

Phosphorus Nutrition of Pecan

by

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Abstract

A fundamental component of pecan management is maintaining phosphorus (P) nutrition throughout the tree's growth, development and production. Pecans have difficulty maintaining adequate P concentrations when managed for high input production, so alternate methods of P fertilization in pecan are of interest in correcting deficiencies of the element. The role of P in root development of pecan is also of interest as increased fibrous root development observed in *Arabidopsis* in response to low P concentrations would be desirable for container production of pecan.

Phosphorus is relatively immobile within the soil profile making broadcast applications ineffective for rapid correction of P deficiency in pecans. Banded applications have been used to successfully correct P deficiency quickly. An experiment was conducted to determine effectiveness of banded P applications at differing rates within irrigated and non-irrigated plots on P movement within the soil, P uptake and movement within pecan trees, and yield and quality of nuts. On March 20, 2015, P at 0 kg ha⁻¹ (0x), 19.6 kg ha⁻¹ (1x), 39.2 kg ha⁻¹ (2x), and 78.5 kg ha⁻¹ (4x) was applied in bands of triple superphosphate to a 'Desirable' pecan orchard.

Soil test P decreased linearly over time in non-irrigated and irrigated environments when P was applied at 2x and 4x rates by 34.7% and 54.0% and 41% and 58.6%, respectively. There was no change in soil test P over time at the 0x application rate for both irrigation regimens. Soil test P decreased 44.4% for the 1x treatment in the irrigated plot but did not change in the non-irrigated plot. The largest linear decrease in soil test P from experiment start to finish was

measured in the top 0–7.6 cm of the soil profile dropping 53.4% and 61.2% in the non-irrigated and irrigated plots, respectively. In contrast, soil test P did not decrease in the irrigated plot at the 15.0–22.5 cm soil depth for the entirety of the experiment but decreased linearly by 23.2% in the non-irrigated plot.

Increasing P application rate increased foliar P concentration quadratically in the non-irrigated plot, but only the 4x application rate increased foliar P modestly compared to the 0x control. In the irrigated plot, foliar P concentrations decreased linearly from 0.133% in 2015 to 0.121% in 2017 and foliar P concentrations were not influenced by P application rate. Foliar Fe and Cu decreased with increasing P application in the irrigated plot rate while foliar B increased. Foliar B concentrations were influenced by the application side of the P band in the irrigated plot as well, increasing on the proximal side and decreasing on the distal side compared to the application site. No differences in pecan yield or quality were observed in either irrigated or non-irrigated plots. Overall, P banding may not be the most sustainable way to increase concentrations of P quickly or to maintain foliar concentrations of the nutrient long term.

Pecan seedlings are known to have strong tap-rooting tendencies, a characteristic that can hinder containerized production of trees. In *Arabidopsis*, a reduction of P reduced vertical root development and encouraged root branching. An experiment was conducted to determine if decreased P rates would increase root branching and decreased tap rooting in container grown pecan seedlings. Beginning at 47 days after germination, varying P rates (0, 3.1, 7.75, 15.5, 23.25, and 31 mg L⁻¹ P) in modified Hoagland's solution were applied in 250-mL aliquots to randomly-assigned seedlings twice per week for the duration of the experiment. Seed retention or removal was added as a factor 47 days after germination as well. At the conclusion of the experiment, foliar P concentrations were within the recommended sufficiency range for all P

rates and P rate did not influence plant dry weight or network surface area. Seed retention increased plant dry weights and network surface area but decreased foliar P concentrations. These findings indicate that seedlings with greater biomass would be produced if growers maintained seed attachment when transplanting, which would be beneficial for getting plants large enough to graft sooner in containerized production than those without seed attachment. Further research would be beneficial in determining the long-term effect of seed attachment in pecan seedling development and how long it is influential in growth characteristics. The increased fibrous root development observed in *Arabidopsis* in response to low P rate was not observed in pecan seedlings during the experiment.

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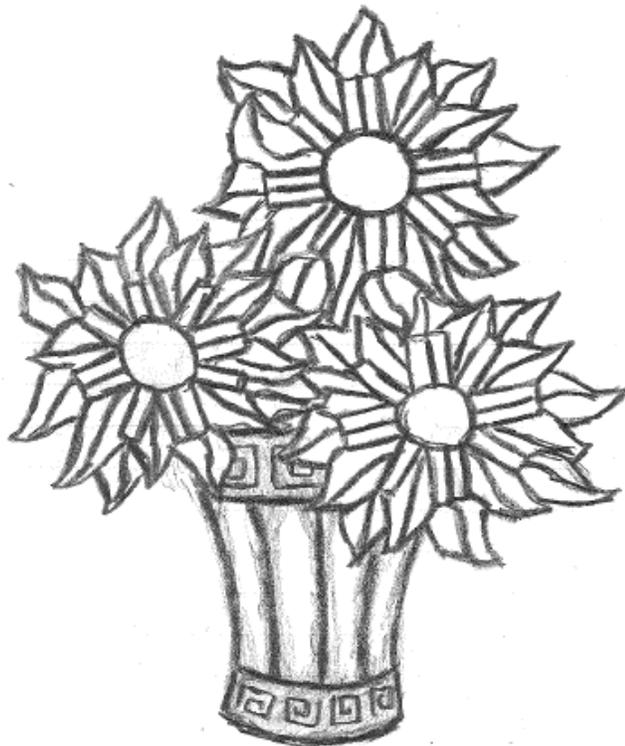
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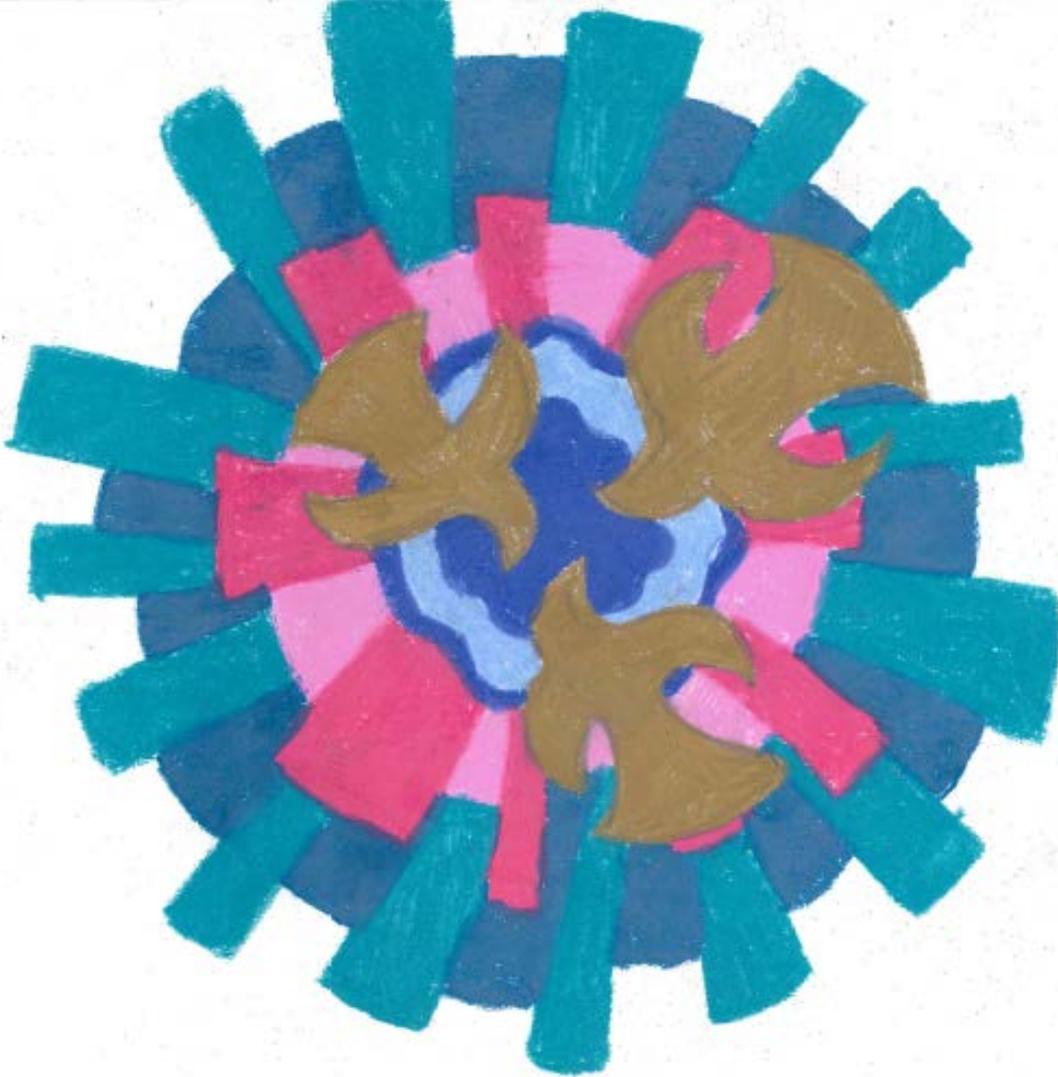
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Gloria Patri, et Filio, et Spiritui Sancto.

Sicut erat in principio, et nunc, et semper, et in sæcula sæculorum. Amen



Praise God, from whom all blessings flow;

Praise Him, all creatures here below;

Praise Him above, ye heavenly host;

Praise Father, Son, and Holy Ghost. Amen.

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List of Abbreviations

ac	Acre
Al	Aluminum
B	Boron
C	Celsius
Ca	Calcium
cm	Centimeter
Ca	Calcium
conc.	Concentration
Cu	Copper
DAT	Days After Transplanting
d	Days
F	Fahrenheit
Fe	Iron
ft	Foot
GA	Georgia
gal.	gallon
h.	Hours
ha	Hectare
in	Inch

Jan.	January
Jul.	July
K	Potassium
kg	Kilogram
L	Linear
L	Liters
lbs.	Pound
m	Meter
Mar.	March
Mg	Magnesium
min	Minutes
Mn	Manganese
N	Nitrogen
N	North
Na	Sodium
NASS	National Agricultural Statistics Service
Nov.	November
NS	Non-significant
O	Oxygen
P	Phosphorus
P	Probability
Q	Quadratic
S	South

S	Sulphur
SAS	Statistical Analysis System
Sept.	September
U.S.	United States
USDA	United States Department of Agriculture
USGS	U.S Bureau of Mines and United States Geological Survey
Zn	Zinc

Chapter 1: Literature Review

Introduction

Pecan [*Carya illinoensis* (Wangenh.) K. Koch] has the distinction of being both a commodity native to the North American continent and one of economic importance in several states. Factors contributing to successful pecan production include irrigation, nutrition, and disease management: the relative importance of each factor varies greatly by location, especially in the southern United States. Much research in the region focuses on disease management, particularly of pecan scab, which is rightly identified as one of the most limiting factors to the expansion of the pecan industry within the region. While pecan scab is of great importance to pecan growers within the region there are other issues of concern including irrigation and fertilizer use efficiency. Orchards in the region are often established on sandy soils that are naturally low in Phosphorus (P). Although irrigation is known to improve pecan production, many growers forgo the use of irrigation due to implementation cost. Therefore, it is of interest to determine the effects of irrigation and P fertilization rate using a novel banding technique on pecan yield, quality and P movement in pecan soils.

The morphology of pecan roots in container production is important as their tendency to form long taproots reduces production efficiency. There were promising results using P concentrations to modify root structures in the classic plant model organism *Arabidopsis thaliana* (Bates and Lynch 1996; Ma et al., 2001). These results have yet to be applied to pecans grown in containers to see if similar results could be replicated in the species. It would be beneficial to be able to reduce the tap rooting characteristic of pecan seedlings and increase root branching for long term container production.

Natural Habitat and Range of Pecan and Related Species

The natural habitats of *Carya* species vary greatly both globally and within North America. Pecan is the most cultivated member of the genus and is native to the Mississippi River Basin, preferring the floodplains in the region. Fralish and Franklin (2002) provided an extensive review of pecan's natural habitat, which can be summarized in two main points. First, the majority of *Carya* species in North America thrive in similar locales ranging from river banks to part of the mature *Quercus-Carya* mix found in the central states, and second, the adaptability of *Carya* has allowed different species to migrate to soil types from sand to clay dominated profiles to fill ecological niches.

One consistent characteristic of *Carya* is their ability to tolerate moderate flooding, but not prolonged water-logged soils. Some species in Asia are able to thrive in tropical conditions but are now threatened by habitat reduction. *Carya* species tend to prefer soils with adequate moisture and do not thrive under drought conditions. They are not considered a companion plant but depending on their adaptation are associated with oaks and willows in many habitats (Fralish and Franklin, 2002). Most of the natural range of pecan is in the central and eastern United States with minor pockets in Mexico. They often provide a source of food for wildlife and, historically, have been a staple of Native American diets ensuring their ultimate spread and introduction into new habitats

Pecan has demonstrated great adaptability in recently introduced plantings outside of their native range. The pecans in modern day Mexico and some parts of south Texas are the direct result of Native American plantings along trade routes and camp sites. Most plantings have survived to the present with minimal human input and with some selections being incorporated into the USDA breeding program with hopes that they will impart stress tolerance

to modern selections (L. J. Grauke, USDA-ARS Pecan Breeding, personal communication). Pecan plantings established in western states have benefited from the reduction of disease due to reduced humidity but have required extensive irrigation to be successful. The greatest success story of pecan being introduced out of their native range is Georgia. Georgia is consistently the largest producer of pecans within the U.S., accounting for about a third of U.S. pecan production. The average pecan harvest in Georgia is about 88 million pounds—enough to make 176 million pecan pies, which is an entertaining way to keep count. The estimated value of pecan production to the state of Georgia in 2016 was \$355,854,324 (University of Georgia, 2017)

Alabama has a small area where pecan is considered a native species, but most of the state's production occurs in areas where the species was introduced. Within Alabama there are 22,000 acres planted in pecans centered in Mobile and Baldwin counties (Browne, 2013). Alabama consistently ranks 8th in U.S. pecan production (Browne 2013; USDA, 2017) and has great potential for future expansion outside of its traditional production regions. The future of the pecan industry within the state may depend on its migration further north. Rising costs of real estate within Mobile and Baldwin counties combined with adverse tropical weather events are increasing the cost of pecan production and reducing the commodity's presence in the area. In addition, pecan production in the southeast often occurs in sandy soils and this is especially true in Mobile and Baldwin counties. Pecan production in sandy soils is characterized with difficulty in maintaining adequate P nutrition and growers within the region struggle to maintain recommended foliar P concentrations.

