EFFECT OF NITROGEN APPLICATION RATES ON PECAN ABOVE GROUND PHYSIOLOGY AND ROOT TRAITS

A Thesis

by

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Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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May 2014

Major Subject: Horticulture

ABSTRACT

The United States pecan industry is experiencing a rapid acreage increase. Many growers believe high rates of nitrogen (N) accelerate tree development and increase yields; however, a high rate of N application may not be cost-effective and can be harmful to the environment. The aim of this project was to evaluate the effect of N application rate on young pecan seedling performance as measured through photosynthesis, water use efficiency (WUE, defined as the ratio between net assimilation rate and transpiration rate), growth, and to determine if and when N application rates had an effect on root system, especially specific root length (SRL) and root length density (RLD) following five N application rates (0x, 1/4x, 1/2x, 1x, and 2x, where x corresponded to the recommended rate, as reported by Texas AgriLife Extension). Soil sampling was performed five days after N application and continued throughout two growing seasons for evaluation of soil nitrate and SRL and RLD. Diameter of the seedlings was measured at two locations on each tree throughout both years of the experiment. Gas exchange was measured every 3-4 weeks using an infrared gas analyzer. Results showed that soil N was higher in the 1x and 2x treatments than in the other treatments. However, N treatment did not affect amount of WUE, net photosynthesis rate, trunk growth, SRL, or RLD. Therefore, it is possible that the most effective rate of N application for young pecan seedlings is likely much lower than recommended rates.

DEDICATION

I would like to dedicate this thesis to my parents, Richard and Terri Hannah. I am very thankful for the love and support they have given me throughout my Master's process, and am so blessed to have them as parents.

ACKNOWLEDGEMENTS

I would like to thank my committee chairs Dr. Leonardo Lombardini and Dr. Astrid Volder for their patience, dedication, support and guidance to see this project come to completion. I would also like to thank my committee members Dr. Mike Arnold and Dr. Cristine Morgan for their knowledge and help on this project. I would also like to thank Dr. George Ray McEachern and Monte Nesbitt for all of their guidance and help.

I would also like to thank Paige Graves, Alison Bingham, Kirk Laminack, Dominique Conrad, Stephanie Clay, Sean Carver, Ockert Grevenstien, Cassie Warren, Alexandra Garcia and Clint Taylor for helping me with data collection. I would like to thank Scott Hansen, Grant Clay, John Ainsworth, Jared Cobb, Nathan Walton, Dianna Bagnall, Cody Bagnall and Brad Coffman for all of their help collecting soil samples. For moral support I would like to thank Carol Nesbitt, Travis Britt, Andrew King, Cate Simpson, Catherine Stroo, Jessica McDonald, Lindy Reese, and Morgan Burcham.

I would like to acknowledge the USDA-CSREES Specialty Crop Research Initiative Grant #2008-57780-19563, the Salopek Foundation, and Wylie Hatcher for their monetary support for this research.

Finally, I would like to thank my parents for their constant love, support, and encouragement throughout this entire process.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Nitrogen (N) is an essential element that, compared to other elements, is needed in relatively large amounts for plant function. It is a building block for many key molecules such as amino acids, proteins, and nucleic acids. In order for gaseous (or atmospheric) N to be available to plants, it must undergo a process known as N fixation which can be a natural or industrial process. The two inorganic N forms available to plants for uptake from the soil solution are nitrate (NO_3^-) and ammonium (NH_4^+). Because of cation exchange in soils, ammonium is held by soil particles more tightly than nitrate which is quickly leached through the soil (Emmett et al., 1995).

Nitrate pollution can cause eutrophication of streams and coastal zones and decrease the amount of oxygen available to other organisms living in aquatic systems (Tuncer et al., 1998). The major source of nitrate pollution often comes from agriculture watersheds (Spalding and Exner, 1993). High concentrations of nitrates in drinking water have been correlated with health issues such as stomach cancer and methemogloninemia (blue baby syndrome) in infants (Wolfe and Patz, 2002). Minimizing N input from agricultural practices can help reduce nitrate runoff and associated pollution problems (Dinnes et al., 2002).

For young pecan (*Carya illinoinensis*) trees it is important to monitor the effect N may have on growth during establishment. In a fertigation study, one-year-old pecan trees received calcium nitrate ($CaNO_3$) at five different rates (0, 2, 10, 20, and 40 g of N per seedling) over one growing season (May-October) (Conner, 2007). Results showed that the different N application rates did not induce differences in diameter growth, except for the highest rate which caused a decrease in diameter growth (Conner, 2007). When orange (*Citrus sinensis*) seedlings were fertilized with 0, 794, 1589, 2384, 3179, and 3976 $kg \cdot ha^{-1}$ N of 8N-0P-6.6K (4% ammonium, 4% nitrate) per season, trunk diameter was greatest in the N application of 794 $kg \cdot ha^{-1}$ but was actually reduced when trees received more than 1589 $kg \cdot ha^{-1}$ (Davies and Zalman, 2008). Thus, it is possible that current N application practices in pecan orchards may exceed the demand of young trees and could have negative effects on pecan growth.

1.2 Role of nitrogen in the establishment of a pecan orchard

Pecan is an important nut crop in Texas and other parts of the southern United States. Commercial production requires large amounts of fertilizers to ensure profitable nut yield (Kim et al., 2002). Nitrogen application is an essential management practice for physiological processes such as photosynthesis (Evans, 1989), and, ultimately, for kernel development and yield (Marquard, 1987). Growers apply between 112 to 280 $kg \cdot ha^{-1}$ each growing season (Kim et al., 2002). Applying N in excess can be harmful to the environment as well as the tree. Since nitrate is highly mobile in the soil, application of excessive amounts of nitrate fertilizer can lead to N leaching into nearby water sources instead of uptake by trees (Baker and Johnson, 1981). Additionally, an excessive amount of N can reduce yield, delay kernel maturation, as well as prolong growth at the end of the growing season, which will make the tree more susceptible to frost damage (Kraimer et al., 2001). Nitrogen applications can also increase canopy growth which can reduce sunlight penetration within the canopy itself and between surrounding trees, thus reducing photosynthesis per unit leaf area (Lombardini et al., 2009) as well as yield and nut quality (Lombardini, 2006). Applying excessive amounts of N during establishment of seedlings can also damage trees by inhibiting growth of the root system (Jacobs et al., 2004). However, applying inadequate amounts of N can be harmful as well. A study done on olive (*Olea europea*) trees showed that N-deficient plants had less chlorophyll *a* and reduced photosynthetic capacity, thus reducing yield. Nitrogen-deficient plants also showed a decrease in total biomass (Boussadia et al., 2010).

For establishing a pecan orchard, recommendations by Texas A&M Agrilife Extension are to apply ammonium sulfate [$(NH_4)_2SO_4$] or ammonium nitrate (NH_4NO_3) in the month of June at the rate of 0.23 kg/tree during the first year, but if trees are not increasing in height and canopy size, fertilizer should not be applied (McEachern, 2007). According to the same recommendations, in the second year, N should be applied in the months of April, May and June at the rate of 0.23 kg/tree per application if trees are growing rapidly, for the third and fourth year at 0.5 kg, and for years five though seven 1 kg should be applied per tree.

Nitrogen fertilization at the time of transplant reduces transplant shock and encourages plant growth (Carlson and Preisig, 1981). However, studies have shown that roots preferentially proliferate in areas of high N when N availability in the soil is heterogeneous (Friend et al., 1990), thus if too much N is applied in the area of planting, roots may not expand far enough for tree survival (Coutts and Philipson, 1976). In a study on establishment of Douglas-fir (*Psedotsuga menziesii*), a high rate of N application reduced the amount of root growth partially because of N toxicities and because biomass was preferentially allocated to shoots and not roots. An improperly established root system can increase drought stress in young trees (Jacobs et al., 2004).

There are three major sources of N for growth of perennial plants: N that is already present in the soil, N fertilizer applied during the growing season, and N that is located in plant reserves (Cheng and Fuchigami, 2002). For efficient use of N, it is critical that supply of N matches demand. In order to estimate the amount of N needed by the plant, it is important to consider how much N can be remobilized from reserves stored from the previous season (Neilsen et al., 2001). Before leaf senescence, N is translocated as amino acids to root and stem tissues and stored to support next season's growth (Cheng and Fuchigami, 2002). Smith et al. (2007) determined that pecan trees do not respond to applied N from the current year, but respond to N applied in the previous year. Thus, large N applications may not be necessary during spring, since there is an abundance of N stored inside perennial tissues. This finding indicates that a multi-season N management strategy needs to be maintained for optimal N utilization efficiency.

Applying a lower rate of N early in the growing season could be just as efficient as applying a standard rate and could decrease the amount of N losses as non-point source pollution. For example, a study on apple (*Malus* x *domestica*) showed that N requirements were low for young trees and early season N demand was met by remobilization from stored reserves (Neilsen et al., 2001). However, this may not work for all species; in almond (*Prunus dulcis*), N reserves were low for young trees and N application was crucial for their establishment (Bi et al., 2003).

1.3 Role of roots in nitrogen uptake

Applying fertilizer when roots are maximizing nutrient uptake and nutrient storage may help reduce over-application of fertilizer and reduce N leaching (Lucash et al., 2005). It appears that most N uptake is driven by plant demand and studies have shown that there are seasonal trends for N uptake in seedlings (Acuña-Maldonado et al., 2003). These uptake patterns are also species-dependent. One study on *Pinus radiata* showed that N uptake was greater in the spring and summer than in autumn and winter (Smethurst and Nambiar, 1989). In another study on *Pinus taeda,* nutrient uptake was greatest in the months of April and October and the least in July (Lucash et al., 2005). For pecan, previous research has shown that N absorption was greatest during the time of rapid shoot and leaf expansion (April and May) followed by a second peak of absorption during the dormant season (November to February) (Acuña-Maldonado et al., 2003).

Another factor affecting N uptake is the amount of N already present in the soil. Roots exposed to soils high in N generally have a lower uptake capacity (Siddiqi et al., 1990). A study on seedlings of Douglas-fir showed that total root biomass, root length, and specific root length were reduced in areas with high levels of ammonium-based fertilizer (Olsthoorn et al., 1991).

Lateral roots have an important role in absorption of water and nutrients from soil. Nutrient uptake rates may be impacted by primary root architectural and morphological characteristics such as lateral root initiation, root length, and root age (Casimiro et al., 2003; Glass, 2003; Volder et al., 2005). In soils with patchy nutrient distribution, there is an abundance of lateral roots in high nutrient areas compared to the bulk soil, indicating a positive relationship between nutrient availability and development of lateral roots (Casimiro et al., 2003). Presence of patches with high nutrients has been found to increase root growth and branching in these patches in a range of studies (Casimiro et al., 2003; Forde and Lorenzo, 2001). The ability to grow preferentially in these patches allows plants to take advantage of greater nutrient availability (Forde and Lorenzo, 2001). However, Casimiro et al. (2003) found that lateral roots in high N patches had increased length, but not increased numbers. They also found that soils with highly uniform nitrate levels had a number of lateral root primordia similar to soils with low nitrate, despite the fact that lateral roots in greater N soils failed to elongate. Increasing specific root length (length per mass) in response to N can improve N uptake by increasing the amount of surface area the root system can cover without sacrificing carbon allocation to above ground portions of the plant (Marschner, 1995). Root age has an influence on the root’s ability to absorb N with young roots usually showing greater activity in nutrient uptake processes (Glass, 2003; Volder et al., 2005). Younger grape (*Vitis vinifera*) roots were more effective at taking up N and had higher rates of respiration than older roots (Volder et al., 2005). Thus, determining the effect of management practices on the dynamics of new root production can help orchard managers make informed decisions about the timing of fertilization application.

