Smith, 1991). The most common minimum sufficiency concentration is 2.5 %. Differences in the minimum sufficiency concentration among the states reflect differences in growing seasons (rainfall, light intensity, temperature, humidity), tree type (native vs. cultivar), dominant cultural practices (irrigated vs. non-irrigated, orchard floor management, intensity of pest control), soil types, and grower and scientist management objectives. Although the minimum sufficiency concentrations for N are different among states, there is a great deal of overlap in the sufficiency ranges among states.

Deficiency is visually evident when mid summer foliage concentration is < 1.7%. As a highly mobile element, the oldest leaves on the shoot are the first to exhibit symptoms of chlorosis. Leaf size and shoot length is reduced in severe cases. Deficient leaves senescence earlier in autumn and exhibit brighter coloration. N uptake by roots can be either as NO_3^- or NH_4^+ and the uptake rates vary throughout the annual growth cycle. Rapid correction is typically by ammonium nitrate, ammonium sulfate, urea, and blends of urea with ammonium nitrate. In commercial orchards, rates of 56-300 kg/ha of N typically correct deficiencies. Excessive leaf N concentration in combination with low leaf K can produce marginal necrosis, especially on young trees. It is avoided by applying K along with N.

Nitrogen application can cause a reduction in the concentration of other elements in the tree. This occurs when tree growth is stimulated by N application, but there is an inadequate supply of another element to meet the needs of increased growth. In one study, N rate was negatively related to leaf K and P concentration (Smith and Cotten, 1985). High N application rates can cause marginal chlorosis or necrosis of leaves called nitrogen scorch (Sparks, 1976). Scorch symptoms, which develop during June or July, occur more frequently on young vigorous trees than on mature trees, and are more common on Desirable than other cultivars. These symptoms appear to be common in

Georgia, rare in Louisiana, and have not been reported in Oklahoma, Texas, Arizona or New Mexico (Smith, 1991). Sparks (1976) reported that leaf scorch appears to be caused by a shortage of K induced by excess N fertilization, but Worley (1990) found that leaf N and K concentration were not closely related to leaf scorch.

Nitrogen applications vary from about 100 to 250 pounds per acre (Sparks, 1991). High applications should be applied only to non prolific cultivars and then only if soil moisture is maintained at an optimum level. Otherwise, more fruit will be set than the tree can adequately develop (Sparks, 1989). Unfortunately, the concept that N is the major growth promoting factor has been elevated to a horticultural principle without supporting data. Consequently, N becomes a cure all and more N is often recommended when low vigor is due to other causes. There are two other misconceptions concerning N. 1) N application, especially late in the growing season, will increase the susceptibility of the tree to winter injury, and 2) N application delays nut maturity. There are no data to support either supposition. In fact, winter injury is not increased by late N application (Sparks, 1991) and time of fruit maturity in pecan is directly dependent on heat units in the springtime (Sparks, 1989). However, as in the case of N vs. tree vigor, the idea that N increases winter injury and delays nut maturity have also unfortunately been elevated to horticultural principles.

Legumes were an extensively utilized N source in pecan orchards in the 1950's and earlier; however, inexpensive commercial N sources caused a decline in their use. Interest in legumes as a cover crop has been renewed by rising N fertilizer prices, environmental issues, and their potential use in integrated pest management programs (Wood et al., 1983). The N fixation potential of legumes is influenced by the legume species, type of *Rhizobium*, environmental conditions, and availability of soil N (White et al., 1981). Studies on pecan indicate that some legumes can supply about 100 kg N / ha to the pecan (White et al., 1981; Wood et al., 1983). Additional research is needed to identify legume species and cultivars that will meet the requirements of an orchard management program.

1.2.2. Phosphorus

Total soil P content is variable in the U.S., but P is generally lower in humid regions of the southwest than in the central and western states (Tisdale et al., 1985). Fertilized soils can be quite high in P since little of this element is lost in percolating water and crop removal is small compared to N and K.

P deficiency symptoms include pale green foliage that may develop interveinal chlorosis. Necrotic tissue near the leaf margin may develop when the shortage is severe, followed by defoliation (Sparks, 1988). These symptoms are more pronounced on shoots with fruit than on shoots without fruit (Sparks and Madden, 1977).

Pecan fruit contain a substantial amount of P that is transported into the fruit in greatest quantities during kernel development (Diver et al., 1984). Most of the P in the fruit is located in the kernel, and the shuck P content is higher than the shell (Sparks, 1975; Diver et al., 1984). Sparks(1977) found that leaves on shoots with fruit had less N, P, K and Zn than leaves on shoots without fruit, and that leaf scorch (necrotic tissue on the leaf margins) and defoliation were more severe when fruit were present on the shoot. Later research by Sparks (1988) implicated low leaf P as the major cause of leaf scorch and defoliation. The greater severity of P deficiency on shoots with fruit appears to result form large quantities of P transported to the fruit during kernel development (Diver et al., 1984).

Leaf P concentrations have also been related to cold damage. Smith and Cotten (1985) found that cold damage of 32-year-old Western trees decreased as leaf N increased from 1.9% to 2.5% and leaf P increased from 0.11% to 0.17%. Trees also had more cold damage as yield per tree increased.

Response of pecan to applied P has been inconsistent. The chief reasons for variable responses are lack of P movement into the root zone from surface applications thus limiting P absorption by the tree (Worley, 1974; Worley et al., 1974; Worley, 1977), the native P content of the soil (Worley et al., 1974) and the nutrient status of the tree (Worley, 1974).

Minimum leaf P concentration recommended for pecan range from 0.1% in Arizona to 0.18% in Georgia (Smith, 1991). Most states use 0.12 % as the minimum acceptable leaf P concentration. Sparks (1986; 1988), based on greenhouse and field tests, suggested the minimum sufficiency level of P for pecan is too low. He found that maximum vegetative growth of greenhouse-grown pecan seedlings occurred when leaf P concentrations were 0.19% to 0.22%. Field studies with adult trees showed that greatest nut growth and minimum leaf scorch and defoliation occurred when leaf P was greater than 0.14% and 0.16%, respectively.

1.2.3. Potassium

Typically, soils of the southeastern and southern coastal plains of the United States are low in K because they are formed from marine sediments that are highly leached (Tisdale et al., 1985). Soils of the mid-south are usually low in K although they are from high K bearing minerals. Because of their age and the climate, leaching has decreased their K content. Soils from the mid and far-western states are typically high in K, except where high rainfall has leached K.

Potassium comprises about 58% of the total elemental content of the fruit (Sparks, 1975). The shuck contains the majority (86%) of the K (Diver et al., 1984), which is returned to the soil when the shucks abscise. Potassium removal by nut harvest is about 10 g/kg of nuts (Smith, 1991).

The most rapid transport of K into the fruit is during the final 30 days before the fruit ripen (Diver et al., 1984). This high demand for K by the fruit decreases leaf K concentrations (Sparks and Madden, 1977; Diver et al., 1984), and can induce K shortage causing leaf scorch or defoliation (Sparks, 1976).

Early symptoms of K shortage are irregular interveinal chlorosis which appears first on older leaves and leaflets. As the shortage becomes more severe, the chlorotic symptoms progress to the younger leaves. Severe K shortage causes a marginal scorch which can spread over most of the leaf margin, eventually causing leaf death (Sparks,

1976). These symptoms frequently developed on young Desirable trees, particularly if N fertilization was high (Smith, 1991). Desirable s greater susceptibility to leaf scorch, and responsiveness to K fertilization (Worley, 1974; Worley et al., 1974) suggest that Desirable may have a greater K requirement than most other cultivars.

Potassium shortages occur frequently in pecan, and when severe may require several years to correct (Smith and Cotten, 1985). Potassium applications to the soil surface of a fine-textured soil required 5 years before leaf K concentrations of mature Western trees were consistently increased (Smith and Cotten, 1985), but only 1 year s application was required to increase leaf K concentrations on young Desirable trees grown in a sandy soil (Worley et al., 1974). Foliar application of K_2SO_4 or KNO_3 either applied with or without NH_4NO_3 and/or urea were not effective in supplying K to adult pecan trees (Smith et al., 1987).

1.2.4. Calcium

Calcium shortages are normally associated with low pH soils. Frequently, either excesses of some micronutrients or shortages of certain macronutrients will occur because of low soil pH before Ca is in short supply. Normally, Ca shortages are corrected by the application of lime which also increases the soil pH. In those instances when the soil pH is within an acceptable range but Ca is low, calcium sulfate can be used without affecting the soil pH.

1.2.5. Magnesium

Mg shortages have been identified in the acid soils of Georgia and Florida (Worley et al., 1975). These shortages of Mg were more severe when orchards had been heavily fertilized with K fertilizer, since K reduces Mg absorption. Worley (1975) found dolomitic limestone was more effective than foliar Mg sprays in correcting these shortages. Similarly, Sparks (1986) reported that foliar Mg applications were not as

effective in increasing leaf Mg concentration and growth of pecan seedlings as root supplied Mg.

1.2.6. Sulfur

Sulfur deficiencies are rare among the nut crops. This is because S is often included in other fertilizers applied to the crop such as P, $(NH_4)_2SO_4$, and $ZnSO_4$. In addition, S is added to the soil from atmospheric pollution. Sulfur deficiency symptoms are similar to those of N deficiency. Leaves have a pale green color and trees lack vigor.

Minimum recommended sufficiency concentrations for S range from 0.25% in Georgia, to 0.1% in Arizona and New Mexico. There has been little research to support these minimum S concentrations, which contributes to the wide range in recommended concentrations among locations. Leaf S concentrations below 0.2% causes a decline in tree growth, and deficiency symptoms were apparent at 0.1% S (Smith, 1991).

1.2.7. Zn

Typical Zn deficiency symptoms include small leaves with wavy margins and interveinal chlorosis. In severe cases, internodes are shortened and shoot tips die, causing a bunchy growth habit. Yields are frequently reduced by Zn shortages prior to visual symptoms.

Zn shortages occur in all areas where pecans are grown. Pecan trees in the southeastern U.S. have responded to soil applied Zn (Worley et al., 1972; Sparks and Payne, 1982) in contrast to in western regions. The major difference is that soils in the western region have a higher soil pH with greater Ca and Mg concentrations which renders the Zn unavailable soon after application. In the southeastern U.S., soil application of 2.2 to 4.4 kg of 36% Zn sulfate per tree, depending on tree size and severity of the Zn shortage has corrected Zn problems (Sparks, 1976). In the western U.S., Zn is applied using 3 to 6 foliar sprays. Storey et al. (1979) reported that $ZnNO_3$ was more

efficient than $ZnSO_4$ in increasing leaf Zn concentration, especially if tank mixed with a urea/ammonium nitrate solution.

1.2.8. Iron

Most Fe shortages that have been observed were induced by cool, wet spring weather but as temperatures increased, deficiency symptoms were alleviated (Smith, 1991).

1.2.9. Boron

Boron excess is a more common problem than B shortage. Symptoms typical of excess B include marginal leaf scorch which extends toward the midrib. Leaves may fall prematurely and if the excess is severe, shoot dieback may occur. Excess B is usually associated with high B levels in the irrigation water (Smith, 1991). In these cases, the only correction method is an irrigation source with acceptable B concentrations.

Recommended boron sufficiency ranges vary among states. New Mexico and Arizona sufficiency ranges are greater than those from other states. These high ranges probably reflect greater B in soils and irrigation water, and pecan tolerance to B rather than the concentration needed for maximum growth and production.

1.2.10. Copper

Little work has been conducted in the field to confirm copper sufficiency ranges(Smith, 1991). Occasional leaf Cu concentrations below sufficiency ranges have been observed, and $CuSO_4$ soil applications have increased leaf Cu concentrations into the sufficiency range.

1.2.11. Mn

No cases of Mn shortage have been documented on pecan. Greenhouse studies have indicated pecan trees require at least 50 ppm leaf Mn for normal growth. In the

field, pecan trees with up to 2500 ppm leaf Mn have been identified with no apparent damage (Smith, 1991).

2. Plant Nutrition

2.1. General

The beneficial effect of adding mineral elements (e.g., plant ash or lime) to soils to improve plant growth has been known in agriculture for more than 2000 years (Marschner, 1997). Justus von Liebig (1803-1873) compiled and summarized the scattered information concerning the importance of mineral elements for plant growth. Liebig s conclusion that the mineral elements N, S, P, K, Ca, Mg, Si, Na, and Fe are essential for plant growth was based on observation and speculation. From the extensive investigations on the mineral composition of different plant species growing on various soils, it was realized that neither the presence nor the concentration of a mineral element in a plant is a criterion for essentiality. Plants have a limited capability for the selective uptake of those mineral elements which are essential for their growth. They also take up mineral elements which are not necessary for growth and which may even be toxic.

The term essential mineral element (or mineral nutrient) was proposed by Arnon (1950). These authors concluded that, for an element to be considered essential, three criteria must be met:

1. A given plant must be unable to complete its life cycle in the absence of the mineral element
2. The function of the element must not be replaceable by another mineral element
3. The element must be directly involved in plant metabolism - for example, as a component of an essential plant constituent such as an enzyme - or it must be required for a distinct metabolic step such as an enzyme reaction.

Most micronutrients are predominantly constituents of enzyme molecules and are thus essential only in small amounts. In contrast, the macronutrients either are constituents of organic compounds, such as proteins and nucleic acids, or act as osmotica. These differences in function are in plant shoots that are sufficient for adequate growth. The values can vary considerably depending plant species, plant age, and concentration of other mineral elements.

2.2. Nitrogen

2.2.1. Function

Depending on the plant species, development stage, and organ, the N content required for optimal growth varies between 2 and 5% of the plant dry weight. When the supply is sub-optimal, growth is retarded; N is mobilized in mature leaves and retranslocated to areas of new growth. Typical N deficiency symptoms, such as enhanced senescence of older leaves, can be seen. An increase in the N supply not only delays senescence and stimulates growth but also changes plant morphology in a typical manner, particularly if the N availability is high in the rooting medium during the early growth. An increase in shoot-root dry weight ratio with increase in N supply takes place in both perennial and annual plant species (Levin et al., 1989; Olsthoorn, 1991), and is more distinct in wheat than maize (Hocking and Meyer, 1991). This increase in shoot-root ratio might even be larger in terms of shoot and root length, a shift which is unfavorable for acquisition of nutrients and water from soil at later growing stages.

N alters plant composition much more than any other mineral nutrient. For example, the dry matter production of ryegrass is increased by N in a typical yield response curve. Simultaneously, the total N content increases, but the contents of the two main storage carbohydrates in grasses, polyfructosans and starch, decrease drastically. In contrast to the carbohydrate content, lignin content in grasses may increase rather than decrease with high N supply as the amino acids phenylalanine and tyrosine are precursors

of lignin synthesis. However, part of this enhancement effect on lignin content in mature tissues may be counteracted by enhanced formation of new leaves with their typically lower lignin content.

The shift in plant composition with increasing N supply reflects a competition for photosynthates among the various metabolic pathways. This competition is modulated by internal and external factors. When the N supply is suboptimal, ammonia assimilation increases both protein content and leaf growth and correspondingly the leaf area index (LAI). As long as the increase in LAI is correlated with an increase in net photosyntheis, the requirement of carbon skeletons for ammonia assimilation does not substantially depress other biosynthetic pathways related to carbohydrates (sugars, starch, cellulose, etc.), storage lipids, or oils. In this N concentration range, the plant composition does not change substantially, but the total production of plant constituents per unit surface area increases. As the N supply is further increased, however, a higher proportion of the assimilated N is sequestered in storage pools, as amides, for example, but in C_3 plants it is also stored in the chloroplasts in the form of Rubisco (Millard, 1988).

2.2.2. Ammonium vs. nitrate

In most soils, ammonium and nitrate are the predominant sources of N that are available for plant nutrition. Although the average ammonium concentrations of soils are often 10-1000 times lower than those of nitrate, the difference in soil concentrations does not necessarily reflect the uptake ratio of each N source. Indeed, the role of ammonium in plant nutrition has probably been under estimated, because most plants preferentially take up ammonium when both forms are present - even if ammonium is present at lower concentrations than nitrate. Ammonium requires less energy for uptake and assimilation than nitrate mainly because nitrate has to be reduced prior to assimilation (Bloom et al., 1992). Optimal plant growth is, however, usually achieved when N is supplied in both forms. The exclusive supply of N as ammonium is harmful to many plant species, and can

cause poor root and shoot growth, and reduced mineral cation contents relative to those of plants receiving nitrate or ammonium nitrate nutrition (Marschner, 1997). In part, growth depression is directly related to ammonium uptake, as the assimilation of ammonium is accompanied by about equimolar proton production. These protons are excreted, most probably due to an increased H^+-ATPase activity leading to an acidification of the rhizosphere. Nitrate is not only an important osmoticum, but also an essential counter-ion for cation translocation in the xylem, and a signal that induces organic acid metabolism and starch synthesis (Stitt, 1999). Moreover, changes in the N source can modify the hormonal balance in the xylem sap, thereby affecting the growth of the shoot (Peuke et al., 1998). Following the transfer of plants from mixed N nutrition to ammonium alone, newly formed leaves are smaller and have fewer cells than those formed before the changes in N nutrition; these changes coincide with a strong decline in cytokinin concentrations in the xylem sap. In addition, exclusive ammonium nutrition increases the xylem concentrations of abscisic acid, a hormone that potentially contributes to the stunted growth phenotype (Peuke et al., 1998).

2.2.3. Genotypic preference of N sources

Plants vary greatly in their ability to absorb and use ammonium and nitrate as their sources of N. Experimental observations indicate that some calcifuge (acid-loving) species are adapted to, or at least tolerate a N supply predominantly in the form of ammonium (Gigon and Rorison, 1972; Ingestad, 1976). Krajina et al. (1973) supplied N in nutrient solutions as nitrate, ammonium, or in a 7: 1 nitrate: ammonium combination buffered at pH 5.5 ± 0.1 and evaluated growth in four species of conifer seedlings: *Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*, *Pinus contorta* Dougl. Ex Loud. var. *contorta*, *Tsuga heterophylla* and *Thuja plicata* Dorm ex D. Don in Lamb. The *P. menziesii* grew best under the nitrate treatment, having heavier leaves and larger leaf areas than plants grown in the combined treatment. Plants receiving the ammonium

treatment either died or were small with the least leaf areas. *P. contorta* was tolerant of all treatments; however, plants supplied with ammonium were significantly larger in terms of dry weight and total leaf area than under other treatments. Fresh weights of the shoots and roots of *T. heterophylla* in the combined and ammonium treatments were similar and significantly larger than plants under nitrate treatment. Like *P. menziesii*, *T. plicata* grew poorly in the ammonium treatment while the nitrate plants were significantly heavier than the seedlings given the combined treatment. Of the four species studied, two preferred nitrate (*P. menziesii* and *T. plicata*) while one preferred either ammonium or a combination (*T. heterophylla*) and one had a preference for ammonium (*P. contorta*). The results of this work reflect the habitats in which these plants have evolved. *P. menziesii* and *T. plicata* are found naturally in soils where nitrification takes place while *P. contorta* and *T. heterophylla* grow better where nitrification does not actively occur (Krajina, 1965).

There seems little doubt that ionic imbalances are a contributing factor to the preferences of coniferous plants for ammonium or nitrate. Those plants which prefer nitrate exhibit symptoms of calcium deficiency when supplied with ammonium (Krajina et al., 1973) whereas plants which display a preference for ammonium show no such deficiencies under the same regime. Nelson and Selby (1974) associated preference for ammonium in *Picea sitchensis* and *Pinus sylvestris* with nitrate-facilitated Fe deficiency, in as much as Fe supplied as Fe chelate to nitrate-grown plants partially alleviated the chlorosis. It was suggested that the chlorosis could be explained by the competitive chelation hypothesis, whereby the excess organic anions produced under nitrate nutrition compete for Fe-binding sites in the cells of both roots and leaves and at the root surface, thereby interfering with the functions of Fe.