Global Phosphorus

Phosphate reserves are defined by the U.S. Bureau of Mines and United States Geological Survey (USGS) as deposits that can be economically extracted with current mining

practices (USGS, 2015). The USGS defined phosphate reserves as those retrievable below \$35 per ton in 1998 (Steen, 1998). This figure was updated in 2001 with the economic threshold being redefined as those extractable for \$40 per ton or less (McClellan and Van Kauwenbergh, 2004). Phosphate resources are distinct from phosphate reserves with resources being defined as reserves plus all other deposits that may be accessed at some point in the future (Roberts and Stewart, 2002).

Calculations of total global phosphate reserves can become a byzantine process due to rapidly changing variables. One view is that at current production levels, global commercial phosphate reserves will last approximately 50-100 years (Cordell et al., 2009). It has also been speculated that the United States' phosphate ore reserves would last less than 20 years at the current extraction rate (Roberts and Stewart, 2002), but this prediction made in 2002 has yet to materialize as of 2017. With the definition of reserves being based on economics, the time frame could change as sources previously uneconomical become viable. If expanded to include sources currently considered marginal, the base life of phosphate reserves can be extended to nearly 100 years within the U.S. and 300 years globally (Roberts and Stewart, 2002).

The life expectancy of global and domestic phosphate reserves and resources are open to differing interpretations, but the growing trend is that the quality of mined phosphate rock is declining (Cordell et al., 2009; Roberts and Stewart, 2002; Smil, 2000; Steen, 1998). An exception appears to be Moroccan reserves that are characterized as high-quality ores. Other phosphate rock producers are known to supply lower quality phosphate rock. When considering the projected phosphate rock shortage and the current reduction in quality, it is apparent that the discovery and development of renewable, high quality P fertilizer sources is an important

endeavor (Wells, 2013; Wells et al., 2017). Until that time, the most sustainable course of action is to utilize P for agricultural purposes as wisely and conservatively as possible.

Phosphorus Application and Soil Interactions

Surface broadcasting of P, particularly in the form of poultry litter, concentrates nutrients at the soil surface (Watts et al., 2010). This can be undesirable as it can lead to increased P losses in surface runoff (Gaston et al., 2003; He et al., 2009). Banding of inorganic P fertilizers can result in highly localized soil P concentrations that can be used to reduce surface P buildup (Fernández and Schaefer, 2011). A similar trend was observed in banded applications of poultry litter that resulted in increased P retention, especially in the top 5 cm of the soil profile (Watts et al., 2015).

Phosphorus banding has proven to be an effective alternative to broadcast applications of the nutrient that can lead to increased yields in maize (Welch et al., 1965). While P band application increased the total and labile P supply at the center of the band, it was mainly confined to a relatively small area within ~5 cm of the band (Kar et al., 2012). It was also determined that early beneficial effects of banding are primarily obtained from the placement of the fertilizer where contact by active roots is likely, as opposed to increased availability that may be obtained from decreased soil-fertilizer contact associated with banding (Sleight et al., 1983).

Previous research has indicated that soil type influences the efficacy of P banding. It was reported that in a loamy fine sand movement of P and increased the probability of root-fertilizer contact while no such movement occurred in a silt loam (Sleight et al., 1983). In contrast, the best method for applying P to in soils that adsorb large quantities of P was determined to be an

initial broadcast application of P with subsequent band applications of P for each crop in maize for maximum yield (Yost et al., 1978).

Phosphorus Nutrition in Pecans

Phosphorus is an important element for energy storage and is fundamental to the production of wood and nuts (Wells, 2007). One of the clearest signs of P deficiency in pecan is the occurrence of dark green foliage with no interveinal chlorosis (Wells, 2007). Phosphorus deficiency can also be expressed as a marginal leaf scorch beginning 7–10 days before shuck split and can cause premature defoliation. This was traced to phloem-mobile P decreasing rapidly in leaves as it is transported to developing cotyledons in the latter part of the growing season (Diver et al., 1984; Kim and Wetzstein, 2005; Krezdorn, 1955; Smith, 2009). The P accumulation in the cotyledons is primarily in the form of phospholipids that are used as a substrate in unsaturated fatty acid synthesis and is stored as inositol hexaphosphate (Chesworth et al., 1998). This rapid uptake of P in the fruit and resulting depletion of P from the leaves is what causes leaf necrosis and partial defoliation when P is limiting. (Hunter and Hammer, 1957; Krezdorn, 1955; Smith, 2010; Sparks, 1977). This is exacerbated in very prolific cultivars that are more likely to experience severe leaf scorch and defoliation (Sparks, 1977).

The recommendations for adequate foliar P percentage vary. It has been consistently observed that a deficiency of the nutrient can be expected at levels below ~0.11% (Alben, 1947; Sparks 1978; Sparks 1986). The most recent and widespread recommendation for adequate foliar P in high input orchards is 0.14% (Smith, 2010; Smith et al., 2012; Wells, 2007) with the further urging that this should be the minimum level in July (Smith, 2010). Smith proposed this threshold in 2010 when he found that leaf, kernel, and shuck concentrations of P were positively correlated with weight per nut and the percentage of grade 1 kernels. Sparks observed a similar

trend in ‘Mahan’ and ‘Grabohls’ where P application increased nut volume, weight per nut, and shell weight. This led to his recommendation of 0.16% as the ideal P concentration in 1988, and at one point, an even higher level of 0.30% was suggested (Jones, 1972). Correcting a P deficiency should take into account other essential plant nutrients since high concentrations of P can inhibit the uptake of nitrogen (N), iron (Fe), zinc (Zn), and copper (Cu) by the pecan trees (Sparks, 1988; Wells, 2007).

Experiments growing pecan seedlings in alternative substrates have been useful for determining field fertilizer and nutrient recommendations. Phosphorus is readily absorbed by pecan seedlings grown in perlite and deficiency of the element is easily induced when it is withheld (Sparks, 1977; Sparks, 1986; Sparks, 1988). A modification of this strategy was used with hydroponically grown pecan seedlings to study N and Zn nutrition in pecan seedlings (Kim et al., 2002a; Kim et al., 2002b). Comparisons of field and greenhouse studies have indicated that critical values derived from greenhouse studies are transferable to the field in pecan for N (Alben, 1946; Alben, 1947; Kim et al., 2002a; Sparks, 1968; Sparks, 1978; Sparks and Baker, 1975; Wood et al., 1983; Worley, 1974), Magnesium (Mg), (Alben, 1947; Sharpe et al., 1951; Sparks, 1978; Sparks, 1986), Zn, (Lane et al., 1965; Kim et al., 2002b, Núñez-Moreno et al., 2009; Sparks and Payne, 1982) and even P (Sparks, 1988) though the conclusion from the P study of 0.16% is higher than the standard 0.14% recommended currently (Smith, 2010).

Phosphorus is relatively immobile within the soil profile, so P incorporated at planting can last for several years when applied at the recommended rate, 11.89–19.82 kg ha⁻¹ (26.22–43.70 lbs. ac⁻¹) (Wells, 2007). This immobility of P is likely the reason it has been observed that broadcast applications of P lack efficacy when correcting a short-term deficiency of the element in pecan (Alben and Hammar, 1964; Hunter and Hammar, 1947, 1952, 1957; Smith et al., 1960;

Sparks, 1988; Worley,1974). The ineffectiveness of broadcast applications of P was reinforced when Sparks (1988) observed that it took 2.20 kg/6 m²/tree P to increase P adsorption.

Banded applications of P were shown to increase foliar P when applied every year at the rate of 127.3 kg ha⁻¹ (113.6 lbs. ac⁻¹) P (Smith and Cheary, 2013). Banded P applications ameliorated leaf deficiency symptoms by increasing leaf phosphorus concentrations and improving flowering, but was correlated with darker kernels in control treatments in Oklahoma (Smith and Cheary, 2013). This result was not ideal as it led to more grade 3 pecans, and contrasts with a previous study where P application increased the percentage of grade 1 pecans (Smith, 2010). It is unknown whether kernel darkening was due to cultivar response, drought stress conditions, phosphate banding, or some combination thereof. This same result has not thus far been observed by many commercial growers who have adopted banding applications of P (Smith 2010).

The role of irrigation and banded P applications in pecan have not been thoroughly studied. A previous comprehensive study using the technique by Smith and Cheary (2013) was performed under drought stress conditions. A meta-analysis of drought stress effects on plant P concentrations found that P could reduce by ~9.18% (He and Dijkstra, 2014). It is well known that irrigation is one of the most important management tools in pecan production and results in increased nut size, yield, nut quality, and precocity (Alben, 1957; Brison, 1974; Daniell, et al., 1979; Stein et al, 1989; Wells, 2015; Worley, 1982). The current recommended irrigation schedule for pecan in the southeast was established by Wells (2007) based on data from Daniell (1985). In 2015, Wells reported that water stress was highest during the kernel-filling stage regardless of soil moisture. This period of high water stress corresponds to when pecans are more

likely to experience leaf necrosis and defoliation due to depletion of the nutrient as it is being shuttled into the fruit (Smith, 2010; Sparks, 1977).

Root Architecture and Phosphorus

Plant root systems develop asymmetrically and this is an expression of the roots' ability to adjust growth and development to environmental factors (Robinson, 1994; Forde and Lorenzo, 2001). The topsoil in the soil profile has the greatest P bioavailability, and when limiting, root foraging was observed among genotypes of maize and bean (Bonser et al., 1996; Ge et al., 2000; Liao et al., 2001; Ho et al., 2005; Zhu et al., 2005). Root hairs, subcellular protrusions of root epidermal cells, that aid in acquisition of relatively immobile nutrients, are crucial to this foraging (Clarkson, 1985; Peterson and Farquhar, 1996; Jungk, 2001) since they can represent up to 70% of the total root surface area (López-Bucio et al., 2003). In *Arabidopsis thaliana*, root hairs become longer and denser under low P availability conditions (Bates and Lynch 1996; Ma et al., 2001). Pecans lack root hairs (Woodruff and Woodruff, 1934) so they are without this fundamental tool used by most plants to increase P uptake. Root-hair-less mutants of *Arabidopsis* were used to demonstrate the importance of root hairs for P uptake (Bates and Lynch 2000; Bates and Lynch, 2001). When P was limiting, root hairs on wildtype *Arabidopsis* plants led to better plant growth, biomass production, P uptake, and reproductive output when compared to the mutant without root hairs. In contrast, when P availability was high, wildtype *Arabidopsis* plants and mutants without root hairs had similar growth patterns, P acquisition, and fecundity (Bates and Lynch, 2001)

In addition to the effects on root hairs, it was observed that moderate concentrations of P favor lateral root growth over primary root growth. *Arabidopsis* seedlings grown in media containing 100 μ M P had increased lateral root density when compared to seedlings grown in

media containing 2.5 mM P, which was considered a high level of P (Williamson et al., 2001). It was observed that concentrations of P less than 50 μ M in *Arabidopsis* growth medium had a dramatic effect on root architecture. The number of lateral roots were up to five times greater in plants grown at a limiting P concentration of 1 μ M rather than in plants grown at an optimal P level of 1 mM (López-Bucio et al., 2002)

The root architecture of *Arabidopsis* plants grown in low P concentrations are characterized by having lateral roots arising near each other, having determinate growth, and being densely covered by root hairs (López-Bucio et al., 2003). In addition, it was reported that the low-P-induced determinate root growth in *Arabidopsis* was linked to the inhibition of root meristematic activity (Sánchez-Calderón et al., 2005). Root tip contact was also essential to this modification of the root architecture (Svistonoff et al., 2007). Anatomical and biochemical analyses showed that *Arabidopsis* roots grown in low P condition had mature roots lacking a normal apex and with an increased expression of P transporter genes. In contrast, *Arabidopsis* roots grown in high P conditions had high meristematic auxin concentrations and with cells that expressed high mitotic activity, which correlated with reduced gene expression encoding high affinity P transporters (López-Bucio et al., 2003).

No research has observed if the root expression in *Arabidopsis thaliana* under low and high P conditions can be replicated in pecan. Previous findings indicated that since pecans are phreatophytes, plants that root to the water table, there is a low probability of altering the root: shoot ratio by fertilization (Conner 2006; White, 1980). This characteristic of pecans is vital to its success in native areas where competition between tree species is frequent (Fletcher et al., 2012, Sparks, 2002) and could very well cause differences in root expression. Though not directly analogous, low P grove soils did not limit growth of trees or hinder seedling

establishment of *Microberlinia bisulcata* (Newbery et al., 2002), and pecan may be expected to show similar results, though the climatic environments of the two species is differ.

Conclusion

Previous research on P banding has led to its rising prominence within the southeast (Smith and Cheary, 2013). This research conditions in Oklahoma differed greatly from the more humid areas where the technique is becoming more prevalent. Of great concern is the difference in rainfall between the two regions. South Alabama receives significantly more rainfall than Oklahoma during the pecan growing season that may change the efficacy and longevity of the technique. The study was performed during a period of great drought stress which may have influenced its findings, but led to widespread adoption of the practice. Another factor of note is that the same study applied P annually at a high rate which did not address how long more moderate rates could maintain efficacy with a single application. This led to the conclusion that a replication of the experiment in irrigated and non-irrigated settings with P applied once at experiment initiation at more moderate rates in the region would be insightful in forming grower recommendations.

As mentioned earlier, there has been no significant research done in pecan to reproduce or distinguish differences in root expression between *Arabidopsis thaliana* and *Carya illinoensis* in relation to low and high P conditions. If pecans seedlings exhibit the same response to P fertilization recorded in *Arabidopsis*, it could be of interest to container production. Reduced tap rooting and increased fibrous roots would be more beneficial in containerized production than pecan seedlings' natural tendency to taproot heavily. A study was conducted to determine if different P regiments could affect said root characteristics during pecan seedling development.