1.4 Methods for studying root growth

There are several methods for measuring standing root mass and root production. Coring techniques include sequential coring and ingrowth soil cores. Sequential soil cores are used to measure temporal changes of standing fine root biomass or length, and thus measure net production. Ingrowth cores are used to measure new root production in root-free soil over a defined period of time (Aber et al., 1985). The problem with both the ingrowth core technique and the sequential coring technique is that they are destructive and thus do not allow for repeated observations through time of the same soil volume. In addition, they assume that little heterogeneity exists in root distribution and root production in space, which is an assumption that is rarely true. Thus, estimating net or gross root production through time using soil cores requires a high number of replicates as root distribution and patterns of root production in space are generally very uneven (Vogt et al., 1998). Another method of understanding root development is the use of a minirhizotron to track root initiation, growth, and turnover rates. A minirhizotron is a clear tube inserted in the soil that can be used to view small sections of soil and observe root development via 360° scanned images. Minirhizotrons are more accurate in determining root turnover than sequential soil cores, ingrowth cores, and carbon/N budget approaches because they allow researchers to examine root development rates by conducting multiple observations of a specific section of soil with minimal or no disturbance (Tierney and Fahey, 2001). One disadvantage is that only one small portion of the root system is observed (Yao et al., 2009).

1.5 Summary

Nitrogen is an important nutrient for tree establishment. Uptake of N by plants is regulated by the concentration of N in the soil, as well as by shoot demand and the size, age, soil moisture, and activity of the root system. Understanding how much N is required for establishment of young pecan trees and determining when young pecan trees are most capable of high N uptake rates can help improve N fertilizer use efficiency and reduce N runoff.

In this study, five N application rates were used to determine the optimal application rate for establishment of young pecan seedlings. The hypothesis was that a direct relationship exists between N application rate and trunk diameter growth, photosynthesis, and standing root mass, where increasing N application rates would stimulate trunk diameter growth, photosynthesis, and water use efficiency, but would reduce root production at application rates greater than the recommended rate.

CHAPTER II

MATERIALS AND METHODS

2.1 Orchard site

This experiment was conducted from Apr. 2009 through Nov 2012 at the Texas A&M University pecan research orchard (lat. 30°31′N, long. 96°24′W, elevation 67 m), located in Burleson County, TX. This orchard is situated on Westwood silt loam soils (0% to 1% slope, fine-silty, mixed, thermic Fluventic Ustochrepts, pH 8.1). Forty second-leaf (second production of leaves since planting), open-pollinated, bare-rooted ‘Mohawk’ seedlings were bought bare-root from Womack Nursery (De Leon, TX). Seedlings roots were cut back to a length approximately 15 cm, and planted at a spacing of 3.8 × 15.2 m with the soil compacted around existing root system in early 2009. From May until October of each year, trees were irrigated with micro-sprinklers located between each tree (2/tree) (Mini-Wobbler®, Senninger Irrigation Inc., Lubbock, TX,) with an output of 95 $L \cdot hr^{-1}$ for 24 h every 1 to 2 weeks, depending on weather conditions and the judgment of a professional orchard manager.

2.2 Environmental data

Soil water content was measured twice per week over a 0-20 cm depth using buried TDR (time-domain reflectometer) probes vertically inserted into the soil 40 cm from the base of the tree and a MiniTrase TDR probe reader (Soilmoisture Equipment

Corp., Santa Barbara, CA). Daily weather conditions data were recorded by a weather station located in an adjacent field.

In 2011, pre-dawn and mid-day leaf water potentials for all treatments were collected on the mornings (pre-dawn) and afternoons (mid-day) of 4 May, 2 June, 1 July, 28 July, 24 Aug., 23 Sept., and 20 Oct. 2011 using a pressure chamber (Soilmoisture Corp., Santa Barbara, CA).

2.3 Nitrogen treatments

On 21 May and 21 June 2010, and 16 May and 13 June 2011, ammonium sulfate [$(NH_4)_2SO_4$] was applied at a rate of 0 (0 N), 57.4 (0.25 N), 114.8 (0.5 N), 229.5 (1 N) [standard rate according to the recommendations from the Texas A&M AgriLife Extension Service (McEachern, 2007)], and 459.0 (2 N) $kg{\cdot}ha^{-1}$. To control weeds and maximize N availability to the trees, the orchard floor was sprayed with glyphosate (Round-up, Monsanto, St. Louis, MO) on a weekly basis and weeds were mowed close to the ground using a string trimmer three days before each N application. The fertilizer was applied using a hand-held spreader on a 3 × 3 m plot with each tree in the center of each plot. The size of the plot was chosen because a pecan seedling root system typically grows approximately 1 m in a horizontal direction in the first year (Woodroof and Woodroof, 1934). When soil samples were collected 5 d after the initial fertilizer application (i.e., 16-17 months after planting) at 90 cm from the base of the tree, few pecan roots were recovered from the cores, indicating that the root system extended that far but not much further. After each fertilizer application, the orchard was irrigated with micro-sprinklers (95 $L \cdot hr^{-1}$) for 24 h to maximize soil penetration and minimize ammonia volatilization. Within a week of each first annual N application, new growth was marked on multiple branches by tying strings on petioles of fully expanded leaves, thus enabling a separation of growth and physiology of leaves developed prior and post fertilizer application. Soil samples were collected using an AMS Soil Core Sample Kit with 5.9-kg hammer attachment and a 7.5”x 2” auger with a volume of 343.47 cm^3 (Forestry Supplier, Jackson, MS) 90 cm away from the tree trunk at two depth intervals (0-20 and 20-40 cm). On 25 May 2010, 5 July, 7 Sept., and 1 Nov. 2011 three samples were collected per depth per tree and were composited and homogenized before root extraction; on 19 Aug. and 1 Oct. 2010, one sample was collected per depth interval per tree. After the initial core extraction on May 25, 2010, the number of cores per plot per depth was reduced to one core to decrease processing time. However, this did not yield enough root sample material for nutrient analysis and, consequently, in 2011 the number of core samples collected was increased back to three samples per depth. Sub-samples of root-free soil samples were air-dried, ground, and analyzed for extractable N content using a 1 N KCl solution (Texas A&M Agrilife Extension Service Soil, Water and Forage Testing Laboratory, College Station, TX). Pre-dawn and mid-day water potentials were collected on seven dates (4 May, 2 June, 1 July, 28 July, 24 Aug., 23 Sept., and 20 Oct. 2011) using a pressure chamber (Soilmoisture Equipment Corp., Santa Barbara, CA) to measure tree water status. Soil samples remained in the refrigerator for up to 12 weeks. Samples were treated uniformly throughout the study and cold degradation of fine roots over such a period is minimal (Watson et al., 2000) and unlikely to differ between treatments. Root samples were removed from soil cores by hand using a 2-mm sieve before the soil was dried and kept separated according to the same depth intervals. Pecan roots from a young seedling not included in this study, as well as several common weed roots found in the orchard, were excavated so that the difference between pecan and weed roots could be determined by size, color, and branching pattern. Pecan roots were separated from weed roots and then sub-divided into fine (<0.5 mm diameter) and coarse roots (>0.5mm diameter). Root length, mean diameter, and length per diameter class were determined using a precision scanner and image analysis software (WinRhizoPro 2009, Regent Instruments Inc., Ottawa, Canada). Root samples were then dried and weighed after a minimum drying period of 72 hours at 70 ºC. Root data were then used to calculate SRL (root length per root dry mass, $m \cdot g^{-1}$) and RLD (standing root length per core volume, $km \cdot m^{-3}$).

In Oct. 2012 an additional set of soil cores was collected to document extractable nitrate availability throughout the profile (up to 200 cm), as well as the mass of the roots throughout the soil profile. We extracted these soil cores using a hydraulic corer (Giddings Machine Company, Windsor, CO) mounted on a truck. After extraction from the soil, cores were laid on a sheet of plastic and divided in 20 cm sections ranging from 0-200 cm. We sieved the roots from a portion of the soil sample; air dried the root free soil samples and then analyzed the soils for extractable N content using a 1 N KCl solution (Texas A&M Agrilife Extension Service Soil, Water and Forage Testing Laboratory, College Station, TX). We sieved the roots from remaining portion of the soil sample, combined these with the roots from the root-free subsample and dried these roots to constant mass at 70 ^{o}C (minimum 72 h).

2.4 Above ground measurements

Leaf gas exchange was monitored on leaflets of recently matured leaves every 3 weeks during the 2010 and 2011 growing seasons using a portable infrared gas analyzer (LI-6400XT, LI-COR, Lincoln, NE).

After each gas exchange measurement, the leaflet used was removed, placed in a re-sealable plastic bag and stored on ice. Once in the laboratory, leaflets were scanned for projected leaf area in the chamber (cm^2) (WinRhizoPro 2007 software, Regent System, Quebec, Canada). A color-reader (SPAD, Konica Minolta, Tokyo, Japan) was used as an indicator of leaf greenness (Gianquinto et al., 2003). Leaflets were then dried and weighed and specific leaf area ($m^2{}_{\text{leaf area}} \cdot g^{-1}{}_{\text{leaf dry mass}}$, SLA) was calculated.

2.5 Tree growth

Trunk diameter was measured at the same point each time at two locations: the base of the tree, approximately 0.5 m from the soil surface (established trunk at transplant) and the central leader of the tree (new growth after transplanting). The central leader was established in the 2010 growing season by removing all lateral shoots except for one, the central leader. Relative growth rate was measured and calculated as:

$$\ln(\text{diameter}_2) - \ln(\text{diameter}_1)/(\text{time}_2 - \text{time}_1)$$

where diameter_1 and diameter_2 were the diameter (mm) measured at time_1 (days) and time_2 (days), respectively.

2.6 Data analysis

This experiment was conducted as a completely randomized design with five N application rates (0, 57.4, 114.8, 229.5, and 459.0 $\text{kg}\cdot\text{ha}^{-1}$) with eight single-tree replications for each treatment. Treatment effect (N treatment, sampling data, soil depth) were analyzed using analysis of variance (ANOVA) with N treatment as a continuous factor using the JMP 10 statistical software package (SAS Institute Inc., Cary, NC). Effects of nitrogen treatment, sampling date or soil depth, or their interaction, with a $P < 0.05$ were considered statistically significant.

CHAPTER III

RESULTS

3.1 Soil nitrogen

For the 2010 growing season (Table 1 and Fig. 1A, C) there was significantly more nitrate in May in the 1 N and 2 N treatments, than in the lower N application treatments. There was also significantly more N in the 0-20 cm depth than in the 20-40 cm depth in May.

For the 2011 growing season (Table 1 and Fig. 1B, D) there was significantly more extractable nitrate in the soils in Sept than in May and July and significantly more extractable nitrate in the soils in the 1 N and 2 N treatments than in the lower application rate treatments ($P_{\text{date x N treatment}} = 0.005$). There was significantly more extractable nitrate in the 0-20 depth than in the 20-40 cm depth for the 1 N and 2 N treatments, but not the lower application rates. This effect was stronger in May and September than in July ($P_{\text{date x N x depth}} < 0.001$).

