Recovery of Late-season ^{15}N -labeled Fertilizer Applied to Pecan

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Abstract. The recovery of late-season (September) ^{15}N -labeled fertilizer (N at 55 kg-ha⁻¹) was followed in mature pecan trees [*Carya illinoensis* (Wangenh.) K. Koch] and soil (0–270 cm) from 1996 (application year) through 2001 (end of study). Recovery of late-season applied ^{15}N was compared to the recovery of six ^{15}N applications (March through June, N at 221 kg-ha⁻¹) of a previously reported study. By Nov. 1996, both fertilizer schedules exhibited considerable ^{15}N accumulation below the rooting zone and just above the water table (280 cm), with 43.4% and 35.3% ^{15}N recovered from the soil sampling profile of the September and March–June schedules, respectively. ^{15}N recoveries from perennial storage tissues (root and wood) were 20.6% and 10.1% under the September and March–June schedules, respectively. The ^{15}N recoveries from annual abscission tissues (leaf, shuck, and nut) were 1.4% and 10.6% under the September and March–June schedules, respectively. By the end of the 2001 growing season, 4% and 9% of the ^{15}N remained in the soil following the September and March–June applications, respectively. Under both fertilizer schedules, >80% of the fertilizer-N was lost to the environment through natural processes and very little was removed during harvest. Nearly 6 years following application, perennial storage of ^{15}N remained greater in the September application (4.3% of the ^{15}N applied) than in the March–June application (2.7% of the ^{15}N applied). Late-season application of fertilizer-N during the kernel filling stage was stored in perennial tissues for use the following year; very little was used for current year growth of annual tissues. Increased accumulation of perennial storage N by late-season application may reduce the depletion of N caused during a heavy-cropping on-year and may moderate the alternate-bearing trend in pecan by providing a greater reservoir of N the following year.

The fate of fertilizer-N depends, in part, upon the timing of its application relative to the nutritional demands of the plant. Because N is mobile in both the soil and plant tissues, proper timing of N fertilizer application serves to optimize N use efficiency while meeting plant demands. Although late winter or early spring are traditional times to apply N to fruit and nut trees (Smith et al., 1995), previous research has led to conflicting conclusions. Weinbaum et al. (1984a) found that fertilizer-N in almond [*Prunus dulcis* (Mill.) D.A. Webb] is first preferentially partitioned into vegetative growth in the year of application, and is then later recycled to support the early stages of reproductive growth in the following year. Others (Grasmanis and Nicholas, 1971; Tromp and Ovaa, 1973) concluded that early spring growth in apple (*Malus pumila*) was heavily dependent on the movement of newly absorbed N to the growing tissues and that the total N of storage tissues was not significantly depleted. McEachern (1993) postulated that the late-season application of N to pecan [*Carya illinoensis* (Wangenh.) K. Koch] could stimulate growth and cause serious freeze damage, but Smith et al. (1995) found that

the amount of cold damage sustained by two pecan cultivars was unaffected by the timing of N application. Previous recommendations emphasized timing N application to meet the current-year pecan tree growth requirement (Kilby, 1982). However, the nutritional status of the tree and soil from the previous year may be just as important in determining pecan yield as is the nutritional status during the current growing season as in apples and grapes (*Vitis vinifera*) (Conradie, 1983; Oland, 1959).

Some researchers have suggested that nutrient uptake is dependent on tree demand rather than the concentration of the nutrient in the soil (Imssande and Touraine, 1994; Millard, 1996; Weinbaum et al., 1994). This demand can be met, in part, from storage pools in perennial tree organs (Weinbaum, 1994). A large N demand during pecan kernel filling, about mid-August through September, may reduce current-year allocation of N to pecan storage tissues and, therefore, limit the availability of N to annual tissues the subsequent season. Nitrogen fertilization during kernel filling, especially during an on-year in the alternate-bearing cycle, could mitigate storage-N depletion and better prepare the tree for the following season's growth. In light of the demand-driven nutrient uptake hypothesis, application of N during flowering and biomass accumulation and especially during kernel filling would be compatible with both the current season and the following season's production. In addition, it is both environmentally and economically desirable to apply N that is adjusted to the

actual need of the plant (Láng, 1996). That is, the movement of fertilizer-N, in the form of nitrate, from the root zone represents an economic loss to the grower while the entry of nitrate to the underlying groundwater may present an environmental hazard (Timmons and Dylla, 1981).