An alternative hypothesis has been suggested in relation to members of the family Ericaceae. Colgrove & Roberts (1956) observed better growth of *Azalea* and *Rhododendron obtusum* in ammonium than in nitrate substrate. Ammonium reduced the uptake of other cations, thus reducing tissue pH while nitrate increased base absorption

hence, increasing tissue pH. The increased pH caused Fe inactivation in the plants. Thus Fe chlorosis and other deficiency symptoms appeared. The same principle has been used to explain the inferiority of *Vaccinium corymbosum* L. when grown on nitrate (Cain, 1952, 1954). Chlorosis has also been noticed in young leaves of *Vaccinium myrtilla* L. and *Vaccinium vitis idaea* L. in connection with nitrate nutrition (Ingestad, 1976).

A large number of plants belonging to the family Ericaceae grow predominantly in acid soils and are said to be acid-loving plants or calcifuges . These plants also show a preference for ammonium rather than nitrate N, thus causing confusion and controversy over whether preference for ammonium is really an indirect requirement. For instance, both highbush blueberry and low-bush blueberry grow better with ammonium N than nitrate N (Townsend, 1973).

The literature suggests that most calcifuge plants growing naturally in acid soils, where little nitrification takes place, are adapted to use ammonium in preference to nitrate (Kirkby, 1967; Wiltshire, 1973). For example, Wiltshire (1973) observed that climax species of perennial grass from Rhodesia yielded much more when supplied with ammonium versus nitrate, and showed a much greater preference for ammonium than several other species. It was suggested that grassveld climax in the highveld is dominated by calcifuge species, which are more tolerant of ammonium than their precursors. He noted that the N source affected yield much more than did acidity, and that soils of the highveld are acid and contain few nitrifying bacteria and little nitrate for most of the year. Woolhouse (1966) showed that seedlings of the calcifuge grass *Deschampsia flexuosa* show a much higher rate of fixation of carbon dioxide in the dark than related species which grow naturally on soils where appreciable nitrification takes place. It was suggested that this high fixation rate in the dark may be an adaptation to a predominantly ammonium nutrition, since the tricarboxylic acid cycle intermediately used to detoxicate absorbed ammonium, are most produced through fixation of carbon dioxide in the dark.

2.3. Zinc

The mechanisms responsible for higher Zn acquisition in efficient genotypes of a species are poorly understood. In soybean only a few genes seem to control uptake efficiency or inefficiency (Hartwig et al., 1991). In bean, in the Zn-inefficient cultivar Sanilac, typical Fe deficiency-induced root responses were enhanced under Zn deficiency and the Fe content in the leaves increased (Jolley and Brown, 1991). In the Zn-efficient cultivar Saginaw this behavior was not observed. In graminaceous species such as wheat and barley, release of phytosiderophores may also be enhanced under Zn deficiency (Zhang et al., 1991). It is not clear whether this enhanced release is an expression of a separate regulation of phytosiderophore biosynthesis by Fe and Zn, or more likely, of impaired Fe metabolism under Zn deficiency. In wheat cultivars differences in Zn efficiency found on calcareous soils are related to differences in release of phytosiderophores as occur under Zn deficiency under controlled conditions. The Zn efficient Aroona released much higher quantities of phytosiderophores under Zn deficiency as compared with the Zn inefficient Durati . In contrast, under Fe deficiency both cultivars release similar amounts of phytosiderophores. It well might be that in the Zn efficient Aroona Fe metabolism is more severely imparied under Zn deficiency that in the Zn-inefficient Durati .

Lowland rice cultivars also differ very much in Zn efficiency, particularly when grown in soils of high pH. In Zn-efficient cultivars, high bicarbonate concentrations as well as low root zone temperatures have only small effects compared to Zn inefficient cultivars. Thus Zn efficiency in rice seems to be causally related to increases in root growth by bicarbonate and a better control of organic acid accumulation in the roots as compared with the Zn inefficient cultivar

Several mechanisms have been studied to understand the physiological basis of Zn efficiency: enhancement in root growth (Dong et al., 1995), root uptake and root-to-shoot translocation of Zn (Cakmak et al., 1996; Rengel and Graham, 1996), release of Zn-

mobilizing phytosiderophores from roots (Cakmak et al., 1996; Erenoglu et al., 1996) and internal utilization of Zn (Rengel, 1995).

Although several physiological mechanisms involved in Zn efficiency have been well documented, very little information is available on the genetic control of these mechanisms and identification of the chromosomes affecting Zn efficiency.

2.4. Processes in plant nutrition

2.4.1. Uptake

In the past, studies on ion uptake by roots have mainly concentrated on the role of the free space, carrier mechanisms, ion selectivity, and occurrence of single- or multiple-binding sites at the plasmalemma or the dual mechanism of ion uptake. However, in the last decade increasing attention has been paid to electrophysiological aspects, as well as to physiological and anatomical gradients along the roots and their consequences for uptake transport of ions.

Another field of new interest in ion uptake and mineral nutrition in general is the consequences which arise at the root surface. For example, as in the result either removal of ions (development of a depletion zone at the root surface), difference in cation/anion uptake and the corresponding induced change in pH around the root. These factors are of less importance in a stirred nutrient solution, but can be dominant in ion uptake and in the mineral nutrition of plants growing in solid substrates such as soils.

Additionally certain plant species and genotypes of particular species suffering from Fe deficiency are able to decrease the pH of the substrate by increasing proton efflux (Brown, 1978). In sunflower, for example, this Fe deficiency-induced decrease in the substrate pH is mainly restricted to the apical zone and is correlated with anatomical changes in the roots and marked increase in Fe uptake. Supplying a sparingly soluble inorganic Fe-III-compound (e.g., Fe-III- hydroxide), this Fe-stress-induced pH decrease leads to the mobilization of the substrate with correspondingly periodical changes in both

the Fe nutritional status of the plants and pH fluctuations in the substrate (Venkat-Raju and Marschner, 1972). These effects can be depressed in amplitude but not prevented by an increase in phosphate concentration. The ability of a plant to increase the uptake rate of Fe in response to stress is causally related to an increase in the reducing capacity of its roots (Brown, 1978; Romheld and Marschner, 1979). This response phenomena is typical for so-called Fe-efficient plant species. This stress response offers an interesting insight into a regulatory mechanism of ion uptake controlled by the nutritional status (internal concentration) .

In addition to the pH in the rhizosphere of the root solid substrate interface, ion concentration at the root surface can also be quite different from the average concentration in the substrate. For mineral nutrients such as P and K, the diffusion rate to the root surface usually limits uptake rate, and a depletion zone can occur around the root (Brewster and Tinker, 1970). Tills may restrict the uptake of these ions to the apical region of the roots regardless of a potentially high uptake rate in the basal regions. Both root growth in general and root hair formation in particular (Bhat and Nye, 1974) are therefore of overwhelming importance for mineral nutrition in solid substrates. In contrast they are of much less significance in solution culture where exhaustion zones are of limited importance.

Ion concentration at the root surface can also be much higher than that in the average substrate. This occurs when the uptake rate of an ion (e.g., Ca) is relatively low as compared to the mass flow of solution to the root surface induced by transpiration (Mengel et al., 1969). As a result of this process, the availability of both mineral nutrients (e.g., by precipitation of calcium phosphates) and water (e.g., saline soils) can be depressed. For plants growing in solid substrates and transpiring at a high rate, the concentration of soluble salts at the root surface can be several times higher than in the bulk substrate (Sinha and Shingh, 1976).

Results from studies in solution culture investigating the effect of high salt concentrations, e.g., NaC1, on ion uptake and water status of plants cannot therefore be

extrapolated to solid substrate conditions even when average isoosmotic concentrations in both substrates are considered.

Ion concentration and ion availability at the root surface can also be affected indirectly by microorganisms in the rhizosphere. This subject is of increasing interest in mineral nutrition in general and especially in relation to the actual and potential role of certain associations for N_2 fixation and symbiosis for phosphate uptake (e.g., mycorrhiza by crop plants).

2.4.2. Transport

Our understanding of long-distance transport from roots to shoots is based mainly on analysis of xylem exudate from decapitated plants. The composition of this exudate, however, does not necessarily reflect conditions occurring at sites of excretion of the ions into the root xylem, as resorption by the surrounding xylem can induce considerable changes in ion concentration and relation of ions in the xylem during long-distance transport. Furthermore the contribution made by phloem in vivo to long-distance transport of mineral nutrients from roots to shoots cannot be determined by this method. Only indirect evidence is available for this in vivo transport, as shown in studies comparing forms of N supply (Martin, 1971). The possibility must also be considered that diurnal fluctuations (day-night) occur in the relative importance of mineral element transport in the phloem from roots to shoots, as has been demonstrated for phloem transport into fruits (Hocking et al., 1978).

For some mineral nutrients such as P (Jager de, 1979) and K (Barber, 1979) the uptake rate by the roots seems to be controlled by the shoot rather than by the roots. The mechanism of this feed-back effect from the shoots is not fully understood as yet. For phloem-mobile mineral elements such as P and K, the transmission of information concerning the nutritional status of the shoot may be achieved by the intensity of retranslocation of these mineral elements in the phloem to the roots. In plants where circulation of K from the shoots into the roots as salts of organic acids is important, a regulation of K uptake by the K content of the shoots and thus intensity of K

retranslocation would be a consequence of this model. In the case of phloem-immobile mineral elements such as Ca the removal from exchange sites of the xylem might offer another type of control mechanism.

2.4.3. Allocation

The transpiration rate of a plant organ (e.g., water loss g^{-1} dry wt.) is one the determining factors in the ratio of mineral element import via xylem versus phloem. Compared to fully expanded leaves, the transpiration rate and thus mineral element import via the xylem can be expected to be much lower in developing leaves and particularly fruits, seeds and storage organs (e.g., potato tubers).

During recent years Pate and co-workers (Pate et al., 1974; Pate and Hocking, 1978) have developed a method to calculate the relative importance of xylem and phloem in the transport of mineral elements into the fruits of various legumes. The question of whether uncontaminated phloem sap is obtained by this method is discussed later. In general, mineral element import into legume fruits occurs predominantly via the phloem (Pate and Hocking, 1978). However, for certain elements (e.g., Cu and Fe) considerable diurnal fluctuations occur in the ratio of xylem/phloem import (Hocking et al., 1978), and the ratio may also be dependent on the availability of particular mineral elements in the root medium. This has been demonstrated for Mn. Mn concentrations in the phloem sap below 0.5 ppm are associated with a marked increase in occurrence of a 4 split seed disorder, involving discoloration, spliting, and deformity of seeds (Hocking and Pate, 1977).

In this legume fruit system no data are presented for the two micronutrients, B and Mo. At least for B there is evidence that this element is quite mobile in the phloem of stems and leaves (Oertli and Richardson, 1970) and that fruits and storage organs may be supplied mainly via the phloern. The same is true for growing potato tubers where the influx of xylem fluid is small or unimportant. Only Ca, and no other mineral element, including B, needs to be supplied directly to the tuber surface to maintain a high growth

rate (Krauss and Marschner, 1975). Similarly, during fruit development of *Arachis hypogaea* and *Trifolium subterranean* net influx of xylem fluid is very low, but the B demand for fruit and seed growth is completely met by internal supply, most probably by phloem import (Campell et al., 1975).

2.4.4. Retranslocation

This process is particularly important (a) in perennials in autumn, (b) in both perennials and annuals when uptake is inadequate, and (c) during reproductive stages or storage organ growth. The extent of retranslocation also depends upon the particular mineral element. Occurrence of deficiency symptoms in older leaves (N, K, Mg, P) reflects a high retranslocation rate, whereas the occurrence of deficiency symptoms in only the young leaves (Ca, Fe, Mn, Cu, Zn, Mo, B) demonstrates insufficient retranslocation rates of these mineral elements. This retranslocation from the older leaves takes place in the phloem. It cannot be concluded, however, that the occurrence of the deficiency symptoms on younger leaves reflects inadequate phloem mobility of these mineral elements. Retranslocation from the older leaves involves several steps: (1) mobilization within the leaf cells, (2) transport to the sieve elements, (3) phloem loading, and (4) mobility within the sieve tubes. Phloem mobility is thus only one factor which determines the retranslocation rate of a mineral element from the leaves.

Retranslocation from leaves is particularly important during seed development, as in this growth stage the carbohydrate supply to the roots and thus root activity and ion uptake generally decline rapidly. The extent of retranslocation of certain mineral elements during fruit and storage growth depends upon various factors, such as uptake rate by the roots and sink-size , particularly in relation to source-size .

The high retranslocation rate of N, P, K, and Mg from leaves is in accordance with the occurrence of deficiency symptoms in older leaves insufficiently supplied by the root system. Although somewhat lower than for the macronutrients, the retranslocation rate of the micronutrients Fe, Zn, Mn and Cu is still surprisingly high, considering the

fact that deficiency symptoms of these elements occur only on young, growing parts (leaves), but not on fully expanded leaves.

For the micronutrients, only a certain fraction can be mobilized from fully expanded leaves (Loneragan et al., 1976; Loneragen, 1978). It appears that when the shoot apex or fruits are inadequately supplied, the demand cannot be transformed into a signal strong enough to bring about extensive mobilization and retranslocation from fully expanded leaves. These leaves therefore do not show micronutrient deficiency symptoms. In contrast, in the case of the macronutrients N, K and Mg, this signal seems to be strong enough to induce exhaustion of the older leaves in these particular elements.

A close correlation between concentration and retranslocation rate of mineral elements from older leaves has been clearly demonstrated by Hill et al. (1978; 1979). In wheat, for example, during grain development, leaves with high Cu content lost more than 70% of their Cu, whereas for leaves of Cu-deficient plants the value was less than 20%. This proportion of Cu retranslocation can be increased, however, particularly from Cu- deficient leaves, for example, by shading (Hill et al., 1979). During senescence, induced either by shading or by N deficiency (Hill et al., 1978), a further fraction of Cu is mobilized in these older leaves and is retranslocated. The relatively high retranslocation rate of micronutrients during fruit development is perhaps the result of leaf senescence. The extent of retranslocation may also be causally involved in the development of S deficiency symptoms preferentially either on young or old leaves depending on the level of N supply (Loneragan et al., 1976).

The extent of remobilization of macro- and micronutrients is attracting increasing interest also in connection with selection and breeding of genotypes of high nutrient efficiency . Genotypes which are able to grow well on substrates (soils) with relatively low nutrient availability may differ not only in the uptake and translocation rate of a particular mineral nutrient, but also in mineral nutrient effectivity at cellular levels (compartmentation, solubility etc.) and higher retranslocation rates from older leaves, seeds, and storage organs to younger leaves.

2.4.5. Seasonal variations of carbohydrates

Carbohydrate reserve accumulation and depletion are related to the growth stage of plants. Seasonal variation of carbohydrate in perennial woody plants can be characterized by accumulation and depletion of carbohydrates, in that carbohydrates become depleted with new growth and accumulate throughout the growing season. Leaves in different developmental stages vary in photosynthetic capacity, and there are also temporal changes in source-sink relation with growth phase (Loescher et al., 1990). The rate of photosynthesis is low in very young leaves, increases with leaf age, reaches maximum levels around full leaf expansion, then declines. Early in the season, the growing plant parts obtain their nutrients and carbohydrates by redistribution from deposits stored the previous year in perennial tissues, but after leaf maturation, these organs become material exporters. After fruit ripening, a second extensive redistribution of nutrients and carbohydrates takes place from the senescing leaves of deciduous trees before leaf fall (Smith, 1987; Loescher et al., 1990). Seasonal variation of carbohydrate reserve in apple (*Malus domestica*) is well described in Hansen and Grauslund s report (1973). Carbohydrates are depleted in late spring by the flush of new growth. Sucrose begins to accumulate shortly afterwards and reaches a maximum in late summer. Starch reaches a maximum in early fall, then disappears with the coming of cold weather in late fall, while sucrose level is retained in high concentration throughout the winter.

Carbohydrate reserves decline during early spring when new growth occurs and respiration increases. During the period of rapid new growth, reserved carbohydrates in twigs, stems, and roots decline, and the pattern of export varies with the growth characteristics of the plant species. Hilgeman et al. (1967) studied seasonal variation of carbohydrate in Valencia orange. In their study, total carbohydrates in leaves decreased in early spring when the development of the extremely large number of spring leaves, blossom formation, and initial setting of fruit occur in Valencia orange. Additional demands for carbohydrate during fruit maturation also induced a carbohydrate deficit.

During this period, competition for carbohydrate between young leaves and fruit and mature fruit induced reduced rates of solid accumulation in fruit. After harvest, accumulation of carbohydrates in leaves was reported.

In general, carbohydrate reserve accumulation in perennial parts occurs when the carbohydrate production exceeds its utilization (Kramer and Kozlowski, 1979). An accumulation of reserves in the form of sugar and alcohol-insoluble compounds takes place in the course of the autumn. The level of carbohydrate in perennial parts reach a maximum value just before leaf drop (Siminovitch et al., 1953). With decreasing temperatures, a conversion of starch to sucrose occurs (Hansen and Grauslund, 1973). Worley (1979) reported that soluble sugar and starch exhibited different accumulation patterns in above ground tissues during the winter with starch decreasing and sugar increasing in about ground tissue. Later on, total carbohydrate contents in perennial part decreases during winter, and again rapidly in early spring when new growth occurs and respiration increases.

Seasonal variation of carbohydrate in evergreen trees differs from those of deciduous trees (Kramer and Kozlowski, 1979). Evergreen species accumulate carbohydrates much later into winter and in general seasonal variations in carbohydrate reserves are much smaller in evergreen than in deciduous trees. Trees which grow in seasonal tropical climates, such as *Antiaris africana* (Olofinboba, 1969) and *Theobroma cacao* (Humphries, 1947), show seasonal variation in carbohydrates, although they generally exhibit a limited range of variation in carbohydrate level compared to trees of cooler climates.

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CHAPTER 3

Studies on the Effect of Zinc Supply on the Growth and Nutrient Uptake in Pecan.[1]

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Abstract

Zinc deficiency is a nutrient disorder observed in pecan (*Carya illinoinensis* Wangenh. K. Koch) under field conditions, and can cause distorted leaf growth and severe rosetting of shoots. Conducting zinc studies with pecan in the field have been problematic because zinc nutrition is difficult to control. In this study, zinc nutrient disorders were induced in greenhouse-grown pecan seedlings using hydroponic culture. Zinc efficiency was compared in two pecan seedstocks, Stuart and Curtis by evaluating growth response, nutrient uptake, and leaf nutrient analysis. Zinc deficiency symptoms appeared in plants grown in the absence of zinc after 6 weeks. Deficiency symptoms were characterized by interveinal mottling, followed by interveinal chlorosis, interveinal necrosis, and marginal curling. Symptoms were confined to the youngest most distal three to five leaves. Differences in zinc efficiency between the two seedstock were observed. Stuart exhibited more severe deficiency ratings than Curtis . Zinc supply also had an differential effect on the foliar concentration and content of zinc and other nutrient. Stuart seedstocks grown under minus zinc versus plus zinc conditions exhibited significantly higher foliar concentration of P, Ca, Mg, and Cu, while Curtis leaves contained significantly higher Mn and lower S. Results of this study concur with the observed frequency of zinc deficiencies of the cultivars in the field, i.e. Stuart shows zinc deficiency more frequently then Curtis . This study verifies that in pecan, there are genotypic differences in zinc efficiency, and that hydroponic culture can be utilized for screening and selection.

