INFLUENCE OF NITROGEN APPLICATION TIME ON NITROGEN CONCENTRATION AND STORAGE RELATED TO FRUITING OF PECAN

By

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INTRODUCTION

Mineral nutrition is a frequent problem affecting yield and quality of plants. There are many factors that influence the fertility of crops, including the availability of nutrients in the soil, their absorption and utilization by the crop, the type of fertilizer, and the time and method of application.

Nitrogen is considered the most frequently needed nutrient for pecan [*Carya illinoinensis* (Wangenh.) C. Koch] growth. Nitrogen deficiencies in pecan include chlorotic leaves, abnormal growth, and reduction in crop yield (Smith, 1991). Symptoms of excess N fertilization in pecan had been reported as necrotic leaves or "nitrogen scorch" that is caused by an induced potassium or phosphorous deficiency (Sparks, 1976).

Nitrogen fertilization time is of particular importance for managing a successful pecan orchard. In Oklahoma, pecan trees are usually fertilized before budbreak, i.e. from February through March, (McCraw, 1994) and typical N applications rates for improved orchards range between 92 to 138 kg $N \cdot ha^{-1}$ annually.

The recommendation for N application in the spring was done by Gammon and Sharpe (1955). They reported that competition for N would be minimized if N was applied while the grass in the orchard was dormant. Another benefit of spring N application is less flower abortion (M. W. Smith, unpublished data).

Some studies indicate that other N application times could be beneficial. In pecan, Smith et al., (1995) showed that October N applications increased the yield of 'Hayes' 37% over 7 years, compared to March N applications.

The effects of N application time on yield, depends on several factors including nitrogen uptake, nitrogen status of the tree, and developmental stage of the tree (Hill-Cottingham, 1963; Williams, 1963; Hill-Cottingham and Williams, 1967; Imsande and Touraine, 1994). Several studies have reported improved performance characteristics of apple [*Malus domestica* (L.) Mill. (Borkh.) Mansf.] and pear (*Pyrus communis* L.) with summer and fall N fertilizations. For instance, Hill-Cottingham and Williams (1967) and Delap (1967) found that fall N application increased apple fruit set compared to spring or summer application. The effect of early fall N application on apple was studied also by Williams (1965) who found that a fall application ameliorate blossom quality of certain cultivars compared with a spring N application. Research on pear showed that autumn N was efficiently absorbed, whereas spring applications are poorly assimilated, probably due to insufficient available carbohydrates (Taylor et al., 1975).

Hunter and Lewis (1942) reported that time of fertilizer application had no significant influence on pecan yield. Their study showed that the poorest quality nuts resulted from a split N application in the spring and summer. They speculated that the low quality nuts were caused by the new growth stimulated by the summer N application that depleted carbohydrates for the filling process. Another study in apple found no long-term differences in fruit production related to the kind of N fertilizer used or the time that it was applied (Goode and Higgs, 1977).

Nitrogen nutrition affects flower development of many species (Kinet et al., 1985). The N influence on flowering is of special importance since alternate bearing is a major problem for pecan. Alternate bearing in pecan has been widely investigated, but the mechanisms that regulate this are still debated. Depletion of carbohydrate reserves

may be the sole trigger for alternate bearing in pecan (Davis and Sparks, 1974; Sparks, 1974; Smith and Waugh, 1938). Another hypothesis suggests that flowering is controlled at two levels (Smith et al., 1986; Wood, 1991). First, carbohydrates act as a threshold; below a certain concentration the tree is incapable of flowering, and above the minimum concentration a balance of endogenous phytohormones regulates flowering. Wood (1995) later suggested that photoassimilates are the primary regulator of flowering, although phytohormones likely play a role in flower regulation. Alternate bearing seems to be alleviated by factors that favor carbohydrate accumulation, such as healthy leaves, longer leaf retention after maturity (Hinrichs, 1962; Worley, 1979a, 1979b), longer shoot growth with greater leaf area (Amling, 1968; Malstrom and McMeans, 1982; Wood, 1995), and by factors that reduce the production of flower inhibitors (Hill-Cottingham, 1968; Smith et al., 1986), for example, fruit thinning (Smith et al., 1993).

Studies examining the effect of N application time in apple flowering have concluded that that the quality of blossoms is determined by the extent that reserve carbohydrates and N have been accumulated during the previous year (Hill-Cottingham, 1963). She suggested that the high quality of apple blossoms from the fall N application resulted from prolonged leaf retention. Later studies showed that application of N to apple during the summer and fall increased N content of the scion and leaves, without inducing additional growth or retarding leaf senescence (Hill-Cottingham and Williams, 1967).

Translocation of nutrients from leaves before defoliation has been reported by Hill-Cottingham (1968). Apple leaves lost about half of their pre-senescence N content in

a short period of time before abscission. She suggested that the N lost from the leaves was mobilized to the perennial plant parts.

Smith and Waugh (1938) reported that N content in pecan roots decreased during rapid spring growth and increased during winter while trees were dormant. These findings are in agreement with those of Hill-Cottingham and Williams (1967) who found that N content in apple roots doubled during winter if N was applied in the fall. This indicates that N, if present, is absorbed at any season. However, the subsequent distribution of N within the tree varies with application time and is primarily restricted to the roots after leaf-fall. Experiments with ^{15}N by Lindemann et al., (1998) indicated that pecan tree utilizes the N accumulated from the previous year for growth during the current year.

Nitrogen absorption varies depending on sink strength that is affected by the developmental stage, nitrate availability in the soil, and carbohydrate reserves in the plant (Hill-Cottingham, 1963). Rosecrance et al., (1996) found that nutrient uptake occurred primarily during nut filling in both on-year and off-year pistachio (*Pistacia vera* L.) trees suggesting that sink demand regulates the uptake and distribution of nitrogen.

