RATE AND DEPTH OF LIQUID P FERTILIZER PLACEMENT AFFECTS ROOT ARCHITECTURE AND ARBUSCULAR MYCORRHIZAL FUNGAL ASSOCIATIONS IN GRAIN CORN

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ABSTRACT

A two-site, two-year study was conducted on corn root response to liquid phosphorus (P) fertilizer applications as a function of rate and depth of placement. Corn planted into no-till at two locations (Thrall & Snook, TX) was fertilized with banded liquid P applied 15 cm off seed row at different rates and depths. The upper portion of the root systems were excavated along with all top plant matter at the V10 - V12 growth stage. Digital images of washed roots were analyzed for spatial density of roots relative to the zone of concentrated P fertilizer. Images were processed using the free-ware application 'ImageJ'. Results for two years at both sites indicated decreased root densities associated with the volume of soil nearest the banded zone. Yield differences, when present did not show a relationship to rate of P application. However, there was a positive relationship between both rate of phosphorus and depth of application to concentration of P in corn leaf tissue collected at the same time. In the second year, the association between arbuscular mycorrhizal fungi (AMF) and roots was examined. Root-AMF colonization was negatively affected by both rate and depth of P fertilizer application.

INTRODUCTION

Phosphorus (P) is an essential primary macronutrient for crops. Grain crops such as corn (*Z. mays*) have a high relative P requirement, and exhibit characteristic yield responses to application of fertilizer P when soil supply is less than sufficient (Grant et al., 2001; Dodd and Mallorino, 2005; Veneklaas et al., 2012). However, Plant uptake of P fertilizer is constrained by poor solubility of phosphate (PO_4^{3-}), low mobility, and high favorability of chemical reactions that precipitate or fix PO_4^{3-}, in soil. Furthermore, the mineral source supply of P is finite and is becoming more costly each year. Therefore, improvements to P fertilizer use efficiency are key to ensuring food security and to the sustainable production of crops worldwide (Schröder et al., 2011).

A number of strategies for improving efficiency and sustainability in the use of P resources in agricultural production have been identified, i) including intensification of recycling of nutrients from manures, ii) reducing inputs via adoption of better fertilizer source, timing, placement and rate of application practices, iii) erosion prevention, iv) improvement of crop genotypes, v) modification of root systems, and vi) enhancement of mycorrhizal symbioses (Shenoy, and

Kalagudi, 2005; Ramaekers et al., 2010; Schröder et al., 2011). Plant root architecture is a major determinant of soil P acquisition, and P availability in and of itself is known to affect root architecture. When experiencing stress from low P availability, plants respond by shifting photosynthetic product to the root system to improve access to soil P supplies (Péret et al., 2011). Lopez-Bucio et al. (2002) reported a negative relationship between phosphorus availability and both lateral root and root hair formation in *Arabidopsis.*

Plant root architecture and P acquisition can be enhanced through mutualistic association with arbuscular mycorrhizal fungi (AMF). Kabir et al., (1998) showed that AMF density in corn roots was positively associated with phosphorus uptake, and that AMF activity was greater in no till, as compared to moldboard plowed, soils. The level of mutualism between corn and AMF is negatively related to the plant availability of phosphorus in soils (Ryan et al., 2000). The paradigm in this system appears to favor greater activity in soil P acquisition by root / AMF combined activity under P stress conditions. Yet there is no evidence to suggest that this system can, in the absence of P fertilization, produce yields competitive with conventional fertilizer practice.

Work in Texas has shown that the use of banded liquid P fertilizer as *source* (e.g. ammonium polyphosphate) can increase use-efficiency and yield in grain production (Miller, 1998; Coker et al., 2007). The primary goal of this study was to further investigate the effect of depth of placement of ammonium polyphosphate (APP) on P fertilizer use efficiency in corn production. The second goal of this study was to examine the effect of fertilizer rate and placement on corn root architecture in the field. In the second year of the study, additional supporting data was collected to examine the effect of the treatments on root / AMF associations.

MATERIALS AND METHODS

Study Location

Field experiments were conducted at the Bottom Farm (Snook, TX; 30.524669, -96.439846) and at the Stiles Farm (Thrall, TX; 30.595260, -97.283772) during the spring and summer of 2016 and 2017. The soil at the Bottom farm is a Weswood series (fine-silty, mixed, superactive, thermic Udifluventic Haplustept). The soil at the Stiles farm study site is a Burleson series (fine, smectitic, thermic Udic Haplustert).

Experimental Design

Treatments for rate and depth of P fertilizer placement are shown in Table 1. All other nutrients were supplied at the Texas A&M AgriLife Extension Soil Water and Forage Testing Laboratory recommended rate according to soil test results (SWFT Laboratory, College Station TX). Nitrogen contained in the APP applied at different rates was accounted for. The field experimental design was randomized complete block with a minimum of four replicates for each site-year. In the second year, 2017, the only change was to move the plots to an immediately adjacent area to avoid carryover from different P fertilizer applications in 2016.