Introduction

As one of the essential elements in plants, zinc plays a number of roles. Zinc in plants is reported to be involved in membrane integrity, enzyme activation, gene expression and regulation, carbohydrate metabolism, anaerobic root respiration, protein synthesis, structural integrity of ribosomes, detoxification of superoxide radicals, phytohormone activity (e.g., auxin and gibberellic acid), gene structure (zinc finger motif), and disease resistance (Marschner, 1997).

Differences in zinc efficiency of crop species are well documented. Also, within a given species there are distinct differences between cultivars in zinc efficiency. These genotypic differences are closely related to higher uptake rates of zinc by efficient cultivars when grown in zinc-deficient soils (Marschner, 1997). Genetic differences in zinc efficiency are reported in maize (Shukla and Raj, 1976), wheat (Shukla and Raj, 1974), (Graham et al., 1992), barley (Graham et al., 1992) and soybean (Hartwig et al., 1991). Several mechanisms have been studied to understand the physiological basis of zinc efficiency including enhancement in root growth (Dong et al., 1995), root uptake and root-to-shoot translocation of zinc (Cakmak et al., 1996; Rengel and Graham, 1996), release of zinc-mobilizing phytosiderophores from roots (Cakmak et al., 1996; Erenoglu et al., 1996), and internal utilization of zinc (Rengel, 1995).

In pecan (*Carya illinoinensis* Wangenh. K. Koch), nutritional problems frequently limit orchard productivity (Smith, 1991). Zinc deficiency of pecan has been reported since the early 1900's, and is related to soil pH and liming (Sparks, 1976). Zinc deficiency is evident when the mean foliar zinc concentration is less than 40 ppm (Sparks and Payne, 1982). Rosette, a disorder in pecan resulting from inhibition of shoot elongation, has been recognized as a major cultural problem since the early part of last century and discovered to be caused by zinc deficiency (Sparks, 1989). Researchers have reported the physiological impact of zinc deficiency in pecan, which includes diminishing chlorophyll synthesis and gas exchange (Hu and Sparks, 1991), inhibition of reproductive

development (Hu and Sparks, 1990), and decreased carbonic anhydrase activity (Snir, 1983).

Differences in the frequency of Zn deficiency and efficiency in orchards have been observed in different pecan cultivars. However, comparing zinc efficiencies between cultivars in the field is problematic. Zinc nutrition is difficult to control in the field. Plants require minute amounts of zinc for their growth, and it is difficult to prevent zinc input from meteological sources or chemical sources such as herbicides, pesticides and fertilizers. Other micro-environmental differences, such as variations in soil properties, can make zinc treatment differences obscure. Nonetheless, the identification of genotypes with enhanced zinc efficiency would be of great interest to pecan breeding programs if a means of assessing and/or screening genotypes could be developed.

This work presents a method which can be used to assess zinc efficiency in pecan using hydroponic culture. A range of zinc nutrient disorders were induced in greenhouse-grown seedlings collected from Stuart and Curtis pecan trees. Growth responses were evaluated, and nutrient uptake data were combined with leaf nutrient analysis. This method was effective in evaluating differences in zinc utilization.

Materials and Methods

Plant materials. Seedstocks from 2 pecan cultivars were used for this study: Curtis , and Stuart . Stuart has been reported to exhibit zinc deficiency under field conditions, whereas Curtis rarely shows zinc deficiency symptoms (Sparks, personal communication). About 400 seeds from each cultivars were collected and stratified. Seeds were stratified in moist vermiculite at 4 C, in the dark for several months. Seeds were germinated in 12 cm x 12 cm x 20 cm pots filled with perlite. Nutrition were supplied with full strength modified Hoagland s solution (Hoagland and Arnon, 1950) modified for pecan seedling growth as described later. Seedlings 10-15 cm tall with 3 - 5

leaves were obtained 3 weeks after planting. Seedlings used in the study were selected for uniformity.

Hydroponic culture. Seedlings were carefully removed from the perlite, and roots were washed with deionized water. Cotyledons were removed to eliminate them as a source of Zn supply to seedlings. Seedlings were transferred to hydroponic culture conditions in 15 L translucent pots (Rubbermaid Commercial Products, Winchester, VA) that are painted on the outside with stainless steel emulsion paint (Zn free) to exclude light from the root zone. To aerate the root zone, tubing was placed in pots and air was bubbled continuously. Three plants per pot were suspended by foam collars in holes drilled through the lid. Plants were acclimated to solution culture for 1 week in deionized water followed by 1 week in 1/4 strength Hoagland s solution. Elemental concentrations of the 1/2 strength Hoagland's solution were (in M (mg/L)): NO_3 -N 2700 (37.8); NH_4^+-N 2700 (37.8); P 500 (15.5); K 1925 (75); Ca 1500 (60); Mg 1000 (24.3); S 3063 (98); B 8 (0.1); Cl 300 (10.7); Cu 0.16 (0.01); Fe 0.14 (0.01); Mn 4.6 (0.25); Mo 0.06 (0.01). In addition, the Hoagland s solution was modified to contain Zn at either 0 or 1.53 M (i.e. 0.1 mg/L). Nutrient solutions were changed every week.

Experimental design. A randomized complete block design was used with 96 plants (2 seedstocks x 8 replications x 2 Zn concentrations x 3 plants per pot) for the experiment. Data was analyzed using GLM procedure in SAS program (SASInstitute, 1985).

Plant growth parameters. Visual symptoms of zinc deficiency in leaves were rated on a scale with 0 = no symptoms; 1 = interveinal mottling at the tip of leaves; 2 = overall interveinal chlorosis with some necrosis, and 3 = overall interveinal chlorosis, necrosis, and marginal curving. Plant height from the top of the pot, number of leaves, and number of nodes were measured. The dry weight of shoots and roots were recorded

separately. Leaf surface area was measured using a LI-3000 leaf area meter (LiCor, Lincoln, NB) at the conclusion of the experiment prior to drying.

Element analysis. Leaf samples were pooled by pot for analysis in seedstock comparisons. All tissues were washed in deionized water with a mild soap added, then rinsed in deionized water to remove any dust or contamination which could affect nutrient concentrations. Samples were oven dried at 80 C to constant weight then ground with a Wiley mill to pass a 20-mesh sieve. A 0.5 g sample was ashed at 500 C for 8 hours, then dissolved in 20% aqua regia + 10 ppm Cd solution. To determine nutrient uptake by plants, samples were aliquoted from the hydroponic culture solutions before and after plant growth. Uptake was calculated as the difference in concentration between the initial and final nutrient solution samples. Al, B, Ca, Cu, Fe, K, Mg, Mn, Mo, Na, P, and Zn were quantified in both leaf tissues and nutrient solutions using an ICAP (Inductively coupled argon plasma spectrophotometer; Jarrel-Ash, Franklin, Mass.). NO_3^--N and NH_4^+-N were quantified by a continuous flow nitrogen analyzer.

Symptom rating comparison. An additional study was conducted to relate zinc deficiency symptom ratings with zinc foliar concentration. Plants from Curtis seedstocks were prepared and grown under plus zinc and minus zinc nutrition as described above. For symptom rating comparisons, leaves with same symptom rating were pooled and analyzed after 8 weeks grow. Leaf tissue was prepared for elemental analysis as described above.

Results and Discussions

None of the seedlings grown under plus zinc nutrition exhibited any deficiency symptoms. The minus zinc treatment began to exhibit deficiency symptoms after 6 weeks of hydroponic culture, and were confined to the youngest, most distal 3-5 leaves. The

older, more proximal leaves in the no zinc treatment did not exhibit any nutritional disorder symptoms. Visual symptoms of zinc deficiency in leaves were rated on a scale with 0 = no symptoms; 1 = interveinal mottling at the tip of leaves; 2 = overall interveinal chlorosis and some necrosis and 3 = overall interveinal chlorosis, necrosis, and marginal curving (Fig. 1). These characteristics correspond with zinc deficiency symptoms observed in the field by others (Sparks, 1989).

Zinc deficiency symptom ratings were related to zinc concentration when leaves were separated and grouped by severity of deficiency symptom prior to analysis (Table 1). Zinc concentration decreased as the deficiency symptom rating increased. The concentration of zinc in severely chlorotic leaves exhibited necrosis and marginal curling, and was only 65% of zinc levels in leaves showing no symptoms. This confirmed that the aberrant visual characteristics of leaves were induced by low levels of zinc in leaves.

In both Stuart and Curtis seedstocks, plants grown under minus zinc conditions exhibited significantly higher deficiency symptom ratings than corresponding plus zinc plants (Table 2). Seedstocks exhibited a differential response to zinc nutrition. Under minus zinc nutrition, Stuart exhibited symptoms that were significantly more severe than those for Curtis . The 2.2 mean deficiency rating of Stuart leaves in the minus zinc treatment was characterized by leaves exhibiting overall interveinal chlorosis and some marginal curving. In contrast, the 1.5 mean deficiency rating of Curtis was associated with leaves exhibiting only partial interveinal chlorosis and interveinal mottling. In Stuart , foliar zinc concentration was 24% lower in minus zinc than plus zinc treatments, and were significantly different. In contrast, with Curtis , although zinc concentration decreased by 19% in the minus versus plus zinc treatment, the difference was not significant. Lack of significance in foliar zinc concentration between treatments in Curtis are likely a result of dilution by nutrient-sufficient leaves. It should be noted that these data are mean values for whole plant foliar zinc concentrations, and thus included the basal leaves exhibiting no deficiency symptoms.

The differential effects observed between the two seedstocks in this study corresponds with the observed frequency of zinc deficiencies of these cultivars in the field. Stuart shows zinc deficiency more frequently than Curtis . Difference in visual zinc deficiency symptoms between zinc-efficient and zinc-inefficient genotypes have been reported in a number of plant species. In alfalfa (*Medicago sativa* L.), Grewal and Williams (1999) evaluated 13 genotypes and reported that alfalfa genotypes differed markedly in ranking based on zinc deficiency scores that corresponded with the genotype s zinc efficiency. Ambler and Brown (1969) reported that, in navy bean, visual zinc deficiency symptoms, i.e., necrotic and discolored leaves, were more pronounced in Sanilac a zinc-inefficient genotype than in Saginaw a zinc-efficient genotype.

In general, zinc nutrition had little effect on plant growth parameters (Table 3). An exception was total leaf area, that was significantly lower in Curtis minus zinc versus plus zinc treatments, but not significantly different between Stuart zinc treatment. In Stuart , stem dry weight was significant higher in minus zinc than plus zinc nutrition. No significant difference was observed in root dry weight between treatment in both seedstocks. Although shorter internode length and smaller leaves are typical zinc deficiency symptoms in the field (Sparks, 1976; Sparks, 1993), no significant difference was observed in internode length between zinc treatments. In our study, Stuart exhibited more extensive root growth than Curtis regardless of whether zinc was present or absent. Stuart also exhibited a higher root/shoot ratio than Curtis in both treatment. However, no significant differences in root/shoot ratio were observed between zinc treatment in either seedstocks. In wheat, Rengel and Graham (1996) reported that no difference was found between the relative production of root and shoot dry matter at deficient compared to sufficient zinc supply for the two genotypes tested. Dong et al. (1995) reported a significant effect of zinc application on dry matter production in wheat, where a reduction in both root and shoot growth occurred, but of similar magnitude, so that no changes in root/shoot ratio were detected. However, they reported changes in root morphology of a zinc-efficient genotype under zinc-deficient

conditions. The zinc efficient genotype developed roots that were longer and thinner at the early seedling stage. In pecan, however, no differences in root morphology was observed between zinc treatments (data not shown). In wheat, zinc deficiency resulted in an increase in the root/shoot fresh weight ratio, with zinc-inefficient genotypes being affected to a greater extent than zinc-efficient genotypes (Pearson and Rengel, 1997). They suggest that this may reflect the need for the zinc-inefficient genotype to increase the absorptive root surface area proportionally more than the zinc-efficient genotype in order to take up the required amount of zinc. In oilseed rape (Lu et al., 1998), two genotypes exhibited different response of root/shoot ratio between high and low zinc treatments.

Zinc supply had an effect on the foliar content of other nutrients (Table 4). Stuart seedstocks exhibited significantly higher foliar contents of P, Ca, Mg, S, B, and Fe under minus zinc versus plus zinc nutrition. In contrast, Curtis leaves contained significantly higher levels of only Mn. Thus, the content of other nutrients was more affected by zinc nutrition in Stuart than Curtis seedstock, and concurs with the overall more severe response of Stuart in terms of visual symptoms and zinc content. The effect of zinc supply on foliar concentration (Table 5) exhibited similar patterns observed with foliar content (Table 4) except for S, in which no significant difference was observed. Studies evaluating the effect of zinc on the content of other nutrients are limited. Generally, uptake and accumulation of zinc is related with P and other heavy metal elements including Fe, Cu, and Mn (Marschner, 1997). Rengel and Graham (1996) reported that shoot concentrations of Fe, Mn and Cu were higher when plants were grown at deficient than at sufficient zinc conditions in both zinc-efficient and zinc-inefficient wheat genotypes. In our study, mean values of foliar nutrient concentration except N, K, S, and Zn were higher in the minus zinc compared to plus zinc treatment. Decreased mean leaf weight may be a contributory factor.

We were able to induce zinc deficiency in pecan using hydroponic culture and to distinguish zinc efficient- and inefficient-genotypes using hydroponic culture. Our study

shows that hydroponic culture can be utilized for screening genotypic differences in nutritional efficiency, and can be applied to studies with other nutrients. This study verifies that there are genotypic differences in zinc efficiency. Selection and identification of efficient genotypes and their utilization in pecan breeding program would be useful in crop improvement.

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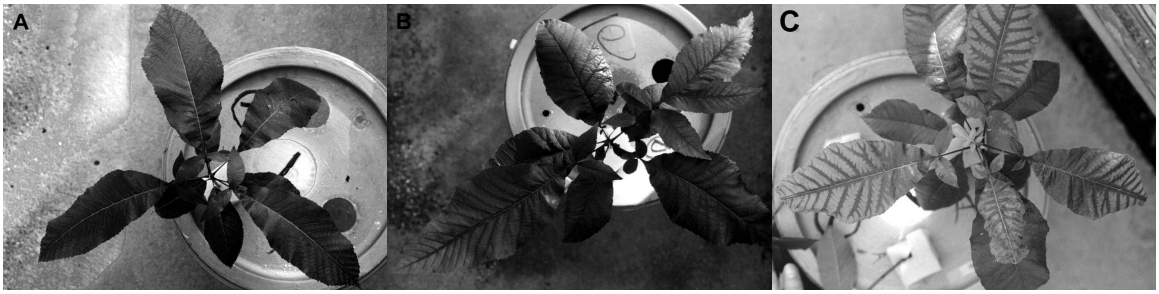

Fig. 1. Foliar zinc deficiency symptoms exhibited in pecan plants grown under minus zinc condition. A) Plant with a deficiency rating of 1, interveinal mottling at the tip of leaves; B) plant with a deficiency rating of 2, overall interveinal chlorosis with some necrosis; C) plant with a deficiency rating of 3, overall interveinal chlorosis, necrosis, and marginal curving.

Table 1. Foliar zinc deficiency symptom ratings and foliar zinc concentration

Deficiency rating	Symptom description	Zinc concentration (ppm)
0	no symptoms	11.15
1	interveinal mottling at the tip of leaves	9.79
2	overall interveinal chlorosis with some necrosis	9.56
3	overall interveinal chlorosis, necrosis, and marginal curving	7.21

Table 2. Effect of zinc supply on visual deficiency symptom ratings, and foliar zinc concentrations in two pecan seedstocks.

Seedstock	Zinc treatment	Deficiency symptom rating [z]	Foliar zinc concentration (ppm)
Stuart	(+) Zinc	0.0	20.7
	(-) Zinc	2.2**	15.8**
	% change [y]		-24
Curtis	(+) Zinc	0.0	26.2
	(-) Zinc	1.5**	21.1
	% change		-19

** significantly differ from control at alpha = 0.05

* significantly differ from control at alpha=0.1

[z] Visual symptoms of zinc deficiency in leaves were rated on a scale with 0 = no symptoms; 1 = interveinal mottling at the tip of leaves; 2 = overall interveinal chlorosis and some necrosis, and 3 = overall interveinal chlorosis, necrosis, and marginal curving.

[y] percentage decrease in minus from plus zinc treatment

Table 3. Effect of zinc supply on mean internode length, leaf area, leaf area index, biomass, and root/shoot ratio.

Seedstock	Zinc Treatment	Total leaf area (cm^2)	Leaf area index (cm^2/g)	Leaf weight (g)	Stem weight (g)	Root weight (g)	Mean internode length (cm)	R/S ratio
Stuart	(+) Zinc	881	229	3.8	21	2.9	2.4	0.12
	(-) Zinc	756	212	3.6	23**	3.1	2.4	0.12
Curtis	(+) Zinc	874	208	4.2	22	2.6	2.6	0.1
	(-) Zinc	638*	199	3.2	22	2.5	2.6	0.1

** significantly differ from control at alpha = 0.05

* significantly differ from control at alpha=0.1

Table 4. Effects of zinc supply on area based foliar content of essential elements in pecan.

Seed stock	Zinc supply	Macro nutrients (%)						Micro nutrients (ppm)			
		N	P	K	Ca	Mg	S	B	Cu	Fe	Mn
Stuart	(+)Zinc	69.0	15.0	69.7	47.9	14.0	6.42	0.20	10.3	1.4	0.63
	(-)Zinc	71.9	21.8***	71.2	63.1***	17.2***	7.14**	0.25**	7.9	1.9***	0.85
	%[z]	+4	+45	+2	+31	+22	+11	+25	-24	+35	+34
Curtis	(+)Zinc	64.9	15.5	63.2	54.6	17.4	6.98	0.21	13.1	2.2	0.52
	(-)Zinc	64.6	17.6	64.1	57.6	18.3	6.55	0.24	10.5	2.6	0.86**
	%	-1	+13	+1	+5	+5	-7	+14	-20	+18	+65

*** significantly differ from control at alpha = 0.01

** significantly differ from control at alpha = 0.05

[z] percentage increase/(+) or decrease/(-) in minus zinc compared to plus zinc treatment

Table 5. Effects of zinc supply on foliar concentration of essential elements in pecan.

Seed stock	Zinc supply	Macro nutrients (%)						Micro nutrients (ppm)			
		N	P	K	Ca	Mg	S	B	Cu	Fe	Mn
Stuart	(+)Zinc	1.61	0.35	1.62	1.11	0.33	0.150	47.7	4.1	344	152
	(-)Zinc	1.53	0.47**	1.52	1.35**	0.37**	0.151	54.7	5.9**	411	187
	%[z]	-5	+34	-6	+22	+12	+1	+15	+44	+19	+23
Curtis	(+)Zinc	1.35	0.32	1.31	1.12	0.36	0.144	45.1	4.8	462	109
	(-)Zinc	1.33	0.36	1.31	1.17	0.37	0.135*	49.6	4.8	548	181*
	%	-1	+13	-	+4	+3	-6	+10	-	+19	+66

**: significantly differ from control at alpha = 0.05

*: significantly differ from control at alpha=0.1

[z] percentage increase/(+) or decrease/(-) in minus zinc compared to plus zinc treatment

CHAPTER 4

Cytological and Ultrastructural Evaluations of Zinc Deficiency in Leaves[2]

[2] Tehryung Kim, Harry A. Mills, and Hazel Y. Wetzstein. To be submitted to *Journal of the American Society for Horticultural Science*

Abstract

A microscopic evaluations of zinc deficiency were conducted of pecan leaves (*Carya illinoinensis* Wangenh. K. Koch) using light and transmission electron microscopy. Zinc deficiency was induced using hydroponic culture. Zinc deficiency symptoms varied with severity ranging from interveinal mottling, overall chlorosis, necrosis, to marginal curving. Changes in leaf thickness and mesophyll cell organization were associated with zinc deficiency. Cells in zinc deficient leaves had limited cytoplasmic content and accumulated phenolic compounds in vacuoles. Extensive starch accumulation was observed in chloroplasts. This work represents the first detailed microscopic evaluations of zinc deficiency in leaves.