Various studies on hardwood temperate species (Wetzel et al., 1989; Coleman et al., 1991; 1992; 1994; Sauter et al., 1989) have shown that a certain group of proteins, termed bark storage protein (BSP), are accumulated at high concentrations in the inner bark tissue of many woody plants during dormancy. These concentrations then decline during spring when growth begins. The accumulation of BSP seems to be induced by photoperiod (Coleman et al., 1992). BSP may function as a source of stored nitrogen that is mobilized from the leaves to the bark before leaf abscission. BSP may be synthesized

when nitrogen availability, either from the soil or from senescing leaves, exceeds the growth requirement for the tree. Storage-protein deposition would then not be related to frost hardiness or overwintering, but rather act as temporary nitrogen storage (Wetzel et al., 1989; Coleman et al., 1994).

According to Kang and Titus (1980), the majority of reserve nitrogen in leaves and bark is stored as protein. Sauter et al., (1989) suggested that these proteins are stored in the form of intravacuolar protein aggregates. Their distribution in a poplar (*Populus deltoides* Bart. ex Marsh.) indicate that younger parts of the branch and stem wood store much more protein than the older parts. Protein content of the bark was 2 to 4 times higher than in the wood because of the high amount of living cells in the bark compared to wood. Pregitzer (1990) found large diameter structural poplar roots as the major site of nitrogen storage following leaf fall.

Pecan pistillate flowers are formed terminally on current season's growth that arises from terminal or lateral buds on 1-year-old branches. In Oklahoma, pistillate flower induction is in October (Hinrichs, 1962; Worley, 1979a). Wetzstein and Sparks (1983) correlated the beginning of pecan pistillate flower initiation with bud development. Initiation occurred between the time of outer bud scale-shedding and inner bud swelling. Although bud developmental stage is a more accurate indicator of flower development, Woodroof and Woodroof (1926) observed that differentiation begins between mid February and March. Budbreak is about mid April in Oklahoma. Anthesis, according to Sparks (1986), occurs 3 to 4 weeks after budbreak. The number of pistillate flowers per inflorescence vary depending on the cultivar, but averages three to six (Sparks, 1986). During the growing season, three fruit or pistillate flower drops take

place (Sparks, 1986). He reports that the first drop occurs immediately after full bloom, and is associated with weak flowers or underdeveloped flowers. The second drop begins about 2 weeks after pollination and continues for about 40 days. This drop is caused by lack of pollination or failure of endosperm development. About a week after cessation of the second drop, the third drop begins and may continue until fruit maturity. This drop is the cause of failure of the zygote. The pecan fruit consists of the shuck (involucre), shell (ovary wall plus packing tissue), and kernel (seed coat, embryo, storage carbohydrates and remains of the endosperm). The two halves of the kernel are the cotyledons (Sparks, 1986).

The objectives of this study are (1) to determine the distribution and concentration of nitrogen in the tree during different seasons, (2) determine the effect of nitrogen fertilization time on nitrogen concentration and storage, and (3) determine the effect of nitrogen fertilization time on return bloom (fruiting).

Results of this study may be of importance in explaining the effect of nitrogen on flowering, the effect of nitrogen fertilization on storage of nitrogen and the relationship of nitrogen concentrations to return bloom. The practical application of this study will determine if a split N application is more effective in terms of N uptake efficiency compared to the conventional single application in February or March, and the potential of fall N application to partially regulate production and reduce alternate bearing.

MATERIALS AND METHODS

Ten 15-year-old 'Maramec' pecan trees growing on a Teller sandy loam soil (fine-loamy, mixed, active, thermic Udic Argiustoll) at the Fruit Research Station near Perkins, OK were selected based on uniformity of size, vigor and location within the 0.75-ha orchard. Trees were spaced 12.2 x 12.2 m and were 9.3 ± 1.2 m tall with 29 ± 3 cm diameter trunks measured at 1.4 m above ground. A 7.3 m wide weed-free area was maintained the entire length of the row. Trees were irrigated using drip irrigation, except a traveling gun was used following nitrogen application. Pests were managed according to Oklahoma Cooperative Extension Service recommendations (von Broembsen et al., 1997).

Treatments were a single nitrogen application annually in March or a split application with 60% applied in March and 40% in October. Trees received 125 kg $N \cdot ha^{-1}$ on 13 Mar. 1998 and 12 Mar. 1999 or 75 kg $N \cdot ha^{-1}$ on 13 Mar. 1998 and 12 Mar. 1999 plus 50 kg $N \cdot ha^{-1}$ on 2 Oct. 1997 and 7 Oct. 1998. Trees were bordered with trees receiving like treatments. Ammonium nitrate was used during 1997 and 1998, and in 1999 urea was used due to the lack of the availability of the former.

Nitrogen was uniformly broadcast from the trunk to the adjacent border tree and the application was not incorporated. Irrigation with a traveling gun was immediately after the nitrogen application. Each treatment had five single-tree replications arranged in a randomized complete block design.

Total nitrogen in the tree was determined from samples of selected tree components. Samples included leaves and current season's growth from vegetative and reproductive shoots, one-year-old branches, trunk bark and wood, and roots $< \text{or} \geq 1$ cm

diameter. Ten vegetative and reproductive shoots, along with their leaves and five one-year-old branches were collected at random canopy positions each sample date. Bark and wood samples were collected from the trunk at 1.0-1.3 m above ground by taking a core with a spade bit. Bark included the inner bark, and wood samples were about 2.5-5 cm deep on the interior side of the bark. The bark was distinguished from the wood tissue by differences in color and texture.

Root samples were collected using a backhoe from a 1.5 x 1.0 m hole 0.5 m deep that was 2-3 m from the trunk. Roots were separated into those < 1 cm and ≥ 1 cm in diameter, then washed in tap water. Twenty fruit per tree were collected on 27 Oct. 1997, consisting of the shuck (involucre), shell (pericarp) and kernel (cotyledons, seed coat, embryo, and remains of the endosperm). Samples were dried at 60C, ground to pass a 3 mm screen (leaves 850 µm screen) and stored in air-tight glass jars until analyzed. Kernels were ground using a kitchen blender. Fruit were harvested and weighed.

Samples were collected during selected phenological events. These were pre-fall nitrogen application (1 Oct. 1997, 6 Oct. 1998), defoliation (11 Nov. 1997, 12 Nov. 1998), pistillate flower development (19 Feb. 1998, 24 Feb. 1999), stage 4 budbreak (Wetzstein and Sparks, 1983) (8 Apr. 1998, 19 Apr. 1999), post-pollination (22-28 May 1998) and leaf and shoot maturation (23 July 1998).