Table 1. P-values for the effects of N treatment, date, and depth on extractable soil nitrate for the 2010 and 2011 growing seasons (*n* = 8).

Source	2010	2011
Date	0.005	0.022
N Treatment	0.154	<0.001
Date × N Treatment	0.005	0.008
Depth	<0.001	<0.001
Date × Depth	0.013	0.124
N Treatment × Depth	0.364	0.020
Date × N Treatment × Depth	0.161	<0.001

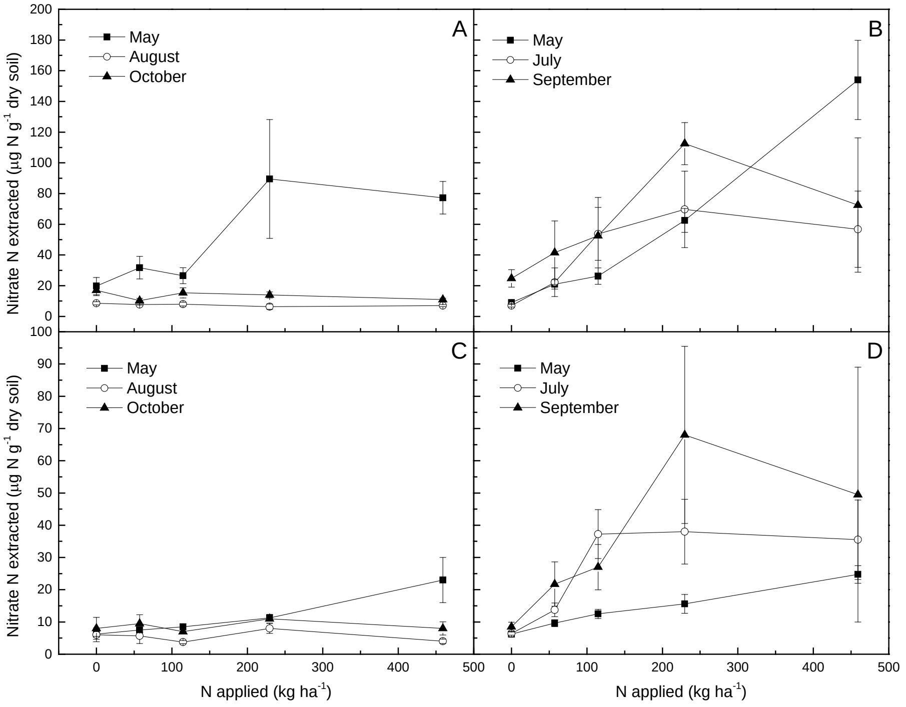

Fig. 1. Average amount of nitrate-N extracted from soil within 2 weeks of $(NH_4)_2SO_4$ application using a 1 N KCl solution for 0-20 (A, B) and 20-40 cm (C, D) depths in 2010 (A, C) and 2011 (B, D). Data are average ± SE (*n* = 8).

About 15 months after the last N application, the effect of double N application (last applied in May 2011) was still strong (Fig. 2), with up to 15 times greater amounts of extractable nitrate-N in these plots than in the plots with the recommended and zero application rates. However, there was no difference in extractable nitrate between zero application and the recommended application rate in the 0-100 cm soil depth. Samples collected below 100 cm depth revealed an increase in extractable nitrate compared to a low at 40-60 cm in the 0 N treatment. Available extractable nitrate in the 120-160 cm depth was comparable to that in 0-20 cm depth (Fig. 2).

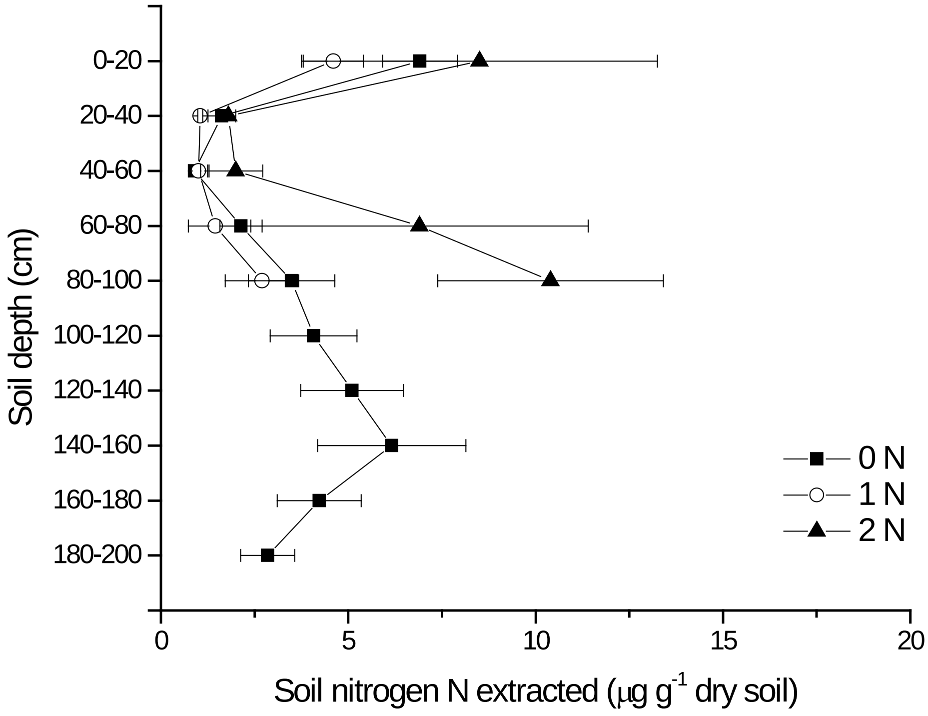

Fig. 2. Extractable soil NO_3^- as a function of fertilizer application rate, where 0 N, 1 N and 2 N correspond to 0, 229.5, and 459 kg·ha^{-1}, respectively. Data were collected in October 2012, 15 months after the last fertilizer application, and are average ± SE (*n* = 8 for 0 N, *n* = 4 for 1 N and 2 N).

3.2 Environmental data

Volumetric soil water content ranged from 14.1% to 30.1% with an average level of 21.4% (Fig. 3). Pre-dawn water potentials ranged from -0.07 to -1.10 MPa with a mean of -0.35 MPa and mid-day water potential ranged from -0.40 to -2.55 MPa with a mean of -1.50 MPa (Fig. 4). Temperature was lowest in late October for both years and highest in August for both years (Table 2).

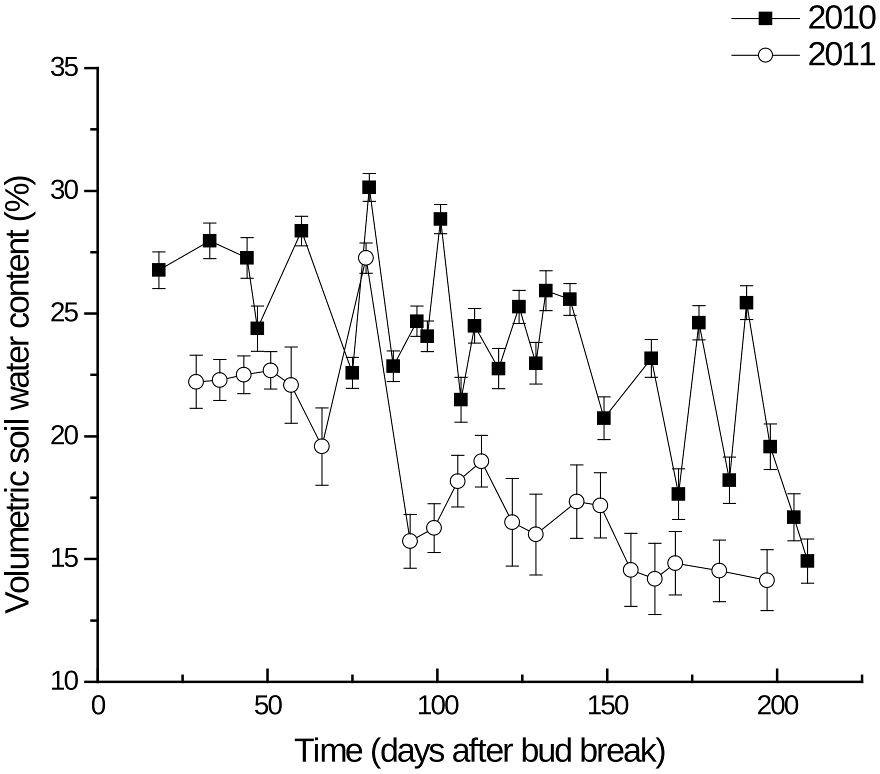

Fig. 3. Volumetric soil water content throughout the growing season in 2010 and 2011. Data are average ± SE ($n = 16$).

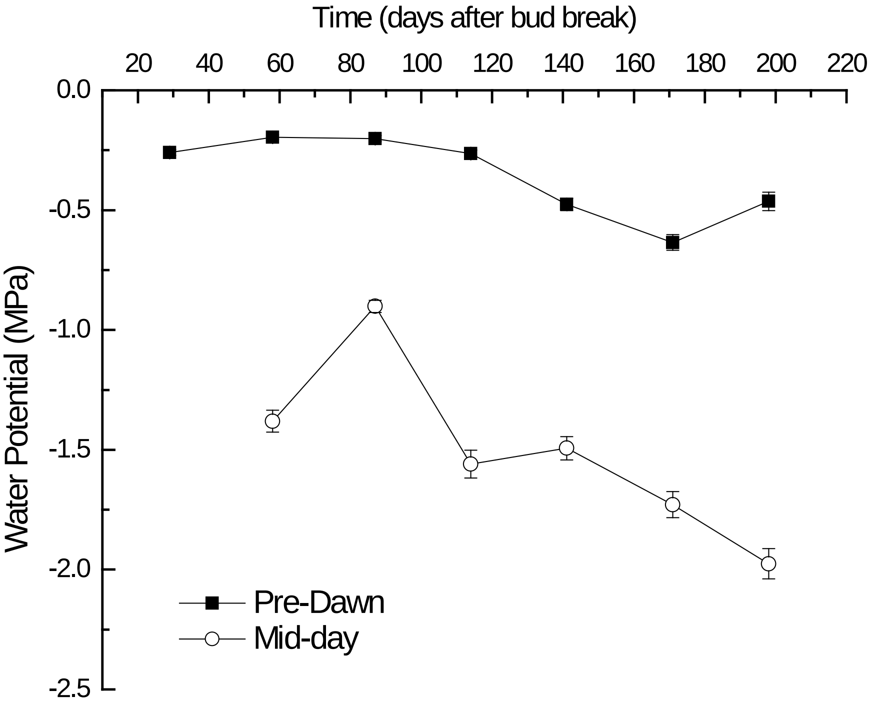

Fig. 4. Pre-dawn and mid-day water potential of fully developed pecan leaves for all treatments during the 2011 growing season. Data are average ± SE (*n* = 8).

Table 2. Air temperatures for each date gas exchange data were collected. Bulk air temperatures were measured at 1 and 2 meters above ground at a local weather station. Air temperature was also measured in the Li-Cor 6400 chamber (*n* = 8), showing that on most dates measurement temperature corresponded to the bulk air temperature.