^{15}N -labeled fertilizer was used in this study in order to provide information about the fate of residual fertilizer-N from one growing season to the next, unconfounded with non-labeled fertilizer-N and mineralized organic N. The objectives of this study were to: 1) describe the fate of ^{15}N in soil and pecan tissues following a single September application of ^{15}N -labeled ammonium sulfate; and 2) compare the fate of ^{15}N in soil and pecan tissues in a September application to that of March–June split applications.

Materials and Methods

The study was initiated in Sept. 1996 in a commercial orchard (6.1 ha) ≈12 km south of Las Cruces, N.M. The 15-year-old 'Western' trees were growing on a level, well-drained Agua loam [fine-loamy over sandy, mixed (calcareous), thermic Typic Torrifluent] that formed in mixed alluvium in the Rio Grande flood plain. The loam surface graded into a fine loamy sand at a depth of ≈45 cm. The water table was ≈280 cm below the surface.

Management of the orchard was typical for the area. Flood irrigation supplied ≈150 cm of water per year and the area received ≈20 cm of annual precipitation per year. Nonlabeled ammonium sulfate fertilizer (N at 373 kg-ha⁻¹) was applied in six split applications from March through mid-June. This annual rate of N fertilization was greater than the recommended rate (N at 242 kg-ha⁻¹) for pecan in the Las Cruces area (Herrera, 1994). Additionally, the orchard received yearly applications of phosphorus, potassium, and foliarly applied zinc. Typical pesticide application practices were used to control weeds and insects. The average yield of the orchard from 1992 to 2001 was 2662 kg-ha⁻¹. With respect to the alternate-bearing trend in pecan, 1996 was an on-year.

Three research trees were selected in different rows and were representative of other trees in the orchard with respect to canopy cover, trunk diameter, height (average values of 116.8 m², 32.8 cm, and 14.2 m, respectively), and tree spacing (99 trees/ha). The three trees were managed as were those in the rest of the orchard except that a single application of ^{15}N -labeled ammonium sulfate (N at 55 kg-ha⁻¹) was applied on 18 Sept. 1996. Trees in this study were in the same orchard and in close proximity to trees in a companion study (Kraimer et al., 2001). Each of the research trees was located at the center of a 9 × 9-m plot to which the ^{15}N -fertilizer was uniformly hand-applied. Before ^{15}N application, the three plots were rototilled to roughen the surface and to ensure quick entry of water and fertilizer. The labeled fertilizer was immediately raked in, and the orchard was flood-irrigated within 48 h to minimize ammonia volatilization. The

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^{15}N -labeled ammonium sulfate (ISOTEC, Miamisburg, Ohio) had a total N content of 20.9% and a ^{15}N content of 10.4 atom%.

Background ^{15}N concentrations for soil and tissue samples were randomly collected from untreated areas within the same orchard. Prior to the start of the experiment and three times during the 1996 and 1997 growing seasons, background soil samples were collected and similar depths composited; concentrations of ^{15}N were averaged at each depth for use in calculations. Background leaf samples were collected 15 times during the course of the experiment and their ^{15}N concentrations were averaged for use in calculations. Background ^{15}N concentrations for other tissues (shuck, shell, kernel, wood, and root) were collected when the respective tissue was sampled from the three research trees.

Soil samples were collected with a 3.8-cm bucket auger at depths of 0–30, 30–60, 60–90, 90–180, and 180–270. Three sample holes were augered within each research plot at random distances (ranging from 1 m to 4 m) from each tree. Samples from the same depth from each of the three holes were composited.

Leaf samples were collected from the middle pair of leaflets at the middle position of the compound leaf of current-year growth. Shuck, shell, leaf, and kernel samples were simultaneously collected within a few days prior to leaf drop. The wood (including bark) sample was collected by coring to the center of the trunk after harvest. Roots were removed from soil samples just before or after harvest and brushed clean of soil particles before grinding. Root samples of varying sizes were collected at various depths (0 to 270 cm) from each tree; no attempt was made to distinguish roots collected from different depths.