Samples were redried at 80C, then nitrogen concentration was determined by macro-Kjeldahl (Horowitz, 1980). Nitrate-N was determined for all samples, except leaves, using the cadmium reduction method. The extraction and decoloration procedures were according to Jackson (1980). Modifications to Jackson's procedures were the use of 2.0 M potassium chloride instead of water as the extraction agent and the ratio of sample

to activated carbon was changed from 0.4 g sample : 1 g activated carbon to 0.25 g sample : 1 g activated carbon for root tissue and 1.25 g sample : 1 g activated carbon for the other tissues. The NO_3 analysis followed methods of Page et al. (1982), with absorption at 540 nm determined with a spectrophotometer (Sequoia-Turner, model 340).

Seventy-five vegetative and reproductive shoots per tree were tagged in Oct. 1997 and 1998 for determination of return bloom. Shoots were selected at random canopy positions. The number of shoots per one-year-old branch and the number of flowers per current season shoot were recorded for each tagged shoot on 19-21 May 1998 and 1999, about two weeks after pollination.

Three 15-year-old 'Maramec' growing on a Port silt loam soil, with a 2 m water table, were selected based on trunk diameters similar to those trees in the study at the Perkins Research Station, OK. Trees were spaced 10.7 x 10.7 m and fertilized with 125 kg $N \cdot ha^{-1}$ on 7 Apr. 1998. Whole-trees were harvested on 16-23 Oct. 1998 and each treetop was separated into fruits, leaves, current season shoots and one-year-old branches.

A complete disk about 8 cm thick was separated from the trunk and branch for each tree. Disk diameters were recorded. Outer bark from discs was removed, then the remaining wood and inner bark were dried and weighed. Inner bark was then removed and the wood was weighed again. Finally, the wood was divided into equal 20 mm parts from the perimeter to the core of the disk. Nitrogen concentrations were determined on each segment.

Two 51 cm wide x 4.7 m long areas were excavated to about 2 m deep. The water table was found about 2 m from the surface soil and no roots were observed below this point. The total root area allotted a 10.7 x 10.7 m spaced pecan tree is 114 m^2, thus the

root area sampled was 4.8%. Roots were separated into < 1 cm and ≥ 1 cm in diameter, then washed in tap water. The rest of the above ground perennial parts were mechanically chipped. Tree components were oven dried at 70C and weighed until a constant weight was reached.

Complete tree weights were obtained and biomass regression equations developed for selected tree components. These included leaves, current season shoots and one-year-old branches. The regression model took the form of the equation $Y= ax^b$ using diameter at breast height (1.4 m) as the independent variable as developed by King and Schnell (1972) and Brenneman et at. (1978), where Y is the weight in kg, and x is the trunk diameter in cm.

The biomass regression equation obtained for leaves, current season shoots and one-year-old branches are shown below in their respective order.

$Y = 0.000547\ X^{3.4067}$ $R^2 = 0.99$

$Y = 0.002461\ X^{2.3373}$ $R^2 = 0.99$ **

$Y = 0.006592\ X^{2.1752}$ $R^2 = 0.99$ *

Root biomass and bole weight (above ground tissue except leaves) were determined using King and Schnell (1972) regression equation developed for black oak (*Quercus velutina* Lam.). Bark and wood weights were calculated from the bole weight based on the proportion of wood (83%) and bark (17%) determined from the sampled disks.

Nitrogen content of each plant part was calculated from total dry weight for each sample component and its nitrogen concentration. Wood values were predicted according to a regression equation developed to predict the nitrogen concentration of the wood at various distances from the perimeter ($Y= 0.755\ X^{-0.3756}$; $R^2= 0.62$ ***; where Y= % nitrogen and X= distance from perimeter in %). The wood weight was divided into ten equal parts, multiplied by the adjusted nitrogen concentration, then nitrogen contents summed to determine total nitrogen contained in the wood.

RESULTS

The influence of nitrogen application time and sample date on Kjeldahl-N concentration in selected tissues. Kjeldahl-N concentration was not affected by N treatment in large roots, current season shoots and leaves (Table 1). The split application increased trunk wood and bark Kjeldahl-N in Apr. 1998, but decreased Kjeldahl-N in small roots during May 1998 and Apr. 1999, and in 1-year-old branches during Apr. 1999, compared to the single application in March.

Between the first week of Oct. and the first killing frost in Nov. 1997, Kjeldahl-N increased 34% in roots $\geq$ 1cm diameter, 23% in roots < 1cm diameter, 21% in trunk bark, and 29% in current season shoots (Table 1). Nitrogen concentration remained similar for trunk wood and 1-year-old branches, and decreased 13% in leaves. During the following season, Kjeldahl-N increased 28% in 1-year-old branches and decreased 29% in the trunk wood, and 40% in leaves between Oct. and Nov. 1998, but was not significantly different in the roots, trunk bark and current season shoots. The reduction in leaf N concentration from October to November probably reflects N translocation to perennial parts of the tree.

No significant changes in Kjeldahl-N concentration were found between defoliation (November) and pistillate flower development (February) in the first season (Table 1). However, during the same time of year in the second season, current season shoots and roots < 1 cm diameter increased 29% and 19% respectively, in N concentration; the rest of the tissues did not exhibit a significant difference. This suggests that N is absorbed and stored while the tree is dormant; however, the amount of absorption varied substantially between years.

Between pistillate flower development (February) and budbreak (April), Kjeldahl-N decreased in 1-year-old branches during both years, at the same time, N concentration increased in the small roots, trunk wood and bark and current season shoots in 1998 (Table 1). Small roots and current season shoots decreased the N concentration in the following year, but it increased in trunk wood. Nitrogen concentration was not significantly different between these two sample times for the other tissue sampled.