Introduction

Zinc is an essential micronutrient and involved in a number of important physiological activities. Zinc plays a role in membrane integrity, enzyme activation, gene expression and regulation, carbohydrate metabolism, anaerobic root respiration, protein synthesis, structural integrity of ribosomes, detoxification of superoxide radicals, phytohormone activity (e.g., auxin and gibberellic acid), gene structure (zinc finger motif), and disease resistance (Marschner, 1997). Reduced carbonic anhydrase level (Edwards and Mohamed, 1973; Randall and Bouma, 1973), and impaired Hill reaction activity (Spencer and Possingham, 1960) have been reported in zinc-deficient plants. Zinc deficiency is one of the most widespread nutritional disorders in plants grown under a wide range of soils, and is a common feature of both tropical and temperate climates (Graham et al., 1992; Rengel, 1995; Cakmak et al., 1996). However, earlier studies have generally been limited to evaluations of growth, yield, and visual symptoms. In spite of its importance in physiological and developmental aspects of plant function, little information on the cytological and ultrastructural effects of zinc deficiency exist.

In pecan (*Carya illinoinensis* Wang K.Koch), nutritional problems frequently limit orchard productivity (Smith, 1991). Zinc deficiency in pecan has been reported since the early 1900's, and is related to soil pH and liming (Sparks, 1976). Rosette, a disorder in pecan resulting from inhibition of shoot elongation, has been recognized as a major cultural problem since the early part of the last century and discovered to be caused by zinc deficiency (Sparks, 1989). Researchers have reported the physiological impact of zinc deficiency in pecan, which includes diminishing chlorophyll synthesis and gas exchange (Hu and Sparks, 1991), inhibition of reproductive development (Hu and Sparks, 1990), and decreased carbonic anhydrase activity (Snir, 1983). However, anatomical changes associated with the zinc deficiency have not been studied. One of the main reasons is that zinc nutrition is difficult to control in the field. Plants require minute amounts of zinc for their growth, and it is difficult to prevent zinc input from

meteological sources or chemical sources such as herbicides, pesticides and fertilizers. Furthermore, uptake of applied zinc is variable.

In our previous work, zinc deficiency was induced in pecan seedlings using hydroponic culture (Kim et al., In prep.). The nutrient supply to plants during their development was critically controlled using this method. Plants exhibited a range of zinc deficiency symptoms, and foliar levels of zinc were related to the severity of deficiency symptoms. The objectives of this study were to define cytological and ultrastructural characteristics associated with zinc deficiency symptoms.

Materials and Methods

Plant materials. Pecan (*Carya illinoinensis* Wang. K. Koch) seeds collected from Curtis trees were stratified in moist vermiculite at 4 C, in the dark for several months. Seeds were germinated in 12 cm x 12 cm x 20 cm pots filled with perlite. Nutrition were supplied with full strength Hoagland s solution (Hoagland and Arnon, 1950) modified for pecan seedling growth as described later. Seedlings 10-15 cm tall with 3 - 5 leaves were obtained 3 weeks after planting. Seedlings used in the study were selected for uniformity.

Hydroponic culture. Seedlings were carefully removed from the perlite and roots were washed with deionized water. Cotyledons were removed to eliminate them as a source of zinc supply to seedlings. Seedlings were transferred to hydroponic culture conditions in 15L translucent pots (Rubbermaid Commercial Products, Winchester, VA) that are painted on the outside with stainless steel emulsion paint (zinc free) to exclude light from the root zone. To aerate the root zone, tubing was placed in pots and air was bubbled continuously. Three plants per pot were suspended by foam collars in holes drilled through the lid. Plants were acclimated to solution culture for 1 week in deionized water followed by 1 week in 1/4 strength Hoagland s solution. Elemental concentrations of the 1/2 strength Hoagland's solution were (in M (mg/L)): NO_3 -N 2700 (37.8); NH_4^+-

N 2700 (37.8); P 500 (15.5); K 1925 (75); Ca 1500 (60); Mg 1000 (24.3); S 3063 (98); B 8 (0.1); Cl 300 (10.7); Cu 0.16 (0.01); Fe 0.14 (0.01); Mn 4.6 (0.25); Mo 0.06 (0.01). In addition, the Hoagland s solution was modified to contain zinc at either 0 or 1.53 M (i.e. 0.1 mg/L). Nutrient solutions were changed every week.

Microscopy. Tissues were obtained from the middle region of the leaf blades adjacent to the main veins. Samples were fixed in 2% glutaraldehyde with 0.1 M sodium cacodylate buffer (pH 7.2), then post-fixed in 1% osmium tetroxide with 0.1 M sodium cacodylate buffer (pH 7.2). Samples were dehydrated in a graded ethanol series after several washes with the 0.1M sodium cacodylate buffer, then embedded in Spurr s medium (Spurr, 1969). Blocks were sectioned with an MT6000-XL ultramicrotome (RMC Inc., Tuscon, AZ). For light microscopy, thick sections (about 300 nm) were collected on glass microscope slides and stained with toluidine blue. The slides were examined with a Zeiss microscope (Carl Zeiss, Thornwood, NY). For transmission electron microscopy, thin sections (<100 nm) were collected on Formvar-coated, gilded copper slot grids (Ted Pella, Redding, CA) and placed on Formvar-coated bridges to dry. The sections were poststained with 4% (w/v) aqueous uranyl acetate and Reynold s lead citrate (Reynold, 1963). The sections were examined with a Zeiss EM 902A transmission electron microscopy (LEO Electron Microscopy, Thornwood, NY) at 80 kV.

Results and Discussions

None of the seedlings grown with added zinc nutrition exhibited any deficiency symptoms. Leaves from plants grown in the presence of zinc (Fig. 1A) were uniformly dark green in color, characteristics of leaves with sufficient nutrient levels. Deficiency symptoms began to appear after 6 weeks of hydroponic culture plants grown in the absence of zinc, and were confined to the youngest, most distal 3-5 leaves. The older, more proximal leaves of the shoot in the no zinc treatment failed to exhibit any nutritional

disorder symptoms. Lack of zinc supply induced deficiency symptoms that ranged from mild to severe. Leaves with mild deficiency symptoms (Fig. 1B) were predominantly green but had interveinal mottled regions. Chlorosis was limited to the tip of the leaf, but more than 50% of the leaf surface was a healthy dark green color. Leaves with intermediate symptoms (Fig. 1C) exhibited overall interveinal chlorosis. Some necrotic spots were observed also. Leaves with severe symptoms (Fig. 1D) had overall chlorosis, necrosis, puckering, and distorted cup-shaped leaf blades. These characteristics correspond with zinc deficiency symptoms observed in the field by others (Sparks, 1993). Zinc is known as an immobile element in plants (Mengel and Kirkby, 1982). Symptoms occur at the tip of young leaves first. Size decrease, including small leaves and rosetting, due to zinc deficiency have been reported in peach (Arce et al., 1992), citrus (Bell et al., 1997), apple (Ganai et al., 1982), tomato (Parker, 1992), navy bean (Jolley and Brown, 1991), and wheat (Cakmak et al., 1996).

Leaves which developed under complete nutrient conditions exhibited typical features of pecan leaves (Fig. 2A). The upper and lower epidermis were uniserate, and mesophyll cells were differentiated into distinct palisade and spongy regions. The palisade cells had few intercellular spaces. Both palisade and spongy mesophyll cells had abundant cytoplasm, numerous chloroplasts, and few vacuoles. Spongy cells had extensive intercellular spaces. In contrast, zinc deficient leaves (Fig 2B-D) exhibited divergent cell arrangements. Leaves showing mild visual symptoms (Fig. 2B) had palisade cells which were wider, shorter, and more vacuolate than leaves with sufficient zinc. Intercellular spaces were more numerous. Epidermal and spongy mesophyll cell size increased in deficient leaves. Spongy cells were highly vacuolate. In leaves exhibiting intermediate zinc deficiency ratings (Fig. 2C), cellular deficiency characteristics became more exaggerated. Palisade cells were progressively shorter and wider. Additionally, phenolic components were found in the vacuoles, especially in palisade cells. In leaves with severe deficiency symptoms (Fig. 2D), mesophyll cells developed numerous small vesicles. Loss of cellular integrity was observed in some area.

Anatomical characteristics of zinc deficient leaves are shown in Table 1, where leaf thickness, palisade layer thickness, spongy layer thickness, epidermis cell height, and palisade cell width were quantitated in leaves with different symptom ratings. As symptoms became more severe, leaf thickness and the height of the palisade and spongy layers decreased. Epidermal cell height increased with symptom rating. The number of palisade cells per length along the leaf surface decreased as a result of wider palisade cell interspersed with more extensive intercellular spaces. Anatomical differences were more marked as the symptoms in leaves progressed up to intermediate-deficiency ratings. Leaves exhibiting intermediate versus severe deficiency symptoms showed little difference in anatomical characteristics.

Transmission electron microscopy of leaves with intermediate symptoms are presented to illustrate the ultrastructural characteristics of zinc deficiency (Fig. 3). In leaves with mild deficiency symptoms, structures varied depending upon the location of cells evaluated. Leaf chlorosis was mottled and interspersed with predominantly green sectors exhibiting no deficiency symptoms. Likewise, descriptions of leaves with severe deficiency were deemed less informative in that. Severely deficient leaves had increasing necrotic areas, characterization of which would provide limited insight into changes induced by lack of zinc. Furthermore, only minor anatomical differences were observed in comparisons between leaves with intermediate versus severe deficiency (Table 1).

Leaves from plants grown with zinc had elongate palisade cells which were closely packed with few intercellular spaces (Fig 3A). Cells had a high cytoplasmic content and limited vacuolar space. Palisade cells exhibited well-delimited nuclear structures, and mitochondria were prevalent (Fig 3B). Chloroplasts had osmiophilic lipid globules, and internal membranes were organized into granal stacks (Fig. 3C). Starch was observed occasionally in the chloroplasts. Cells in the spongy mesophyll also exhibited high cytoplasmic content and dominant chloroplasts with occasional starch grain formation (Fig. 3D). In zinc deficient leaves, palisade cells were more irregularly shaped and had more extensive intercellular spaces (Fig. 4A). Chloroplasts were still

prevalent but characterized by extensive starch deposits (Fig. 4A, B). Internal membranes maintained their integrity and osmiophilic globules were observed. Cells had extensive vacuolar spaces which frequently had extensive accumulations of phenolic compounds (Fig. 4A, C). The number of mitochondria were lower than in zinc sufficient leaves. Spongy cells in zinc-deficient leaves exhibited significant formation of vacuoles with the cytoplasm restricted to parietal areas (Fig. 4D). Phenolic accumulation occurred within the vacuoles. In some cases, loss of cell integrity was associated with precipitates in cytoplasm region (Fig. 4E). The plasma membrane separated from the cell wall in some areas (Fig. 4F).

Prior to the current study, cytological and ultrastructural characteristics associated with zinc deficiency have not been well defined. Samsidar and Gomez (1980) conducted structural evaluations of a number of macro- and micro-nutrient deficiencies in *Hevea*. Similar to our results, they reported that zinc deficient leaves exhibited decreased palisade cell length, increased intercellular space, and extensive accumulation of starch grains in chloroplasts. However, their microscopic evaluations were limited, and induction of deficiencies was not described.

The accumulation of starch observed in zinc deficient pecan leaves concurs with biochemical studies reporting an accumulation of sucrose and starch in other species including cabbage and bean (Sharma, 1983; CakMak et al., 1989). Accumulation of carbohydrates was attributed to impaired phloem export. In contrast, zinc deficiency decreased sucrose concentration and sucrose synthetase activity in sugarbeet (Brown et al., 1993) and maize (Shrotri et al., 1993). Brown et al. (1993) attributed decreased starch formation under zinc deficiency to a reduction in starch synthetase activity.

Plants exhibited various zinc deficiency symptoms ranging from mild (interveinal mottling), intermediate (overall interveinal chlorosis and necrotic spots) to severe (extensive necrosis and marginal curving) conditions. Structural differences associated with zinc deficiency include changes in leaf thickness and mesophyll cell organization. Cells in zinc deficient leaves had limited cytoplasmic content and accumulated phenolic

compounds in vacuoles. Extensive starch accumulation was observed in chloroplasts. Unlike studies using field-collected material, the differences observed were definitely due to zinc deficiency in that the current study controlled the level of other nutrients using hydroponic culture and were confirmed by nutrient analysis in another study. This work represents the first detailed microscopic evaluations of zinc deficiency in leaves.

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Table 1. Leaf thickness, palisade layer height, spongy layer height, epidermis cell height, and number of palisade cells per unit area for different zinc deficiency symptom ratings.

Symptom rating	Leaf thickness (um)	Palisade layer height (um)	Spongy layer height (um)	Epidermis cell height (um)	# palisade cell per 100 um
No symptom	95 ± 2.2 [z]	40 ± 1.6	39 ± 1.4	8 ± 0.7	12 ± 0.7
Mild	89 ± 2.4	36 ± 1.0	38 ± 2.5	10 ± 0.4	11 ± 0.9
Intermediate	82 ± 4.3	29 ± 2.6	36 ± 2.2	11 ± 1.0	10 ± 0.6
Severe	83 ± 1.5	30 ± 2.2	35 ± 1.5	11 ± 0.6	8 ± 0.2

[z] mean ± standard error

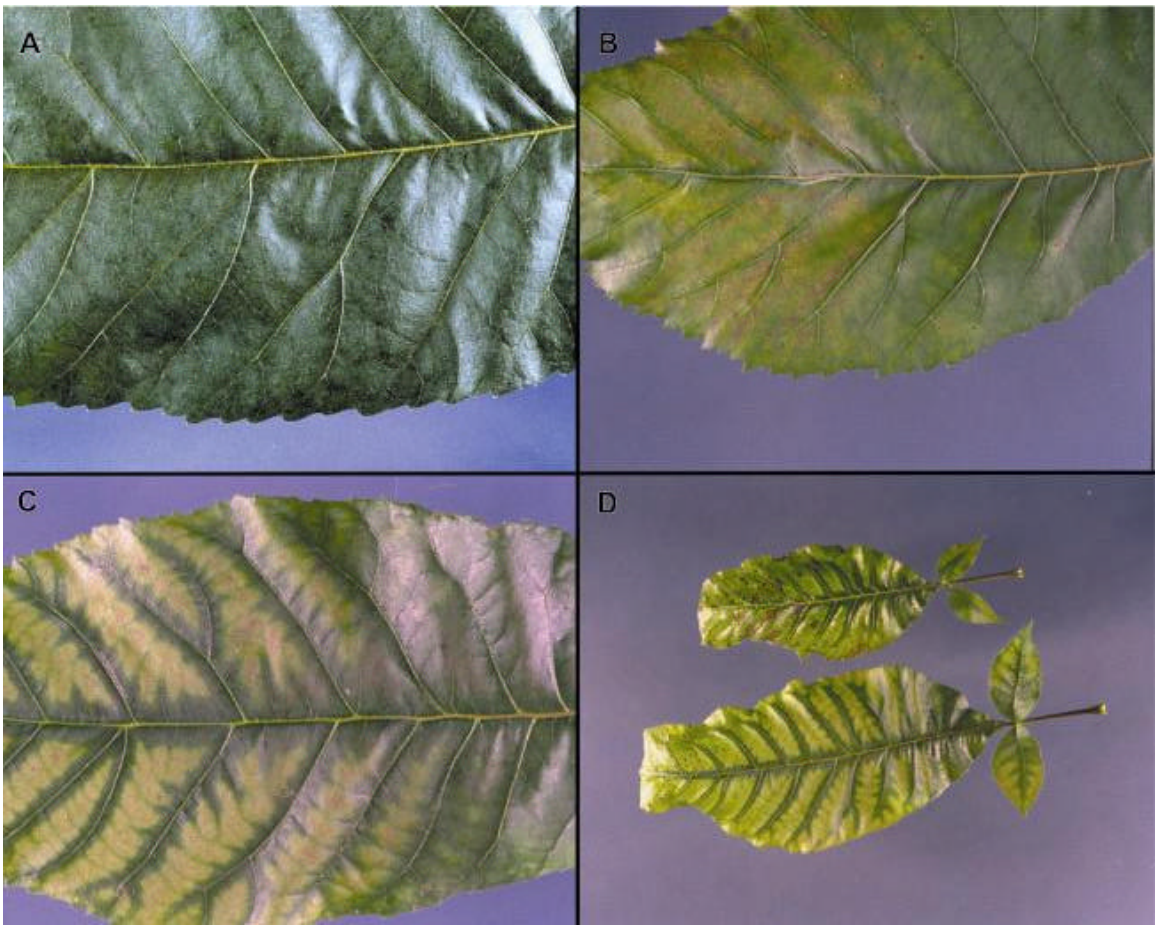

Fig. 1. Foliar symptoms of zinc deficiency. A: no symptom, B: mild, C: intermediate, D: severe.

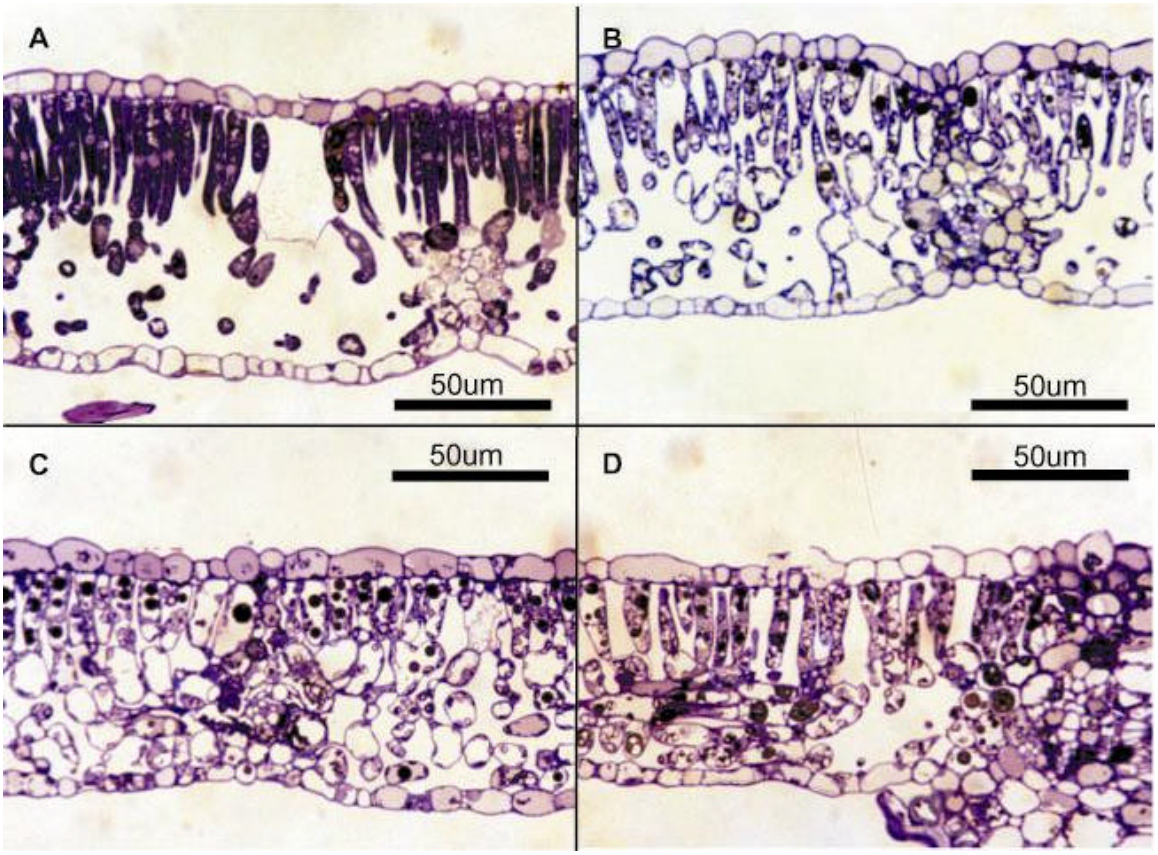

Fig. 2. Light micrographs of cross sections from leaves exhibiting different zinc deficiency symptoms. A: control leaf grown in the presence of zinc and exhibiting no deficiency symptoms, B: leaf with mild deficiency symptoms, C: leaf with intermediate deficiency symptoms, D: leaf with severe deficiency symptoms.