Date	Air Temp 1 m (°C)	Air Temp 2 m (°C)	Air Temp Li-Cor (°C)
11 Jun 2010	33.0	33.2	30.7
25 Jun 2010	34.4	35.6	30.8
16 Jul 2010	35.8	36.8	34.3
9 Aug 2010	36.9	37.7	34.4
26 Aug 2010	36.0	36.5	34.8
17 Sep 2010	34.7	35.9	31.1
9 Oct. 2010	30.5	31.0	30.6
30 Oct 2010	26.9	27.3	29.2
4 May 2011	29.1	30.2	29.2
2 June 2011	37.2	38.0	30.6
1 July 2011	36.6	37.7	35.0
28 Jul 2011	38.3	39.6	35.2
24 Aug 2011	39.3	41.0	35.4
23 Sep 2011	30.7	31.1	30.0
20 Oct 2011	26.5	27.0	27.5

3.3 Leaf nitrogen concentration

There were no effects of N treatment or year on leaf N concentration (*P* = 0.639 and *P* = 0.306 respectively). Mean leaf N concentration was 20.2 $mg \cdot g^{-1}{}_{\text{dry mass}}$ and 20.7 $mg \cdot g^{-1}{}_{\text{dry mass}}$ for 2010 and 2011, respectively (Table 3).

Table 3. Minimum, maximum, mean and standard error (SE) for total leaf nitrogen (N) for each N treatment applied [$(NH_4)_2SO_4$] on pecan (*Carya illinoinensis*) seedlings for the years 2010 and 2011 (*n* = 8).

N treatment (kg/ha)	Total leaf nitrogen ($mg \cdot g^{-1}$ dry mass)							
	2010				2011			
	Min.	Max.	Mean	SE	Min.	Max.	Mean	SE
0	17.9	23.4	19.6	0.70	17.6	25.1	20.5	0.92
57.4	18.4	23.9	21.2	0.74	19.1	21.6	20.4	0.38
114.8	16.2	23.0	20.0	0.78	18.4	26.1	22.0	0.98
229.5	18.7	21.6	19.9	0.44	18.0	24.3	20.6	1.28
459	17.4	25.2	20.2	0.96	15.4	22.8	20.1	0.85

3.4 Tree growth

There was no effect of N treatment (*P* = 0.947) on the rate of base diameter increase through time nor was there an interaction between N treatment and time (*P* =

0.579) indicating that base diameter relative growth rate (RGR) was not affected by the N treatment (Fig. 5B). There also were neither differences in changes in central leader diameter through time due to N treatment (*P* = 0.079) nor was there a N treatment × time interaction indicating that central leader diameter RGR was not affected by N treatment (*P* = 0.585; Fig. 5A). There was a difference in mean diameter in each year (*P* < 0.001), where mean base diameter was 37.4 mm in 2010 and 45.3 mm in 2011 and mean central leader diameter was 18.2 mm in 2010 and 31.2 mm in 2011. The slopes for the base and central leader in the 2010 growing seasons were not statistically significant (*P* = 0.7432 and *P* = 0.9594 respectively). However for the 2011 growing season the base diameter slope was statistically significant (*P* = 0.001) with a slope of 0.0804 (µm mm^{-1}·day^{-1}) (SE = 0. 211) and central leader slope was also statistically significant (*P* = <0.0001) with a slope of 1.3 (µm mm^{-1}·day^{-1}) (SE = 0. 263).

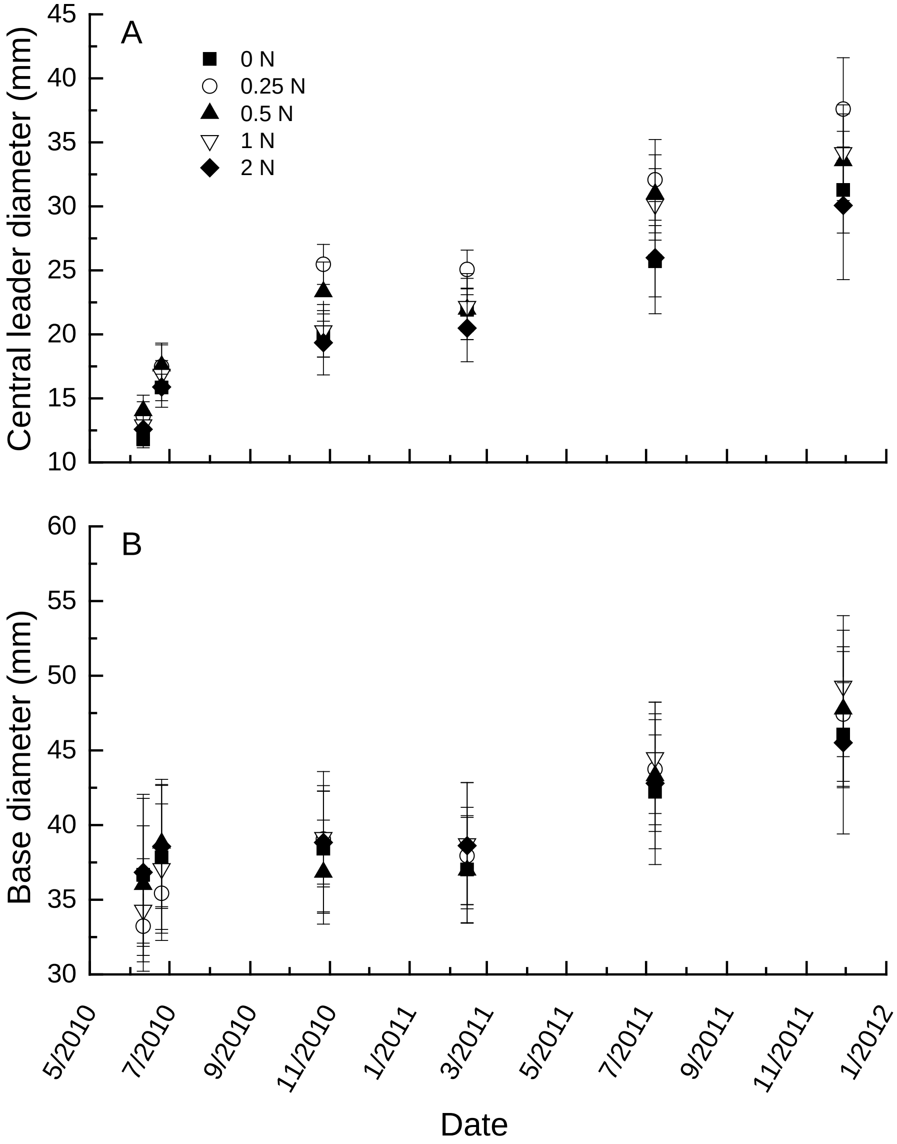

Fig. 5. Central leader (A) and base (B) diameter of pecan seedlings through time as affected by N application rates (where 0 N, 0.25 N, 0.5 N, 1 N, and 2 N correspond to 0, 57.4, 114.8, 229.5, and 459.0 kg·ha^{-1}, respectively) for both 2010 and 2011 growing seasons. Data are average ± SE (*n* = 8).

3.5 Specific leaf area

There was no significant effect (*P*=0.397) of N treatment on specific leaf area (SLA) ($m^2 \cdot g^{-1}$) but there was a significant effect of date on SLA (*P* < 0.001, Fig. 6). SLA decreased about 38 % in 2010 and 34 % in 2011 as the leaves aged throughout the growing season.

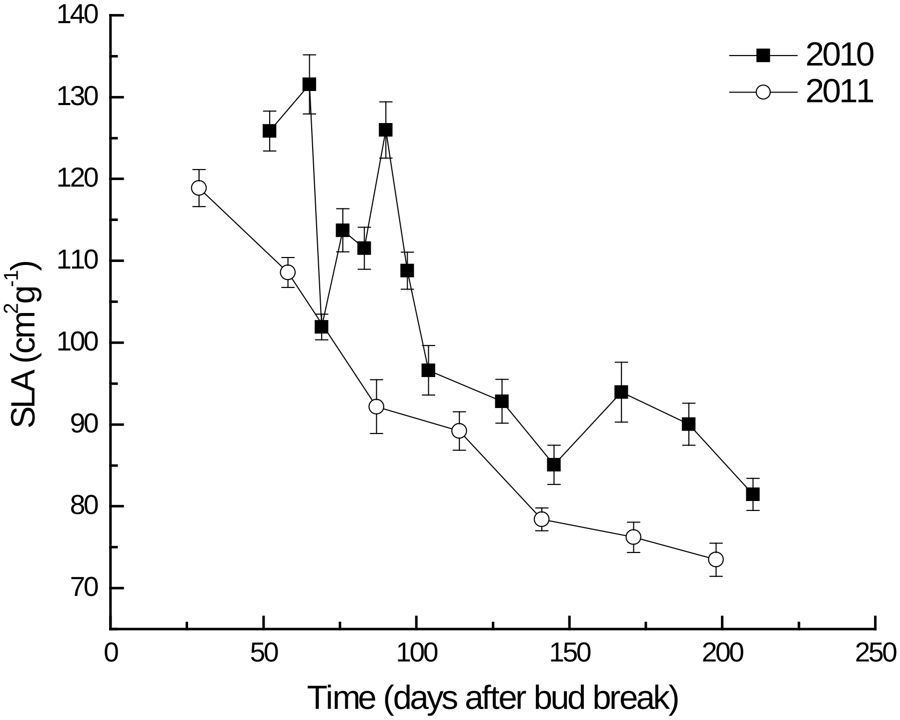

Fig. 6. Average specific leaf area (SLA) of pecan seedlings over both 2010 and 2011 growing seasons. Data are average ± SE (*n* = 8).

3.6 Gas exchange

There was no effect of N treatment on net photosynthetic rate (*P* = 0.876) (Fig. 7). However, net photosynthetic rate showed a hyperbola throughout each season, where rates were low early and late in the season and reached maximum rates in late July and early August (Fig. 7).

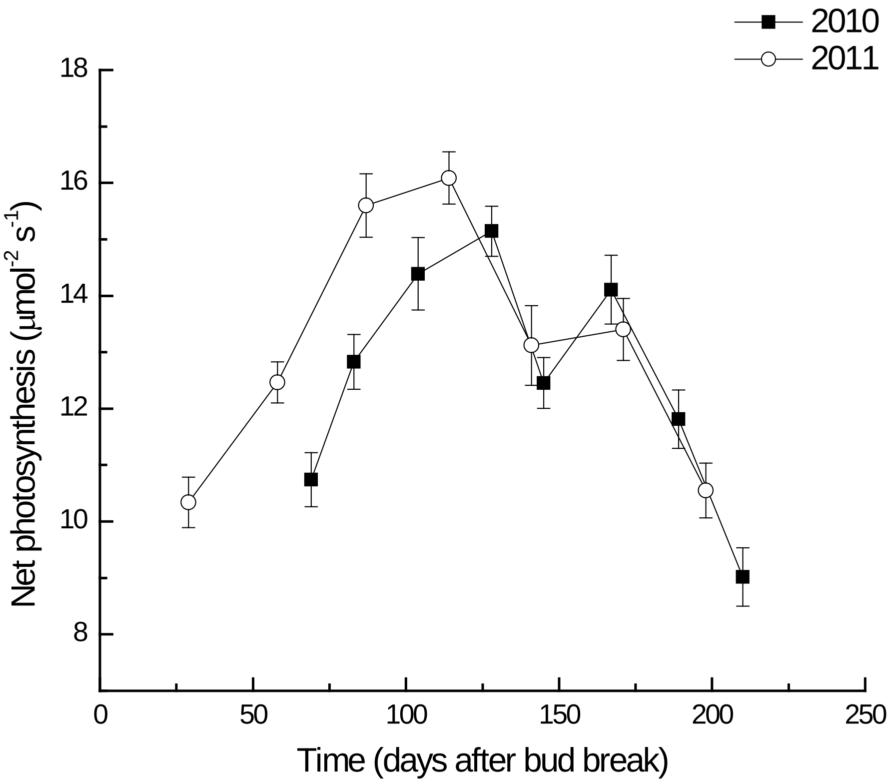

Fig. 7. Light-saturated net photosynthesis rates of fully developed pecan leaves during the 2010 and 2011 growing seasons. Data are average ± SE (*n* = 8).