References:

- Alben, A.O. 1946. Nitrogen deficiency of pecans in the spring of 1946. Proc. Texas Pecan Growers Assn. 25:24–27.
- Alben, A.O. 1947. Analysis of pecan leaves as an aid in fertility studies. Proc. Oklahoma Pecan Growers Assn. 25:24–27.
- Alben, A.O. 1957. Results of an irrigated experiment on Stuart pecan trees in East Texas in 1956. Proc. Texas Pecan Growers Assn. 36:16–23.
- Alben, A.O. and H.E. Hammar. 1964. Soil penetration and uptake of P and K in a 10 year NPK fertilizer experiment with ‘Schley’ pecan trees. Soil Sci. 97:179–182.
- Bates, T.R. and J.P. Lynch. 1996. Stimulation of root hair elongation in *Arabidopsis thaliana* by low phosphorus availability. Plant Cell Environ. 19:529–538.
- Bates, T.R. and J.P. Lynch: 2000. Plant growth and phosphorus accumulation of wild type and two root hair mutants of *Arabidopsis thaliana* (Brassicaceae). Am. J. Bot. 87:958–963.
- Bates, T.R. and J.P. Lynch 2001. Root hairs confer a competitive advantage under low phosphorus availability. Plant Soil 236:243–250.
- Bonser, A.M., J. Lynch, and S. Snapp. 1996. Effect of phosphorus deficiency on growth angle of basal roots in *Phaseolus vulgaris*. New Phytol. 132:281–288.
- Brison, F.R. 1974. Pecan culture. Capital Printing, Austin, TX.
- Brown, C. 2013. Encyclopedia of Alabama: Pecan Industry in Alabama. 11 October 2014. <<http://www.encyclopediaofalabama.org/face/Article.jsp?id=h-1647>>.
- Chesworth, J.M., T. Stuchbury, and J.R. Scaife. 1998. Agricultural biochemistry. Chapman and Hall, New York, NY.
- Clarkson, D. 1985. Factors affecting mineral nutrient acquisition by plants. Ann. Rev. Plant Physiol. 36:77–115.
- Conner, P. 2006. Effects of nitrogen fertigation on first-year pecan seedling growth. HortScience 41:1062.
- Cordell, D., J. Drangert, and S. White. 2009. The story of phosphorus: global food security and food for thought. Global Environmental Change 19:292–305.
- Daniell, J.W. 1985. The cumulative production through the 12th year (overgrown at that time) of pecan trees in a high density planting with and without irrigation. Proc. Georgia Pecan Growers Assn. 16:11–18.

- Daniell, J.W., R.B. Moss, and B. Deal. 1979. The use of irrigation in pecan orchards. *Pecan South* 6:84–85.
- Diver, S.G., M.W. Smith, and R.W. McNew. 1984. Influence of fruit development on seasonal elemental concentrations and distribution in fruit and leaves of pecan. *Commun. Soil Sci. Plant Anal.* 15:619–637.
- Fernández, F.G., and D. Schaefer. 2011. Assessment of soil phosphorus and potassium following real time kinematic-guided broadcast and deep-band placement in strip-till and no-till. *Soil Sci. Soc. Amer. J.* 76:1090–1099.
- Fletcher III, E.H. M. Thetford, J. Sharma, and S. Jose. 2012. Effect of root competition and shade on survival and growth of nine woody plant taxa within a pecan [*Carya illinoensis* (Wangenh.) C. Koch] alley cropping system. *Agroforestry. Syst.* 86:49–60.
- Fralish, J.S. and S.B. Franklin. 2002. Taxonomy and ecology of woody plants in North American forests. John Wiley & Sons,. New York, N.Y.
- Forde, B. and H. Lorenzo. 2001. The nutritional control of root development. *Plant Soil* 232:51–68.
- Gaston, L.A., C.M. Drapcho, S. Tapadar, and J.L. Kovar. 2003. Phosphorus runoff relationships for Louisiana Coastal Plain soils amended with poultry litter. *J. Environmental Quality* 32:1422–1429.
- Ge, Z., G. Rubio, and J.P Lynch. 2000. The importance of root gravitropism for inter-root competition and phosphorus acquisition efficiency: results from a geometric simulation model. *Plant Soil* 218:159–171.
- He, M. and F. A. Dijkstra. 2014. Drought effect on plant nitrogen and phosphorus: a meta-analysis. *New Phytologist* 204:924–931.
- He, Z., C.W. Honeycutt, I.A. Tazisong, Z.N. Senwo, and D. Zhang. 2009. Nitrogen and phosphorus accumulation in pasture soil from repeated poultry litter application. *Commun. Soil Sci. Plant Anal.* 40:587–598
- Ho, M., J. Rosas, K. Brown, and J. Lynch 2005. Root architectural tradeoffs for water and phosphorus acquisition. *Functional Plant Biol.* 32:737–748.
- Hunter, J.H. and H.E. Hammar. 1947. The results of applying different fertilizers to the Moore variety of pecan over a 10 year period. *Proc. Southeastern Pecan Growers Assn.* 40:10–32.
- Hunter, J.H. and H.E. Hammar. 1952. Influence of cultivation, mulching, and fertilizers on chemical composition of pecan leaves and their relation to yield and quality of nuts. *Soil Sci. Proc.* 16:346–349.

- Hunter, J.H. and H. E. Hammer. 1957. Variation in composition of pecan leaves. *Better Crops Plant Food* 41(2):18–25.
- Jones, Jr., J.B. 1972. General fertilizer recommendations for pecans. *Proc. Southeastern Pecan Growers Assn.* 65:101–112.
- Jungk, A. 2001. Root hairs and the acquisition of plant nutrients from soil. *J. Plant Nutr. Soil Sci.* 164:121–129.
- Kar, G., D. Peaka, and J. J. Schoenau. 2012. Spatial distribution and chemical speciation of soil phosphorus in a band application. *Soil Sci. Soc. Amer. J* 76: 2297–2306.
- Kim, T., H.A. Mills, and H.Y. Wetzstein. 2002a. Studies on effects of nitrogen form on growth, development, and nutrient uptake in pecan. *J. Plant Nutr.* 25:497–508.
- Kim, T., H.A. Mills, and H.Y. Wetzstein. 2002b. Studies on the effect of zinc supply on growth and nutrient uptake in pecan. *J. Plant Nutr.* 25:1987–2000.
- Kim, T. and H.Y. Wetzstein. 2005. Seasonal fluctuations in nutrients and carbohydrates in pecan leaves and stems. *J. Hort. Sci. Biotechnol.* 80:681–688.
- Krezdorn, A.H. 1955. The nutrient status of pecan leaves in relation to alternate bearing. *Proc. Texas Pecan Growers Assn.* 34:43–53.
- Lane, R., H.F. Perkins, and F.E. Johnstone, Jr. 1965. Studies on the relationship of calcium, zinc and pH in pecan nutrition. *Proc. Southeastern Pecan Growers Assn.* 58:21–24.
- Liao, H., G. Rubio, X.L. Yan, A.Q. Cao, K.M. Brown, and J.P. Lynch. 2001. Effect of phosphorus availability on basal root shallowness in common bean. *Plant Soil* 232:69–79.
- López-Bucio, J., A. Cruz-Ramirez, and L. Herrera-Estrella. 2003. The role of nutrient availability in regulating root architecture. *Current Opinion Plant Biol.* 6:280–287.
- López-Bucio, J., E. Hernández-Abreu, L. Sánchez-Calderón, M.F. Nieto-Jacobo, J. Simpson, and L. Herrera-Estrella. 2002. Phosphate availability alters architecture and causes changes in hormone sensitivity in the *Arabidopsis* root system. *Plant Physiol.* 129:244–256.
- Ma, Z., D.G. Bielenberg, K.M. Brown, and J.P. Lynch. 2001. Regulation of root hair density by phosphorus availability in *Arabidopsis thaliana*. *Plant Cell Environ.* 24:459–467.
- McClellan, G.H. and S.J. Van Kauwenbergh. 2004. World phosphate deposits. In: *Use of phosphate rocks for sustainable agriculture: FAO Fert. Plant Nutri. Bul. Food and Agricultural Organization of the United Nations, Rome, Italy.*
- Newbery, D.M., G.B. Chuyong, J.J. Green, N.C. Songwe, F. Tchuenteu, and L. Zimmermann. 2002. Does low phosphorus supply limit seedling establishment and tree growth in groves of ectomycorrhizal trees in a central African rainforest? *New Phytologist*, 156:297–311.

- Núñez-Moreno, H., J.L. Walworth, and A.P. Pond. 2009. Manure and soil zinc application to 'Wichita' pecan trees growing under alkaline conditions. *HortScience* 44:1741–1745.
- Peterson, R.L. and M.L. Farquhar. 1996. Root hairs: specialized tubular cells extending root surfaces. *Botanical. Rev.* 62:1–40.
- Roberts, T.L. and W.M. Stewart. 2002. Inorganic phosphorus and potassium production and reserves. *Better Crops.* 86(2):6–7.
- Robinson, D: 1994. The responses of plants to non-uniform supplies of nutrients. *New Phytologist.* 127:635–674.
- Sánchez-Calderón, L., J. López-Bucio, A. Chacón-López, A. Cruz-Ramírez, F. Nieto-Jacobo, J.G. Dubrovsky, and L. Herrera-Estrella. 2005. Phosphate starvation induces a determinate developmental program in the roots of *Arabidopsis thaliana*. *Plant Cell Physiol.* 46:174–184.
- Sleight, D.M., D. H. Sander, and G. A. Peterson. 1983. Effect of fertilizer phosphorus placement on the availability of phosphorus. *Soil Sci. Soc. Amer. J.* 48:336–340.
- Sharpe, R.H., G.H. Blackmon, and N. Gammon, Jr. 1951. Magnesium deficiency of pecans. *Proc. Southeastern Pecan Growers Assn.* 45:81–85.
- Smil, V. 2000. Phosphorus in the environment: natural flows and human interferences. *Annu. Rev. Energy Environ.* 25:53–88.
- Smith, C.L., O.W. Harris, and H.E. Hammar. 1960. Comparative effects of clean cultivation and sod on tree growth, yield, nut quality, and leaf composition of pecan. *Proc. Amer. Soc. Hort. Sci.* 75:313–321.
- Smith, M.W. 2009. Partitioning phosphorus and potassium in pecan trees during high- and low-crop seasons. *J. Amer. Soc. Hort. Sci.* 134:399–404.
- Smith, M.W. 2010. Relationship of leaf necrosis and defoliation to phosphorus and potassium concentrations in selected tissue and to certain fruit quality parameters of pecan. *Scientia Hort.* 125:117–122.
- Smith, M.W. and B.S. Cheary. 2013. Response of pecan to annual soil band applications of phosphorus and potassium. *HortScience* 48:1411–1415.
- Smith, M.W., C.T. Rohla, and W.D. Goff. 2012. Pecan leaf elemental sufficiency ranges and fertilizer recommendations. *HortTechnology* 22:594–599.
- Sparks, D. 1968. Some effects of nitrogen on young pecan trees. *Proc. Southeastern Pecan Growers Assn.* 61:93–102.
- Sparks, D. 1977. Effects of fruiting on scorch, premature defoliation, and nutrient status of 'Chickasaw' pecan leaves. *J. Amer. Soc. Hort. Sci.* 102:669–673.

- Sparks, D. 1978. Nutrient concentrations of pecan leaves associated with deficiency symptoms and normal growth. *HortScience* 13:256–257.
- Sparks, D. 1986. Growth and nutrition of pecan seedlings from potassium phosphate foliar sprays. *HortScience* 21:451–452.
- Sparks, D. 1988. Growth and nutritional status of pecan in response to phosphorus. *J. Amer. Soc. Hort. Sci.* 113:850–859.
- Sparks, D. 2002. Rainfall governs pecan stand homogeneity in native, wild habitats. *J. Amer. Soc. Hort. Sci.* 127:860–868.
- Sparks, D. and D. H. Baker. 1975. Growth and nutrient response of pecan seedlings, *Carya illinoensis* Koch, to nitrogen levels in sand culture. *J. Amer. Soc. Hort. Sci.* 100:392–399.
- Sparks, D. and J.A. Payne. 1982. Zinc concentration in pecan leaflets associated with zinc deficiency symptoms. *HortScience* 17:670–670.
- Steen, I. 1998. Phosphorus availability in the 21st century: management of a non-renewable resource. *Phosphorus and Potassium.* 217:25–31.
- Stein, L.A., G.R. McEachern, and J.B. Storey. 1989. Summer and fall moisture stress and irrigation scheduling influence pecan growth and production. *HortScience* 24:607–611.
- Svistoonoff, S., A. Creff, M. Reymond, C. Sigoillot-Claude, L. Ricaud, A. Blanchet, L. Nussaume, and T. Desnos. 2007. Root tip contact with low-phosphate media reprograms plant root architecture. *Nature Genet.* 39:792–796.
- University of Georgia, UGA Extension. 2017. Pecans. 13 October 2017
<<http://extension.uga.edu/topic-areas/fruit-vegetable-ornamentals-production/pecans.html>>
- USGS. 2006. Phosphate rock. In: Mineral commodity summaries. United States Geological Survey.
- Watts, D.B., H.A. Torbert, S.A. Prior, and G. Huluka. 2010. Long-term tillage and poultry litter impacts soil carbon and nitrogen mineralization and fertility. *Soil Sci. Soc. Amer. J.* 175:474–486.
- Watts, D.B., T. R. Way, H. A. Torbert, and S. D. Armstrong. 2015. Subsurface band application of poultry litter and its influence on phosphorus concentration and retention after runoff from permanent pastures. *J. Environ. Quality.* 44:1930–1937.
- Welch, L. F., D. L. Mulvaney, L. V. Boone, G. E. McKibben, and J. W. Pendleton. 1965. Relative efficiency of broadcast versus banded phosphorus for corn. *Agron. J.* 58: 283–287.