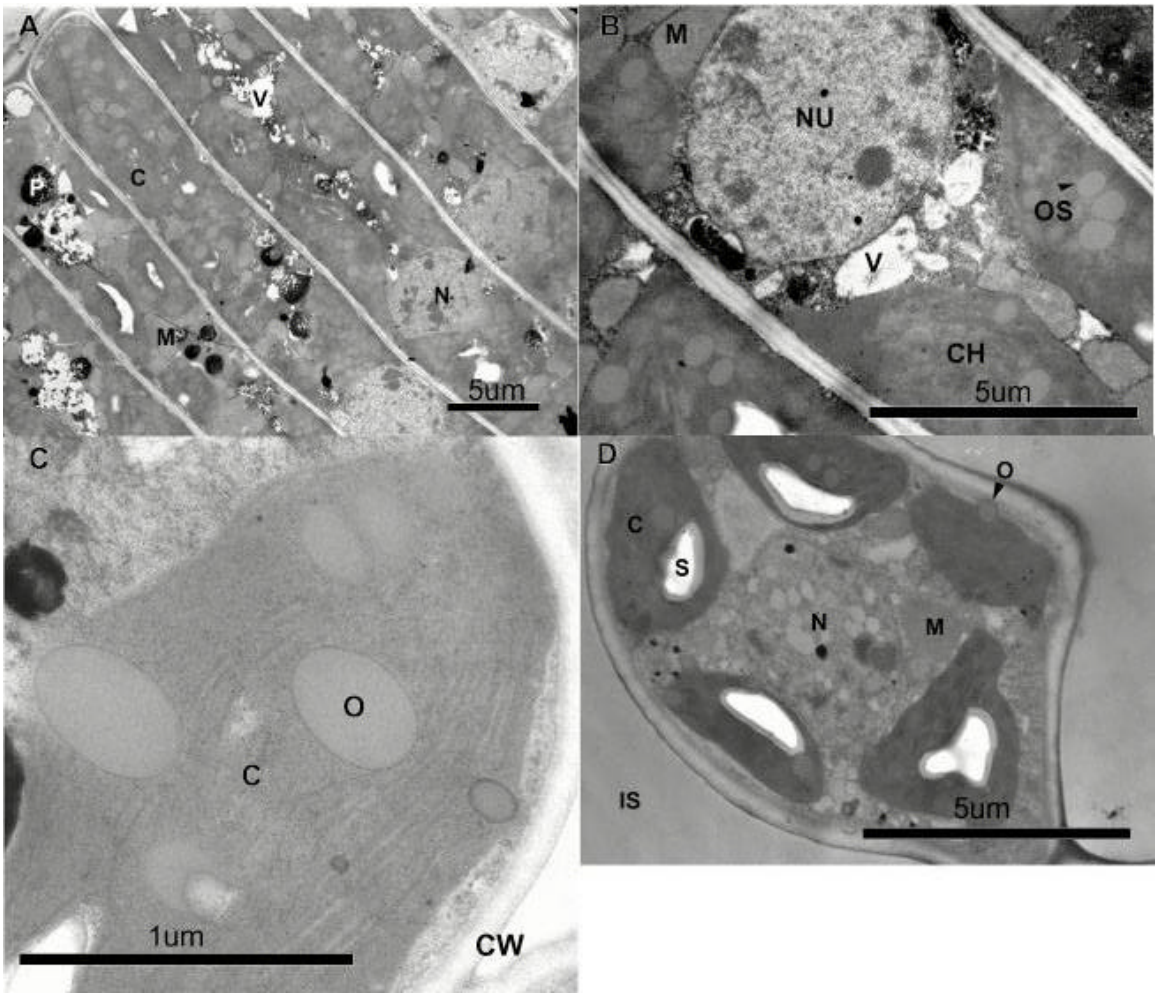

Fig. 3. Transmission electron micrographs of control leaves grown in the presence of zinc. A: elongated palisade parenchyma cells were closely packed with few intercellular space. Cells had a high cytoplasmic content and limited vacuolar spaces. B: Higher magnification showing nucleus and numerous chloroplasts. C: Chloroplast with osmiophilic globules and granal stacks. D: Spongy mesophyll cell showing nucleus and chloroplasts with occasional starch grain formation.

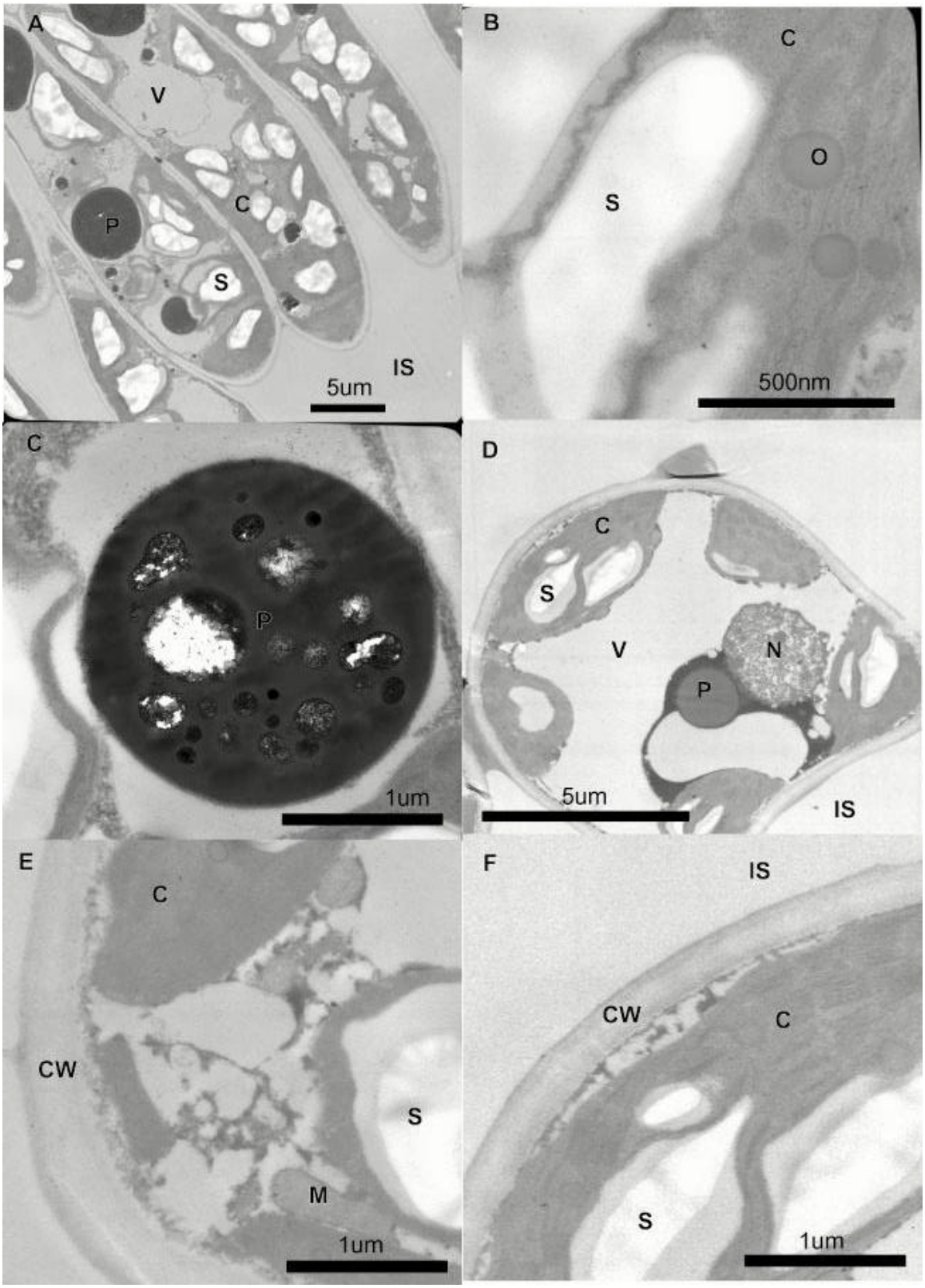
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Fig. 4. Transmission electron micrographs of zinc deficient leaves with intermediate deficiency symptoms. A: Palisade parenchyma were irregularly shaped and had extensive intercellular spaces. B: Higher magnification of a chloroplast showing numerous starch deposits. C: Vacuolar region showing accumulation of phenolic components in a palisade parenchyma cell. D: Spongy mesophyll cell with large central vacuole and parietal cytoplasm; chloroplasts had numerous starch deposits. E: Region of a spongy parenchyma cell showing vesicle formation and breakdown of cytoplasm. F: Region adjacent to cell wall showing separation of the plasma membrane.

CHAPTER 5

Studies on the Effects of Nitrogen Form on Growth, Development and Nutrient Uptake in Pecan[3]

[3] Tehryung Kim, Harry A. Mills, and Hazel Y. Wetzstein To be submitted to *Journal of Plant Nutrition*

Abstract

Leaf nutrient analysis coupled with fertilizer applications are routinely used in commercial pecan production. Nitrogen can be applied in a range of formulations. However, definitive studies to establish nitrogen uptake preference in pecan are lacking, as are definitive studies on the effects of nitrogen form on plant biomass production. In this study, we evaluated the effects of nitrogen form on plant growth and nutrient uptake in pecan (*Carya illinoinensis* Wangenh. K. Koch) using hydroponic cultures. Plants were grown under three nitrogen ratios of ammonium:nitrate (25:75, 50:50, and 75:25). Results from this study indicate that increasing ammonium nutrition inhibited seedling growth. Plants grown with the 75:25 ratio (ammonium:nitrate) exhibited significantly lower biomass, decreased root/shoot ratio, and lower specific leaf weight than observed in the other treatments. The highest root growth was observed in the 50:50 ratio treatment (ammonium:nitrate). Levels of Ca, Mg, and Mn in leaves were higher in plants grown under 25:75 (ammonium:nitrate) than in 75:25. Total nitrogen uptake on a dry weight basis was highest in the 75:25 ammonium:nitrate treatment. Plants exhibited preferential uptake of ammonium nitrogen under all nitrogen regimes. Ammonium nitrogen is generally applied in pecan orchard practices. Our data suggest that further studies evaluating the effects of nitrogen form are warranted to determine if similar detrimental effects on pecan growth occur in the field. Such studies would be useful for optimizing current fertilization practices.

Introduction

Pecan growers have been able to improve growth and yield with improved fertilization regimes. However, nutritional problems frequently limit orchard productivity (Smith, 1991). In commercial pecan production, leaf analysis and fertilizer applications are routinely used. Nitrogen applications vary from about 100 to 250 pounds per acre (Sparks, 1991). The recommended nitrogen sufficiency range in leaves varies. For example, levels of 2.3 - 3% are recommended in Oklahoma compared to 2.7 - 2.9% in Georgia and Alabama (Smith, 1991). Nitrogen deficiency is visually evident when mid summer foliage concentration is lower than 1.7%. As a highly mobile element, the oldest leaves on the shoot are the first to exhibit symptoms of chlorosis. Leaf size and shoot length is reduced in severe cases. Rapid correction is typically by application of ammonium nitrate, ammonium sulfate, urea, and blends of urea with ammonium nitrate. In commercial orchards, rates of 56-300 kg/ha of nitrogen typically correct deficiencies.

Nitrogen application can cause a reduction in the concentration of other elements in pecan. This occurs when tree growth is stimulated by nitrogen application, but there is an inadequate supply of another element to meet the needs of increased growth. Smith (1985) reported that nitrogen rate was negatively related to leaf potassium and phosphorus concentration. High nitrogen application rates can cause marginal chlorosis or necrosis of leaves called nitrogen scorch (Sparks, 1976). Growth in pecan is soil pH sensitive (Sparks and Madden, 1977). Growth increases strikingly between a soil pH of 4.5-6.1. A very rapid and pronounced change in pH due to application of nitrogen fertilizers has been reported. Those side effects of nitrogen application are related to the form of nitrogen form.

Nitrate and ammonium are the major forms of inorganic nitrogen taken up by higher plants. Whether ammonium or nitrate as a form of nitrogen supply is better for plant growth and yield depends on the plant species (Haynes and Goh, 1978; Marschner, 1997). Ammonium and nitrate comprise about 80% of the total cations and anions taken

up by plants. Thus the form of nitrogen supply has a strong but inverse impact on the uptake of other cations and anions, on cellular pH regulation, and on rhizosphere pH (Wiren et al., 2000). Moreover, changes in the nitrogen form can modify the hormonal balance in the xylem sap, thereby affecting the growth of the shoot (Gao et al., 1992).

Nutritional studies evaluating the effects of nitrogen form on growth, development and nutrient uptake in pecan are lacking, in part due to difficulties inherent to field studies. The objectives of this study were to ascertain if pecan exhibits a nitrogen form uptake preference, and to evaluate the effects of nitrogen form on biomass accumulation and the uptake of other essential elements. Such information can be used to refine fertilization schemes and our understanding of pecan nutrition.

Materials and Methods

Plant materials. Seeds were collected from 'Stuart' pecan trees. To eliminate the need to stratify seed, the shell was carefully removed without damaging the embryo/cotyledons. Seeds were germinated in 12-inch pots filled with perlite with 10 seeds planted per pot. Nutrition was supplied with full strength modified Hoagland's solution for pecan. To prevent fungal infection, 0.05% Captan was applied with watering. Seedlings 10-15 cm tall with 3 - 5 leaves were obtained 3 weeks after planting. Uniform seedlings were selected for transfer to hydroponic culture.

Hydroponic culture. Seedlings were carefully removed from the perlite media, roots were washed with water, and the cotyledons were removed to eliminate these as a form of nutrients. One seedling was transferred into each 4 L container with light excluded, suspended by a foam collar. Air was bubbled continuously into each pot to provide aeration. Plants were acclimated to solution culture for 1 week in deionized water followed by 1 week in 1/4 strength Hoagland's solution. Thereafter, plants were transferred and maintained in a 1/2 strength Hoagland's solution. Elemental

concentrations of the 1/2 strength modified Hoagland's solution were (in M (mg/L)): N 5400 (75.6); P 500 (15.5); K 1925 (75); Ca 1500 (60); Mg 1000 (24.3); S 3063 (98); B 8 (0.1); Cl 300 (10.7); Cu 0.16 (0.01); Fe 0.14 (0.01); Mn 4.6 (0.25); Mo 0.06 (0.01); Zn 1.53 (0.1). Keeping nitrogen levels constant, three ratios of ammonium:nitrate were used: 25:75, 50:50, and 75:25. Nutrient solutions were changed every week. Each treatment was replicated 5 times in a completely randomized design. Data was analyzed using GLM procedure in SAS program (SAS Institute, 1985).

Plant growth parameters. After 6 weeks of growth, plants were destructively harvested. Plant height and number of leaves (> 1cm) were recorded. Leaf surface area was measured using a LI-3000 leaf area meter (LiCor, Lincoln, NB). The roots and shoots were separated, and oven dried. Leaves were pre-washed and used in elemental analysis as described below.

Element analysis. Leaf samples from each plant was pooled for analysis. All tissues were washed in deionized water with a phosphate-free mild soap added, then rinsed in deionized water to remove any dust or contamination which could affect nutrient concentrations. Samples were oven-dried at 80 C to constant weight then ground with a Wiley mill to pass a 20-mesh sieve. A 0.5 g sample was ashed at 500 C for 8 hours, and then dissolved in 20% aqua regia + 10 ppm Cd solution. To determine nutrient uptake by plants, samples were aliquoted from the hydroponic culture solutions before and after plant growth. Uptake was calculated as the difference in concentration between the initial and final nutrient solution samples. Al, B, Ca, Cu, Fe, K, Mg, Mn, Mo, Na, P, and Zn were quantified in both leaf tissues and nutrient solutions using an ICAP (Inductively coupled argon plasma spectrophotometer; Jarrel-Ash, Franklin, Mass.). Ammonium and nitrate were quantified by a continuous flow nitrogen analyzer. Foliar accumulation of essential elements was calculated by leaf dry weight x leaf concentration.

Results and Discussions

Growth parameters.

Plants grown under the highest ammonium treatment exhibited stunted growth with smaller, thicker and darker green-colored leaves than other treatments. However, foliar nutrient disorder symptoms including chlorosis and necrosis were not observed. Roots grown under the highest ammonium treatment exhibited reduced growth, with thinner, less branched, shorter and darker colored lateral roots than other treatments (Fig. 1). Similar detrimental effects of growth due to enhanced ammonium nutrition has been reported in a number of plant species including artichoke (Elia et al., 1996), swiss chard, fennel, celery (Santamaria et al., 1999), rocket salad plant, chicory (Santamaria et al., 1998), pansy (Hamlin et al., 1999), and poinsettia (Scoggins and Mills, 1998). General deleterious effects of high ammonium are characterized by an immediate restriction in growth rate, wilting, marginal necrosis and interveinal chlorosis of terminal leaves, and finally death of the entire plants. In addition, high ammonium has been reported to change root morphology. Artichoke (*Cynara scolymus* L.) plants grown with ammonium treatments of 100% and 70% were stunted, with signs of marginal leaf necrosis, progressive wilting of leaves and exhibited poor root growth (Elia et al., 1996). Similar stunted growth due to ammonium have been reported in swiss chard, fennel, celery (Santamaria et al., 1999), rocket salad plant, chicory (Santamaria et al., 1998), pansy (Hamlin et al., 1999), and poinsettia (Scoggins and Mills, 1998). Warncke and Barber (1973) reported darker and less branched roots in corn plants grown with ammonium nitrogen. They attributed the observed decrease in root production to greater acidity around the roots. Elia et al. (1996) reported typical pH changes in artichoke hydroponic culture: the nutrient solution became acidified with higher ammonium. Similar pH change due to ammonium has been reported in fennel, celery, swiss chard (Santamaria et al., 1999), rocket salad plant, and chicory (Santamaria et al., 1998). However, in our

study, no significant changes in pH were observed in any treatment, and pH of hydroponic solutions ranged from 5.5 - 5.8.