However, N treatment and time did have a significant effect on SPAD measurements (*P* = 0.046 and *P* < 0.001 respectively) (Fig. 8). The lowest mean SPAD value was recorded in the 2 N treatment (32.7) and the highest mean SPAD value was recorded for the 0.5 N treatment (35.4). There was a significant correlation between photosynthesis rate and SPAD (*P* = 0.009) (data not shown).

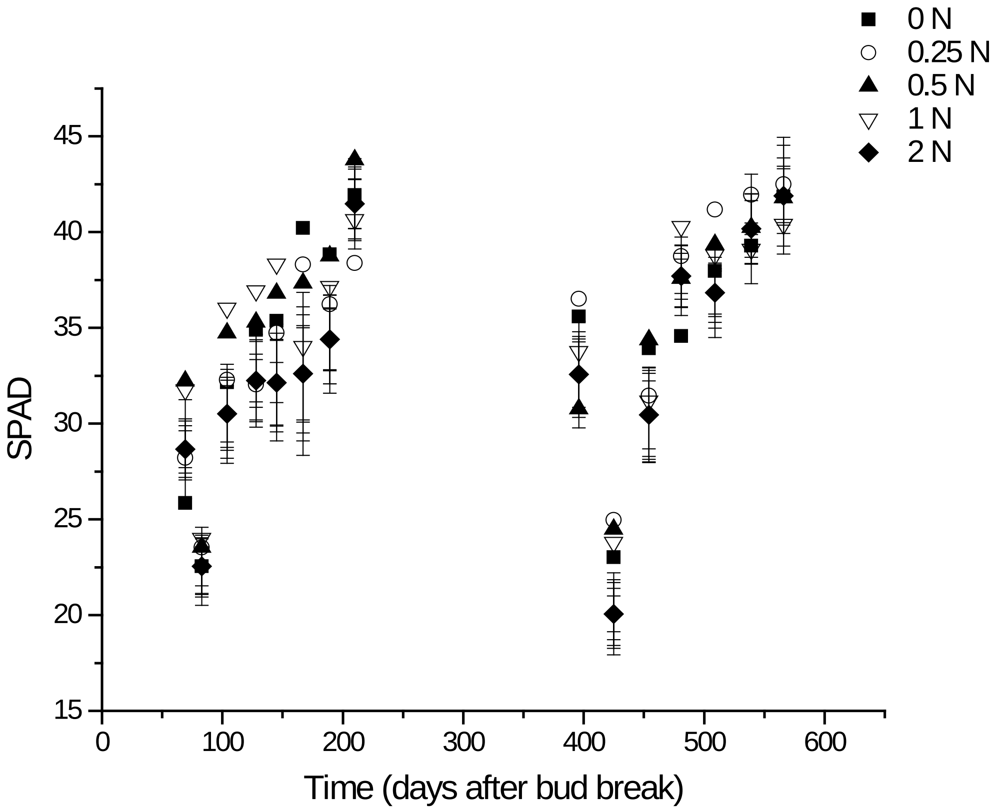

Fig. 8. SPAD measurements for pecan at each date for both 2010 and 2011 growing seasons. Data are average ± SE (*n* = 8).

There was no effect of N treatment on stomatal conductance (*P* = 0.665) or transpiration (*P* = 0.761) (Fig. 9). However, there was a difference in transpiration rate between the two growing seasons, with a higher overall transpiration rate in 2011 ($P <$ 0.001). There was no effect of N treatment on WUE (P = 0.950) (Fig. 10).

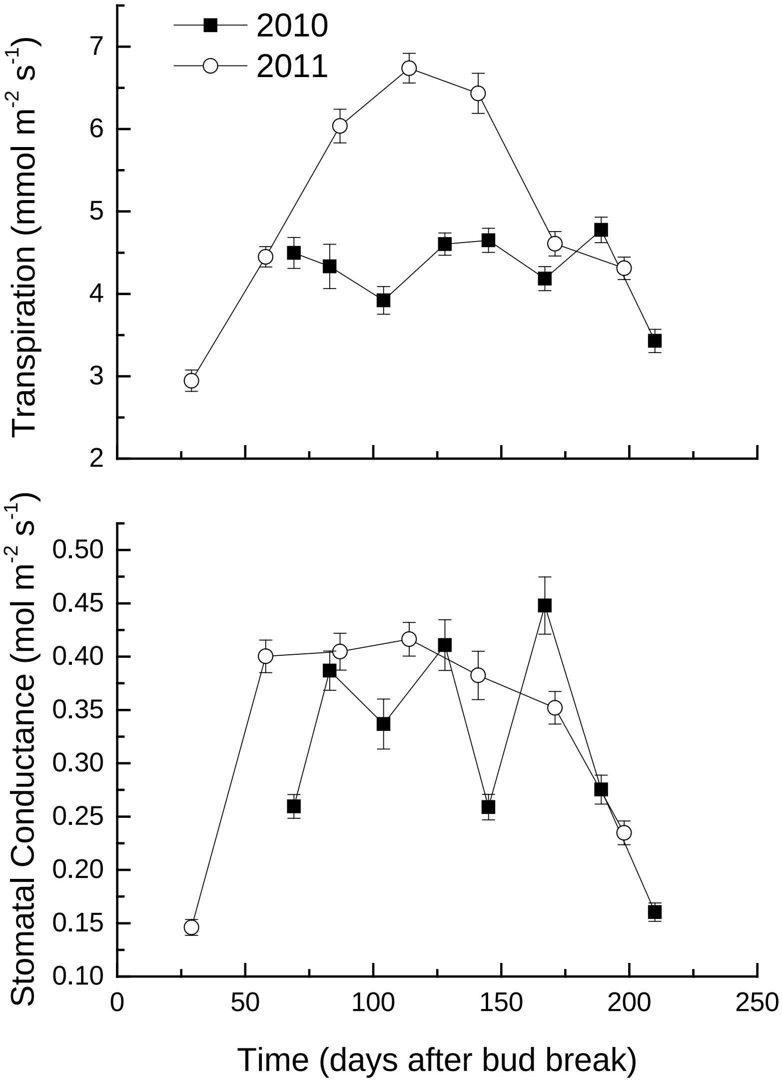

Fig. 9. Stomatal conductance and transpiration of pecan seedlings for 2010 and 2011 growing seasons. Data are average ± SE (n = 8).

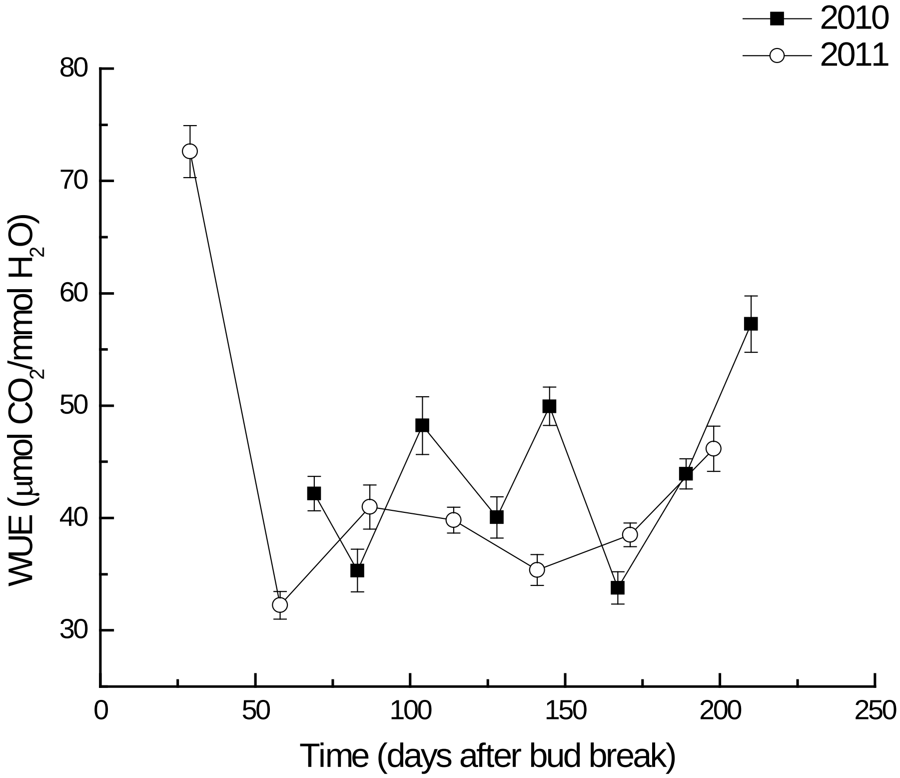

Fig. 10. Water use efficiency (net assimilation rate/ transpiration rate, WUE) of pecan seedlings for the 2010 and 2011 growing seasons. Data are average ± SE (*n* = 8).

3.7 Root length density

There was no effect of N application rate on root length density (RLD) (Table 4), however RLD was overall higher in the 2010 growing season than in the 2011 growing season with a mean of 0.45 $km \cdot m^{-3}$ versus 0.25 $km \cdot m^{-3}$. In both years RLD declined from a maximum value 2.79 $km \cdot m^{-3}$ in May to a minimum in September 0.011 $km \cdot m^{-3}$ (P_{date} = 0.001, Fig. 11). In 2010, RLD was lower in the deeper soil layer, but this pattern was reversed in 2011 when the highest RLD was found in the 20-40 cm depth ($P_{\text{depth x year}}$ = 0.014, Fig. 11, Table 4).

Table 4. P-values for the effects of N treatment, core depth, year and date on root length density of pecan seedlings for the 2010 and 2011 growing seasons.