- Wells, D. E. 2013. Poultry litter ash as a phosphorus source for greenhouse crop production. LSU Doctoral Dissertations. 1849.
<http://digitalcommons.lsu.edu/gradschool_dissertations/1849>
- Wells, D.E., J.S. Beasley, E.W. Bush, and L.A. Gaston. 2017. Poultry litter ash rate and placement affect phosphorus dissolution in a horticultural substrate. *J. Environ. Hort.* 35:117–127.
- Wells, M. L. (Ed). 2007. Southeastern pecan growers' handbook. Univ. Georgia Coop. Ext. Publ. 1327. 1965.
- Wells, L. 2015. Irrigation water management for pecans in humid climates. *HortScience* 50:1070–1074.
- Williamson, L., S. Ribrioux, A. Fitter, and O. Leyser. 2001. Phosphate availability regulates root system architecture in *Arabidopsis*. *Plant Physiol.* 126:1–8.
- White, Jr., A.W. 1980. Effects of topsoil-subsoil fertilizer and lime amendments on top and root growth of 'Stuart' pecan seedlings in a simulated Norfolk soil profile. *HortScience* 17:380–381.
- Wood, B., W. White, and W. L. Tedders. 1983. Use of legumes in pecan orchards. *Oklahoma Pecan Growers Assn.* 53:30–35.
- Woodruff, J.G. and N. C. Woodruff. 1934. Pecan root growth and development. *J. Agricultural Res.* 49:511–531.
- Worley, R.E. 1974. Effect of N, P, K, and lime on yield, nut quality, tree growth, and leaf analysis of pecan (*Carya illinoensis* W.). *J. Amer. Soc. Hort. Sci.* 99:49–57.
- Worley, R.E. 1982. Tree yield and nut characteristics of pecan with drip irrigation under humid conditions. *J. Amer. Soc. Hort. Sci.* 107:30–34.
- Yost, R.S., E. J. Kamprath, E. Lobato, and G. Naderman. 1978. Phosphorus response of corn on an oxisol as influenced by rates and placement. *Soil Sci. Soc. Amer. J.* 43:338–343.
- Zhu J., S.M. Kaeppler., and J.P. Lynch 2005. Topsoil foraging and phosphorus acquisition efficiency in maize (*Zea mays*). *Functional Plant Biol.* 32:749–762.

Chapter 2: Long-Term Response to Phosphorus Banding in Irrigated and Non-Irrigated Pecan Production

Abstract

An experiment was conducted to determine the effectiveness of banded phosphorus (P) applications at differing rates within irrigated and non-irrigated pecan plots on P movement within the soil, P uptake and movement within pecan trees, and yield and quality of nuts. On March 20, 2015, P at 0 kg ha⁻¹ (0x), 19.6 kg ha⁻¹ (1x), 39.2 kg ha⁻¹ (2x), and 78.5 kg ha⁻¹ (4x) was applied in bands of triple superphosphate to randomly selected trees in non-irrigated and irrigated plots of a ‘Desirable’ orchard bordered by ‘Elliot’ trees. Soil core samples measuring 22.5-cm (9-in) deep were collected bi-monthly within each band (tree) and divided into 7.5-cm (3-in) increments. Foliar samples were collected annually on 20 July.

When P was applied at 2x and 4x rates, total soil test P decreased linearly by 35% and 54%, respectively, in non-irrigated plots and by 41% and 59%, respectively, in irrigated plots over the course of the experiment. There was no change in soil test P over time at the 0x rate for either irrigation regimens, but at the 1x rate, soil test P decreased 44.4% in the irrigated plot but did not change in the non-irrigated plot. The largest linear decrease in soil test P from experiment start to finish was in the top 0–7.6 cm. In contrast, soil test P in the 15.2–22.9 cm depth decreased linearly by 23.2% in the non-irrigated plot but did not decrease over time in the irrigated plot.

Increasing P application rate increased foliar P quadratically in the non-irrigated plot, but only the 4x application rate increased foliar P compared to the 0x control. In the irrigated plot, foliar P concentrations decreased linearly from 2015 to 2017 and foliar P concentrations were not influenced by P application rate. Foliar Fe and Cu concentrations decreased with increasing P

application rate in the irrigated plot while foliar B concentrations increased. Foliar B concentrations were influenced by the application side of the P band in the irrigated plot as well, increasing on the proximal side and decreasing on the distal side compared to the application site. No differences in pecan yield or quality were observed in either irrigated or non-irrigated plots. Overall, P banding may not be the most sustainable way to increase concentrations of P quickly or to maintain foliar concentrations of the nutrient long term.

Introduction

Approximately 3.1 million kg (6.8 million lbs.) of pecans were produced in Alabama in 2013 (Brown, 2013). The majority of production was located in the southwest corner of the state in Mobile and Baldwin counties. Soils in those counties are mostly sandy loams and are typical of pecan orchards throughout the southeastern United States. Growers often experience difficulties maintaining recommended foliar phosphorus (P) levels in their orchard trees due to naturally low P soils and the nature of movement and adsorption of P in those soils.

Recommendations for adequate foliar P concentrations in pecan vary, though it was consistently observed that visual deficiency symptoms can be expected at foliar concentrations below ~0.11% (Alben, 1947; Sparks, 1978; Sparks, 1986). The current standard recommendation for adequate foliar P is 0.14% (Smith, 2010; Smith et al., 2012). Pecan fertilizer recommendations are often based on a combination of the current year's soil test and the previous year's foliar nutrient concentration data, with the latter considered more important. When correcting a P deficiency, it is important to consider other essential plant nutrients since high concentrations of P can inhibit the uptake of nitrogen, iron, zinc, and copper in pecan (Sparks, 1988; Wells, 2007).

Phosphorus is relatively immobile in most soils and, though often applied in combination with nitrogen (N) and potassium (K), is required by plants in much lower quantities. As a result, a single broadcast application of 29.4–49 kg ha⁻¹ (26.2–43.7 lbs. ac⁻¹) P incorporated at planting can be adequate for several years during orchard establishment (Wells, 2007). The immobility of P in soils may result in the observed ineffectiveness of broadcast applications in correcting short-term deficiencies in established orchards (Alben and Hammar, 1964; Hunter and Hammar, 1947, 1952, 1957; Smith et al., 1960; Sparks, 1988; Worley, 1974). As a result, Sparks (1988) reported that 2.2 kg/6 m²/tree P was required to significantly increase P concentrations in pecan leaves. Due to the consideration of tree spacing, the rate reported by Sparks was not easily interpreted in its initial publication and later interpretations ranged from 3,670 kg ha⁻¹ (3,274 lbs. ac⁻¹) (Smith and Cheary, 2013) to 14,985 kg ha⁻¹ (13,369 lbs. ac⁻¹) P per year (Worley, 2002). Broadcast applications at extremely high rates should be discouraged due to potential environmental contamination.

Previous research indicated that banded applications of P increase foliar P when applied annually at the rate of 127.3 kg ha⁻¹ (113.6 lbs. ac⁻¹) P (Smith and Cheary, 2013). Banded P applications increased leaf P concentrations, ameliorated foliar deficiency symptoms, and increased return bloom. However, Smith and Cheary (2013) also reported kernel darkening in response to repeated P banding. The reported kernel darkening contrasts with a previous study in which P application improved color quality of pecans (Smith, 2010). It is unknown whether the reported kernel darkening was due to cultivar response, drought stress conditions, P banding, or some combination thereof (Smith and Cheary, 2013). The reported positive benefits of P banding have outweighed the negative for many growers in the southeast who have adopted it as a

standard practice. Thus far, there have been no reports of observed kernel darkening due to P banding in Alabama.

The positive effects of irrigation in pecan are well-known and include precocity, increased nut size and yield, and improved nut quality (Alben, 1957; Brison, 1974; Daniell, et al., 1979; Stein et al, 1989; Wells, 2015; Worley, 1982). The current recommended irrigation schedule for pecan in the Southeast was established by Wells (2007) based on data from Daniell (1985). Adequate soil moisture is important for pecans in the nut filling stage in August and September (Wells 2015). Adoption of irrigation practices among Alabama pecan growers has been slow, but larger growers have invested in the infrastructure required.

A previous study of P banding by Smith and Cheary (2013) was conducted in an irrigated orchard, but under drought conditions. Irrigation efficacy was compromised at the height of the drought as the irrigation source became unusable (Smith, personal communication). According to a separate, unrelated meta-analysis of the effects of drought stress on plant P concentrations, He and Dijkstra (2014) reported that drought stress may reduce P concentrations in plants by up to 9.18%. Despite the negative effects of drought stress, increased foliar P was observed from annual banded applications of 127.3 kg ha⁻¹ (113.6 lbs. ac⁻¹) P (Smith and Cheary, 2013)

Phosphorus banding has yet to be replicated in an irrigated or non-irrigated environment like that present in the southern portion of Alabama. Additionally, the effects of a one-time band application have not been observed nor those of lessor, more sustainable application rates. An experiment was designed to determine the efficacy of a single P band at selected rates on soil test P and P uptake by plants over multiple years in a typical non-irrigated and irrigated Alabama pecan orchard.

Materials and Methods

The experiment was conducted at the Gulf Coast Research and Extension Center in Fairhope, AL. The orchard's soil type was a mixture of a Greenville loam and an Orangeburg fine sandy loam. The mature orchard was comprised of 'Desirable' trees planted at a 12 by 12 m (40 by 40 ft) spacing or ~66 trees ha⁻¹ (27 trees ac⁻¹). Six, 49m (160 ft.) rows of 'Desirable' trees were bordered on all sides by a row of 'Elliot' trees. All trees in the orchard were originally grafted to open-pollinated 'Elliot' seedling rootstocks. The orchard had a recorded history of difficulty maintaining adequate foliar P concentrations, which along with the reduced alternate bearing of 'Desirable', made it ideal for the research conducted. The orchard was scouted frequently for pathogens and treated as necessary. This was especially important as 'Desirable' is susceptible to pecan scab which is endemic in the region.

For irrigation, the orchard was split into equal halves with each half (plot) being treated as a separate experiment. A border row of 'Desirable' trees separated the plots to prevent water from the irrigated plot crossing over into the non-irrigated plot. The existing sub-surface drip irrigation system in the orchard was turned off in the non-irrigated plot, so the trees only received natural rainfall. Trees in the irrigated plot, in addition to natural rainfall, were supplemented with the irrigation system to meet the requirements outlined by Wells (2007). The irrigation system was a sub-surface drip system with five emitters per tree and each emitter delivered 3.8 L h⁻¹ when in use.

Phosphorus was applied in the form of triple superphosphate (0N-20.1P-0K). The rates applied were based on the standard broadcast recommendation to correct a deficiency of P, 19.6 kg ha⁻¹ (17.5 lbs. ac⁻¹) of P (Wells, 2007). Treatment levels were equivalent to 0 kg ha⁻¹ (0 lbs. ac⁻¹), 19.6 kg ha⁻¹ (17.5 lbs. ac⁻¹), 39.2 kg ha⁻¹ (35 lbs. ac⁻¹), and 78.5 kg ha⁻¹ (70 lbs. ac⁻¹) P and

hereafter referred to as 0x, 1x, 2x, and 4x, respectively. Bands of triple superphosphate at each rate were applied on 20 March 2015 under the south side dripline of each tree located approximately 3 m (10 ft.) from the base of the trunk. Each band measured 6 m (20 ft.) long and was ~10 cm (4 in.) wide. No other sources of P were applied for the duration of the experiment, but nitrogen (N) and potassium (K) were applied to maintain or achieve recommended foliar concentrations. (Smith et al., 2012).

Soil samples were collected within the application strip from three trees at each treatment level starting 2 months after initiation on 20 May 2015 and continuing at 2-month intervals until 20 July 2017. Three 22.5-cm (9-in.) core samples were collected within each band (tree) and were pooled in three 7.5-cm (3-in.) increments, hereafter referred to as top, middle, or bottom. Soil samples were not collected on 20 September 2016 for non-irrigated trees due to moderate drought conditions. Standard soil analysis was performed to determine pH (McLean, 1982), organic matter (Schulte and Hopkins, 1996), estimated nitrogen release (Schulte and Hopkins, 1996), Bray I phosphorus (Bray and Kurtz, 1945), exchange capacity (Gavlak et al., 2003), percent base saturation of cation (Gavlak et al., 2003), and Mehlich III extractable phosphorus (P), manganese (Mn), zinc (Zn), boron (B), copper (Cu), iron (Fe), aluminum (Al), sulphur (S), calcium (Ca) magnesium (Mg), potassium (K), sodium (Na) (Mehlich, 1984) (Brookside Laboratories, New Bremen, OH).

Foliar samples were collected from all experimental trees once per year on 20 July 2015, 2016, and 2017, which falls within the standard recommendation time for collection of foliar samples (Wells, 2007; Smith et al. 2012). Samples were collected from both the south (treated) and north (untreated) sides of the canopy. Each sample contained 20 middle leaflet pairs of the current season's growth. One gram after drying was used for analysis. Samples were digested

according to procedures for wet acid digestion using nitric and perchloric acids described by Mills and Jones (1996). Concentrated samples were diluted in 20 mL deionized water and analyzed for elemental concentrations using inductively coupled plasma optical emission spectroscopy (Brookside Laboratories, New Bremen, OH).

Nut yield data were collected at the 50% shuck date which for ‘Desirable’ was the first week of November in 2015 and 2016. Yield data from 2017 were omitted due to a tropical weather system that caused the crop to fall early and mix on the orchard floor. The wedge method was used to determine total yield (Worley and Smith 1984). Quality data were collected from 40-nut samples taken in conjunction with those collected for yield in 2015 and 2016. Pecans were graded according to USDA guidelines (USDA, 1976; Goff et al., 1989).

An analysis of variance was performed on soil and foliar responses using PROC GLIMMIX in SAS version 9.4 (SAS Institute, Cary, NC). Non-irrigated and irrigated data were analyzed as separate experiments. Soil data were analyzed as a split-split plot with P rate in the main plot, sampling depth in the sub-plot, and sampling period in the sub-sub plot. Foliar data were analyzed as a split-plot with year in the main plot and application rate and side of application in the sub-plot. Where residual plots and a significant covariance test indicated heterogeneous variance among treatments, a RANDOM statement with the GROUP option was used to correct heterogeneity. Presented are least squares means. Linear and quadratic trends over P rate, sampling depth, and sampling period were tested using model regressions. All significances were at $\alpha = 0.05$.

Results

The soil depth by P rate, P rate by sample date, and soil depth by sample date interactions were significant for soil test P in irrigated and non-irrigated plots. Over the course of the experiment, soil test P decreased linearly with increasing soil depth by 30.8% and 41.7% at the 1x and 2x application rates, respectively, and decreased quadratically by 40.1% at the 4x rate (Table 2.1). In contrast, there was no change in soil test P over depth at the 0x application rate. Soil test P increased quadratically as application rate increased at each soil depth. Similar trends were observed in the irrigated plot (Table 2.2).

Soil test P increased linearly or quadratically, as P application rate increased, for all sampling periods (Table 2.3). On the first collection period, 20 May 2015, soil test P increased linearly by 1,624% from the 0x to the 4x P rates. By the last collection period, 20 July 2017, this trend had changed to a quadratic increase of 727% from the 0x to the 4x P rates. Soil test P decreased linearly over time by 34.7% and 54.0% when P was applied at 2x and 4x rates, respectively, but there was no change at the 0x and 1x rates. Similar trends were observed in soil P over P application rate and sampling date in the irrigated plot with two notable exceptions (Table 2.4). In the irrigated plot, soil test P increased quadratically with increasing P rate, but the increase was lower (402%) as a percentage compared to the increase in the non-irrigated plot (727%). Soil test P did not change over the course of the experiment at the 1x application rate in the non-irrigated plot but decreased quadratically by 44% in the irrigated plot.