The form of nitrogen affected total biomass accumulation with highest dry weights being obtained with plants grown in 25:75 and 50:50 (ammonium:nitrate) treatments (Fig. 2A). Plants grown with the 75:25 ammonium:nitrate treatment exhibited significantly lower total biomass than other treatments. Shoot dry weight was inversely related to ammonium levels in the treatments. Dry weights decreased by 37% at the highest vs. lowest ammonium levels. Highest root mass accumulation occurred at the equimolar treatment (50:50 ammonium:nitrate). Root dry weight accumulation was markedly inhibited with the high ammonium ratio treatment. Growth suppression observed in the high ammonium treatment (Fig. 2A) was associated with lower root:shoot ratios (Fig. 2B) than in other treatments, illustrating that root growth was suppressed more than shoot biomass. Our results indicated that root growth in pecan is more sensitive to high ammonium than shoot growth. Plants in the equimolar treatment exhibited higher mean values of root biomass and root:shoot ratio than other treatments, showing that biomass accumulation was preferentially directed to roots under those conditions. Leaf characteristics were also modified by nitrogen form. Specific leaf weight was lowest in plants grown under high ammonium conditions (Fig. 2b). This concurs with the observations that leaves from plants grown under high ammonium were smaller and thicker than other treatments.

A number of other studies report that shoot and root biomass accumulation is negatively affected by increasing ammonium:nitrate ratios, including those with maize (*Zea mays* cv. Anjou 256) (Schortemeyer and Feil, 1996), sorghum (Traore and Maranville, 1999), celery, fennel, swiss chard (Santamaria et al., 1999), rocket salad plant (Santamaria et al., 1998), pansy (Hamlin et al., 1999), and poinsettia (Scoggins and Mills, 1998). Jeong and Lee (1992) evaluated several bedding plants, and found a range of growth responses to ammonium:nitrate ratios. Most of the species (seven of eleven

species) grew better with equimolar ammonium:nitrate ratios. However, some species grew best with 100% ammonium or 100% nitrate nutrition.

Our observation of lower root:shoot ratios with high ammonium treatments concurs with previous reports. Maynard and Barker (1969) found that root:shoot ratios decreased with higher ammonium:nitrate ratios in bean and cucumber. In contrast, with high ammonium treatments, root:shoot ratio increased in corn, and remained unchanged in pea (Maynard and Barker, 1969) and maize (Schortemeyer and Feil, 1996).

Nutrient analysis.

The foliar concentration of some nutrients was affected by nitrogen form (Table 1). In our study, foliar concentrations of Ca, Mg, and Mn exhibited significant differences with nitrogen ratios. Ca and Mn concentrations were about 2 times higher and Mg concentration was about 1.5 times higher with 25:75 ammonium:nitrate treatments than equimolar treatments. No significant differences in any of the other foliar concentrations of essential elements were observed.

Figure 3 shows foliar accumulation of essential elements in pecan leaves grown under different ammonium:nitrate ratios. In the 75:25 ammonium:nitrate treatments, mean foliar accumulation values were lower for all nutrients except for Cu compared to other nutrient regimes. Ca, Mg, P, Fe, Mn, and Zn decreased in 75:25 (ammonium:nitrate) treatments, with decreases ranging from 62% for P to 45% for Zn. N, K, S, and B accumulation was highest in 50:50 (ammonium:nitrate).

Effect of nitrogen form on the levels of other elements may be due to in part to the uptake process of the other elements. Cox and Reisenauer (1973) using *Triticum aestivum* demonstrated that increasing rates of nitrate uptake were associated with increasing rates of Ca, Mg, and K accumulation, but with increasing rates of ammonium uptake, accumulation of Ca and Mg decreased. Higher levels of nitrate increased K, Ca and Mg, and decreased P contents, and vice versa for higher levels of ammonium in sugar beet

(Harada et al., 1968). In artichoke, total inorganic anions increased and total inorganic cations decreased with ammonium:nitrate ratio (Elia et al., 1996).

Pecan exhibited differential uptake of nitrogen with form. Effects of nitrogen form on ammonium and nitrate uptake are shown in Fig. 4. Total nitrogen uptake on a dry weight basis increased with ammonium:nitrate ratio from 10.0 mg nitrogen per g dry weight to 20.9 mg nitrogen per g dry weight. Uptake of ammonium nitrogen also increased with ammonium:nitrate ratio while uptake of nitrate remained stable. Uptake of nitrogen was preferentially as ammonium under all ammonium:nitrate ratios. Increased ammonium ratio decreased the percentage of nitrate uptake of the total nitrogen uptake from 20% in 25:75 (ammonium:nitrate) to 14% in 75:25 (ammonium:nitrate). Inhibition of nitrate uptake by high ammonium in mixed nitrogen forms has been reported in a number of plants, including *Picea abies* (Peuke and Tischner, 1991), barley (Rao and Rains, 1976), and *Arabidopsis thaliana* (Doddema and Telkamp, 1979). In general, most crop plants (Crawford and Glass, 1998) and forest trees (Eltrop and Marschner, 1996) will take up a higher proportion of ammonium to nitrate in a mixed nitrogen form. However, the form of nitrogen uptake is determined by plant preferences for certain nitrogen forms. For examples in tree species, white spruce was reported to utilize ammonium-nitrogen with strict preference over nitrate (Kronzucker et al., 1997) and loblolly pine showed an increased preference for nitrate versus ammonium at elevated CO_2 levels (Bassirirad et al., 1996). The ability of plants to regulate ammonium or nitrate uptake was found to be related to their relative soil adaptations. Haynes and Goh (Haynes and Goh, 1978) reviewed that plants vary greatly in their ability to absorb and use ammonium and nitrate as their forms of nitrogen, and experimental observations indicate that some calcifuge species, i.e. plants that grow poorly in lime-rich soil, are adapted to, or at least tolerate, a nitrogen supply predominantly in the form of ammonium. Most calcifuge plants grow naturally in acid soils, where little nitrification takes place, and are adapted to use ammonium in preference to nitrate. In contrast, calcicoles, i.e. plants that thrive in soil rich in lime, utilize nitrate preferentially (Kirkby, 1967).

Up to now, nutritional studies in pecan have been conducted in the field. Limitations to this approach are that nitrogen form cannot be controlled and nutrient uptake cannot be measured. By using hydroponic culture, we were able to detect subtle changes in uptake and control the form and amount of nitrogen available to the plants. Results of this study show that applications of nitrogen as ammonium cause strong detrimental effects on pecan growth that are characterized by decreased total biomass and root growth. Current pecan production practices are to apply ammonium form nitrogen fertilizers, such as ammonium nitrate, ammonium sulfate, urea, and blends of urea with ammonium nitrate, in early spring. This study shows that pecan preferentially takes up ammonium over nitrate. Thus, the dominant form of nitrogen taken up by pecan is ammonium during the period that resumption of shoot and root growth occurs. Locally high ammonium concentrations, such as in the vicinity of fertilizer bands, may even impose ammonium stress on plants. Recurrent applications of ammonium nitrogen fertilizer may have cumulative effects. This study suggests that current fertilization practices may be improved by modification of nitrogen form in fertilization practices.

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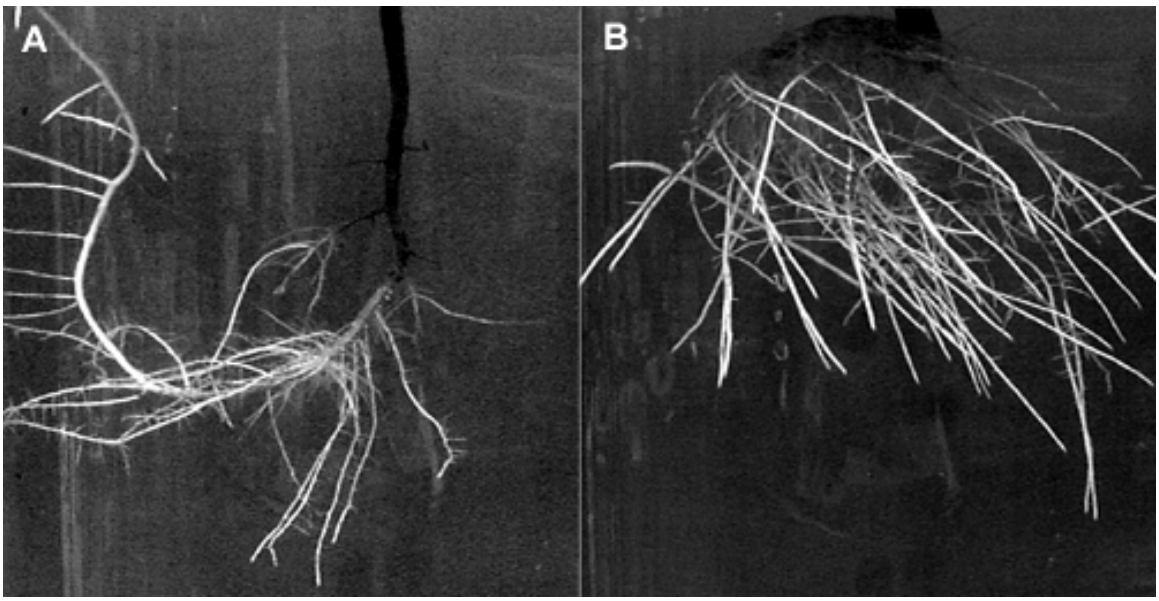
Table 1. Effects of nitrogen form on nutrient concentration in pecan leaf tissue.

Element	Treatment (NH_4 : NO_3)		
	75:25	50:50	25:75
N (%)	1.4 a [z]	1.6 a	1.3 a
P (%)	5.4 a	4.9 a	6.1 a
K (%)	1.4 a	1.6 a	1.3 a
Ca (%)	1.4 b	1.0 c	2.1 a
Mg (%)	3.9 ab	3.1 b	4.7 a
S (%)	0.1 a	0.2 a	0.1 a
B (ppm)	88.6 a	83.6 a	123.2 a
Cu (ppm)	0.1 a	0.1 a	0.1 a
Fe (ppm)	170.6 a	139.3 a	205.2 a
Mn (ppm)	77.1 ab	51.4 b	98.7 a
Mo (ppm)	0.4 a	0.5 a	0.4 a
Zn (ppm)	12.9 a	12.8 a	19.9 a

[z] Means within a column were separated using Duncan Multiple comparison at alpha = 0.1.

Fig. 1. Changes in root morphology in plants grown under different ammnoium:nitrate



ratios. A) 75:25 ammonium:nitrate, B) 25:75 ammonium:nitrate

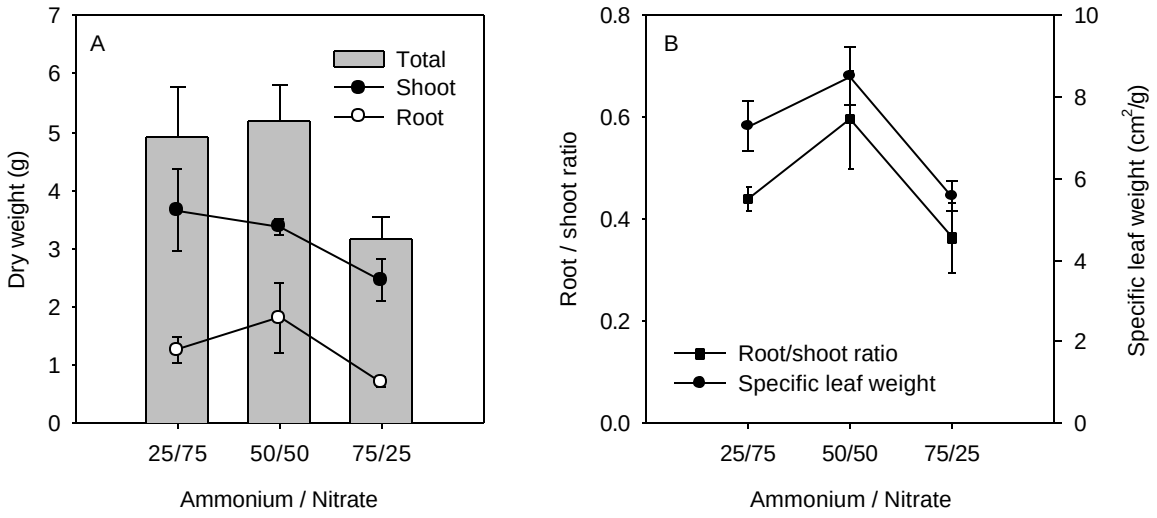


Fig. 2. Effect of ammonium:nitrate ratio on biomass, root/shoot ratio, and specific leaf weight.

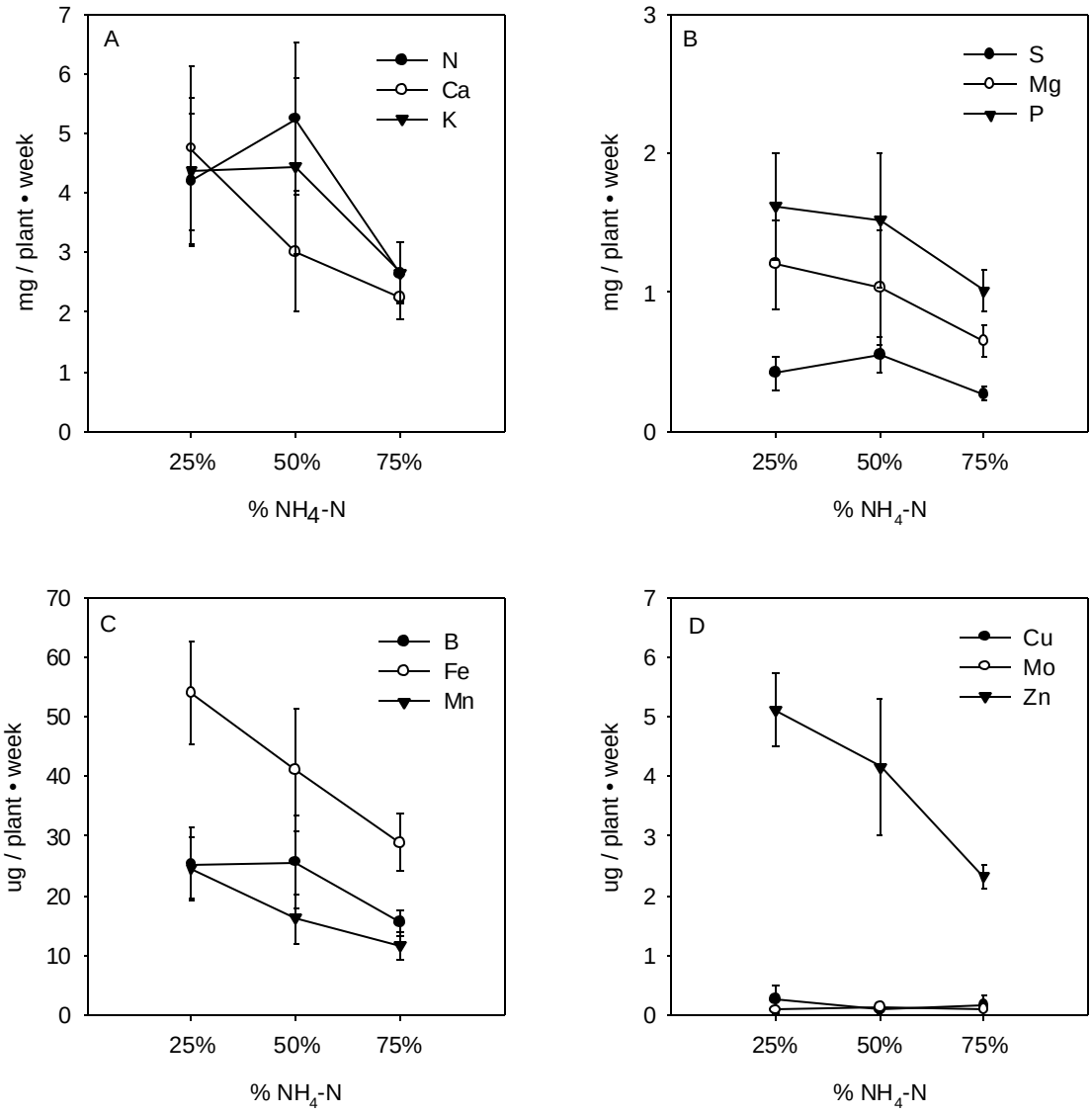


Fig. 3. Foliar accumulation of essential elements in pecan

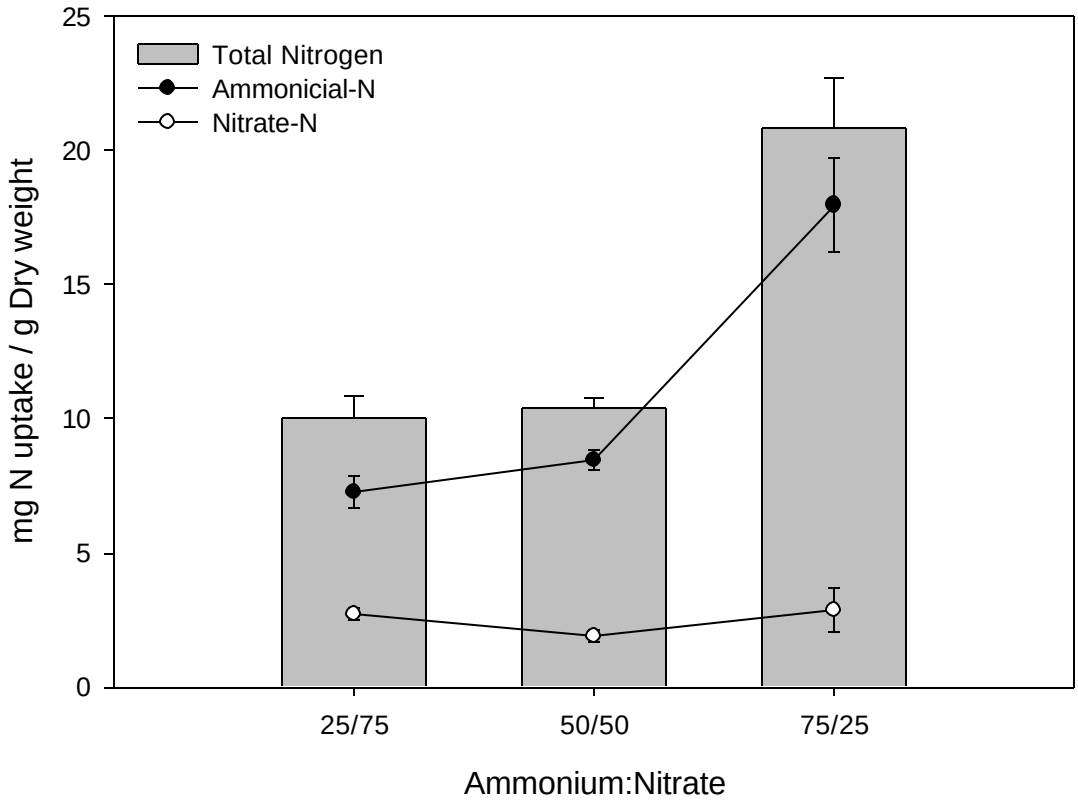


Fig. 4. Effect of nitrogen form on uptake of ammonium and nitrate in pecan.

CHAPTER 6

Seasonal Fluctuations in Nutrients and Carbohydrates in Pecan[4]

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Abstract

It has been shown that perennial woody plants exhibit marked seasonal changes in nutrient content, carbon metabolism, and organ development. An integrated study of temporal carbohydrate, nutrient and structural changes in pecan leaves and shoots would provide insight into differences in seasonal requirements for this crop. Changes in soluble carbohydrate levels were related to leaf and fruit developmental stages. Soluble sugars in leaves declined during fruit development. Glucose level declined during cotyledon elongation and reserve deposition in fruit. Fructose levels declined during fruit storage reserve accumulation. After fruit maturation, stems exhibited a dramatic increase of sucrose along with a marked decrease of starch. Seasonal variation of essential element levels in leaves indicated developmental stage-related nutrient movement. During leaf development, all essential elements accumulated in leaves. During fruit development leaves exhibited a net export of many nutrients which suggests that fruit is a strong sink for not only carbohydrates but also essential elements.