Source	P-value
N Treatment	0.452
Depth	0.053
N Treatment × Depth	0.588
Year	0.001
N Treatment × Year	0.739
Depth × Year	0.014
N Treatment × Depth × Year	0.657
Date	0.001
N Treatment × Date	0.448
Depth × Date	0.134
N Treatment × Depth × Date	0.148
Year × Date	0.329
N Treatment × Year × Date	0.616
Depth × Year × Date	0.200
N Treatment × Depth × Year × Date	0.289

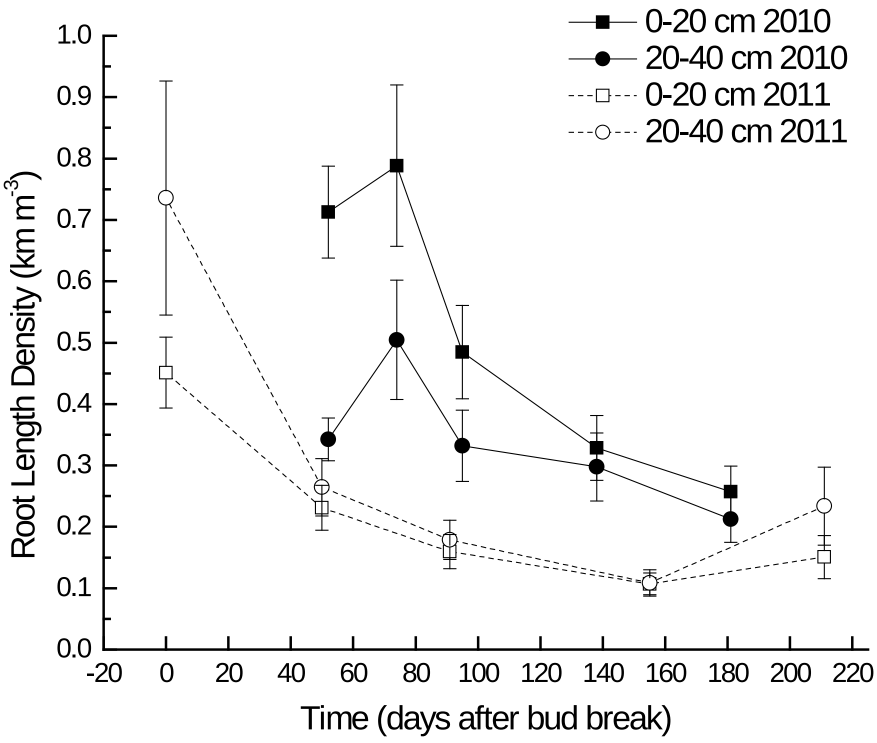

Fig. 11. Root length density for pecan seedlings at each soil depth through time for both the 2010 and 2011 growing seasons. Data are average ± SE (*n* = 8).

3.8 Specific root length

Specific root length was greater at the deeper soil depth ($P = 0.015$); with a mean of 51.1 m·g^{-1} in the 20-40 cm soil depth compared to 46.3 m·g^{-1} in the 0-20 cm depth. The seasonal pattern was opposite in the two years, (year × date interaction $P < 0.001$) (Table 5), mean SRL increased through time in 2010, while it decreased over a similar period in 2011 (Fig. 12).

Table 5. P-values for the effects of N treatment, core depth, year and date on specific root length of pecan seedlings for the 2010 and 2011 growing seasons.

Source	P-value
N Treatment	0.655
Depth	0.015
N Treatment × Depth	0.716
Year	0.147
N Treatment × Year	0.457
Depth × Year	0.815
N Treatment × Depth × Year	0.601
Date	0.498
N Treatment × Date	0.737
Depth × Date	0.911
N Treatment × Depth × Date	0.553
Year × Date	0.001
N Treatment × Year × Date	0.978
Depth × Year × Date	0.089

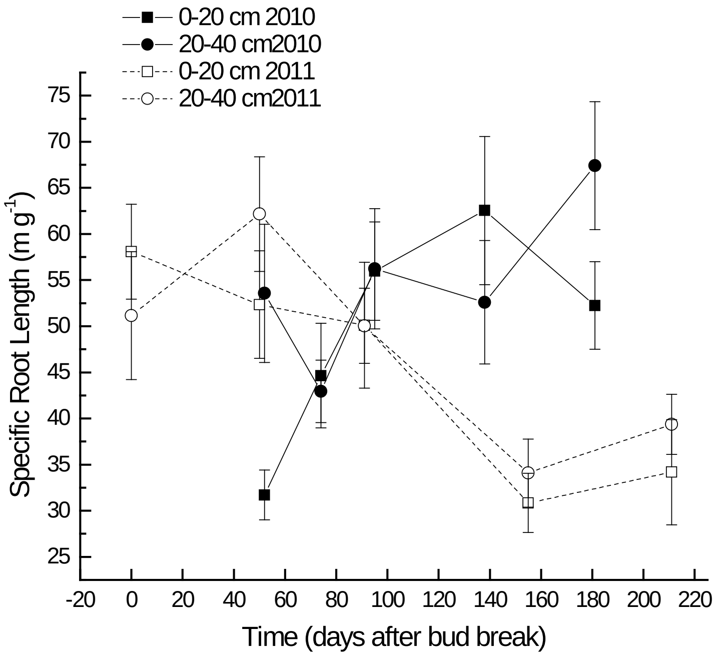

Fig. 12. Specific root length for pecan seedlings at each soil depth through time for both the 2010 and 2011 growing seasons. Data are average ± SE (*n* = 8).

3.9 Root weights for 0-200 cm depths

In October 2012 an additional set of soil cores was collected to determine the amount of roots found at deeper soil depths (Fig. 13). There were no significant differences due to N treatment (*P* = 0.97), depth (*P* = 0.19) or nitrogen by depth (*P* = 0.99).

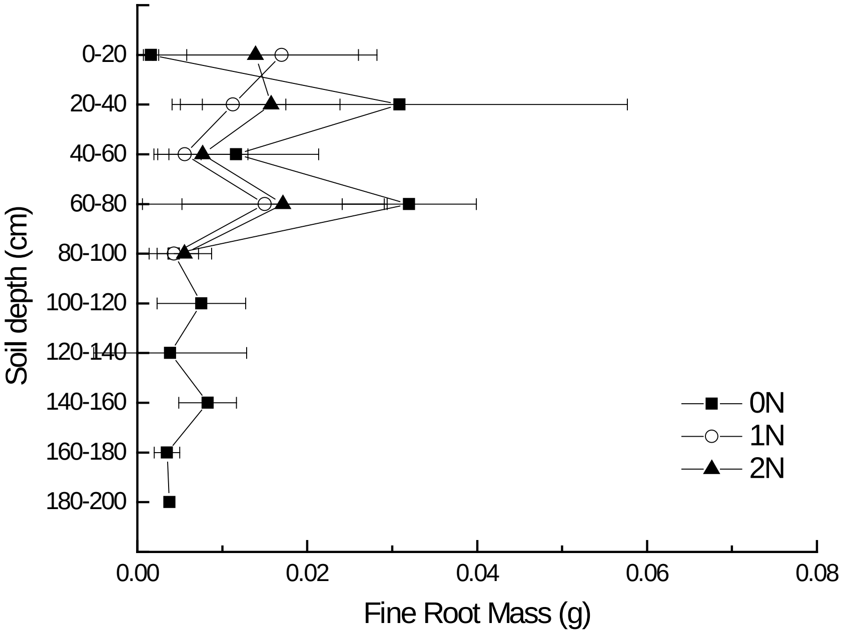

Fig. 13. Root mass for soil samples in 2012 for depths of 0-200 cm. Data are average ± SE (*n* = 8 for 0 N, *n* = 4 for 1 N and 2 N).

CHAPTER IV

DISCUSSION

Even though N fertilization significantly increased nitrate availability in the soil, diameter growth, leaf tissue N concentration and photosynthesis were not affected by N fertilization. The lack of a positive plant response to N application (especially in 1 N and 2 N treatments) suggests that current recommended fertilizer application rates for new pecan orchards are too high. According to Sparks (Sparks, 1968), one-year old pecan seedlings should exhibit visible N deficiency symptoms when the foliar concentration ranges between 1.8 and 2.2 percent. Based on these values, the seedlings in our study should have been N deficient; however there were no visible signs indicating a N deficiency (pale leaf coloration), and the higher rates of N fertilization did not increase foliar N concentrations. Sparks study differs from the present one in age of seedlings, and thus perhaps rate of growth which in turn may influence dilution of N in leaves.

There was no significant effect of N treatment on diameter growth rate of either the base or central leader. A similar study using fertigation on first year pecan seedling growth also found that there were no statistically significant effects of N application (at the rates of 0, 4, 10, 20, and 40 g/tree of N) on diameter growth, except for the highest rate which actually reduced diameter growth (Conner, 2007).

Net photosynthesis was not affected by N application rate. Using five bottomland tree species, Vaitkus et al. (Vaitkus et al., 1993) showed that the effect of N application on photosynthesis was species-specific. In their study, water oak (*Quercus nigra*) was the only species that showed a significant increase in photosynthesis with additional N application. The other four species (horn-beam [*Carpinus caroliniana*], pond pine [*Pinus serotina*], red maple [*Acer rubrum*], and swamp chestnut [*Quercus michauxii*]) did not respond positively to N application even though photosynthesis was positively correlated to foliar N.

In my study, net photosynthesis in increased throughout the summer season and then declined after July in both years. During the month of August there was a dip in net photosynthesis that was followed by an increase in net photosynthesis in September. One explanation might be that high air temperatures in August caused water stress which may have induced stomatal closure, resulting in reduction of transpiration and photosynthesis. However, in 2011 transpiration did not decrease during the August dip while photosynthesis did. Another explanation could be that the second spike in September was correlated with kernel fill. This could be a genetic mechanism used to increase energy production and sugar allocation to the developing fruits when these seedlings enter maturity. A study on five different olive cultivars showed that there was a seasonal trend for all cultivars showing a similar trend of higher CO_2 assimilation rates in spring and autumn and lower assimilation rates in summer and late fall. In the olive study it was hypothesized that a decrease in CO_2 assimilation rates was due to high temperatures in the summer, followed by an increase due to more favorable temperatures and presence of rapidly developing fruit in the fall (Hagidimitriou and Pontikis, 2005).

SPAD is a measure of greenness usually indicating there is more chlorophyll present in the leaves (Marquard and Tipton, 1987). More chlorophyll increases the amount of light captured during the light reaction, causing an increase in ATP and NADPH availability to the Calvin cycle and can thus increase sucrose production (Lu and Zhang, 2000). In my study there was a positive correlation between SPAD measurements and photosynthesis. Nitrogen application up to the recommended rate also increased SPAD measurements, similar to a study on pumpkin where SPAD increased with increasing rates of applied N (Swiader and Moore, 2002). Leaves collected from trees in the 2 N treatment had significantly lower SPAD readings than the other treatments. Studies on tomato (*Solanum lycopersicum*) and potato (*Solanum tuberosum*) showed that the SPAD value had a threshold level below, which a reduction in yield occurred (Gianquinto et al., 2003; Gianquinto et al., 2006). The high rates of N applied could have caused damage to the root system (Jacobs and Timmer, 2005), therefore reducing the health of the foliage due to impairment of nutrient uptake (Maynard et al., 1997).

There was no effect of N application rate on stomatal conductance, transpiration, or WUE. However there were differences in transpiration rates between years. During the second year of this study, transpiration rates were higher potentially because of higher air temperatures (Table 2) that increased the vapor pressure difference between the leaf and the surrounding air.

It is possible that N was stored in perennial plant tissues (roots, branches, and trunk) for later use. It is common for pecan to store N reserves in perennial tree parts (i.e. stems, trunk, roots) and use it for the next year’s growing season (Acuña-Maldonado et al., 2003; Smith et al., 2007). However there were no differences in growth that indicated N from 2010 was utilized in 2011. These aboveground data suggest that there may not be a need to apply much, if any, N to young pecan seedlings during the first two years. Nitrogen applications did not enhance aboveground seedling growth or photosynthesis compared to those where no N was applied. More research needs to be conducted to determine if N reserves still play a key role in N allocation in young pecan seedlings and to determine whether higher N application rates actually damage the root system.