In the non-irrigated plot, soil test P decreased linearly by 54.3% from the top to the bottom depth at the first collection period but decreased quadratically at all other dates (Table 2.5). On the final collection date, the quadratic decrease was 24.5% from the top to the bottom depth. Over time, the top depth had the largest linear decrease in soil test P with an overall

reduction of 53.4%. The middle and bottom depths had lessor linear decreases over time of 16.6% and 23.2%, respectively. Similar trends were observed in soil test P over soil depth and sample date in the irrigated plot (Table 2.6) with the exceptions of a larger linear percentage decrease of soil test P at the middle depth (41.8% compared to 16.6%) and no decrease of soil test P being observed at the bottom depth over time.

The P rate and year main effects were significant for foliar P in the non-irrigated plot but not for the irrigated plot. Foliar P concentrations increased quadratically with increasing P application rates in the non-irrigated plot (Table 2.7). Only the 4x application rate increased foliar P compared to the 0x control. Foliar P concentrations for the 1x and 2x rates were below those observed at the 0x rate.

The year main effects were significant for foliar N, P, K, Mg, S, B, and Cu, but there were no differences for foliar Ca, Fe, and Mn in the non-irrigated plot. Foliar N and K concentrations increased linearly from 2015 to 2017 (Table 2.8). Foliar P concentrations followed a quadratic trend, decreasing from 2015 to 2016, then increasing from 2016 to 2017. Foliar Mg and S had linear or quadratic trends over years, but Mg concentrations were within the recommended range (Smith et al., 2012) and were therefore not considered biologically significant. Zinc is the micronutrient of most concern in pecan production, but it did not change over years. No differences in yield or quality were observed in the non-irrigated plot.

In the irrigated plot, the year main effects were significant for N, P, K, Mg, Ca, and S; the year and P rate main effects were significant for Fe and Cu; and the year, P rate, and application side main effects were significant for B. No differences were observed for Mn and Zn. Foliar N changed quadratically, decreasing from 2015 to 2016, then increasing from 2016 to 2017 (Table 2.9). Foliar K increased while P decreased linearly from 2015 to 2017. Plant nutrients that also

decreased linearly or quadratically over time were Mg, Ca, S, and B. Foliar Fe and Cu concentrations followed a quadratic trend over time similar to N with higher observed concentrations in 2015 and 2017 than in 2016. Zinc was not affected by year. Of the elements that changed over time, only P, K, S, and Fe were lower than sufficiency ranges, and were potentially biologically significant (Smith et al., 2012). Foliar concentrations of B increased linearly by 10% while Cu decreased linearly by 12.7% with increasing P rate (Table 2.10). Foliar Fe decreased quadratically by 9.0% from the 0x to the 4x rate. Foliar B concentrations were influenced by the application side of the P band in the irrigated plot, increasing on the proximal side and decreasing on the distal side compared to the application site (Table 2.11). No differences in yield or quality were observed in the irrigated plot.

Discussion

The irrigated and non-irrigated plots both had linear decreases in soil test P over soil depth at the 1x and 2x rates followed by a quadratic decrease at the 4x rate. This was a confirmation that the majority of the P applied was remaining in the top depth but was moving steadily down to lower depths. As P application rate increased soil test P increased at each depth.

In the non-irrigated plot soil test P concentrations remained higher for the 2x and 4x rates at the end of the experimental period than they were for the 1x rate at the start of the experimental period. This was significant as there was no change in the concentration of soil test P present over time at the 1x rate in the non-irrigated plot. It is likely that, since soil test P concentrations were nearly constant for the experimental period at the 1x application rate, the 1x rate introduced what is or is close to the P-holding capacity of the orchard soil and the soil simply maintained P concentrations at or near the maximum equilibrium concentration. The change in concentrations of soil test P throughout the soil profile at higher application rates

would further support this reasoning. Data collected in this experiment are insufficient to confirm or deny this explanation, but neither was that the intention of this experiment. Future research should address this question. It is likely that if observed further, soil test P concentrations will eventually level off for the 2x and 4x rates and approach those present at the 1x rate.

In the irrigated plot soil test P was higher at the 4x rate at the end of the experimental period than it was for the 1x rate at the start of the experimental period. The same was not true for the 2x rate. There was also a quadratic decrease of soil test P over time observed at the 1x rate in the irrigated plot, which was different than what was observed for the non-irrigated plot. Diffusion is the primary way that P moves within the soil (Lewis and Quirk, 1967), but these data suggest that irrigation played a significant role in moving P within the soil profile. Previous research supports this finding with the observation that residual effectiveness of superphosphate decreases as soil water content increases (Bolland and Baker, 1987). This residual effectiveness may be due to the reduced diffusion of P that occurs in dry soils, though it has been reported that reduced soil moisture does not reduce bioavailability of P (McBeath et al., 2012). It is not known from our data to what extent P movement occurred laterally over the course of the experiment or how potential lateral movement was influenced by irrigation. Previous research into diffusion of P at 10 and 20 kg ha⁻¹ P rates showed that concentrations of P in bands decreased logarithmically from the band center and varied substantially along the direction of band application (Stecker et al., 2001)

For the irrigated and non-irrigated experiments, most foliar plant nutrients remained within the sufficiency ranges published for pecan (Smith et al., 2012) except for P, K, S, and Fe. Sulphur and Fe were just below the sufficiency range and thus an application of the nutrients was not necessary. It has been reported that high concentrations of soil test P can reduce Fe uptake in

pecan (Sparks, 1988). The small decrease in foliar Fe observed in the irrigated plot as P application rate increased could have been caused by the high concentrations of soil test P in the banded area, but the expected increase in foliar P was not observed. Potassium concentrations were corrected by separate fertilization over the course of the study. The increase in foliar concentrations of N during the study was also due to fertilization.

Foliar P concentrations were quadratic over year in the non-irrigated plot being higher in 2015 and 2017 than in 2016. In contrast, foliar P concentrations decreased linearly in the irrigated plot from 2015 to 2017. Trends similar to those observed in the non-irrigated orchard were reported in other studies and has been attributed to the alternate bearing phenomenon observed in pecan (Krezdorn, 1955; Smith, 2009). In those studies, higher foliar P concentrations were observed in higher yielding years than in lower yielding years. The trend in the irrigated plot differs from what was reported in those studies.

It is notable that the differing trends were observed between the experiments since ‘Desirable’ is known for its reduced alternating bearing characteristic (Wells, 2007) and there was no difference in yield between 2015 and 2016 in either setting. ‘Desirable’ trees self-abort flowers each year which reduces yield variance between years (Wells, 2007). Our data indicate that P partitioning within the plant still follows an alternate bearing pattern regardless of self-thinning in a non-irrigated orchard. It may also be that irrigation ameliorates that tendency of P in ‘Desirable’ but comes with the tradeoff of steady use of the nutrient.

A 4x application rate was required in the non-irrigated orchard to increase foliar P concentrations over the 0x rate. Foliar P concentrations at the 4x rate would still be considered deficient for a high input orchard (0.14%) but would be considered sufficient for a low input orchard (0.12%) (Smith et al., 2012). In contrast, none of the P rates increased foliar P

concentrations over the 0x rate in the irrigated orchard and all foliar P concentrations were below those needed in a high input orchard. This either indicates a higher rate of application would be necessary to bring foliar concentrations up to those recommended for a high input commercial orchard in both irrigated and non-irrigated environments, or that 'Desirable' trees have a lower P requirement compared to other cultivars. Foliar sufficiency ranges are reported at the species, not the cultivar level, and it is likely cultivar differences exist. Maybe more importantly, application of exceedingly high rates of P fertilizers to pecan orchards may not be an environmentally sustainable option due to the potential negative impacts of P on surface water quality and aquatic life as agriculture was responsible for 38% of global anthropogenic P loads to freshwater from 2002 to 2010 (Mekonnen and Hoekstra 2018).

Regardless of the application rate and potential cultivar differences, the difficulty in increasing foliar P concentration is likely due to the lack of root hairs in pecan (Woodruff and Woodruff, 1934). In most plant species, uptake of P is greatly aided by root hairs. For example, spring barley plants having root hairs absorbed nearly two times more P than root hair-less mutants (Gahoonia and Nielsen, 2001). The high P application rates that were needed to increase P uptake reported by Sparks (1987) and Smith and Cheary (2013) were likely needed because P uptake in pecan is inherently inefficient due to a lack of root hairs. While there is evidence that P banding can increase return bloom and ameliorate P deficiency (Smith and Cheary, 2013), the exceedingly high rates of P needed due to the lack of root hairs in pecan should induce careful consideration towards the sustainability of those large applications.

Although Fe, Cu, and B foliar concentrations were influenced by P rate when combined with irrigation, the magnitude of these changes are not likely biologically significant. Boron foliar concentrations were also affected by the site of P application with irrigation. The side that

the P band was applied on had higher boron concentrations than side that did not. The practical applications of this phenomenon are unknown as B was available at sufficient amounts even without P application.

Overall it appears that P banding may not be the most sustainable way to increase concentrations of P quickly or to maintain foliar concentrations of the nutrient long term. A very high application rate of the nutrient, 78.5 kg ha⁻¹ (70 lbs. ac⁻¹) of P, was necessary to increase foliar concentration of the nutrient in a non-irrigated setting and it was still below the recommended range. The practicality of P banding could be increased in the region if the 19.6 kg ha⁻¹ (17.5 lbs. ac⁻¹) of P was more effective as it remained stable within the soil profile in the non-irrigated orchard, and though it decreased quadratically over time in the irrigated orchard, its loss of P through the soil profile was the least. Banding may be a valuable tool if used in combination with a foliar application of P. This approach is not unprecedented in pecan and has been used as a more long-term solution in correcting Zn deficiency. Foliar sprays of Zn are common to correct short-term deficiency of the element, but a band application of the nutrient has been shown to have long-term efficacy (Wood, 2007) The adoption of this two-pronged approach has proven to be advantageous for pecan growers with it being common for foliar applications of Zn to be paired with a banded application which eventually makes future foliar applications unnecessary. Research into creating a similar protocol with P in pecan may be beneficial.

References:

- Alben, A.O. 1947. Analysis of pecan leaves as an aid in fertility studies. *Proc. Oklahoma Pecan Growers Assn.* 25:24–27.
- Alben, A.O. 1957. Results of an irrigated experiment on Stuart pecan trees in East Texas in 1956. *Proc. Texas Pecan Growers Assn.* 36:16–23.
- Alben, A.O. and H.E. Hammar. 1964. Soil penetration and uptake of P and K in a 10 year NPK fertilizer experiment with ‘Schley’ pecan trees. *Soil Sci.* 97:179–182.
- Bolland, M.D.A., and M.J. Baker. 1987. Increases in soil water content decrease the residual value of superphosphate. *Aust. J. Exp. Agric.* 27:571–578.
- Bray, H.R. and L.T. Kurtz. 1945. Determination of total, organic, and available forms of phosphorus in soils. *Soil Sci.* 59:39–45.
- Brison, F.R. 1974. Pecan culture. Capital Printing, Austin, TX.
- Brown, C. 2013. Encyclopedia of Alabama: Pecan Industry in Alabama. 11 October 2014. <<http://www.encyclopediaofalabama.org/face/Article.jsp?id=h-1647>>.
- Daniell, J.W. 1985. The cumulative production through the 12th year (overgrown at that time) of pecan trees in a high density planting with and without irrigation. *Proc. Georgia Pecan Growers Assn.* 16:11–18.
- Daniell, J.W., R.B. Moss, and B. Deal. 1979. The use of irrigation in pecan orchards. *Pecan South* 6:84–85.
- Gahoonia T.S. and N.E. Nielsen. 2001. Measuring and modelling phosphorus uptake by root hairs. In: Horst W.J. et al. (eds) *Plant Nutrition. Developments in Plant and Soil Sciences*, vol 92. Springer, Dordrecht
- Gavlak, R., D. Horneck, R. Miller, and J. Kotuby-Amarcher. 2003. Soil, plant, and water reference methods for the western region. *Western Region Ext. Publ. WREP-125.*
- Goff, W.D., J.R. McVay, and W.S. Gazaway. 1989. Pecan production in the southeast. *Alabama Coop. Ext. Publ. ANR-459.*
- He, M. and F. A. Dijkstra. 2014. Drought effect on plant nitrogen and phosphorus: a meta-analysis. *New Phytologist* 204:924–931.
- Hunter, J.H. and H.E. Hammar. 1947. The results of applying different fertilizers to the Moore variety of pecan over a 10 year period. *Proc. Southeast Pecan Growers Assn.* 40:10–32.
- Hunter, J.H. and H.E. Hammar. 1952. Influence of cultivation, mulching, and fertilizers on chemical composition of pecan leaves and their relation to yield and quality of nuts. *Soil Sci. Proc.* 16:346–349.