Introduction

Higher plants can exhibit marked seasonal changes in nutrient content, carbon metabolism and organ development. Critical developmental stages can influence both nutrient and carbohydrate allocation patterns. Leaves in different developmental stages vary in photosynthetic capacity, and there are also temporal changes in source-sink relation with growth phase (Kramer and Kozlowski, 1979; Loescher et al., 1990). The rate of photosynthesis is low in very young leaves, increases with leaf age, reaches maximum levels around full leaf expansion, then declines. Early in the season, the growing plant parts obtain their nutrients and carbohydrates by redistribution from deposits stored the previous year in perennial tissues, but after leaf maturation, these organs become material exporters. After fruit ripening, a second extensive redistribution of nutrients and carbohydrates takes place from the senescing leaves of deciduous trees before leaf fall, based on a general redistribution pattern which has been adopted for deciduous trees (Smith, 1987; Loescher et al., 1990).

Foliar nutrient analysis is the most satisfactory method for studying the plant nutrient element status, especially for woody perennial trees, and can used to indicate the nutrient availability in soil as well as root uptake (Mengel and Kirkby, 1982). Nutrient uptake by roots is affected by a number of factors (Bates, 1971), among them being leaf age, which can be a considerable effect, as it represents the physiological stage of the tissue. In addition, leaf nutrient concentration changes with leaf age.

Efficient nutrient resorption may contribute to the regulation of productivity. During the spring-time flush of growth, intense mitotic activity and cellular growth create a high demand for nutrients. A knowledge of seasonal nutrient allocations, as well as an understanding of temporal accumulation patterns can be useful in the development of fertilization regimes that reflect the biology of a tree crop (Picchioni et al., 1997).

Woody plants accumulate carbohydrate reserves during periods of excess production and deplete them whenever the rate of carbohydrate utilization exceeds the

rate of current production. In particular, reserve carbohydrates are depleted from twigs, stems, and roots during periods of most rapid growth in a pattern that varies with species growth characteristics. A number of studies have followed changes in carbohydrate (Smith et al., 1986) and nutrient content (Cresswell and Wickson, 1986) in pecan leaves. However, the objective of these studies was to assess the effect of various treatments such as fungicide application (Worley, 1973), artificial defoliation (Worley, 1978; Worley, 1979), stem cutting (Smith et al., 1975) and fruit loads (Worley, 1973; Wood and McMeans, 1981; Smith et al., 1986; Wood, 1989). In many cases, sampling strategies were based on calendar dates rather than on seasonal developmental stages. Furthermore, an evaluation of changes in specific types of soluble sugars have not been assessed in pecan. An integrated study of temporal carbohydrate, nutrient and structural changes in pecan leaves and shoots would provide insight into differences in seasonal requirements for this crop.

The objectives of this work were to follow levels of nutrients and carbohydrates in pecan leaves and shoots at different growth stages from bud break up to natural abscission. Temporal changes in nutrients and carbohydrates were related to developmental changes during the growing season.

Materials and Methods

Plant material. Studies were conducted using 'Stuart' pecan trees at the University of Georgia Horticulture farm near Watkinsville, GA in 1999. Leaf and branch samples were collected at 5 different growth stages: full bloom (4/23/99; FB), full expansion of leaves (6/8/99; FE), completion of cotyledon elongation prior to reserve deposition (8/28/99; CE), fruit maturation or completion of reserve deposition (10/14/99; FM), and natural leaf defoliation (11/25/99; NA). To minimized diurnal changes in photosynthesis, samples were collected at approximately the same time of day (i.e., 10:00 AM) on a clear sunny day. Three entire shoots from three trees (9 replications) were sampled at each growth

stage, and each shoot represented a replication. Data were analyzed using GLM procedure in SAS program (SASInstitute, 1985).

Extraction of sugars. One-year-old shoots and leaflets were separated and analyzed. The freeze-dried samples were milled into powder using a Wiley mill to pass a 20-mesh sieve. One hundred mg of the powder was homogenized in 10 ml 80% methanol containing 2.2 mg phenyl- -D-glucopyranose as internal standard for 1 min at a speed of 12,000-17,000 rpm using a Handishear AC Hand-held Homogenizer. The resulting slurry was centrifuged for 5 min at 7500 rpm. Ten ml of supernatant was pipetted into a vial and stored at 4 C until utilization.

Oxime-trimethyl silyl derivatization. Trimethylsilylated extracts were prepared according to Chapman and Horvat (1989) with modification. A 10 ml aliquot of each sugar extract was dried over nitrogen gas. Fifty l of hydroxylamine-HCl (25 mg/ml in pyridine) was added, then heated to 75 C for 30 min for oximation.

GC analysis. The oximes of sugars and acids were then derivatized by injecting 70 l BSTFA + 1% TMCS and heated at 75 C for 20 min. After cooling, a 1 l injection was made into the gas chromatograph using a automatic injector. A mixture containing known amounts of the internal standard and pure reference sugars was analyzed every 10 samples to obtain correction factors.

The GC analyses were performed using a Hewlett-Packard 5890A gas chromatograph equipped with a flame ionization detector (FID). The GC-column was a 30 m x 0.32 mm (i.d.) fused silica, DB-5, 0.25 m film thickness capillary column (J&W, Alltech, Inc., Deerfield, IL). Injector and detector temperatures were 250 C and 300 C, respectively. Oven temperature was initially held at 150 C for 1 min, then programmed to increase from 150 C to 210 C at a rate of 4 C/min , then held at 210 C for 0.5 min, then elevated to 250 C at a rate of 7 C/min rate and held at 250 C for 0.5

min, and finally raised to 280 C at a rate of 4 C/min and held for 5 min. Helium was used for carrier gas at a flow rate of 53.1 ml/min. The splitless mode was used. Air and hydrogen flow rates to the detector were 370-380 ml/min and 28 ml/min, respectively. Nitrogen was used as a make-up gas at a flow rate of 30 ml/min. A 3392A integrator was connected to the GC for the peck area measurement.s Identification of each sugar was based on retention times using authentic standards and by adding standards to samples before extraction and verifying retention time of specific peaks.

Starch analysis. Remaining pellets from sugar analysis were used for starch quantification. For starch quantification, samples were washed 3 times with 80% methanol to remove soluble sugars. Starch was gelatinized in a 100 C water bath for 1 hr, then samples were incubated with 7 units of amyloglucosidase with acetate buffer pH 4.8 at 55 C for 24 hrs. Starch was measured as a equivalent of glucose product.

Element analysis. Leaflets were removed from collected shoots. All tissue was washed in de-ionized water with a mild soap then rinsed in de-ionized water to remove dust or contamination. Samples were freeze-dried then ground with a Wiley mill to pass a 20-mesh sieve. A 0.5 g sample was ashed at 500 C for 8 hours, and then dissolved in 20% aqua regia + 10 ppm Cd solution. Al, B, Ca, Cu, Fe, K, Mg, Mn, Mo, Na, P, and Zn were quantified using an ICAP (Inductively coupled argon plasma spectrophotometer). NO3-N and NH4-N were quantified by a continuous flow nitrogen analyzer.

Results and Discussion

Carbohydrate and nutrient concentrations in leaves and 1-year-old stems were measured at 5 growth stages. Full bloom was observed in late April, leaves were fully elongated in early June, cotyledon elongation was observed in mid-August, fruits were maturated in mid-October, and natural abscission occurred in late November. Leaf area

significantly increased between full bloom and full leaf expansion and remain stable after full leaf expansion. Leaf area data at natural leaf abscission could not be accurately assessed because desiccated leaves exhibited excessive curling which prohibited measurement. Leaf dry weight rapidly increased during the period leading up to leaf full expansion, continued to increase during cotyledon elongation then decreased during the period when fruits accumulated reserve deposit. Decrease in dry weight continued during the period leading, which is an indicative of resorption of nutrients and carbohydrates. The mobilization of nutrients and carbohydrates to support fruit development has been shown in other crops including rabbiteye blueberry (*Vaccinium ashei Reade*) (Darnell and Birkhold, 1996), avocado (Holland et al., 1999), and mandarin orange (Liu et al., 1999). In contrast to our results, leaf dry weight patterns in walnut (Drossopoulos et al., 1996) remained stable during fruit maturation.

Seasonal variation in carbohydrates

In our study, fructose was the most predominant soluble carbohydrate in pecan leaves (Fig. 2A). Fructose content in the leaves increased from full bloom until the end of cotyledon elongation, after which time levels began to decrease. A dramatic decrease in fructose content was observed after fruit maturation. Glucose content in leaves increased with leaf elongation until full leaf expansion, then declined. A dramatic decrease in glucose content was also observed after fruit maturation. Throughout the growing season, sucrose content in leaves was low and showed no significant differences. For the most part, studies which have evaluated seasonal fluctuations of carbohydrate levels in fruit tree crops have not measured differences among individual soluble sugars. An exception is walnut (Drossopoulos et al., 1996), where sucrose was the most predominant soluble carbohydrate in leaves. After the end of leaf development, a maximum value was attained in all the sugars and starch found in the leaves. During the same period, an insignificant increase in glucose and fructose levels in the leaves suggested a rapid transport of these sugars to the growing fruits

In the stem (Fig. 2B), sucrose was the most predominant soluble carbohydrate. Sucrose levels remained relatively stable until after fruit maturation when levels markedly increased. Fructose and glucose content in the stem remained stable from full bloom until full leaf expansion, then decreased until natural abscission.

Starch content (Fig. 2c) in leaves was high at the full bloom stage and dramatically declined until reserve deposition in fruit was completed. Starch content in stems was initially low, then sharply increased between full bloom and full leaf elongation. Levels remained fairly constant until completion of fruit maturation, then rapidly decreased. Wood and McMeans (1981) evaluated seasonal carbohydrate changes in pecan stems and leaves. They reported low starch levels during the liquid stage of ovule development which is similar to the period prior to full cotyledon expansion in our study. This is in contrast to our results where high starch levels were maintained throughout this period. It should be noted that Wood and McMeans evaluated current-season branches rather than one-year-old shoots. It is likely that their differences may be related to shoot developmental changes associated with young expanding shoots.

Annual carbohydrate cycles of recurrently flushing species are characterized by depletion of carbohydrates with each growth flush, followed by carbohydrate replacement. Total carbohydrate content of stems and branches reach a maximum in the autumn about the time of leaf fall, begin to decrease in late winter, and decrease rapidly in early spring when carbohydrates are being depleted by accelerated respiration and used in growth of new tissues. This concurs with the current study, where starch reserves were at their lowest level during the full bloom period. This stage is associated with rapid emergence and expansion of new leaves and both staminate and pistillate inflorescences. Although considerable movement of carbohydrates occurs in leaves, carbohydrate transport is predominantly import. The sources of carbohydrates input are pools of reserve carbohydrates and currently produced photosynthate. The amount moved depends on the carbohydrate requirements and on the proximity of growing tissue to sources of supply. In contrast to our results, most woody plants carbohydrates are

translocated largely or entirely as sucrose. The significance of fructose as the primary soluble carbohydrate in pecan is unknown.

Smith et al. (1986) reported that soluble sugar concentration in one-year-old shoots of pecan was greater during the defoliated period than during fruit maturation and full bloom. They also reported that starch concentrations were inversely related to sugar concentrations and that this may reflect changes in sugar:starch ratios associated with cold hardening. They suggested that carbohydrate levels during fruit maturation may influence the next year s flower production. Worley (1979) reported that soluble sugar and starch exhibited different accumulation pattern in above ground tissues during the winter with starch decreasing and sugar increasing in about ground tissue. Davis and Sparks (1974) conducted a study on assimilation and translocation patterns of carbon using 14C and reported that immature leaves are only partially self-supporting and dependent upon substrates mobilized from reserves stored the previous season. After leaf maturation, translocation of carbohydrate from leaves was bidirectional, and during fruit development, basipetal translocation increased.

Seasonal variation in nutrients

Seasonal changes for the macro- and micro-nutrients were measured during the total life cycle of leaves and are expressed as content (i.e., on a per leaf basis) and as concentration (i.e., on a dry weight basis) (Fig. 3 and 4). Nutrient levels were expressed using both methods becasue nutrient content reflects both import and export of nutrients from the leaf, while concentration expresses the nutritional status of the leaf. Although concentration may be valuable in assessing nutrient status (sufficiency vs. deficiency), it does not reflect movement of nutrients. Likewise, even if there are net changes (import / export) in certain elements, plant nutrient concentration may not correspond because growth rate and translocation rate may differ causing a dilution or accumulation of nutrients.

During leaf expansion (from FB through FE), all essential elements exhibited significant level of accumulation ranging from 14% for zinc up to 113% for boron. Nitrogen, potassium, calcium, boron, and iron exhibited about 80% or higher accumulation rates. However, during the same period, concentrations of phosphorus, magnesium, copper, manganese, and zinc decreased in leaves ranging from 10% for magnesium up to 39% for zinc. The amassment of dry weight exceeded the accumulation of those elements and caused decrease in foliar concentration of those elements. Drossopoulos et al. (1996) reported that nutrient accumulation of nitrogen, phosphorus, potassium, calcium, magnesium, iron, manganese, copper, and zinc in the leaves increased dramatically during leaf development in walnut. Similar to our results, nitrogen, phosphorus, copper, and zinc failed to show corresponding increases in leaf concentration. They attributed this to a dilution caused by nutrient accumulation rates being lower than dry matter accumulation.

During the period of cotyledon formation (from FE through CE), foliar levels of magnesium and zinc decreased 23% and 24%, respectively. Magnesium-related leaf scorch has been reported in trees with heavy fruit loads. Histodifferentiation in fruit is occuring during this time and decreases in foliar magnesium and zinc levels may be associated with the fruit being a strong sink for these two elements.

During the period of reserve deposition in fruit (from CE through FM), large quantities of some nutrients have been reported to be transport to fruit during maturation. Decrease in nitrogen, phosphorus, potassium, sulfur, boron, copper, and manganese were observed with declines ranging from 3% for nitrogen up to 35% for boron. Prior to this time period most of these elements were exhibiting net increases. In pistachio, leaf nitrogen and phosphorus content remained relatively stable during seed fill, in contrast to potassium, calcium, and magnesium which increased during this period (Picchioni et al., 1997). Diver and Smith (1984) found that phosphorus is transported into the fruit in greatest quantities during kernel development. They also found that most rapid transport of calcium into the fruit occurs during the final 30 days before the fruit ripen.

After fruit maturation, withdrawal of nutrients (i.e. resorption) from senescing leaves and their storage in perennial parts occurs in trees. During this period (from FM through NA), nitrogen, phosphorus, potassium, calcium, magnesium, sulfur and copper exhibited resorption ranging from 3% for sulfur up to 14% for phosphorus, indicating that translocation out of the leaves occurred in these nutrients. Resorption is an important strategy to conserve nutrients. Resorbed nutrients are more directly available for future plant growth while those not resorbed are essentially lost in the leaf litter. Resorption was not observed in many of the micronutrients. Boron, iron, manganese, and zinc accumulated in leaves during this period 30%, 13%, 64%, and 20%, respectively. This may reflect low mobility of these nutrients in plants. In pistachio, nitrogen and phosphorus declined during the resorption period (Picchioni et al., 1997).

Seasonal variations of nutrient concentration at different developmental stages in stem is shown in Fig. 5. All the macronutrients except phosphorus remained fairly stable throughout the growing season. Copper and zinc showed very subtle declines during the growing season. Boron levels decreased during cotyledon elongation and increase thereafter. Fe level decreased sharply during leaf elongation and remained stable up to the time of leaf abscission. Mn level increased from leaf full expansion throughout the growing season.

In our study, changes in soluble carbohydrate levels were related to leaf and fruit developmental stages. Soluble sugars in leaves declined during fruit development. Glucose level declined during cotyledon elongation and reserve deposition in fruit. Fructose levels likewise declined during fruit storage reserve accumulation. After fruit maturation, stems exhibited a dramatic increase of sucrose along with a marked decrease of starch. This was associated with the period of cold hardening. Seasonal variation of essential element levels in leaves indicated developmental stage-related nutrient movement. During leaf development, all essential elements accumulated in leaves. During fruit development leaves exhibited a net export of many nutrients which suggests that fruit is a strong sink for not only carbohydrates but also essential elements. During

cotyledon formation, magnesium, and zinc was exported from leaves. During reserve deposition, nitrogen, phosphorus, potassium, sulfur, boron, copper, and manganese were exported. During resorption, nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur were exported.

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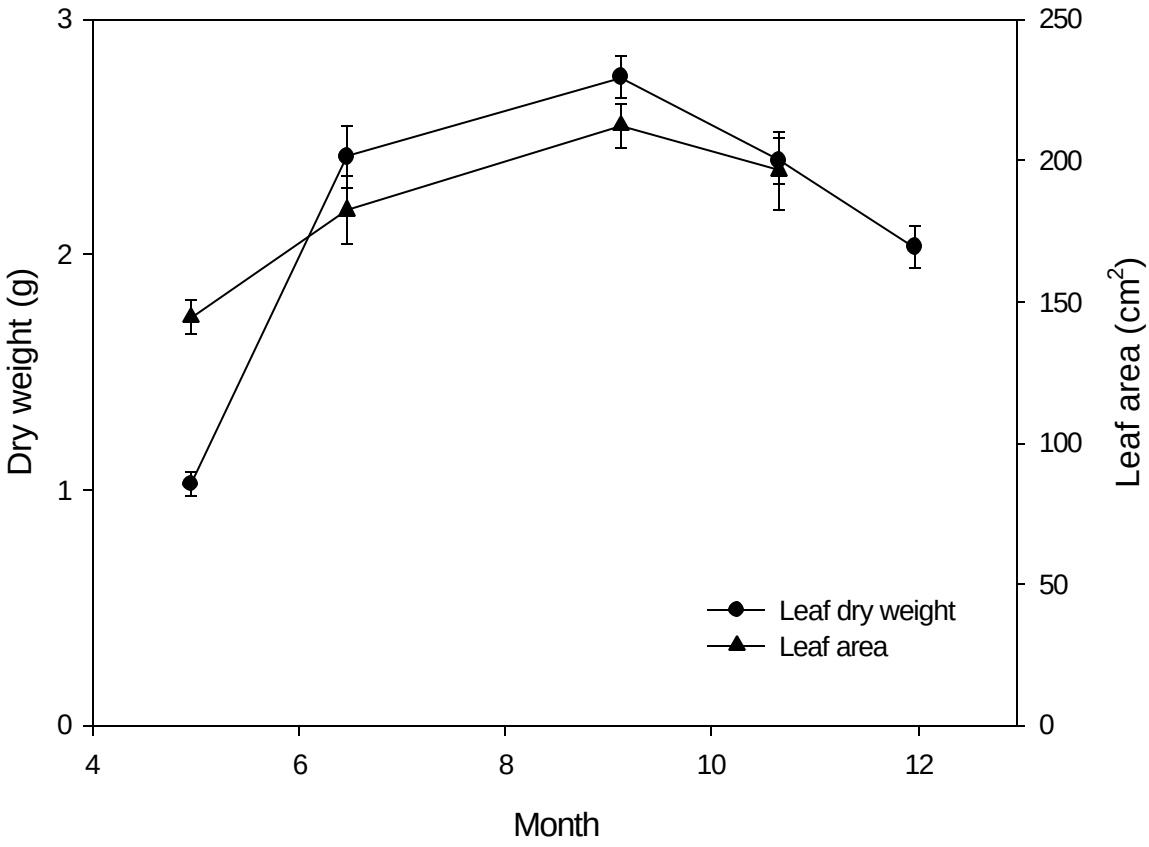

Fig. 1. Seasonal variations in leaf dry weight and leaf area at different developmental stages. Data were collected at full bloom (4/23/1999), leaf full expansion (6/8/1999), complete of cotyledon elongation (8/28/1999), fruit maturation (10/14/1999), and natural abscission (11/25/1999).