Between budbreak (April) and post-pollination (May), N concentration in perennial parts of the tree declined dramatically (32% in roots for the split application and 67% in trunk wood for both treatments), but increased in trunk bark (11%), 1-year-old branches (36%), and current season's growth (38%) (Table 1). This suggests that storage reserves in the roots and wood were mobilized to the new growth. Although the trunk bark also represents a substantial N storage reserve, active radial trunk growth probably increased sink strength resulting in a net N increase.

During fruit development and leaf maturation (July), Kjeldahl-N concentration was reduced by 23% in roots, 14% in bark and 41% in current season shoots, but increased about 40% in trunk wood (Table 1). One-year-old branch concentration remained unchanged during this period.

The influence of shoot type and sample date on Kjeldahl-N concentration of current season shoots and leaves. Reproductive shoots had a higher N concentration than vegetative shoots while the tree was dormant (November to April) in both years (Table 2). The rest of the time, there were no differences in the Kjeldahl-N concentration found in reproductive and vegetative shoots. Leaves from vegetative shoots were significantly

higher in their Kjeldahl-N concentration in Oct. 1997 and 1998 than leaves from reproductive shoots. Nitrogen concentration during other sampled dates from either leaf shoot type was not different. Leaves from reproductive and vegetative shoots lost about 36% and 42%, respectively, of their pre-senescent N concentration in the 4-5 weeks prior to abscission. Since leaves from vegetative shoots had substantially more N than leaves from reproductive shoots, a greater proportion of N form the former was conserved in the tree before defoliation, although leaf N is higher during October in leaves from vegetative than reproductive shoots. Wood (1988) reported leaves from vegetative shoots to have a lower net photosynthetic rate compared to leaves directly associated with developing fruit. Apparently sink demand for photoassimilates limits photosynthesis more than available nitrogen.

The influence of nitrogen application time and sample date on Kjeldahl-N concentration in reproductive and vegetative shoots. Nitrogen treatment influenced the N concentration in current seasons' shoot type (Table 3). At budbreak (1998), reproductive shoots from the split N treatment had a significantly higher N concentration (14%) compared to those from the single application. However, vegetative shoots (Nov. 1998) and reproductive shoots (Apr. 1999) receiving a single application had a significantly higher Kjeldahl-N concentration than that those receiving a split application. There were no significant differences in N concentration, regardless of the N treatment, during other sample dates.

In the first season, vegetative shoots significantly increased in N concentration from Oct. to Nov. 1997 and then from Apr. to July 1998 (Table 3). But there was a

significant decrease in Kjeldahl-N in these shoots during dormancy (November to February) of the first season. Reproductive shoots on the other hand, did not have a significant seasonal change in N concentration during dormancy of the first season, but there was a significant increase in the second season. These results indicate that although vegetative shoots had a significant decrease in Kjeldahl-N concentration from Nov. 1997 to Feb. 1998, this decrease was not consistent with the results from the following season; therefore, both types of shoots may have, in general, similar seasonal N changes.

Reproductive shoots had a greater Kjeldahl-N concentration during defoliation (November) and pistillate flower development (February) in both seasons and during budbreak (April) of the second season, compared to vegetative shoots (Tables 2 and 3). At other sampling dates no differences in the N concentration between shoots were found. These results indicate that a demand for carbohydrates by the fruit increases photosynthesis (Wood, 1988) and translocation of nutrients to the fruit (Smith et al., 1986). Thus, N concentration is higher in bearing than nonbearing shoots.

The influence of nitrogen application time and sample date on the total Kjeldahl-N content in selected tissues. Kjeldahl-N content was significantly affected by N application time in all tissues sampled (Table 4). The split application significantly increased the total Kjeldahl-N content in roots $\geq$ 1 cm diameter during pistillate flower development (Feb. 1998) and budbreak (Apr. 1998, 1999); for the rest of the sampling dates, the N content was similar between N treatments.

Roots < 1 cm diameter receiving a split application had a higher N content at budbreak (Apr. 1998), leaf and shoot maturation stage (July) and pre-fall N application

(Oct. 1998), compared to the single application (Table 4). No differences in N content were seen among other dates, except during budbreak (April) in 1999, when a sharp drop in N content occurred in samples receiving the split application. This abrupt drop may be a random value that will have to be examined after more sampling dates are included in this study. These results suggest that both large and small roots absorbed N while the tree was dormant. N content of roots < 1 cm diameter then decreased 35% from budbreak (April) to leaf and shoot maturation (July), presumably to be utilized for new growth.

The split N application significantly increased the total Kjeldahl-N content in trunk wood during October of both years, Nov. 1997, April of both years and May 1998 compared to the single application. N content was not affected by N treatment during the rest of the sampling dates (Table 4). In 1998, the highest increase (50%) in Kjeldahl-N content was observed on trunk wood between pistillate flower development (February) and budbreak (April), followed by a rapid drop of about 64% from budbreak (April) to post-pollination (May). During this period, the N content decreased substantially not only in trunk wood, but also from both sizes of roots. At the same time N accumulated in 1-year-old branches and current season shoots. This accentuates the evidence that there is a heavy demand for N reserves. Nitrogen content in trunk wood gradually increased during the rest of the season. From Oct. to Nov. 1998, N content decreased in about 31%. No significant N changes occurred for the rest of the sampled dates.

In 1997, a significant increase in N content of trunk bark occurred between October and the first killing frost in November. In 1998, between pistillate flower development (February) and budbreak (April), N was significantly accumulated again. Nitrogen content in trunk bark decreased 13% between post-pollination (May) and leaf

and shoot maturation (July). Total Kjeldahl-N increased significantly between leaf and shoot maturation (July 1998) and budbreak (Apr. 1999) in trunk bark when N application was split. Other sampling dates did not show a significant change in N content.

Kjeldahl-N content of 1-year-old branches in Oct. 1997, Feb., Apr., and May 1998 and Nov. and Feb. 1999 was higher when the N application was split compared to those receiving the single N application. Significant increases in the Kjeldahl-N content were observed between budbreak (April) and post-pollination (May); however, a reduction was noted from post-pollination (May) to leaf and shoot maturation (July). In the second season an increase in the N content occurred from October to November, but there was a reduction from pistillate flower development (February) to budbreak (April). The N content remained unchanged for the rest of the sampling dates.