Soil samples were air-dried for 48 h, then ground to pass a 150- μm sieve. Tissue samples were oven-dried (65 °C) for 48 h, then ground to pass a 180- μm sieve. Ground soil and tissue samples were sent to the U.S. Dept. of Agriculture–Agricultural Research Service (USDA–ARS) laboratory at the Univ. of Nebraska in Lincoln and Isotope Services, Los Alamos, N.M. Atom% ^{15}N and total N were analyzed with the Tracermass Stable Isotope Mass Spectrometer (Europa Scientific, Crewe, Cheshire, U.K.) and included both inorganic N and organic N.

Recovery of fertilizer- ^{15}N in the soil and tissue samples (as a percentage of ^{15}N applied) was determined at the end of each growing season and is described by the following equations:

$$\% \text{ Recovery}_{\text{soil}} = \frac{(\text{total N}) ({}^{15}\text{N}_{\text{sample}} - {}^{15}\text{N}_{\text{BG}})}{(D_B) (\text{Volume}) (100) / {}^{15}\text{N}_{\text{applied}}}$$

$$\% \text{ Recovery}_{\text{tissue component}} = \frac{(\text{total N}) ({}^{15}\text{N}_{\text{sample}} - {}^{15}\text{N}_{\text{BG}})}{(\text{Biomass}) (100) / {}^{15}\text{N}_{\text{applied}}}$$

where total N = mean total N of three research trees (%); $^{15}\text{N}_{\text{sample}}$ = mean ^{15}N of three research trees (atom %); $^{15}\text{N}_{\text{BG}}$ = mean ^{15}N of background samples (atom %); D_B = bulk

density ($\text{kg}\cdot\text{m}^{-3}$); Volume = soil volume of research plot (m^3/plot); $^{15}\text{N}_{\text{applied}}$ = ^{15}N application rate (kg/plot); and Biomass = mean mass of each tissue component of three research trees (kg/plot).

The estimation of aboveground biomass was needed to determine the biomass of each aboveground tissue component. The following model (King and Schnell, 1972) for black oak (*Quercus velutina* Lam.) was used to estimate above- and belowground biomass.

Total biomass:

$$\text{LOG wt} = 1.00005 + 2.10621 \text{ LOG dbh};$$

Root and stump biomass:

$$\text{LOG wt} = 0.38000 + 2.12094 \text{ LOG dbh};$$

where dbh = diameter of trunk at breast height (1.37 m aboveground) in inches; wt = oven-dried weight in pounds.

To confirm the validity of this model for pecan, a tree similar in size to the research trees was sacrificed and all aboveground tissues were collected on a component basis. The leaf and shuck components were dried (65 °C) and weighed directly. The shell and kernel components were also weighed directly. To determine the dry biomass of the wood tissue, representative samples of trunk, large branches (>7.6 cm diameter), medium branches (2.5 to 7.6 cm), small branches (<2.5 cm), and twigs (<1.3 cm) were dried (65 °C) and weighed. The measured aboveground biomass of the sacrificial tree was 8.6% less than its calculated biomass when the dbh of the sacrificed tree was substituted into the model equations and the aboveground biomass determined by difference.

The dbh of each research tree was used to calculate its aboveground dry biomass by using the above models. The proportions of leaf, shuck, and wood tissue of the sacrificial tree were applied to the calculated value of the mean aboveground biomass of the three research trees as described in the following example for the leaf component:

$$\text{Biomass}_{\text{leaf(research)}} = \text{AG Biomass}_{\text{model(research)}} \times \text{Biomass}_{\text{leaf(sacrificial)}}$$

where $\text{Biomass}_{\text{leaf(research)}}$ = mean estimated leaf biomass of three research trees (kg); $\text{AG Biomass}_{\text{model(research)}}$ = mean above ground biomass of three research trees (kg); and $\text{Biomass}_{\text{leaf(sacrificial)}}$ = leaf tissue in aboveground biomass of sacrificial tree (%). The mean aboveground biomass was calculated by substituting the mean dbh of the three research trees into the model equations and determining the aboveground biomass by difference. Estimations of root biomass relied solely on the model equation. The kernel and shell components of the research trees were directly weighed.