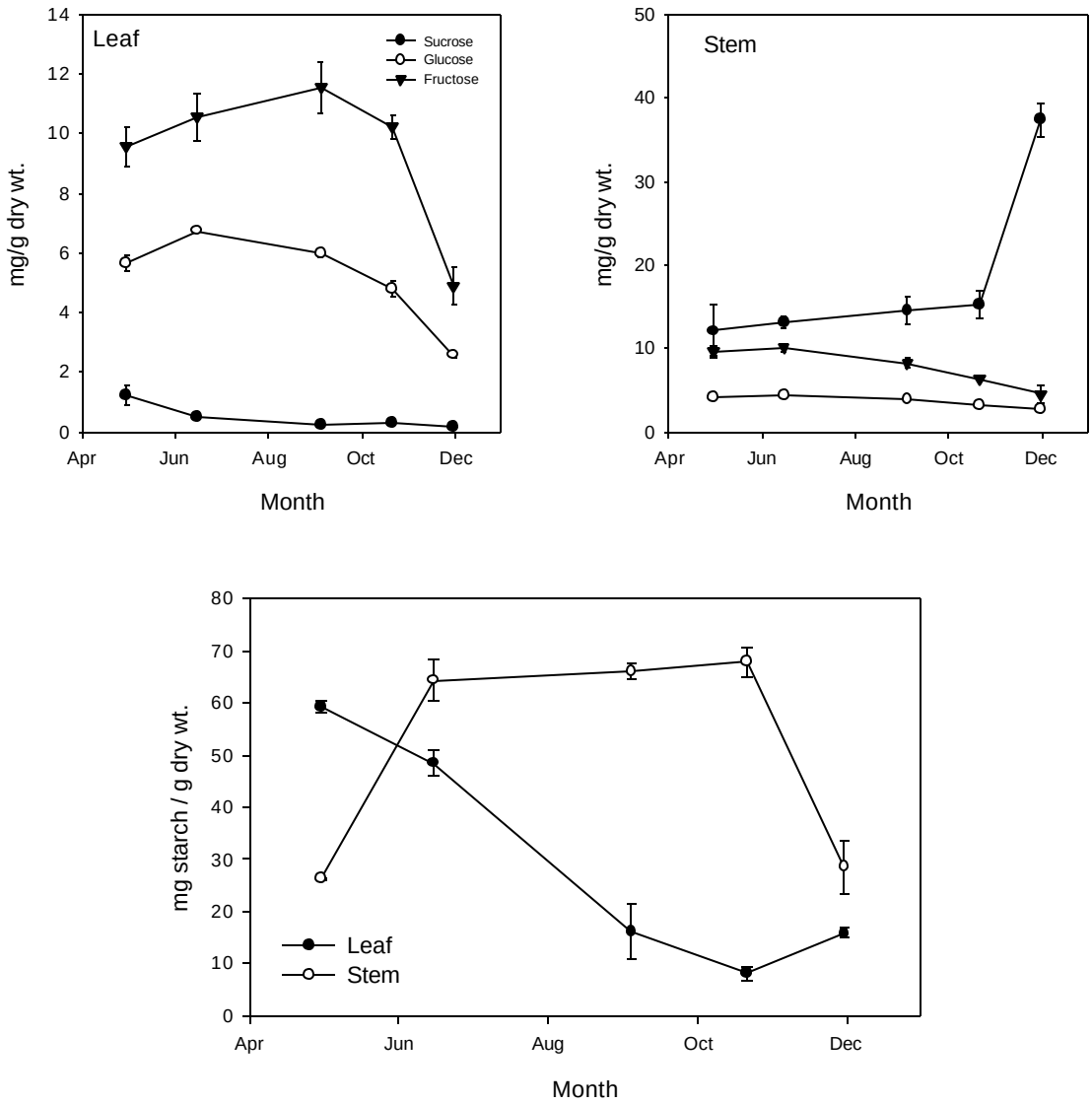

Fig. 2. Seasonal variations in soluble sugar and starch at different developmental stages. Data were collected at full bloom (4/23/1999),leaf full expansion (6/8/1999), complete of cotyledon elongation (8/28/1999), fruit maturation (10/14/1999), and natural abscission (11/25/1999).

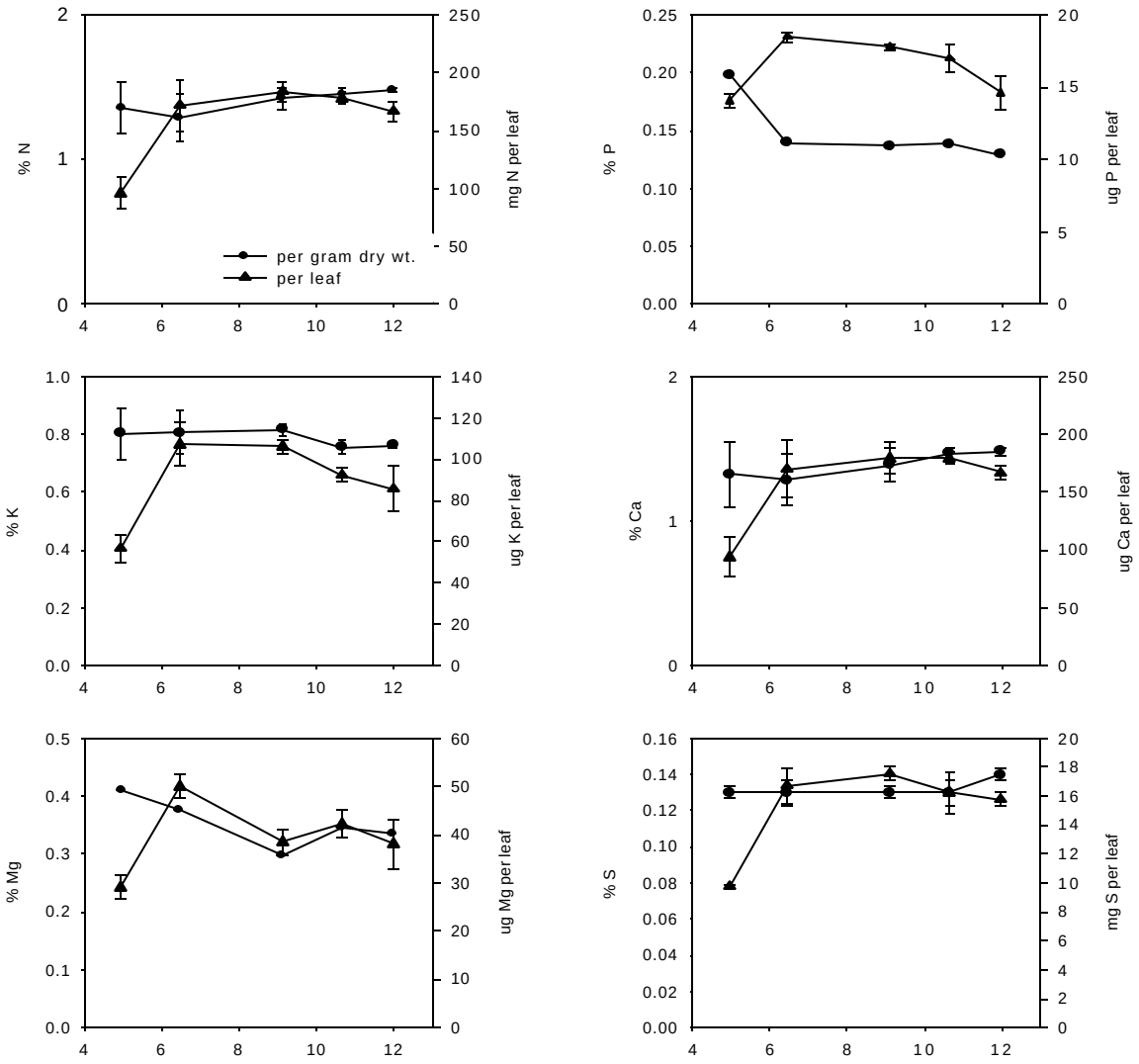

Fig. 3. Seasonal variations in foliar macro nutrient concentration and content at different developmental stages. Data were collected at full bloom (4/23/1999), leaf full expansion (6/8/1999), complete of cotyledon elongation (8/28/1999), fruit maturation (10/14/1999), and natural abscission (11/25/1999).

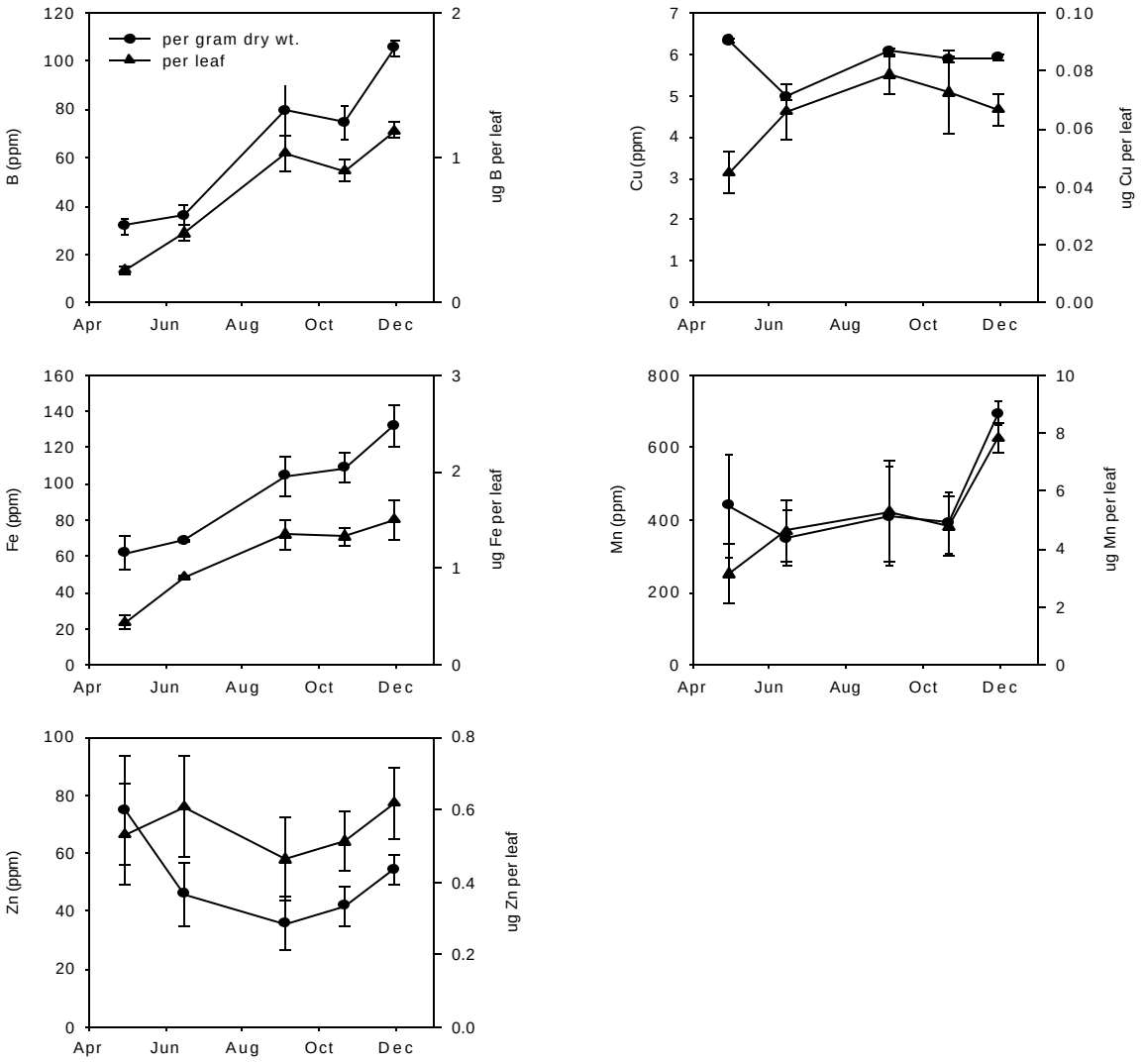

Fig. 4. Seasonal variations in foliar concentrations and contents of micro nutrients at different developmental stages. Data were collected at full bloom (4/23/1999), leaf full expansion (6/8/1999), complete of cotyledon elongation (8/28/1999), fruit maturation (10/14/1999), and natural abscission (11/25/1999).

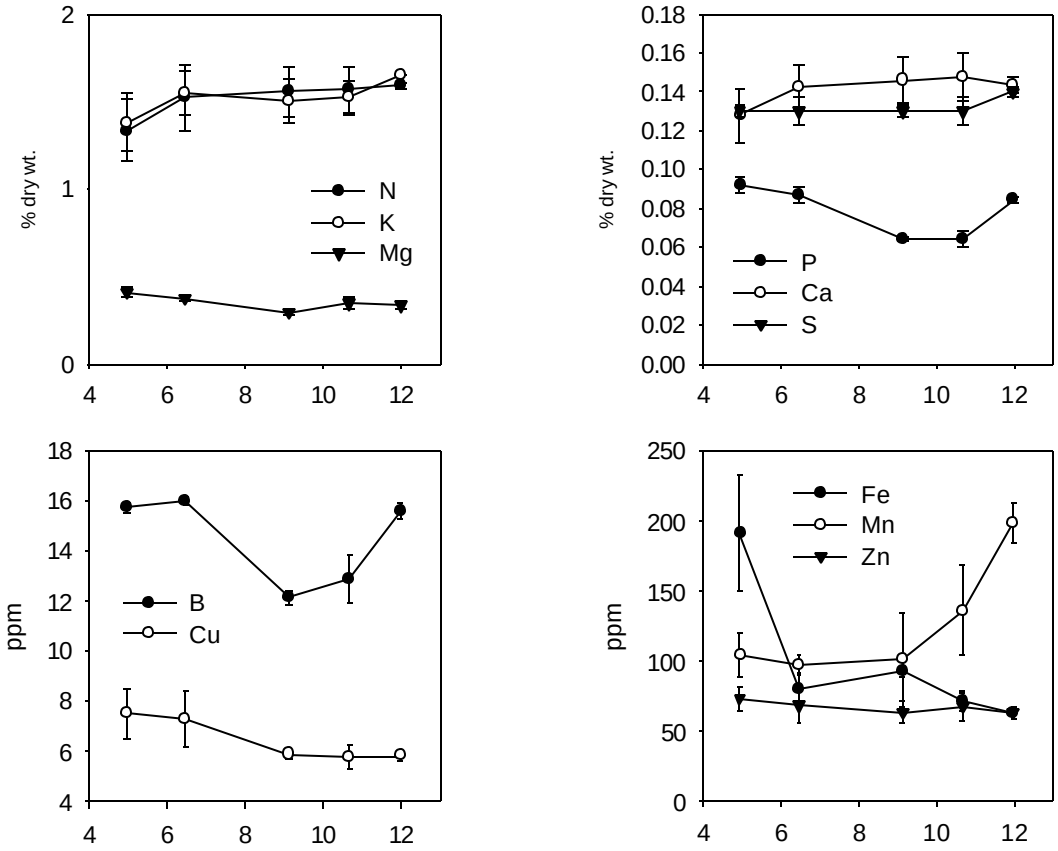

Fig. 5. Seasonal variations in stem nutrient concentrations at different developmental stages. Data were collected at full bloom (4/23/1999), leaf full expansion (6/8/1999),complete of cotyledon elongation (8/28/1999), fruit maturation (10/14/1999), and natural abscission (11/25/1999).

CHAPTER 7

Conclusion

Some specific areas of investigation were conducted in pecan (*Carya illinoinensis* Wangenh. K. Koch). These include studies on 1) the effect of zinc supply on growth, development, and nutrient uptake of pecan seedlings with an evaluation of different seedstocks, 2) evaluation of zinc deficiency at macro- and microscopic levels, 3) the effect of different nitrogen sources on growth, development and nutrient uptake, and 4) seasonal changes in nutrients, carbohydrates, and plant structure. The nutritional studies evaluating nitrogen and zinc used hydroponic culture. The last study was temporal evaluations using mature trees. An advantage of hydroponics is that the uptake and utilization of subtle differences in nutrients can be followed and growth readily assessed.

Zinc deficiency is a nutrient disorder observed in pecan under field conditions, and can cause distorted leaf growth and severe rosetting of shoots. Conducting zinc studies with pecan in the field has been problematic because zinc nutrition is difficult to control. We were able to induce zinc deficiency in pecan using hydroponic culture and to distinguish zinc efficient- and inefficient-genotypes using hydroponic culture. Our study shows that hydroponic culture can be utilized for screening genotypic differences in nutritional efficiency, and can be applied to studies with other nutrients.

In this study, zinc efficiency was compared in two pecan seedstocks, Stuart and Curtis by evaluating growth response, nutrient uptake, and leaf nutrient analysis. Differences in zinc efficiency between the two seedstock were observed. Zinc supply also had an differential effect on the foliar concentration and content of zinc and other

nutrient. This study verifies that in pecan, there are genotypic differences in zinc efficiency, and that hydroponic culture can be utilized for screening and selection.

Plants exhibited various zinc deficiency symptoms ranging from mild (interveinal mottling), intermediate (overall interveinal chlorosis and necrotic spots) to severe (extensive necrosis and marginal curving) conditions. Structural differences associated with zinc deficiency include changes in leaf thickness and mesophyll cell organization. Cells in zinc deficient leaves had limited cytoplasmic content and accumulated phenolic compounds in vacuoles. Extensive starch accumulation was observed in chloroplasts. Unlike studies using field-collected material, the differences observed were definitely due to zinc deficiency in that the current study controlled the level of other nutrients using hydroponic culture and were confirmed by nutrient analysis in another study. This work represents the first detailed microscopic evaluations of zinc deficiency in leaves.

Up to now, nutritional studies in pecan have been conducted in the field. Limitations to this approach are that nitrogen form cannot be controlled and nutrient uptake cannot be measured. By using hydroponic culture, we were able to detect subtle changes in uptake and control the form and amount of nitrogen available to the plants. Results of this study show that applications of nitrogen as ammonium cause strong detrimental effects on pecan growth that are characterized by decreased total biomass and root growth. Current pecan production practices are to apply ammonium form nitrogen fertilizers, such as ammonium nitrate, ammonium sulfate, urea, and blends of urea with ammonium nitrate, in early spring. This study shows that pecan preferentially takes up ammonium over nitrate. Thus, the dominant form of nitrogen taken up by pecan is ammonium during the period that resumption of shoot and root growth occurs. Locally high ammonium concentrations, such as in the vicinity of fertilizer bands, may even impose ammonium stress on plants. Recurrent applications of ammonium nitrogen fertilizer may have cumulative effects. This study suggests that current fertilization practices may be improved by modification of nitrogen form in fertilization practices. Further evaluations of pecan fertilization are areas to pursue to improve crop production.

In our study, soluble carbohydrate level was related to leaf and fruit developmental stages. Glucose level declined after leaf full expansion and fructose level declined after the end of cotyledon elongation. After fruit maturation, during the cold hardening period, a dramatic increase of sucrose and decrease of starch in the stem was observed. Seasonal variation of essential element level in leaves indicated developmental stage-related nutrient movement. During the leaf development period, all essential elements accumulated in leaves. During cotyledon formation period, magnesium and zinc were exported. During reserve deposition, nitrogen, phosphorus, potassium, sulfur, boron, copper, and manganese were exported. Nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur was exported from leaves during the resorption period.