Belowground I had expected to find a decrease in SRL and RLD as N application rates increased (Gorissen et al., 1993), but, surprisingly, we found no effect of N treatment on either SRL or RLD in spite of strongly increased soil nitrate availability. It is possible that the seedlings were still suffering from transplant shock, which will cause a reduction in root production in general (Close et al., 2005).

Root length density ($\mathrm{km}_{\mathrm{root}}\ \mathrm{m}^{-3}{}_{\mathrm{soil}}$) was greatest at the beginning of each season and lowest at the end of each season, presumably due to high root mortality over the summer while few new roots were produced. Increasing fertilization was found to increase RLD in a study on four woody tree species (Coleman, 2007); however, in our study N application rate did not affect RLD. In the second growing season there was a late increase in RLD that coincided with an increase in SRL suggesting that there likely was not a large increase in biomass allocation to the root system, but rather an increase in length per unit mass allocated to the root system (i.e., thinner or less dense roots). A possible explanation for the seasonal pattern is a combination of a response to N availability and an increase in sink demand from shoot to root because shoots are no longer in competition with the root system. It would be advantageous of the root system to utilize this period of higher nutrient availability (Jackson and Caldwell, 1989). The increase in N availability can happen in the spring after soil warms up and mineralization is increasing, and plants with high root growth would have an advantage in nutrient capture. These plants can also produce a flush of root growth in the fall when leaf drop is occurring and more minerizable substrate is available for nutrient uptake and stored in reserves for growth in the spring, which has been observed in pecan (Acuña-Maldonado et al., 2003). The reason there was not an increase in RLD in the first season but the second is perhaps the last round of RLD measurements (1 Oct.) was not taken after leaf drop in the 2010, but was measured in 2011 (1 Nov) (Fig. 11). It is also possible that because SRL increased it caused an increase in RLD as well.

The values of RLD (0.11-0.80 km·m^{-3}) were compared to other reported values (2.00-14.4 km·m^{-3}) (Coleman, 2007). These low values are possibly due to the root system being so young it is still trying to recover from transplant shock (Richardson-Calfee et al., 2010). Low RLD values have also been explained as indicative of low amounts of N available in the soil (Coleman, 2007), however, this is unlikely in the present study since N fertilization did not increase RLD. The greatest root length density was found early in the growing season (May-June) and thus N fertilizer should be applied at that time.

N fertilization did not affect SRL; however depth and date did have an effect on SRL (Table 5, Fig. 12). There was a higher SRL at the depth of 20-40 cm. It was expected to have greater SRL in the soil surface (0-20 cm), because the nutrients tend to be higher in the soil surface and roots proliferate in nutrient-rich areas (Jackson and Caldwell, 1989). A study on *Quercus serrata* (Konara oak) and *Ilex penduculosa* (longstalk holly) showed that the depth effect on SRL is species-specific (Makita et al., 2011). SRL may have been greater in the deeper portion of the soil profile because pecan tends to be a deep-rooted species and its finer roots tend to grow in the top 30-60 cm of the soil, regardless of higher nitrate availability in the top soil (Woodroof and Woodroof, 1934).

In the second season of this study, the SRL was lower later in the season and highest at the beginning (25 May 2011); however, for the first year the SRL was highest at the latest date (10 Oct. 2010) in the season. Specific root length also affects root lifespan, so the higher the SRL the shorter the lifespan of the root (Eissenstat et al., 2000). If there is a flush of N availability in the spring you would have higher SRL during this time and then a decrease in SRL as the season progresses, until a new flush of roots is produced (Eissenstat and Achor, 1999). Pecan roots have been shown to absorb substantial amounts of N between leaf fall and dormancy (Acuña-Maldonado et al., 2003). Since greater SRL indicates greater capacity for nutrient uptake (Eissenstat, 1992), it could be possible for pecan to have greater SRL very late in the season.

The belowground data show that the amount of N applied did not have an effect on root length density and specific root length of pecan seedlings, even though here was substantially more nitrate in soils exposed to the two highest N application rates. Moreover, when applied rates were below the recommended rates, there were very low amounts of nitrate (0-10 ppm; A. Provin, personal communication) present in the soil and thus the trees should have been negatively affected.

The belowground data collected only cover the top 40 cm of the soil. An alternative explanation for the lack of a response to added fertilizer could be that the seedlings were able to meet all their N needs from deeper N reservoirs in the soils, and thus any added N to the topsoil would be superfluous. A recent extension publication shows the presence of substantial amounts of N below 1 m in soil depth in Burleson county (Lemon et al., 2009). Deep soil coring (Fig. 2) shows the presence of nitrate concentrations in 120 – 160 cm soil depths that equal topsoil (0-20 cm) levels. However, the presence of roots in this layer is minimal compared to the presence of fine roots in the 0-40 cm layer (Fig. 13). In addition, adding fertilizer to the topsoil strongly increased available N levels, even compared to the deep N reservoir. If the plants needed additional fertilizer application, they should have responded to the extra N availability, thus, it can still be concluded that in this orchard it was not necessary to apply fertilizer during the establishment of these tree seedlings. Moreover, if the lack of response was due to initial transplant shock, this recommendation can be extended to other orchards as well for at least the first two seasons after transplant. Longer term research is needed to determine at what point in the pecan life cycle it does become advantageous to supply the trees with supplemental N.

CHAPTER V

CONCLUSIONS

My objectives for this study were to determine if the currently recommended nitrogen (N) fertilization rates for recently-transplanted pecan seedlings can be reduced without negatively affecting photosynthesis, WUE, and trunk diameter growth, and to determine if and when different N application rates had an effect on the root system, especially specific root length (SRL) and root length density (RLD), in order to potentially decrease the amount of N that is currently recommended.

It is important to understand how pecan seedlings are utilizing Nitrogen (N) to potentially reduce the amount currently recommended for application, thus reducing the potential for pollution in water systems as well as reducing the production cost for growers.

For the above ground portion of the seedlings it was expected that application of the two highest rates of N [the recommended rate (1 N) and double the recommended rate (2 N)] would lead to the greatest amount of nitrate in the soil, greatest rates of net photosynthesis, highest WUE, and the greatest growth rate of trunk diameter. It was also expected that uniformly applied N would affect the below ground portion of the seedlings by reducing specific root length (SRL) and root length density (RLD).

Even though there were much greater amounts of N available to the seedlings in the two highest N treatments, there were no effects of N application rate on net photosynthesis or trunk growth. The lack of a greater response to higher N application rates (especially in 1 N and 2 N treatments) suggests that current recommended N application rates are potentially too high for young pecan seedlings.

A potential explanation for the lack of response to increased N application rates is that there may have already been an adequate amount of nitrate present in the soil before application of N treatments. However, soil analysis results showed that the untreated control treatment (0 N) did have nitrate present but these amounts were considered to be very low (0-10 ppm Nitrate-N).

A study by Acuña-Maldonado et al. (Acuña-Maldonado et al., 2003), suggested that pecan can use stored N from previous years for growth. Therefore, it is possible that there are substantial amounts of N stored in the seedlings and this stored N can be used for growth for multiple years, which had no direct effect on growth within the first two years after transplant. It would be necessary to continue to monitor growth of these seedlings to see how N treatments affected growth in later seasons.

The SRL and RLD were not affected by N treatment in this study; again, it is possible that transplant shock may have played a role in development of the root system, thus reducing N uptake rates for the seedlings. There were higher values for SRL at the beginning of the 2011 growing season (Fig. 12) which was expected, showing that a high number of roots in the spring will die faster and will continue to decline until a new flush of roots is produced.

Root length density also had a similar pattern to SRL with the highest values at the beginning of the growing seasons and decreasing throughout the season. Root length density decreases throughout the season and then there is another flush of roots in the fall. This has been observed before in pecan (Makita et al., 2011) as well as grape (*Vitis vinifera*) (Bates et al., 2002) and avocado (Ploetz et al., 1993). The greatest root length density was found early in the growing season (May-June) and thus N fertilizer should be applied at that time.

Both SRL and RLD increased at the end of the 2011 growing season. N absorption can be substantial in the period between leaf drop and bud break building up significant reserves to support rapid spring growth (Acuña-Maldonado et al., 2003). During the first year (2010), our last RLD measurement was taken at the beginning of October which may have been too early to measure the fall root flush. However, an increase in RLD and SRL was evident in the second year of the study when root measurements were taken in November, i.e., after leaf drop had already occurred.

Even though there was no effect of N treatment on the seedlings, there was still an interesting pattern of photosynthesis for both growing seasons. This pattern showed that there was a significant drop in August net photosynthetic rates before a second autumn peak in rates in September. More research needs to be conducted to further look into this seasonal pattern of photosynthesis for pecan to determine the reason for this phenomenon.

In conclusion, results of these experiments showed that there may not be a need to apply much, if any, N to young pecan trees during the first two years after establishment/transplant. Nitrogen applications did not enhance aboveground growth, photosynthesis, RLD and SRL of pecan seedlings compared to those where no N was applied. However, transplant shock and establishment rate of seedlings may have had an effect on N absorption. Fertilizer should be applied early in the growing season (May-June) because that is when the greatest amount of root length was present in the soil. The lack of an effect of N application on RLD, SRL, and growth and physiology suggest that no exogenous N is needed until the seedling root system is more adequately established. More research needs to be conducted to determine if N reserves still play a key role in N allocation in young pecan seedlings and to determine whether higher N application rates actually damage the root system.

REFERENCES

Aber, J.D., J.M. Melillo, K.J. Nadelhoffer, C.A. Mcclaugherty, and J. Pastor. 1985. Fine root turnover in forest ecosystems in relation to quantity and form of nitrogen availability: A comparison of two methods. Oecologia 66:317-321.

Acuña-Maldonado, L.E., M.W. Smith, N.O. Maness, B.S. Cheary, B.L. Carroll, and G.V. Johnson. 2003. Influence of nitrogen application time on nitrogen absorption, partitioning, and yield of pecan. J. Amer. Soc. Hort. Sci. 128:155-162.

Baker, J.L. and H.P. Johnson. 1981. Nitrate-nitrogen in tile drainage as affected by fertilization. J. Environ. Qual. 10:519-522.

Bates, T.R., R.M. Dunst, and P. Joy. 2002. Seasonal dry matter, starch, and nutrient distribution in 'Concord' grapevine roots. HortScience 37:313-316.

Bi, G., C.F. Scagel, L. Cheng, S. Dong, and L.H. Fuchigami. 2003. Spring growth of almond nursery trees depends upon nitrogen from both plant reserves and spring fertilizer application. J. Hortic. Sci. Biotech. 78:853-858.

Boussadia, O., K. Steppe, H. Zgallai, S.B. El Hadj, M. Braham, R. Lemeur, and M.C. Van Labeke. 2010. Effects of nitrogen deficiency on leaf photosynthesis, carbohydrate status and biomass production in two olive cultivars 'Meski' and 'Koroneiki'. Scientia Hort. 123:336-342.

Carlson, W.C. and C.L. Preisig. 1981. Effects of controlled-release fertilizers on the shoot and root development of Douglas-fir seedlings. Can. J. Forest Res. 11:230-242.