This study and an expansion of a companion study (Kraimer et al., 2001) were monitored from 1996 through 2001, allowing for the comparison of two different fertilizer application schedules within the same orchard under the same management conditions. In the companion study, ^{15}N -labeled ammonium sulfate (N at 221 $\text{kg}\cdot\text{ha}^{-1}$) was applied in six

split applications from 25 Mar. through 15 June 1996, and the results have been expanded in the present report to include the years 1996 through 2001.

Results and Discussion

Fig. 1 A–D shows the ^{15}N distribution in the soil at the end of the 1996 growing season and throughout the following year (1997) after of a single application (18 Sept. 1996) of ^{15}N -labeled ammonium sulfate (N at 55 $\text{kg}\cdot\text{ha}^{-1}$). Table 1 shows ^{15}N recoveries in both soil and tissue at the end of each growing season from 1996 through 2001. For comparison to a spring application schedule, Fig. 1 E–H and Table 2 refer to the companion study (Kraimer et al., 2001) which was extended in the present report to include 1996 through 2001.

At the end of the 1996 growing season (7 Nov. 1996), both fertilizer schedules (September and March–June) exhibited considerable ^{15}N accumulation below the upper rooting zone (90-cm depth) of pecan and just above the water table (Fig. 1 A and F). About 43.4% of the ^{15}N applied in a single September application remained in the sampling profile (Table 1), while 35.3% was recovered from the sampling profile following the six split March–June applications (Table 2). We consider the upper 90 cm to be responsible for most nutrient sorption by pecan roots. Wood (2002) states that N uptake by pecan is primarily in the top 30 cm of soil. Most N found below 90 cm is highly susceptible to leaching and will eventually be lost to the environment.

During the following winter (1997), the orchard was flood-irrigated with 11.3 cm of water and received an additional 1.5 cm precipitation. On 3 Mar. 1997, 46.4% of the fertilizer applied in Sept. 1996 was recovered to a depth of 270 cm (data not shown). Although ^{15}N enrichment was evident in the 0–90 cm depth of both fertilizer schedules (Fig. 1, B and F), the March–June application resulted in much greater ^{15}N enrichment at all depths throughout the profile. The ^{15}N accumulation in the 0–90 cm depth probably resulted from the replenishment of ^{15}N on the soil surface by the decomposition (recycling) of 1996 ^{15}N -enriched leaves and shucks. Adams and Attiwell (1986) also observed the importance of nutrient cycling by litterfall in Australian eucalypt [*Eucalyptus pauciflora* (Sieber) Sprengel and *E. delegatensis* (R.T. Baker)] forests and found that $\approx 80\%$ of the N cycled from plant to soil is in the form of litter.

The Sept. 1996 application produced a slight ^{15}N enrichment in the soil profile on 26 May 1997 (Fig. 1C), the Mar.–June 1996 applications had pronounced ^{15}N enrichment throughout the profile (Fig. 1G). The notably different levels of ^{15}N enrichment reflect the different fertilizer rates of the two application schedules. However, in both application schedules, recycling of catkin, leaf, and shuck to the soil surface may have contributed to the ^{15}N enrichment in the upper depths of the profile. This is especially true of the March–June applications since by the end of the 1996 growing season, leaves and shucks recovered over 15

Fig. 1. ^{15}N distribution in the soil profile following (A–D) a single application of labeled (10.4 atom percent) $(\text{NH}_4)_2\text{SO}_4$ (N at 55 $\text{kg}\cdot\text{ha}^{-1}$) on 18 Sept. 1996 and (E–H) six split applications of labeled (10.4 atom %) $(\text{NH}_4)_2\text{SO}_4$ (N at 221 $\text{kg}\cdot\text{ha}^{-1}$) from 25 Mar. through 15 June 1996. Standard error of the mean (n = 3) is depicted by error bars.