Kjeldahl-N content was higher in current season shoots receiving the split application than in those receiving a single N application, except in Oct. 1997 before the first fall N application. Kjeldahl-N increased between Oct. 1997 and Nov. 1997 in current season growth of those trees receiving fall N, but not in trees receiving only a spring application (Table 4). Between Nov. 1997 and Apr. 1998, N in the current season's growth increased 48% in both N treatments; however, N content in those receiving a fall application was higher than when N was applied in the spring. In July current season's growth had 25% more N if N application was split. An increase in the N content was observed between Nov. 1998 and Feb. 1999, followed by a decrease from pistillate flower development (February) until budbreak (April). It appears that N was accumulated during budbreak (April) in 1-year-old branches and current season shoots, while in roots

and trunk wood the N content declined. Thus, N was mobilized from roots and wood, to the new growing shoots and leaves.

Kjeldahl-N content was higher in leaves receiving a split N application than a single application for all sampling dates (Table 4). There were no detectable differences in Kjeldahl-N content in leaves between Oct. and Nov. 1997. Conversely, N content decreased about 40% between Oct. and Nov. 1998. Leaf N content decreased between post-pollination (May) and leaf and shoot maturation (July), but increased from July to Oct. 1998 in trees receiving a split application, but not in trees receiving a single N application. Nitrogen increased about 37% in both N treatments between Apr. and May 1998, followed by a decreased in July. The decrease was greater when trees received a single N application compared to the split application.

The influence of nitrogen application time and sample date on NO_3-N concentration in selected tissues. Nitrogen treatment did not affect NO_3-N concentration in the roots during any sample date (Table 5). Nitrate-N tended to accumulate in the roots from February through April, suggesting a period of rapid N absorption. Nitrate-N concentration was higher in the roots than in the other tissues sampled.

Nitrate-N concentration was higher in the trunk wood while trees were actively growing and lowest while trees were entering dormancy or were dormant. Nitrate-N concentration was higher in the trunk wood during Oct. and Nov. 1998 if trees received a split N application.

In the bark NO_3-N was higher during May, July and October than at other sample times. During May trees receiving a split application had a higher NO_3-N concentration than those receiving a single application.

Nitrate-N concentration was higher during May and July in 1-year-old branches and current season's growth than at other times of the year. Nitrogen treatment did not affect NO_3-N concentration in 1-year-old branches during any sample date.

In current season's growth NO_3-N concentration in May was greater if trees received a single N application. N treatment did not affect NO_3-N concentration at other sample times.

The influence of nitrogen application time and sample date on total NO_3-N in selected tissues. Total NO_3-N content was affected by N treatment in trunk wood, bark and 1-year-old branches; however, total NO_3-N in other tissues was not affected by N treatment (Table 6). Nitrate-N content in trunk wood during pre-fall N application (October), defoliation (November), and post-pollination (May), was higher if trees received a split application compared to the single application. Other sampling dates were not affected by N treatment. Since the NO_3-N content was higher during October in trees receiving the split N application, it is unclear if the split application affected the NO_3-N content other times, or if it is an artifact of the initial higher concentration.

Trunk bark samples of the split N application had a higher total NO_3-N than those of the single N application at post-pollination (May) and leaf and shoot maturation (July) stages. Other phenological stages were not closely related to N treatment (Table 6).

The split N application increased NO_3-N content in 1-year-old samples at leaf and shoot maturation (July) stage while other sampling dates were not affected (Table 6).

Roots had the highest total NO_3-N content compared to other tissues (Table 6). Significant fluctuations were seen in the total NO_3-N in roots $\geq$ 1 cm diameter along the sampling period, except during October and November. Between pistillate flower development (February) and budbreak (April), total NO_3-N increased 75% if trees received a split application but only 40% if they received a single application. After post-pollination (May), total NO_3-N decreased from those roots. Total NO_3-N content in roots < 1 cm diameter remained relatively constant throughout the sampled dates.

During pistillate flower development (February), NO_3-N content in trunk wood almost disappeared, then increases over a 100-fold during budbreak (April) followed by about an 80% increase between budbreak (April) and post-pollination (May). After May, NO_3-N began to decline. Nitrate content in roots did not showed significant increase from October trough February; however, between February to April, NO_3-N increased. During the spring flush uptake period, much of the nitrate found in trunk bark, wood, branches and shoots probably came from N absorption.

From October to November, NO_3-N content in bark decreased significantly, then increased from February to April, continue to increase from April to May and after that declined again from May to July (Table 6). A significant increase in the total NO_3-N found in 1-year-old branches occurred between post-pollination (May) and leaf and shoot maturation (July). Nitrate-N did not vary significantly on other sampling dates.

Nitrate-N content increased in current season shoots between defoliation (November) and pistillate flower development (February) in trees receiving a split

application, but not in those receiving a single application (Table 6). The NO_3-N content decreased later from February to April, and then increased from budbreak (April) to post-pollination (May).

The influence of nitrogen application time and sample date on total nitrogen in the tree. Nitrogen application time had a significant effect on Kjeldahl-N and total N (Kjeldahl-N + NO_3-N) in the tree (Table 7). More N was stored in the trees receiving the split application and the effect is consistent along the sampling period. Total N in the tree increased about 20% between the first week of October and the first killing frost in November. Nitrogen accumulated again between pistillate flower development (February) and budbreak (April). Nitrogen content decreased 29% from budbreak (April) to post-pollination (May), and 18% from May to leaf and shoot maturation (July). One problem is the fact that N content was higher in trees receiving a split application than the single application before the fall application was applied. Therefore, it is unclear how effective the fall application was in increasing the N storage pool.

The influence of nitrogen application time and shoot type on return bloom. In 1998 the number of new shoots produced by each terminal branch was not influenced by N application time; however, in 1999 the split N application significantly increased the number of those shoots (Table 8). The split N treatment in 1998, decreased the number of reproductive shoots and total flowers per 1-year-old branch, but had no effect in the following year compared to the single N application. The percentage of fruiting shoots and 1-year-old branches and cluster size were not affected by N application time. About

7% to 12% and 55% to 59% of the shoots bore an average of four fruit per cluster in 1998 and 1999, respectively.