Casimiro, I., T. Beeckman, N. Graham, R. Bhalerao, H.M. Zhang, P. Casero, G. Sandberg, and M.J. Bennett. 2003. Dissecting Arabidopsis lateral root development. Trends Plant. Sci. 8:165-171.

Cheng, L. and L.H. Fuchigami. 2002. Growth of young apple trees in relation to reserve nitrogen and carbohydrates. Tree Physiol 22:1297-1303.

Close, D.C., C.L. Beadle, and P.H. Brown. 2005. The physiological basis of containerised tree seedling 'transplant shock': A review. Austral. For. 68:112-120.

Coleman, M. 2007. Spatial and temporal patterns of root distribution in developing stands of four woody crop species grown with drip irrigation and fertilization. Plant Soil 299:195-213.

Conner, P. 2007. Effect of nitrogen fertigation on first-year pecan seedling growth. HortTechnology 17:491-495.

Coutts, M.P. and J.J. Philipson. 1976. The influence of mineral nutrition on root development of trees. I. The growth of Sitka spruce with divided root systems. J. Expt. Bot. 27:1102-1111.

Davies, F.S. and G. Zalman. 2008. Fertilization and growth of field-grown citrus nursery trees in Florida. HortTechnology 18:29-33.

Dinnes, D.L., D.L. Karlen, D.B. Jaynes, T.C. Kaspar, J.L. Hatfield, T.S. Colvin, and C.A. Cambardella. 2002. Nitrogen management strategies to reduce nitrate leaching in tile-drained midwestern soils. Agron. J. 94:153-171.

Eissenstat, D.M. 1992. Costs and benefits of constructing roots of small diameter. J. Plant Nutr. 15:763-782.

Eissenstat, D.M. and D.S. Achor. 1999. Anatomical characteristics of roots of citrus rootstocks that vary in specific root length. New Phytol. 141:309-321.

Eissenstat, D.M., C.E. Wells, R.D. Yanai, and J.L. Whitbeck. 2000. Building roots in a changing environment: implications for root longevity. New Phytol. 147:33-42.

Emmett, B.A., S.A. Brittain, S. Hughes, J. Gorres, V. Kennedy, D. Norris, R. Rafarel, B. Reynolds, and P.A. Stevens. 1995. Nitrogen additions ($NaNO_3$ and NH_4NO_3) at Aber forest, Wales: I. Response of throughfall and soil water chemistry. Forest Ecol. Manag. 71:45-59.

Evans, J.R. 1989. Photosynthesis and nitrogen relationships in leaves of C-3 plants. Oecologia 78:9-19.

Forde, B. and H. Lorenzo. 2001. The nutritional control of root development. Plant Soil 232:51-68.

Friend, A.L., M.R. Eide, and T.M. Hinckley. 1990. Nitrogen stress alters root proliferation in Douglas-fir seedlings. Can. J. Forest Res. 20:1524-1529.

Gianquinto, G., P. Sambo, and S. Bona. 2003. The use of SPAD-502 chlorophyll meter for dynamically optimising the nitrogen supply in potato crop: a methodological approach. Acta Hort. 607:217-224.

Gianquinto, G., P. Sambo, and D. Borsato. 2006. Determination of SPAD threshold values for the optimisation of nitrogen supply in processing tomato. Acta Hort. 700:159-166.

Glass, A.D.M. 2003. Nitrogen use efficiency of crop plants: Physiological constraints upon nitrogen absorption. Crit. Rev. Plant Sci. 22:453-470.

Gorissen, A., A.E. Jansen, and A.F.M. Olsthoorn. 1993. Effects of a two-year application of ammonium sulphate on growth, nutrient uptake, and rhizosphere microflora of juvenile Douglas-Fir. Plant Soil 157:41-50.

Hagidimitriou, M. and C.A. Pontikis. 2005. Seasonal changes in CO_2 assimilation in leaves of five major Greek olive cultivars. Scientia Hort. 104:11-24.

Hudak, P.F. 2000. Regional trends in nitrate content of Texas groundwater. Journal of Hydrology 228:37-47.

Jackson, R.B. and M.M. Caldwell. 1989. The timing and degree of root proliferation in fertile soil microsites for three cold-desert perennials. Oecologia 81:149-153.

Jacobs, D.F., R. Rose, D.L. Haase, and P.O. Alzugaray. 2004. Fertilization at planting impairs root system development and drought avoidance of Douglas-fir (*Pseudotsuga menziesii*) seedlings. Ann. For. Sci. 61:643-651.

Jacobs, D.F. and V.R. Timmer. 2005. Fertilizer-induced changes in rhizosphere electrical conductivity: relation to forest tree seedling root system growth and function. New Forest 30:147-166.

Kim, T., H.A. Mills, and H.Y. Wetzstein. 2002. Studies on effects of nitrogen form on growth, development, and nutrient uptake in pecan. J. Plant Nutr. 25:497-508.

Kraimer, R.A., W.C. Lindemann, and E.A. Herrera. 2001. Distribution of N-15-labeled fertilizer applied to pecan: A case study. HortScience 36:308-312.

Lemon, R., R. Boman, M. McFarland, B. Bean, T. Provin, and F. Hons. 2009. Nitrogen management in cotton Texas A&M AgriLife Ext. SCS-2009-1.

Lombardini, L. 2006. One-time pruning of pecan trees induced limited and short-term benefits in canopy light penetration, yield, and nut quality. HortScience 41:1469-1473.

Lombardini, L., H. Restrepo-Diaz, and A. Volder. 2009. Photosynthetic Light Response and Epidermal Characteristics of Sun and Shade Pecan Leaves. J. Amer. Soc. Hort. Sci. 134:372-378.

Lu, C.M. and J.H. Zhang. 2000. Photosynthetic CO_2 assimilation, chlorophyll fluorescence and photoinhibition as affected by nitrogen deficiency in maize plants. Plant Sci. 151:135-143.

Lucash, M.S., J.D. Joslin, and R.D. Yanai. 2005. Temporal variation in nutrient uptake capacity by intact roots of mature loblolly pine. Plant Soil 272:253-262.

Makita, N., Y. Hirano, T. Mizoguchi, Y. Kominami, M. Dannoura, H. Ishii, L. Finer, and Y. Kanazawa. 2011. Very fine roots respond to soil depth: biomass allocation, morphology, and physiology in a broad-leaved temperate forest. Ecol. Res. 26:95-104.

Marquard, R.D. 1987. Influence of leaf to fruit ratio on nut quality, shoot carbohydrates, and photosynthesis of pecan. HortScience 22:256-257.

Marquard, R.D. and J.L. Tipton. 1987. Relationship between extractable chlorophyll and an insitu method to estimate leaf greenness. HortScience 22:1327-1327.

Marschner, H. 1995. Mineral nutrition of higher plants. 2nd Ed. Academic Press, London, UK.

Maynard, D.G., K.I. Mallett, and C.L. Myrholm. 1997. Sodium carbonate inhibits emergence and growth of greenhouse-grown white spruce. Can. J. Soil Sci. 77:99-105.

McEachern, G.R. 2007. Planting and establishing pecan orchards. In: G.R. McEachern and L.A. Stein (eds.). Texas pecan handbook. Texas A&M Univ. System, College Station, Texas.

Neilsen, D., P. Millard, L.C. Herbert, G.H. Neilsen, E.J. Hogue, P. Parchomchuk, and B.J. Zebarth. 2001. Remobilization and uptake of N by newly planted apple (*Malus domestica*) trees in response to irrigation method and timing of N application. Tree Physiol. 21:513-521.

Olsthoorn, A.F.M., W.G. Keltjens, B. Vanbaren, and M.C.G. Hopman. 1991. Influence of ammonium on fine root development and rhizosphere pH of Douglas-fir seedlings in sand. Plant Soil 133:75-81.

Ploetz, R.C., J.L. Ramos, and J.L. Parrado. 1993. Periodicity of shoot and root-growth in grafted avocado. Trop. Agr. 70:248-251.

Pregitzer, K.S., J.S. King, A.J. Burton, and S.E. Brown. 2000. Responses of tree fine roots to temperature. New Phytol. 147:105-115.

Richardson-Calfee, L.E., J.R. Harris, R.H. Jones, and J.K. Fanelli. 2010. Patterns of root production and mortality during transplant establishment of landscape-sized sugar maple. J. Amer. Soc. Hort. Sci. 135:203-211.

Siddiqi, M.Y., A.D.M. Glass, T.J. Ruth, and T.W. Rufty. 1990. Studies of the uptake of nitrate in barley. I. Kinetics of $^{13}NO_3^-$ Influx. Plant Physiol. 93:1426-1432.

Smethurst, P.J. and E.K.S. Nambiar. 1989. An appraisal of the insitu soil-core technique for measuring nitrogen uptake by a young *Pinus radiata* plantation. Soil Biol. Biochem. 21:939-942.

Smith, M.W., B.W. Wood, and W.R. Raun. 2007. Recovery and partitioning of nitrogen from early spring and midsummer applications to pecan trees. J. Amer. Soc. Hort. Sci. 132:758-763.

Spalding, R.F. and M.E. Exner. 1993. Occurrence of nitrate in groundwater: A review. J. Environ. Qual. 22:392-402.

Sparks, D. 1968. Some effects of nitrogen on young pecan trees. Proc. Southeastern Pecan Growers Assn.:93-102.

Swiader, J.M. and A. Moore. 2002. SPAD-chlorophyll response to nitrogen fertilization and evaluation of nitrogen status in dryland and irrigated pumpkins. J. Plant Nutr. 25:1089-1100.

Tierney, G.L. and T.J. Fahey. 2001. Evaluating minirhizotron estimates of fine root longevity and production in the forest floor of a temperate broadleaf forest. Plant Soil 229:167-176.

Tuncer, G., T. Karakas, T.I. Balkas, C.F. Gökçay, S. Aygnn, C. Yurteri, and G. Tuncel. 1998. Land-based sources of pollution along the black sea coast of Turkey: Concentrations and annual loads to the black sea. Mar. Pollut. Bull. 36:409-423.

Vaitkus, M.R., T.G. Ciravolo, K.W. Mcleod, E.M. Mavity, and K.L. Novak. 1993. Growth and photosynthesis of seedlings of five bottomland tree species following nutrient enrichment. Amer. Midl. Nat. 129:42-51.

Vogt, K.A., D.J. Vogt, and J. Bloomfield. 1998. Analysis of some direct and indirect methods for estimating root biomass and production of forests at an ecosystem level. Plant Soil 200:71-89.

Volder, A., D.R. Smart, A.J. Bloom, and D.M. Eissenstat. 2005. Rapid decline in nitrate uptake and respiration with age in fine lateral roots of grape: implications for root efficiency and competitive effectiveness. New Phytol. 165:493-501.

Watson, W.T., D.N. Appel, C.M. Kenerley, and M.A. Arnold. 2000. Measurement of apple root losses associated with cold storage and elutriation of soil core samples. HortTechnology 10:580-584.

Wolfe, A.H. and J.A. Patz. 2002. Reactive nitrogen and human health: Acute and long-term implications. Ambio 31:120-125.

Woodroof, J.G. and N.C. Woodroof. 1934. Pecan root growth and development. J. Agr. Res. 49:0511-0530.

Yao, S.R., I.A. Merwin, and M.G. Brown. 2009. Apple root growth, turnover, and distribution under different orchard groundcover management systems. HortScience 44:168-175.