Table 1. Mean ^{15}N recovery at the end of the 1996–2001 growing seasons following a single application of labeled (10.4 atom percent) $(\text{NH}_4)_2\text{SO}_4$ (N at 55 $\text{kg}\cdot\text{ha}^{-1}$) on 18 Sept. 1996.

Component	^{15}N recovery (%)					
	1996	1997	1998	1999	2000	2001
Soil (0–270 cm)	43.4	4.1	7.9	4.5	2.9	4.1
Tree (by tissue component)						
Leaf (recycled)	0.6	6.3	1.5	0.8	1.1	0.4
Shuck (recycled)	0.2	0.3	0.1	0.1	0.1	0.0
Nut (harvested)	0.6	1.1	0.4	0.3	0.3	0.1
Root (stored)	11.5	6.7	2.2	2.3	2.0	1.7
Wood (stored)	9.1	7.3	5.0	2.2	2.7	2.6
Total tree	22.1	21.7	9.2	5.7	6.1	4.7
Previous harvest	---	0.6	1.8	2.2	2.5	2.8
Total (soil + tree)	65.5	26.4	18.9	12.4	11.5	11.6
Lost to environment	34.5	73.6	81.1	87.6	88.5	88.4

Table 2. Mean ^{15}N recovery at the end of the 1996–2001 growing seasons following six split applications of labeled (10.4 atom percent) $(\text{NH}_4)_2\text{SO}_4$ (N at 221 $\text{kg}\cdot\text{ha}^{-1}$) from 25 Mar. through 15 June 1996.

Component	^{15}N recovery (%)					
	1996	1997	1998	1999	2000	2001
Soil (0–270 cm)	35.3	12.5	11.7	11.2	11.3	9.1
Tree (by tissue component)						
Leaf (recycled)	5.6	2.7	1.2	1.2	0.7	0.4
Shuck (recycled)	1.0	0.2	0.1	0.1	0.1	0.0
Nut (harvested)	4.0	0.9	0.4	0.2	0.2	0.1
Root (stored)	3.7	2.9	2.2	1.4	0.7	1.4
Wood (stored)	6.4	4.8	3.4	2.0	1.7	1.3
Total tree	20.8	11.4	7.3	4.9	3.4	3.2
Previous harvest	---	4.0	4.9	5.2	5.4	5.7
Total (soil + tree)	56.1	27.9	23.9	21.3	20.1	18.0
Lost to environment	43.9	72.1	76.1	78.7	79.8	82.0

times more ^{15}N per hectare than following the single September application (calculated from Tables 1 and 2).

After the Sept. 1996 application, ^{15}N concentrations in the soil on all subsequent sample dates through Fall 2001 (data not shown) was very similar to the ^{15}N background level and to the 21 Oct. 1997 soil profile (Fig. 1D). By Fall 1997, most of the ^{15}N applied in Sept. 1996 was leached or absorbed by the tree. By contrast, greater ^{15}N enrichment occurred with the March–June applications (Fig. 1H), particularly in the upper profile. This distinction between the two application schedules was still pronounced nearly 6 years later (Tables 1 and 2). By the end of 2001, over 9% of the ^{15}N applied in the March–June applications remained in the soil, while $\approx 4\%$ ^{15}N remained in the soil following the September application. Some of this difference in soil ^{15}N accumulation may be attributed to biological activity. From September to November, the biological activity in both soil and tree is approaching dormancy, while March through June is a period of increasing biological activity in the soil and tree. We suggest that ^{15}N was immobilized into organic matter in the upper soil profile during this time of increased biological activity. Also, the recycled plant tissues (leaves and shucks) retained much more of the ^{15}N applied in the spring (6.6%) than applied in the fall (0.8%).

Thus, soil accumulation of ^{15}N reflects both the timing of fertilizer application and the amount of recycled N incorporated into the soil surface.