In 1998 and 1999, previous season's fruiting shoots had less reproductive shoots, total shoots, total flowers, and 1-year-old branches producing fruiting shoots the following year (Table 8). Cluster size was the same for both types of shoots in 1998, but in 1999 cluster size was smaller if it was a fruiting shoot.

The fruiting percentage of current season shoots was not altered despite of N application time and previous season's shoot type. Fruiting shoots that received the split N application improved return bloom considerably compared with those receiving the single application (Table 9). Vegetative shoots initiated more pistillate flowers during the following year than reproductive shoots regardless of N application time. Fruiting shoots generally produce fewer flowers in the subsequent year than do vegetative shoots (Malstrom and McMeans, 1982; Reid et al., 1993).

DISSCUSION

This research compared the changes in N absorption, storage and mobilization in various tissues of the pecan tree at selected phenological stages, and return bloom when trees received equal N fertilization either as a single spring application or as a split application between spring and fall. It was hypothesized that fall N application would partially regulate (increase) flower induction during October.

The vast majority of nitrogen in the tree was in the form of organically-bound N whereas NO_3-N represented less than one percent of the total nitrogen in the tree, varying from 0.16% to 0.62%. Although NO_3-N in trees seemed to be negligible, trees receiving the split N application had a higher NO_3-N content in trunk wood, bark and 1-year-old branches than those receiving the single application (Table 6).

Nitrate-N distribution in the tree was mainly restricted to the root system (Table 6). Seventy-five percent of total NO_3-N in the tree during October was localized in the roots and this proportion increased and reached the maximum during defoliation (November) and pistillate flower development (February). Afterwards, NO_3-N content in roots declined for the rest of the season, but it still remained over 50% of total NO_3-N in the tree. Clearly, there is a substantial amount of NO_3-N being absorbed during dormancy. Nitrate-N concentration in feeder roots (roots < 1 cm diameter) was higher than structural roots (roots > 1 cm diameter) suggesting an important role in NO_3 absorption by the former tissues during dormancy.

Trees receiving the split N application had accumulated more total N (total Kjeldahl-N + total NO_3-N) than those receiving the single application in March. Results

from Kjeldahl-N and NO_3-N concentration in selected tree components (Tables 1 and 5) before the October fertilization in 1997 indicated that the N status of all trees were similar. However, trees receiving the split N application had a greater biomass compared with those trees of the single application (Table 4).

During budbreak (April), Kjeldahl-N concentration was higher in trunk bark and wood of trees receiving the split N application but not for those of the single application (Table 1). These results suggest a more effective N absorption by the trees receiving the split application. After spring fertilization in March 1999, Kjeldahl-N concentration in roots < 1 cm diameter and 1-year-old branches was higher in trees receiving the single application than in those receiving the split dosage. These results indicate a good response to the treatments.

Changes in the relative distribution of whole-tree N during the study were associated with phenological stages. The general trend of changes in the N content in the different parts of the pecan tree showed a maximum N level at budbreak (April), after which it gradually dropped to a minimum in October (pre-fall N application)-November (defoliation). Thereafter, the N level increased again to the spring maximum.

It has been established that during senescence significant amounts of N are mobilized from the leaf to perennial woody tissue (Murneek, 1930). In the split N treatment, leaves represented about 25% of total N in the tree in October, but only 21% in November. And for the single N treatment, leaf N accounted for 22% and 17%, respectively, in October and November. This indicates that 19% to 29% of the total leaf N was probably conserved before leaf fall. These results are in agreement with those of Hill-Cottingham and Williams (1967) who found that about 30% of leaf N was mobilized to the

scion of apple trees before defoliation. In pistachio, Rosecrance (1996) reported about 50% of the N was mobilized from senescing leaves.

Translocation of organic nitrogen in woody plants occurs mainly in the xylem (wood), but migration of N from leaves during senescence has been reported to occur in the phloem (bark) (Davis, 1957). The translocation of increased amounts of N in trees was confirmed during October-November period by the presence of significantly higher total Kjeldahl nitrogen levels in the bark. Although N from senescing leaves was associated with the N increase in one-year-old branches in November (Tables 1 and 4), it is possible that mobilization to main storage organs, such as roots and trunk wood, occurred gradually during the following months.

The highest proportion (60%) of total tree N among tissues was found in the root system and occurred between defoliation (November) and pistillate flower development (February) (data not shown). Between the two sizes of roots, those $\geq$ 1 cm diameter were the major site of N storage. Its been established that roots are the main storage site in pecan (Smith and Waugh, 1938; Wood, 1989). In evergreen species, like orange, leaves rather than roots are the major storage reserve (Cameron and Appleman, 1933).

I did not expect to see an increase in total nitrogen content of roots during dormancy in those trees receiving the single application (Table 7). Part of the nitrogen may have been conserved from leaves before defoliation, and was gradually translocated to the roots during dormancy. Nitrogen increase in roots during this period also resulted from root absorption. These findings are in agreement with those of Smith and Waugh (1938) and Hill-Cottingham and Williams (1967) who found that nitrogen content in pecan and apple roots, respectively, increased substantially due to nitrogen absorption while trees

were dormant compared to other times of year. Contrary to these results, in pistachio, N uptake was negligible during dormancy (Rosecrance et al., 1996). They suggested that fertilizer application at this time might not be effective.

Besides roots, trunk wood was another major N storage site in pecan. Total N in trunk wood was higher at budbreak (April) than at any other sample time. Then stored N was mobilized rapidly to leaves and new growth probably triggered by budbreak. During the nut filling-period, however, fruit demand for N drives uptake (Wood, 1988).

Previous research indicates that N removed in nut harvest does not represent a major N loss. A 1000 $kg \cdot ha^{-1}$ crop removes about 10 kg $N \cdot ha^{-1}$ (Diver et al., 1984). This low nutrient removal by the crop may wrongly indicate that the fertilizer requirement of pecan is low.