- Hunter, J.H. and H. E. Hammer. 1957. Variation in composition of pecan leaves. *Better Crops Plant Food* 41(2):18–25.
- Krezdorn, A.H. 1955. The nutrient status of pecan leaves in relation to alternate bearing. *Proc. Texas Pecan Growers Assn.* 34:43–53.
- Lewis, D.G. and J.P. Quirk. 1967. Phosphate diffusion in soil and uptake by plants. *Plant and Soil.* 26: 99–118.
- McBeath T.M., M.J. McLaughlin, J.K. Kirby, and R.D. Armstrong. 2012. Dry soil reduces fertilizer phosphorus and zinc diffusion but not bioavailability. *Soil Sci. Soc. Amer. J* 76:1301–1310.
- McLean, E.O. 1982. Soil pH and lime requirement. p. 199–223. In A.L. Page et al. (ed.) *Methods of soil analysis, part 2.* Agron. Monograph 9, 2nd ed. ASA and SSSA, Madison, WI.
- Mehlich, A. 1984. Mehlich-3 soil test extractant: A modification of Mehlich-2 extractant. *Commun. Soil Sci. Plant Analysis* 15:1409–1416.
- Mekonnen, M. M. and A. Y. Hoekstra. 2018. Global anthropogenic phosphorus loads to fresh water and associated grey water footprints and water pollution levels: A high-resolution global study. *Water Resources Research.* 54: 345-358
- Mills, H.A. and J.B. Jones, Jr. 1996. *Plant analysis handbook II: A practical sampling, preparation, analysis, and interpretation guide.* MicroMacro Publishing, Inc., Athens, GA.
- Schulte, E.E. and B.G. Hopkins. 1996. Estimation of soil organic matter by weight loss on ignition. p. 21-32. In: *Soil organic matter: Analysis and interpretation.* (ed.) F.R. Magdoff, M.A. Tabatabai, and E.A. Hanlon, Jr. Special publication No. 46. *Soil Sci. Soc. Amer.* Madison, WI.
- Smith, C.L., O.W. Harris, and H.E. Hammar. 1960. Comparative effects of clean cultivation and sod on tree growth, yield, nut quality, and leaf composition of pecan. *Proc. Amer. Soc. Hort. Sci.* 75:313–321.
- Smith, M.W. 2009. Partitioning phosphorus and potassium in pecan trees during high- and low-crop seasons. *J. Amer. Soc. Hort. Sci.* 134: 399–404.
- Smith, M.W. 2010. Relationship of leaf necrosis and defoliation to phosphorus and potassium concentrations in selected tissue and to certain fruit quality parameters of pecan. *Scientia Hort.* 125:117–122.
- Smith, M.W. and B.S. Cheary. 2013. Response of pecan to annual soil band applications of phosphorus and potassium. *HortScience* 48:1411–1415.
- Smith, M.W., C.T. Rohla, and W.D. Goff. 2012. Pecan leaf elemental sufficiency ranges and fertilizer recommendations. *HortTechnology* 22:594–599.

- Sparks, D. 1978. Nutrient concentrations of pecan leaves associated with deficiency symptoms and normal growth. *HortScience* 13:256–257.
- Sparks, D. 1986. Growth and nutrition of pecan seedlings from potassium phosphate foliar sprays. *HortScience* 21:451–452.
- Sparks, D. 1988. Growth and nutritional status of pecan in response to phosphorus. *J. Amer. Soc. Hort. Sci.* 113:850–859.
- Stecker, J.A., J.R. Brown, and N.R. Kitchen. 2001. Residual phosphorus distribution and sorption in starter fertilizer bands applied in no-till culture. *Soil Sci. Soc. Amer. J.* 65:1173–1183.
- Stein, L.A., G.R. McEachern, and J.B. Storey. 1989. Summer and fall moisture stress and irrigation scheduling influence pecan growth and production. *HortScience* 24:607–611.
- USDA. 1976. United States standards for grades of pecans in the shell. 41 F. R. 39303.
- Watts, D.B., T. R. Way, H. A. Torbert, and S. D. Armstrong. 2015. Subsurface band application of poultry litter and its influence on phosphorus concentration and retention after runoff from permanent pastures. *J. Environ. Quality.* 44:1930–1937.
- Wells, M. L. (Ed). 2007. Southeastern pecan growers' handbook. Univ. Georgia Coop. Ext. Publ. 1327.
- Wells, L. 2015. Irrigation water management for pecans in humid climates. *HortScience* 50:1070–1074.
- Wood, B.W. 2007. Correction of zinc deficiency in pecan by soil banding. *HortScience* 42:1554–1558
- Woodruff, J.G. and N. C. Woodruff. 1934. Pecan root growth and development. *J. Agricultural Res.* 49:511-531.
- Worley, R.E. 2002. Compendium of pecan production and research. Edwards Brothers Inc. Ann Arbor, Mich.
- Worley, R.E. 1974. Effect of N, P, K, and lime on yield, nut quality, tree growth, and leaf analysis of pecan (*Carya illinoensis* W.). *J. Amer. Soc. Hort. Sci.* 99:49-57.
- Worley, R.E. 1982. Tree yield and nut characteristics of pecan with drip irrigation under humid conditions. *J. Amer. Soc. Hort. Sci.* 107:30–34.
- Worley, R.E. and M. Smith. 1984. A method for estimating pecan yield. *J. Amer. Soc. Hort. Sci.* 19:644.

Table 2.1. Soil phosphorus as affected by soil depth and application rate from 2015 to 2017 after a one-time application of triple superphosphate fertilizer in non-irrigated ‘Desirable’ pecans.^z

Rate ^x	Depth ^y			Sign. ^w
	0–7.5 cm	7.5–15.0 cm	15.0–22.5 cm	
0x	67.5 ^y	41.9	35.3	NS
1x	277.6	235.4	192.0	L**
2x	526.7	373.7	307.3	L***
4x	702.0	485.9	420.5	Q**
Sign. ^w	Q***	Q***	Q***	

^zThe core depth by phosphorus rate interaction was significant at $P < 0.05$.

^ySoil core samples measuring 22.5-cm (9-in.) in depth were collected within the application band and were divided into 7.5-cm (3-in.) increments.

^xRates were the equivalent of 0 kg ha⁻¹ (0 lbs. ac⁻¹), 19.6 kg ha⁻¹ (17.5 lbs. ac⁻¹), 39.2 kg ha⁻¹ (35 lbs. ac⁻¹), and 78.5 kg ha⁻¹ (70 lbs. ac⁻¹) P and referred to as 0x, 1x, 2x, and 4x, respectively.

^wSignificant (Sign.) linear (L) or quadratic (Q) trends using model regressions at $P < 0.01$ (**) or 0.001 (***). NS = not significant.

^vMelich III extractable phosphorus values are reported in milligram per kilogram.

Table 2.2. Soil phosphorus as affected by soil depth and application rate from 2015 to 2017 after a one-time application of triple superphosphate fertilizer in irrigated ‘Desirable’ pecans.^z

Rate ^x	Depth ^y			Sign. ^w
	0–7.5 cm	7.5–15.0 cm	15.0–22.5 cm	
0x	63.2 ^y	46.0	52.4	NS
1x	274.1	234.4	175.7	L***
2x	547.9	404.4	294.0	L***
4x	885.8	594.7	485.7	Q**
Sign. ^w	Q***	Q***	Q***	

^zThe core depth by phosphorus rate interaction was significant at $P < 0.05$.

^ySoil core samples measuring 22.5-cm (9-in.) in depth were collected within the application band and were divided into 7.5-cm (3-in.) increments.

^xRates were the equivalent of 0 kg ha⁻¹ (0 lbs. ac⁻¹), 19.6 kg ha⁻¹ (17.5 lbs. ac⁻¹), 39.2 kg ha⁻¹ (35 lbs. ac⁻¹), and 78.5 kg ha⁻¹ (70 lbs. ac⁻¹) P and referred to as 0x, 1x, 2x, and 4x, respectively.

^wSignificant (Sign.) linear (L) or quadratic (Q) trends using model regressions at $P < 0.01$ (**) or 0.001 (***). NS = not significant.

^yMelich III extractable phosphorus values are reported in milligram per kilogram.

Table 2.3. Soil phosphorus as affected by application rate and collection period from 2015 to 2017 after a one-time application of triple superphosphate fertilizer in non-irrigated ‘Desirable’ pecans.^z

Date ^x	Rate ^y				Sign. ^w
	0x	1x	2x	4x	
20 May 2015	47.1 ^v	287.2	528.6	811.9	L***
20 Jul. 2015	41.7	219.1	527.0	615.1	Q*
20 Sept. 2015	60.2	258.7	541.7	685.9	Q*
20 Nov. 2015	41.4	188.3	423.6	585.3	L***
20 Jan. 2016	49.7	260.4	484.7	577.7	Q*
20 Mar. 2016	47.2	272.2	403.1	655.6	L***
20 May 2016	51.2	293.7	433.7	596.4	L***
20 Jul. 2016	42.9	256.1	378.7	602.4	L***
20 Sept. 2016
20 Nov. 2016	47.9	83.7	178.0	430.2	L***
20 Jan. 2017	82.2	206.4	318.1	338.0	L**
20 Mar. 2017	36.6	210.2	361.8	351.0	Q*
20 Jul. 2017	33.7	219.2	309.2	346.2	Q*
20 Jul.. 2017	45.2	299.5	345.2	373.7	Q*
Sign. ^w	NS	NS	L***	L***	

^zThe phosphorus rate by sample period interaction was significant at $P < 0.05$.

^yRates were the equivalent of 0 kg ha⁻¹ (0 lbs. ac⁻¹), 19.6 kg ha⁻¹ (17.5 lbs. ac⁻¹), 39.2 kg ha⁻¹ (35 lbs. ac⁻¹), and 78.5 kg ha⁻¹ (70 lbs. ac⁻¹) P and referred to as 0x, 1x, 2x, and 4x, respectively.

^xSoil samples were collected from three trees at each treatment level 2 months after initiation starting on 20 May 2015 and continued at 2-month intervals until 20 July 2017. The ninth collection period (20 September 2016) was omitted as collection was prevented by drought conditions.

^wSignificant (Sign.) linear (L) or quadratic (Q) trends using model regressions at $P < 0.05$ (*), 0.01 (**), or 0.001 (***). NS = not significant

^vMelich III extractable phosphorus values are reported in milligram per kilogram.

Table 2.4. Soil phosphorus as affected by application rate and collection period from 2015 to 2017 after a one-time application of triple superphosphate fertilizer in irrigated ‘Desirable’ pecans.^z

Date ^x	Rate ^y				Sign. ^w
	0x	1x	2x	4x	
20 May 2015	64.1 ^v	387.0	498.9	1114.9	L***
20 Jul. 2015	50.3	217.2	413.4	674.0	L***
20 Sept. 2015	47.9	352.7	454.4	747.4	L***
20 Nov. 2015	44.4	177.0	395.4	667.7	L***
20 Jan. 2016	59.8	273.7	385.4	707.2	L***
20 Mar. 2016	53.2	264.0	509.6	862.2	L***
20 May 2016	62.4	177.4	514.0	702.6	L***
20 Jul. 2016	50.3	191.3	427.8	616.6	L***
20 Sept. 2016	50.0	182.2	486.7	606.4	L***
20 Nov. 2016	54.3	128.9	244.7	572.1	L***
20 Jan. 2017	43.4	228.8	399.9	450.3	L***
20 Mar. 2017	37.3	130.8	364.2	466.6	L***
20 May 2017	44.4	266.8	427.3	525.7	L***
20 Jul. 2017	92.0	215.2	294.3	461.8	L***
Sign. ^w .	NS	Q*	L*	L***	

^zThe phosphorus rate by sample period interaction was significant at $P < 0.05$.

^yRates were the equivalent of 0 kg ha⁻¹ (0 lbs. ac⁻¹), 19.6 kg ha⁻¹ (17.5 lbs. ac⁻¹), 39.2 kg ha⁻¹ (35 lbs. ac⁻¹), and 78.5 kg ha⁻¹ (70 lbs. ac⁻¹) P and referred to as 0x, 1x, 2x, and 4x, respectively.

^xSoil samples were collected from three trees at each treatment level 2 months after initiation starting on 20 May 2015 and continued at 2-month intervals until 20 September 2017. The ninth collection period (20 September 2016) was omitted as collection was prevented by drought conditions.

^wSignificant (Sign.) linear (L) or quadratic (Q) trends using model regressions at $P < 0.05$ (*) or 0.001 (***). NS = not significant

^vMelich III extractable phosphorus values are reported in milligram per kilogram.

Table 2.5. Soil phosphorus as affected by soil depth and collection period from 2015 to 2017 after a one-time application of triple superphosphate fertilizer at various rates in non-irrigated 'Desirable' pecans.^z

Date ^x	Depth ^y			Sign. ^w
	0–7.5 cm	7.5–15.0 cm	15.0–22.5 cm	
20 May 2015	627.4 ^y	341.6	287.1	L***
20 Jul. 2015	472.3	322.1	257.8	Q**
20 Sept. 2015	517.5	333.9	308.4	Q*
20 Nov. 2015	392.4	286.8	249.8	Q**
20 Jan. 2016	442.6	329.2	257.6	Q***
20 Mar. 2016	456.3	318.5	258.8	Q**
20 May 2016	423.7	331.9	275.7	Q***
20 Jul. 2016	409.2	292.0	258.9	Q**
20 Sept. 2016
20 Nov. 2016	246.5	173.0	135.3	Q***
20 Jan. 2017	297.6	220.2	190.8	Q***
20 Mar. 2017	277.8	239.3	202.6	Q***
20 May 2017	259.3	221.5	200.5	Q***
20 Jul. 2017	292.2	285.0	220.5	Q***
Sign. ^w	L***	L***	L***	

^zThe sample depth by sample period interaction was significant at $P < 0.05$

^ySoil core samples measuring 22.5-cm (9-in.) in depth were collected within the application band and were divided into 7.5-cm (3-in.) increments.

^xSoil samples were collected from three trees at each treatment level 2 months after initiation starting on 20 May 2015 and continued at 2-month intervals until 20 July 2017. The ninth collection period (20 September 2016) was omitted as collection was prevented by drought conditions.

^wSignificant (Sign.) linear (L) or quadratic (Q) trends using model regressions at $P < 0.05$ (*), 0.01 (**), or 0.001 (***). NS = not significant

^yMelich III extractable phosphorus values are reported in milligram per kilogram.

Table 2.6. Soil phosphorus as affected by soil depth and collection period from 2015 to 2017 after a one-time application of triple superphosphate fertilizer at various rates in irrigated 'Desirable' pecans.^z

Date ^x	Depth ^y			Sign. ^w
	0–7.5 cm	7.5–15.0 cm	15.0–22.5 cm	
20 May 2015	801.8 ^y	429.8	317.1	L***
20 Jul. 2015	488.8	298.3	229.2	Q***
20 Sept. 2015	547.0	370.7	284.2	Q***
20 Nov. 2015	423.0	306.2	234.3	Q***
20 Jan. 2016	459.7	337.8	272.1	Q***
20 Mar. 2016	547.4	396.7	322.7	Q***
20 May 2016	491.6	336.0	264.8	Q***
20 Jul. 2016	420.4	294.8	249.4	Q***
20 Sept. 2016	419.9	322.0	252.0	Q***
20 Nov. 2016	266.9	279.1	204.0	Q***
20 Jan. 2017	344.8	276.4	220.6	Q***
20 Mar. 2017	301.8	245.8	201.7	Q***
20 May 2017	373.8	335.0	239.4	Q***
20 Jul. 2017	311.5	250.1	235.8	Q***
Sign. ^w	L***	L**	NS	

^zThe sample depth by sample period interaction was significant at $P < 0.05$

^ySoil core samples measuring 22.5-cm (9-in.) in depth were collected within the application band and were divided into 7.5-cm (3-in.) increments.