Fig. 2 summarizes the partitioning of ^{15}N into perennial storage tissues (wood and root) and annual abscission tissues (leaf, shuck, and nut) under the two different fertilizer schedules. All samples were collected at the end of each growing season from 1996 through 2001. The data clearly indicate that ^{15}N absorbed late in the season is preferentially stored in storage tissues as a reserve for the next season's growth as well as growth for subsequent seasons, and ^{15}N perennial storage remains higher in the September application than the March–June application through 2001. Perhaps more ^{15}N would have been partitioned into annual abscission tissue if the N fertility status of the tree were lower when entering into the kernel fill stage. The trees in the orchard received fertilizer N at $373 \text{ kg} \cdot \text{ha}^{-1}$ from March–June, >50% above the recommended rate of N application. Remobilization of N from storage in woody tissue supports early spring growth in deciduous trees (Tagliavini et al., 1998; Titus and Kang, 1982; Weinbaum et al., 1984b) because, during the early growth stages, reserve N in perennial tissues contributes more to new growth than does current N uptake (Dong et al., 2001). In citrus, only 23% of fruit N and 21% of leaf N were derived directly from fertilizer; the balance was supplied by N stored in perennial tissue (Feigenbaum et al., 1987). In addition, the timing of N fertilizer applications may have important implications with respect to the alternate-bearing cycle of pecan. The larger accumulation of storage N during off-years may provide the necessary N reservoir demanded during on-years (Weinbaum et al., 1994; Wood, 2002). Picchioni et al. (1997) found that on-year pistachio trees demanded 34% more N during spring growth and over 6-fold more N during seed fill than did off-year trees. We believe that fertilization during kernel filling will better prepare the tree for

the next season's growth and may dampen the alternate-bearing trend in pecan. That is, the increased accumulation of perennial storage N by a late-season application may reduce the depletion of N caused during a heavy-cropping on-year, providing a greater reservoir of N for the following off-year.

By the end of the 1996 growing season, ^{15}N recovery from total tree tissues (Tables 1 and 2) was similar under both fertilizer schedules (22.1% in late- and 20.8% in early-season applications). However, ^{15}N recovery by individual tissue components differed between the two application schedules. The ^{15}N recoveries from perennial storage tissues (root and wood) were 20.6% and 10.1% under the September and March–June fertilizer schedules, respectively. In marked contrast, only 1.4% of the applied ^{15}N was recovered by annual abscission tissues (leaf, shuck, and nut) following the single September application, whereas 10.6% of the applied ^{15}N was recovered by annual abscission tissues following the March–June applications. Late-season absorption of ^{15}N also increased the partitioning of N to roots, compared to spring-absorbed N, in almond (Weinbaum et al., 1984a), pear (*Pyrus communis*) (Sanchez et al., 1991), and peach (*Prunus persica*) (Munoz et al., 1993). Five years following the year of application, ^{15}N recovery from perennial storage tissues was greater than that from annual abscission tissues and greater following the September than the March–June application schedules.

By the end of the 2001 growing season, a greater proportion of applied ^{15}N was lost to the environment, less was cumulatively harvested, less was stored in the soil, and more was stored in the plant with a late season application than with early season split applications. Thus, pecan appears to allocate a greater proportion of fertilizer-N into storage tissues (root and wood) when N is applied late in the season than when applied early in the season. Under both application schedules, >80% of the fertilizer-N was lost through natural processes and very

little was removed during harvest. Although fertilizer-N from some of the abscised tissues is removed in harvest, a portion undergoes mineralization during decomposition on the orchard floor and is available for reabsorption by the tree or susceptible to leaching.

We conclude that, during an on-year, well-fertilized pecan strongly absorbs fertilizer-N applied at the kernel filling stage, and that fertilizer-N is used to replenish endogenous N reserve pools that are likely mobilized to fill the kernels. Under a high N fertilization rate, relatively little of the late-season N is used for current growth of leaves, shucks, or nuts and may have little influence on yield during the year of application. Instead, N fertilizer applied during kernel fill is stored in perennial tissue and used the following year. An ample reservoir of endogenous N at flowering the following year may benefit yields and moderate the alternate-bearing cycle of pecan.

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Fig. 2. Recovery of ^{15}N in pecan tissues after Sept. 1996 application (N at $55 \text{ kg} \cdot \text{ha}^{-1}$) and Mar.–June 1996 application (N at $221 \text{ kg} \cdot \text{ha}^{-1}$). Storage tissue includes root and woody tissue. Abscission tissue includes leaf, shuck, and nut.

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