Return bloom data showed that fewer shoots and flowers were produced in the subsequent year by fruit-bearing shoots than by vegetative shoots. These results are in agreement with those of Malstrom and McMeans, (1982) and Reid et al. (1993).

Nitrogen application time did not improved return bloom in 1998; however, in 1999, a significant increase in the number of shoots produced per 1-year-old branch resulted if trees received a split N application (Table 7). Moreover, a significant treatment by shoot type interaction in 1999 showed that fall N fertilization improved return bloom of fruiting shoots compared with those of the single application (Table 9).

From these results, it is possible to predict the long-term effect that split N applications might have on the potential bearing sites. For instance, after 5 years of treatment, trees receiving a split application might have about 45% more current season shoots than trees receiving a single application. This is based on the increased number of

current season shoots produced when the nitrogen application is split. These results also indicate that under fruiting stress situations, fall N application may improve next year's crop. Thus, N application in the fall may be more beneficial during a heavy crop than a light crop.

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APENDIX

Table 1. The influence of nitrogen application time and sample date on Kjeldahl-N concentration in selected tissues.

N treatment	Kjeldahl nitrogen (Dry weight %)										
	Sample date										
	Oct 97	Nov 97	Feb 98	Apr 98	May 98	Jul 98	Oct 98	Nov 98	Feb 99	Apr 99	LSD .05 Date for same N treatment
					Roots ≥ 1.0 cm diameter						
Single	1.15	1.53	1.37	1.55	1.55	1.25	1.08	1.22	1.35	1.60	
Split	1.05	1.42	1.55	1.71	1.32	1.25	0.97	1.16	1.36	1.59	0.30
					Roots < 1.0 cm diameter						
Single	0.92	1.15	1.33	1.21	1.37	1.12	1.16	1.20	1.43	1.59	
Split	0.96	1.02	1.16	1.42	1.07*	1.13	1.17	1.14	1.23	0.61**	0.23
					Trunk wood						
Single	0.32	0.32	0.35	0.41	0.25	0.35	0.41	0.35	0.36	0.43	
Split	0.33	0.33	0.33	0.49*	0.29	0.34	0.45	0.35	0.36	0.39	0.07
					Trunk bark						
Single	0.59	0.71	0.68	0.74	0.82	0.77	0.78	0.83	0.83	0.85	
Split	0.58	0.71	0.69	0.82*	0.84	0.74	0.72	0.77	0.81	0.82	0.08
					One-yr.-old branch						
Single	0.66	0.64	0.72	0.58	0.80	0.75	0.67	0.82	0.91	0.82	
Split	0.64	0.59	0.68	0.59	0.79	0.72	0.65	0.87	0.92	0.59**	0.12
					Current season shoots						
Single	0.75	0.91	0.83	1.10	1.51	1.06	0.84	0.93	1.16	1.00	
Split	0.70	0.96	0.87	1.04	1.43	1.03	0.80	0.87	1.15	0.92	0.12
					Leaves						
Single	2.12	1.88	---	---	2.51	2.25	2.11	1.55	---	---	
Split	2.09	1.95	---	---	2.51	2.30	2.15	1.50	---	---	0.15

*, ** Significantly different within date and tissue at the 5 % (*) or 1 % (**) level

Table 2. The influence of shoot type and sample date on Kjeldahl-N concentration in current season reproductive (R) and vegetative (V) shoots and leaves from reproductive or vegetative shoots.

Shoot type	Kjeldahl nitrogen (Dry weight %)										
	Sample date										
	Oct 97	Nov 97	Feb 98	Apr 98	May 98	Jul 98	Oct 98	Nov 98	Feb 99	Apr 99	LSD .05 Tissue means for same or different date
					Current season						
R	0.74	1.01	1.01	1.27	1.51	1.06	0.84	1.09	1.33	1.08	
V	0.70	0.86*	0.68*	0.87*	1.43	1.03	0.80	0.70*	1.01*	0.86*	0.11
					Leaves						
R	2.01	---	---	---	2.53	2.23	1.96	1.44	---	---	
V	2.21*	---	---	---	2.50	2.30	2.28*	1.61	---	---	0.15

*, ** Significantly different within date and tissue at the 5 % (*) or 1 % (**) level

Table 3. The influence of nitrogen application time and sample date on Kjeldahl-N concentration in reproductive (R) and vegetative (V) shoots.

Date	N treatment	Shoot type	Kjeldahl nitrogen (dry weight %)
Oct 97	Single	R	0.76
		V	0.74
	Split	R	0.73
		V	0.67
Nov 97	Single	R	1.00
		V	0.81
	Split	R	1.03
		V	0.90
Feb 98	Single	R	1.03
		V	0.62
	Split	R	1.00
		V	0.74
Apr 98	Single	R	1.35
		V	0.84
	Split	R	1.18
		V	0.90
May 98	Single	R	1.53
		V	1.49
	Split	R	1.48
		V	1.38
Jul 98	Single	R	1.10
		V	1.02
	Split	R	1.01
		V	1.04
Oct 98	Single	R	0.87
		V	0.82
	Split	R	0.82
		V	0.78
Nov 98	Single	R	1.06
		V	0.79
	Split	R	1.12
		V	0.61
Feb 99	Single	R	1.29
		V	1.04
	Split	R	1.38
		V	0.98
Apr 99	Single	R	1.17
		V	0.84
	Split	R	0.98
		V	0.87
LSD .05 Date means for same treatment and tissue			0.15
LSD .05 Tissue means for same treatment and same or different date			0.15
LSD .05 Treatment means for same of different tissue and dates			0.15

Table 4. The influence of nitrogen application time and sample date on Kjeldahl-N content in selected tissues.