^xSoil samples were collected from three trees at each treatment level 2 months after initiation starting on 20 May 2015 and continued at 2-month intervals until 20 July 2017. The ninth collection period (20 September 2016) was omitted as collection was prevented by drought conditions.

^wSignificant (Sign.) linear (L) or quadratic (Q) trends using model regressions at $P < 0.01$ (**) or 0.001 (***). NS = not significant

^yMelich III extractable phosphorus values are reported in milligram per kilogram.

Table 2.7. Foliar phosphorus concentrations from 2015 to 2017 as affected by application of a one-time band of triple superphosphate in non-irrigated ‘Desirable’ pecans.^z

Element ^x	Rate ^y				Sign. ^w
	0x	1x	2x	4x	
Phosphorus	0.125	0.122	0.124	0.129	Q*

^zThe phosphorus rate main effect was significant at $P < 0.05$.

^yRates were the equivalent of 0 kg ha⁻¹ (0 lbs. ac⁻¹), 19.6 kg ha⁻¹ (17.5 lbs. ac⁻¹), 39.2 kg ha⁻¹ (35 lbs. ac⁻¹), and 78.5 kg ha⁻¹ (70 lbs. ac⁻¹) P and referred to as 0x, 1x, 2x, and 4x, respectively.

^xData reported in percent of foliar dry weight.

^wSignificant (Sign.) quadratic (Q) trend using model regressions at $P < 0.05$ (*)

Table 2.8. Foliar nutrient concentrations as affected by year after application of a one-time band of triple superphosphate in non-irrigated ‘Desirable’ pecans.^z

Element	Year			Sign. ^y
	2015	2016	2017	
Nitrogen	2.44 ^x	2.47	2.63	L***
Phosphorus	0.128	0.120	0.127	Q***
Potassium	0.913	0.990	1.080	L***
Magnesium	0.445	0.462	0.378	Q***
Calcium	1.587	1.611	1.475	NS
Sulphur	0.187	0.189	0.181	L*
Boron	48.33 ^w	41.84	40.50	Q*
Iron	47.19	48.05	49.50	NS
Manganese	624.58	628.21	542.22	NS
Copper	7.58	6.53	6.90	Q*
Zinc	96.30	84.20	85.20	NS

^zThe year main effect was significant at $P < 0.05$.

^ySignificant (Sign.) linear (L) or quadratic (Q) trends using model regressions at $P < 0.05$ (*) or 0.001 (***).

^xData reported in percent of foliar dry weight.

^wData reported in parts per million foliar of dry weight.

Table 2.9. Foliar nutrient concentrations as affected by year after application of a one-time band of triple superphosphate in irrigated ‘Desirable’ pecans.^z

Element	Year			Sign ^y
	2015	2016	2017	
Nitrogen	2.56 ^x	2.45	2.67	Q**
Phosphorus	0.133	0.125	0.121	L***
Potassium	0.845	0.879	1.017	L**
Magnesium	0.435	0.426	0.374	L*
Calcium	1.64	1.55	1.36	L**
Sulphur	0.190	0.183	0.181	L*
Boron	49.74 ^w	42.00	41.41	Q*
Iron	48.11	42.79	46.89	Q**
Manganese	811.25	744.92	711.71	NS
Copper	7.74	6.85	7.16	Q*
Zinc	89.70	95.66	102.23	NS

^zThe year main effect was significant at $P < 0.05$.

^ySignificant (Sign.) linear (L) or quadratic (Q) trends using model regressions at $P < 0.05$ (*), 0.01(**), or 0.001 (***). NS = not significant.

^xData reported in percent of foliar dry weight.

^wData reported in parts per million foliar of dry weight.

Table 2.10. Foliar iron, copper, boron and aluminum concentrations from 2015 to 2017 as affected by application of a one-time band of triple superphosphate in irrigated ‘Desirable’ pecans.^z

Element ^x	Rate ^y				Sign. ^w
	0x	1x	2x	4x	
Iron	49.07	43.22	46.81	44.63	Q*
Copper	7.43	7.74	7.34	6.49	L**
Boron	43.34	40.46	46.04	47.69	L**

^zThe application main effect was significant at $P < 0.05$.

^yRates were the equivalent of 0 kg ha⁻¹ (0 lbs. ac⁻¹), 19.6 kg ha⁻¹ (17.5 lbs. ac⁻¹), 39.2 kg ha⁻¹ (35 lbs. ac⁻¹), and 78.5 kg ha⁻¹ (70 lbs. ac⁻¹) P and referred to as 0x, 1x, 2x, and 4x, respectively.

^xData reported in parts per million of foliar dry weight.

^wSignificant (Sign.) linear (L) or quadratic (Q) trends using model regressions at $P < 0.05$ (*) or 0.01 (**).

Table 2.11. Foliar boron concentrations from 2015 to 2017 as affected by application side of a one-time band of triple superphosphate in irrigated ‘Desirable’ pecans.^z

Element ^x	Side ^y	
	N	S
Boron	42.95b ^w	45.82a

^zThe tree side main effect was significant at $P < 0.05$.

^yPhosphorus bands were applied on the south (S) side of the trees within the orchard. The north (N) side received no P application.

^yData reported in parts per million of foliar dry weight.

^xLeast squares mean comparison using main effect F-test at $P < 0.05$.

Chapter 3 Phosphorus Nutrition and Pecan Seedling Root Development

Abstract

Pecan seedlings often produce tap roots, hindering containerized production of pecan trees. In *Arabidopsis* a reduction of phosphorus (P) reduced vertical root development and encouraged root branching. An experiment was conducted to determine if lower P rates would increase root branching and decrease tap rooting in container-grown pecan seedlings. Uniform ‘Elliot’ seedlings were transplanted into nursery treepots filled with horticultural-grade perlite and placed under 50% shade. Beginning 47 days after germination 0, 3.1, 7.75, 15.5, 23.25, or 31 mg L⁻¹ P in a modified Hoagland’s solution, were applied in 250-mL aliquots to randomly-assigned seedlings twice per week for the duration of the experiment. Seed detachment or retention was added as a factor at 47 days after germination as well. At 145 days after germination root systems were scanned to determine network surface area. Roots, stems, and leaves were separated, dried, and weighed. Foliar P concentrations were determined using ICP-AES. Foliar P concentrations increased linearly with increasing P rate but were within the recommended sufficiency range for all rates. The rate of P application did not influence plant dry weights or network surface area. Seed attachment increased plant dry weights and network surface area but decreased foliar P concentrations. The increased fibrous root development observed in *Arabidopsis* in response to low P rate was not observed in pecan seedlings during the experiment.

Introduction

The asymmetric development of plant root systems is an expression of the roots’ ability to adjust growth and development to environmental factors (Robinson, 1994; Forde and Lorenzo, 2001). The topsoil of the soil profile has the greatest P bioavailability, and when limiting, root

foraging was observed among genotypes of maize and bean (Bonser et al., 1996; Ge et al., 2000; Liao et al., 2001; Ho et al., 2005; Zhu et al., 2005). Root hairs, subcellular protrusions of root epidermal cells, that aid in acquisition of relatively immobile nutrients, are crucial to this foraging (Clarkson, 1985; Peterson and Farquhar, 1996; Jungk, 2001) since they can represent up to 70% of the total root surface area (López-Bucio et al., 2003). In *Arabidopsis thaliana*, root hairs were longer and denser under low P availability conditions (Bates and Lynch 1996; Ma et al., 2001). Pecans lack root hairs (Woodruff and Woodruff, 1934) thus lacking a fundamental tool used by most plants to increase P uptake. Root-hair-less mutants of *Arabidopsis* were used to demonstrate the importance of root hairs for P uptake (Bates and Lynch 2000; Bates and Lynch, 2001). When P was limiting, root hairs on wildtype *Arabidopsis* plants led to better plant growth, biomass production, P uptake, and reproductive output when compared to the mutant without root hairs. In contrast, when P availability was high, wildtype *Arabidopsis* plants and mutants without root hairs had similar growth patterns, P acquisition, and fecundity (Bates and Lynch, 2001)

In addition to the effects on root hairs, moderate concentrations of P favor lateral root growth over primary root growth. *Arabidopsis* seedlings grown in media containing 100 μM P had increased lateral root density when compared to seedlings grown in media containing 2.5 mM P, which was considered a high level of P (Williamson et al., 2001). Concentrations of P less than 50 μM in *Arabidopsis* growth medium had a dramatic effect on root architecture. The number of lateral roots were up to five times greater in plants grown at a limiting P concentration of 1 μM than in plants grown at an optimal P level of 1 mM (López-Bucio et al., 2002)

Root architecture of *Arabidopsis* plants grown in low P concentrations are characterized by having lateral roots arising near each other, having determinate growth, and being densely

covered by root hairs (López-Bucio et al., 2003). Additionally, low-P-induced determinate root growth in *Arabidopsis* was linked to inhibition of root meristematic activity (Sánchez-Calderón et al., 2005). Root tip contact was also essential to this modification of the root architecture (Svistoonoff et al., 2007). Anatomical and biochemical analyses showed that *Arabidopsis* roots grown in low P conditions had mature roots lacking a normal apex and with an increased expression of P transporter genes. In contrast, *Arabidopsis* roots grown in high P conditions had high meristematic auxin concentrations and with cells that expressed high mitotic activity, which correlated with reduced gene expression encoding high affinity P transporters (López-Bucio et al., 2003).

No research has observed if the root expression in *Arabidopsis thaliana* under low and high P conditions can be replicated in pecan. Previous findings indicated that since pecans are phreatophytes, plants that root to the water table, there is a low probability of altering the root: shoot ratio by fertilization (Conner 2006; White, 1980). This characteristic of pecans is vital to its success in native areas where competition between tree species is frequent (Fletcher et al., 2012, Sparks, 2002) and could very well cause differences in root expression. Though not directly analogous, low P grove soils did not limit tree growth or hinder seedling establishment of *Microberlinia bisulcata* (Newbery et al., 2002), and pecan may be expected to show similar results, though the climatic environments of the two species is differ.

Pecan transplants are often produced in the field. The resulting bareroot seedlings are known for having large taproots with little fibrous root development. This method of seedling production works well for orchard establishment when they can be grafted in the field. For tree replacement or small orchard development, there is a demand for containerized pecan trees that have already been grafted. Pecans produce a large vigorous taproot which often circles the

container causing physiological problems. Although *Arabidopsis* is not a likely candidate as a proxy for pecan, much work has been done to understand the role of P nutrition on *Arabidopsis* root architecture. Influencing root architecture of pecans by manipulating P rates would lead to better production and transplant success of container-grown pecans. Therefore, we conducted an experiment growing pecan seedlings at with various P rates to determine if the desirable characteristic of more fibrous root development expressed by *Arabidopsis* in low P could be replicated in pecan.

Materials and Methods

The germination of seedlings and experiment were conducted in an open sided structure covered with 50% black shade cloth, equipped with overhead irrigation that ran three times per day providing 2.54cm (1 in.) of water per day, located on the Paterson Greenhouse Complex on Auburn University's main campus in Auburn, AL. The experiment was conducted in 2016 and repeated with an additional treatment factor of seed attachment included in 2017. The protocol for the second run was as follows.

Open-pollinated pecan seeds from an 'Elliot' tree were collected at E.V. Smith Research Center located in Shorter, AL in November of 2016. The seeds were stored at room temperature until 3 March 2017 when they were stratified at 7.2 °C (45°F) until 10 May 2017. Seedlings were germinated in 3-gal. containers filled with sand and covered with shade cloth to prevent rodent damage. The shade cloth was raised as the seedlings grew to prevent growth abnormalities. Seedlings were grown in the sand substrate for 47 days before transplanting to treepots filled with horticultural grade perlite. The treepots (TP616, Stuewe and Sons, Tangent, Oregon) measured 40.6 cm (16 in) tall by 15.2 cm (6 in) wide and had a volume of 6.23 L (1.7 gal.) each. Germinated seedlings, of uniform size and root structure, having 3 to 5 true leaves each were

selected on 1 June 2017. This growth size was chosen since previous research by Zhang et al. (2015) reported that pecan seedlings responded better to root pruning for taproot modification at this growth stage. The treepots were held in trays in groups of four to prevent blow over. The seedlings were allowed to acclimate after transplanting until 26 June 2017. After this acclimation period, 60 seedlings of uniform size and quality were selected for the experiment.

Beginning on 26 June 2017, 250 ml of modified Hoagland's solution with various P concentrations [0x (0ppm), 0.1x (3.1 ppm), 0.25x (7.75 ppm), 0.50x (15.5 ppm), 0.75x (23.25 ppm), or 1x (31ppm)] were applied twice weekly to each pecan seedling until experiment termination on 1 October 2017. The seed retention factor was added at experiment initiation on 26 June 2017 with seeds being removed or left intact. This factor was added after the first run in 2016 when all seeds had been left intact and resulted in all seedlings maintaining P concentrations above sufficiency levels (Smith et al., 2012) irrespective of treatment.

At experiment termination, seedlings were destructively harvested. All leaves were removed by hand, placed in a paper bag and dried at 75 °C for 72 h., and weighed. Dried foliar samples from three experimental units were digested according to procedures for wet acid digestion using nitric and perchloric acids described by Mills and Jones (1996). Concentrated samples were diluted with 20 mL deionized water and analyzed for elemental concentrations using inductively coupled plasma optical emission spectroscopy (Waters Agricultural Laboratories, Camilla, GA).

Stems were cut at the soil line after removal of foliar tissue and placed in a paper bag and dried at 75 °C for 72 h and weighed. Root systems were easily removed from the perlite substrate and were carefully submerged in water to remove any lingering substrate. Roots were scanned to measure network surface area using the GiA Roots software (Galkovskyi et al., 2012)

within 15 min of removal from the substrate. Preliminary trials indicated there was a short window to collect root data using this method as pecan seedlings desiccated and started to lose fibrous root structures within 30 min. Therefore, samples were processed in small batches. After scanning, root systems were placed in paper bags, dried at 75 °C for 72 h., and weighed.