N treatment	Total Kjeldahl nitrogen (g/tissue)										
	Sample date										
	Oct 97	Nov 97	Feb 98	Apr 98	May 98	Jul 98	Oct 98	Nov 98	Feb 99	Apr 99	LSD .05 Date for same N treatment
					Roots ≥ 1.0 cm diameter						
Single	1358	1825	1647	1834	1794	1488	1234	1430	1640	1892	
Split	1546	2083	2287**	2535**	1941	1878	1416	1743	1943	2322**	401
					Roots < 1.0 cm diameter						
Single	443	553	629	572	650	536	544	570	680	758	
Split	577	619	697	857**	637	713*	691*	690	754	369**	138
					Trunk wood						
Single	782	782	855	981	629	853	975	859	895	1031	
Split	1001*	1001*	1011	1516**	884*	1035	1398**	1065	1106	1296*	218
					Trunk bark						
Single	505	593	567	616	688	642	650	701	696	712	
Split	613**	753**	742**	865**	888**	784**	761**	815**	852**	864**	77
					One-yr.-old branch						
Single	58	55	61	52	70	60	59	71	79	71	
Split	71*	65	76*	66*	86*	70	71	96**	102**	66	13
					Current season shoots						
Single	41	50	45	61	84	59	47	51	64	56	
Split	50	69**	62**	74**	101**	74**	57*	62*	83**	65*	9
					Leaves						
Single	912	782	---	---	1077	964	906	663	---	---	
Split	1322**	1214**	---	---	1561**	1043**	1331**	936**	---	---	170

*, ** Significantly different within date and treatment at the 5 % (*) or 1 % (**) level

Table 5. **The influence of nitrogen application time and sample date on NO_3-N concentration in selected tissues.**

N treatment	Nitrate as nitrogen (µg/g)						
	Sample date						
	Oct 97	Nov 97	Feb 98	Apr 98	May 98	Jul 98	LSD .05 Date for same N treatment
			Roots ≥ 1.0 cm diameter				
Single	18	69	88	125	91	27	
Split	26	34	78	137	58	46	58
			Roots < 1.0 cm diameter				
Single	47	166	128	132	143	66	
Split	67	77	115	151	135	78	93
			Trunk wood				
Single	1	1	0	8	13	13	
Split	4*	4*	0	8	15	13	3
			Trunk bark				
Single	9	2	2	8	13	7	
Split	10	3	1	8	18**	10	4
			One-yr.-old branch				
Single	4	3	8	15	18	38	
Split	10	1	6	9	16	42	8
			Current season shoots				
Single	4	4	10	4	61	28	
Split	1	2	12	3	47**	19	4

*, ** Significantly different within date and treatment at the 5 % (*) or 1 % (**) level

Table 6. The influence of nitrogen application time and sample date on the total NO_3-N content in selected tissues.

N treatment	Total nitrate as nitrogen (g/tissue)						
	Sample date						
	Oct 97	Nov 97	Feb 98	Apr 98	May 98	Jul 98	LSD .05 Date for same N treatment
			Roots ≥ 1.0 cm diameter				
Single	2	9	10	14	11	3	
Split	4	5	12	21	9	7	8
			Roots < 1.0 cm diameter				
Single	2	9	6	6	7	3	
Split	4	5	7	9	8	4	6
			Trunk wood				
Single	0.30	0.30	0.04	3.26	5.49	5.60	
Split	1.89*	1.89*	0.03	4.08	7.84**	6.37	1.40
			Trunk bark				
Single	0.76	0.14	0.13	0.69	1.07	0.63	
Split	1.12	0.27	0.07	0.87	1.87**	1.06*	0.42
			One-yr.-old branch				
Single	0.03	0.03	0.07	0.14	0.16	0.33	
Split	0.11	0.01	0.07	0.10	0.15	0.47**	0.09
			Current season shoots				
Single	0.02	0.02	0.05	0.02	0.35	0.17	
Split	0.01	0.01	0.09	0.02	0.32	0.14	0.07

*, ** Significantly different within date and treatment at the 5 % (*) or 1 % (**) level

Table 7. The influence of nitrogen application time and sample date on the total nitrogen content in the tree.

Treatment	Sample date										
	Oct 97	Nov 97	Feb 98	Apr 98	May 98	Jul 98	Oct 98	Nov 98	Feb 99	Apr 99	LSD .05 Date for same N treatment
					Total Kjeldahl nitrogen (g/tree)						
Single	3230	3910	3851	4176	4000	3405	3555	3731	4119	4369	553
Split	3908*	4660**	4937**	5988**	4639*	4495**	4439**	4534**	4907**	4775	
					Total NO_3-N (g/tree)						
Single	5.3	18.8	16.7	24.5	25.1	12.2	---	---	---	---	11.5
Split	11.2	11.9	19.0	33.6	27.3	19.8	---	---	---	---	
					Total nitrogen (g/tree)						
Single	3235	3927	3868	4201	4025	3417	---	---	---	---	450
Split	3919**	4671**	4956**	6021**	4666**	4515**	---	---	---	---	

*, ** Significantly different within date and treatment at the 5 % (*) or 1 % (**) level

Table 8. Effect of nitrogen application time and previous year shoot type on return bloom.

	Total shoots/1-yr-old branch	Reproductive shoots/1-yr-old branch	Total flowers/1-yr-old branch	1-yr-old branches fruiting (%)	Cluster size	Current season shoots fruiting (%)
			Nitrogen treatment			
			1998			
Single	2.2	0.25	0.9	22	3.6	12
Split	2.2	0.14*	0.5*	15	4.0	7
			1999			
Single	2.6	1.5	6.4	95	4.0	59
Split	2.8*	1.6	6.5	96	4.0	55
			Shoot type			
			1998			
Vegetative	2.7	0.27	1.0	25	3.7	11
Reproductive	1.7***	0.12*	0.5**	12*	3.9	8
			1999			
Vegetative	2.9	1.7	7.2	98	4.1	59
Reproductive	2.5*	1.4***	5.6***	93**	3.8***	55

*, ** Significantly different within main effects and treatment at the 5 % (*) or 1 % (**) level

Table 9. The influence of nitrogen application time and shoot type on the percent of 1-year-old branches with fruiting shoots in 1999.

N treatment	Shoot type	Branches with fruiting shoots (%)
Single	Vegetative	99
	Reproductive	91
Split	Vegetative	98
	Reproductive	95*
LSD .05 Tissue for same or different treatment		4.3

*, ** Significantly different within tissue and treatment at the 5 % (*) or 1 % (**) level

VITA

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Master of Science

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