An analysis of variance was performed on all responses using PROC GLIMMIX in SAS version 9.4 (SAS Institute, Cary, NC). The two experimental runs were analyzed separately as completely randomized designs. In 2016, the treatment design was 1-way with phosphorus rate, and in 2017, the treatment design was 2-way with phosphorus rate and seed attached or removed. Where residual plots and a significant covariance test indicated heterogeneous variance among treatments, a RANDOM statement with the GROUP option was used to correct heterogeneity. Linear and quadratic trends over phosphorus rate were tested using orthogonal polynomials. Least squares means differences in attached or removed seed were tested using F-tests. All significances were at $\alpha = 0.05$.

Results

The P rate by seed retention interaction was significant in 2017. Foliar P concentrations increased linearly by 49.6% and 47.9% from the 0x to the 1x P rate with increasing P rates in 2016 and 2017, respectively (Table 3.1). Foliar P concentrations were also 10.8% higher in plants where seeds were removed than those with seeds attached (Table 3.2).

The seed retention main effects for foliar, stem, and root dry weight and network surface area were significant in 2017. Phosphorus application rate did not affect plant dry weight (data not shown), but seedlings with seed left intact produced 46.7%, 41.0%, and 50.6% more foliar,

stem and root dry weight, respectively, than plants whose seeds were removed (Table 3.3). Similarly, root network surface area increased 28.7% when seeds were left intact (Table 3.4).

Discussion

It was surprising that seed retention influenced biomass accumulation since Wetzstein et al. (1983) reported that seed attachment was only significant for biomass production up to 3 weeks after germination. Seeds were not detached until 47 d after germination in our study, but foliar, stem, and root dry weights decreased significantly when seeds were detached. This indicates that seed retention is more important in pecan seedling production than previously thought. Growers are generally not concerned about maintaining seed integrity when transplanting pecan seedlings into individual pots after germination. These findings indicate that seedlings with greater biomass would be produced if growers maintained seed attachment when transplanting, which would be beneficial for getting plants large enough to graft sooner in containerized production than those without seed attachment.

In 2016, foliar concentrations were not reduced below sufficiency levels by the P application rates in developing seedlings. It was hypothesized that removing the seed would decrease foliar P concentrations. Thus, the higher P concentrations in seedlings with no seed attached in 2017 had the opposite effect to what was intended. The higher P concentrations may be due to the reduced dry weights associated with seed detachment. Since dry weights were greatly reduced in seedlings with seed removed, in some cases up to 50%, it would take less P to increase overall P concentrations. It can be concluded that seed retention is beneficial to seedling establishment and seed should be left attached if possible.

Phosphorus rates influenced foliar concentrations of the nutrient in 2016 and 2017, but higher application rates did not lead to any measurable benefits. Since rates of P application were not associated with any other measurable benefit, it may be that low rates or possibly no application of P is needed within the first 6 mo. after seedling germination. The increased fibrous root development observed in *Arabidopsis* in response to low P rate (López-Bucio et al., 2003) was not observed in pecan seedlings during these experiments. This may be because the seedlings maintained a P concentration above sufficiency levels, 0.12-0.14%, as established by Smith et al. (2012), across all rates. In contrast, Zhang et al. (2015) was able to increase fibrous root development when seedling roots were pruned at the stage they transplanted for this experiment. It may be that root pruning in combination with retaining seeds on seedlings would be the best way to increase fibrous root development in pecan seedlings and reduce its vigorous tap-rooting characteristic.

References:

- Bates, T.R. and J.P. Lynch. 1996. Stimulation of root hair elongation in *Arabidopsis thaliana* by low phosphorus availability. *Plant Cell Environ.* 19:529–538.
- Bates, T.R. and J.P. Lynch. 2000. Plant growth and phosphorus accumulation of wild type and two root hair mutants of *Arabidopsis thaliana* (Brassicaceae). *Amer. J. Bot.*, 87:958–963.
- Bates, T.R. and J.P. Lynch 2001. Root hairs confer a competitive advantage under low phosphorus availability. *Plant Soil* 236:243–250.
- Bonser, A.M., J. Lynch, and S. Snapp. 1996. Effect of phosphorus deficiency on growth angle of basal roots in *Phaseolus vulgaris*. *New Phytologist* 132:281–288.
- Clarkson, D. 1985. Factors affecting mineral nutrient acquisition by plants. *Annu. Rev. Plant Physiol.* 36:77–115.
- Conner, P. 2006 Effects of nitrogen fertigation on first-year pecan seedling growth. *HortScience* 41:1062.
- Fletcher III, E.H. M., Thetford, J. Sharma, and S. Jose. 2012. Effect of root competition and shade on survival and growth of nine woody plant taxa within a pecan [*Carya illinoensis* (Wangenh.) C. Koch] alley cropping system. *Agroforestry. Syst.* 86:49–60.
- Forde, B. and H. Lorenzo. 2001. The nutritional control of root development. *Plant Soil* 232:51–68.
- Ge, Z., G. Rubio, and J.P. Lynch. 2000. The importance of root gravitropism for inter-root competition and phosphorus acquisition efficiency: results from a geometric simulation model. *Plant Soil* 218:159–171.
- Galkovskyi, T, Y. Mileyko, A. Bucksch, B. Moore, O. Symonova, C.A. Price, C.N. Topp, A.S. Iyer-Pascuzzi, P.R. Zurek, S. Fang, J. Harer, P.N. Benfey, and J.S. Weitz. 2012. GiA Roots: software for the high-throughput analysis of plant root system architecture. *BMC Plant Biology.* 12:116.
- Ho, M., J. Rosas, K. Brown, and J. Lynch 2005. Root architectural tradeoffs for water and phosphorus acquisition. *Functional Plant Biol.* 32:737–748.
- Jungk, A. 2001. Root hairs and the acquisition of plant nutrients from soil. *J. Plant Nutr. Soil Sci.* 164:121–129.
- Liao, H., G. Rubio, X.L. Yan, A.Q. Cao, K.M. Brown, and J.P. Lynch. 2001. Effect of phosphorus availability on basal root shallowness in common bean. *Plant Soil* 232:69–79.
- López-Bucio, J., A. Cruz-Ramirez, and L. Herrera-Estrella. 2003. The role of nutrient availability in regulating root architecture. *Current Opinion Plant Biol.* 6:280–287.

- López-Bucio, J., E. Hernández-Abreu, L. Sánchez-Calderón, M.F. Nieto-Jacobo, J. Simpson, and L. Herrera-Estrella. 2002. Phosphate availability alters architecture and causes changes in hormone sensitivity in the *Arabidopsis* root system. *Plant Physiol.* 129:244–256.
- Ma, Z., D.G. Bielenberg, K.M. Brown, and J.P. Lynch: 2001. Regulation of root hair density by phosphorus availability in *Arabidopsis thaliana*. *Plant Cell Environ.* 24:459–467.
- Mills, H.A. and J.B. Jones, Jr. 1996. *Plant Analysis Handbook II: A practical sampling, preparation, analysis, and interpretation guide*. MicroMacro Publishing, Inc., Athens, GA.
- Newbery, D.M., G.B. Chuyong, J.J. Green, N.C. Songwe, F. Tchuenteu, and L. Zimmermann. 2002. Does low phosphorus supply limit seedling establishment and tree growth in groves of ectomycorrhizal trees in a central African rainforest? *New Phytologist*, 156: 297–311.
- Peterson, R.L. and M.L. Farquhar. 1996. Root hairs: specialized tubular cells extending root surfaces. *Botanical. Rev.* 62:1–40.
- Robinson, D. 1994. The responses of plants to non-uniform supplies of nutrients. *New Phytologist*. 127:635–674.
- Sánchez-Calderón, L., J. López-Bucio, A. Chacón-López, A. Cruz-Ramírez, F. Nieto-Jacobo, J.G. Dubrovsky, and L. Herrera-Estrella. 2005. Phosphate starvation induces a determinate developmental program in the roots of *Arabidopsis thaliana*. *Plant Cell Physiol.* 46:174–184.
- Smith, M.W., C.T. Rohla, and W.D. Goff. 2012. Pecan leaf elemental sufficiency ranges and fertilizer recommendations. *HortTechnology* 22:594–599.
- Sparks, D. 2002 Rainfall governs pecan stand homogeneity in native, wild habitats. *J. Amer. Soc. Hort. Sci.* 127:860–868.
- Svistoonoff, S., A. Creff, M. Reymond, C. Sigoillot-Claude, L. Ricaud, A. Blanchet, L. Nussaume, and T. Desnos. 2007. Root tip contact with low-phosphate media reprograms plant root architecture. *Nature Genet.* 39:792–796.
- Wetzstein, H., D. Sparks, and G. Lang. 1983. Cotyledon detachment and growth of pecan seedlings. *HortScience* 18:331–333.
- White, Jr., A.W. 1980. Effects of topsoil-subsoil fertilizer and lime amendments on top and root growth of ‘Stuart’ pecan seedlings in a simulated Norfolk soil profile. *HortScience* 17:380–381.
- Williamson, L., S. Ribrioux, A. Fitter, and O. Leyser. 2001. Phosphate availability regulates root system architecture in *Arabidopsis*. *Plant Physiol.* 126:1–8.
- Woodruff, J.G. and N.C. Woodruff. 1934. Pecan root growth and development. *J. Agricultural Res.* 49:511–531.

- Zhang, R., F.R. Peng, P. Yan, F. Cao, Z.Z. Liu, D.L. Le, and P.P. Tan. 2015. Effects of root pruning on germinated pecan seedlings. *HortScience* 50:1549–1552
- Zhu, J., S.M. Kaeppler., and J.P. Lynch 2005. Topsoil foraging and phosphorus acquisition efficiency in maize (*Zea mays*). *Functional Plant Biol.* 32:749–762.

Table 3.1. Foliar phosphorus concentrations in ‘Elliot’ pecan seedlings as affected by twice-weekly applications of various phosphorus rates in 250 ml of modified Hoagland’s solutions in 2016 and 2017.

Year	Phosphorus Rate ^z						Sign ^y
	0x	0.1x	0.25x	0.50x	0.75x	1x	
2016	0.125 ^x	0.122	0.140	0.151	0.200	0.248	L***
2017	0.150	0.198	0.210	0.263	0.263	0.288	L***

^zThe phosphorus rate main effects were significant at $P < 0.05$. Phosphorus rates were the equivalent of 0ppm (0x), 3.1ppm (0.1x), 7.75ppm (0.25x), 15.5ppm (50x), 23.25ppm (0.75x), and 31ppm (1x).

^ySignificant (Sign.) linear (L) trend using orthogonal contrasts at $P < 0.001$ (***).

^xData reported in percent of dry weight.

Table 3.2. Foliar phosphorus concentrations in ‘Elliot’ pecan seedlings as affected by seed retention in 2017.^z

Element	Seed Attached ^y	
	Yes	No
Phosphorus	0.216b ^w	0.242a

^zSeed retention rate main effect was significant at $P < 0.05$.

^ySeeds were removed (No) 47 d after germination or left intact (Yes).

^xData reported in percent of foliar dry weight. Least squares mean comparison using main effect F-test at $P < 0.05$.

Table 3.3 Dry weight of ‘Elliot’ pecan seedling tissues as affected by seed retention in 2017.^z

Dry weight (g)	Seed Attached ^y	
	Yes	No
Foliar	3.83a ^x	2.04b
Stem	1.22a	0.72b
Root	7.71a	3.81b

^zSeed retention rate main effect was significant at $P < 0.05$.

^ySeeds were removed (No) 47 d after germination or left intact (Yes).

^xLeast squares mean comparison using main effect F-test at $P < 0.05$. Values within rows sharing a common letter were analogous while those with different letters were not.

Table 3.4. Root network surface area as affected by seed retention in ‘Elliot’ pecan seedlings in 2017.^z

	Seed Attached ^y	
	Yes	No
Network Surface Area (cm ²)	241.41a ^x	172.16b

^zSeed retention rate main effect was significant at $P < 0.05$.

^ySeeds were removed (No) 47 d after germination or left intact (Yes).

^xRoot network surface areas were determined using GiA Roots software. Least squares mean comparison using main effect F-test at $P < 0.05$.

Chapter 4: Final Conclusions

Overall it appears that P banding may not be the most sustainable way to increase concentrations of P quickly or to maintain foliar concentrations of the nutrient long term. A very high application rate of the nutrient, 78.5 kg ha⁻¹ (70 lbs. ac⁻¹) of P, was necessary to increase foliar concentration of the nutrient in a non-irrigated setting and it was still below the recommended range. The practicality of P banding could be increased in the region if the 19.6 kg ha⁻¹ (17.5 lbs. ac⁻¹) of P was more effective as it remained stable within the soil profile in the non-irrigated orchard, and though it decreased quadratically over time in the irrigated orchard, its loss of P through the soil profile was the least. Banding may be a valuable tool if used in combination with a foliar application of P. This approach is not unprecedented in pecan and has been used as a more long-term solution in correcting Zn deficiency. Foliar sprays of Zn are common to correct short-term deficiency of the element, but a band application of the nutrient has been shown to have long-term efficacy (Wood, 2007) The adoption of this two-pronged approach has proven to be advantageous for pecan growers with it being common for foliar applications of Zn to be paired with a banded application which eventually makes future foliar applications unnecessary. Research into creating a similar protocol with P in pecan may be beneficial.

The increased fibrous root development observed in *Arabidopsis* in response to low P rate (López-Bucio et al., 2003) was not observed in pecan seedlings during the experiment. This may be due to the seedlings maintaining a P concentration above sufficiency levels, 0.12-0.14% as established by Smith et al. (2012), across all rates. In contrast, Zhang et al. (2015) were able to increase fibrous root development when seedling roots were pruned at the stage we transplanted them. It may be that root pruning in combination with leaving seeds attached to seedlings would

be the best way to increase fibrous root development in pecan seedlings and reduce its vigorous tap rooting characteristic.

Wetzstein et al. (1983) reported that seed retention was only significant for biomass production up to 3 weeks after germination, but our study proved otherwise. Seeds were not detached until 47 d after germination in our study, but foliar, stem, and root dry weights decreased significantly when seeds were detached. This indicates that seed retention is more important in pecan seedling production than previously thought. Growers are generally not concerned about maintaining seed integrity when transplanting pecan seedlings into individual pots after germinating. These findings indicate that seedlings with greater biomass would be produced if growers maintained seed attachment when transplanting, which would be beneficial for getting plants large enough to graft sooner in containerized production than those without seed attachment. Further research would be beneficial in determining the long-term effect of seed attachment in pecan seedling development and how long it is influential in growth characteristics.