Table 1. Corn P fertilizer rate and depth treatments at 4 site-years

#	Treatment Abbr. rate-depth	Treatment Description
1	0-0	Control - No P fertilization
2	1-0	Surface applied APP at the recommended rate
3	0.5-10	APP at half soil test recommended rate at 10 cm below soil
4	1-10	APP at 0.5x soil test recommended rate at 10 cm below soil
5	1.5-10	APP at 1.5x soil test recommended rate at 10 cm below soil
6	0.5-20	APP at 0.5x soil test recommended rate at 20 cm below soil
7	1-20	APP at 1x soil test recommended rate at 20 cm below soil
8	1.5-20	APP at 1.5x soil test recommended rate at 20 cm below soil

Soil and Tissue Sampling and Analysis

Soil samples were collected as 0-20 cm samples for routine nutrient recommendations, and as 20-61 cm depth samples for residual NO_3-N. Routine nutrients were analyzed at the SWFT Laboratory in College station for pH, EC, P, K, Ca, Mg, and S (Mehlich III/ICP-AES), and NO_3-N (1M KCl/ cd reduction colorimetry). Tissue samples were collected at V6-V10 growth stage range as time and conditions allowed, and again after tasseling but before the onset of dry down. Ten uppermost unfolded leaves were collected for the early sampling, and ten ear leafs were collected at the later sampling events. Total nitrogen was measured by combustion. Nutrient concentrations (e.g. P, K, Ca, Mg, S) were measured by first digesting (microwave assisted aacid digestion) and measurement on ICP-AES.

Root Sampling and Analysis

Three replicates from each treatment were excavated by hand shovel at V8-V10 growth stage as a 'column' of soil and roots centered at the base of the stalk, at least 30 cm in diameter and 40 cm deep. The columns were allowed to soak in water in 20 liter plastic buckets for 24 to 48 hours before washing with gentle pressure. The band side of the plant was tracked using a wooden skewer with a blue marking on the band side inserted through the stalk. The entire root mass was imaged with a digital camera on a black background. The image was separated into 4 quadrants situated relative to the axis of the stalk. Each quadrant was 10 cm x 10 cm (Figure 1.)

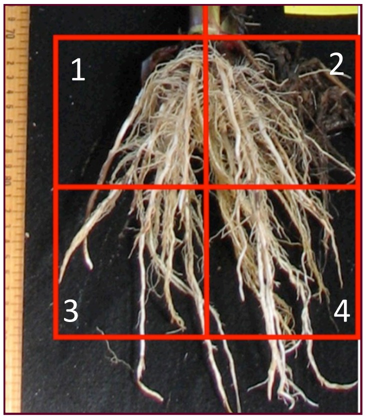
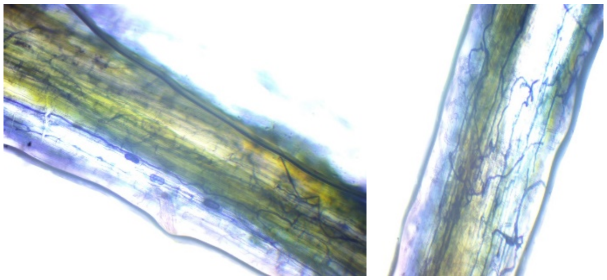


Figure 1. (to left) 10 x 10 cm quadrants were imposed on the corn root crown. A ruler placed in the frame was used to establish scale. Percent coverage within each quadrant was measured using ImageJ freeware. (https://imagej.nih.gov/ij/).

AMF Measurement

Ten 1 cm segments of from each quadrant from each of the excavated plants were cut for AMF staining and imaging. The segments were boiled in 10% KOH for 13 minutes and stained with an ink-vinegar method (Vierheilig et al., 1998). The roots were digitally imaged on the microscope, and the density of AMF quantified as percentage of 10 root subsections for each segment with evidence of colonization (Figure 2.) The mean of each of the 10 sections was used for statistical comparison.

Figure 2. (to right) root images with evidence of AMF hyphae as blue-dyed 'tendril' like structures and arbuscules as blue 'kidney' shaped structures.



Statistical Analysis

Yield, leaf tissue P content, root density by quadrant, and AMF colonization percentage were all tested as response variables for effect of treatments imposed in Table 1. The GLM procedure in SAS software (SAS Institute Inc., Cary, NC, USA) was used to calculate analysis of variance at $\alpha = 0.1$. The model used was response variable = rate depth rate*depth. Post-hoc analysis was performed as comparison of means (Dunnett's in PROC GLM) at $\alpha = 0.1$ and multiple linear regression analysis (PROC REG) whenever ANOVA results were significant for rate or depth.

RESULTS AND DISCUSSION

Corn Grain Yield

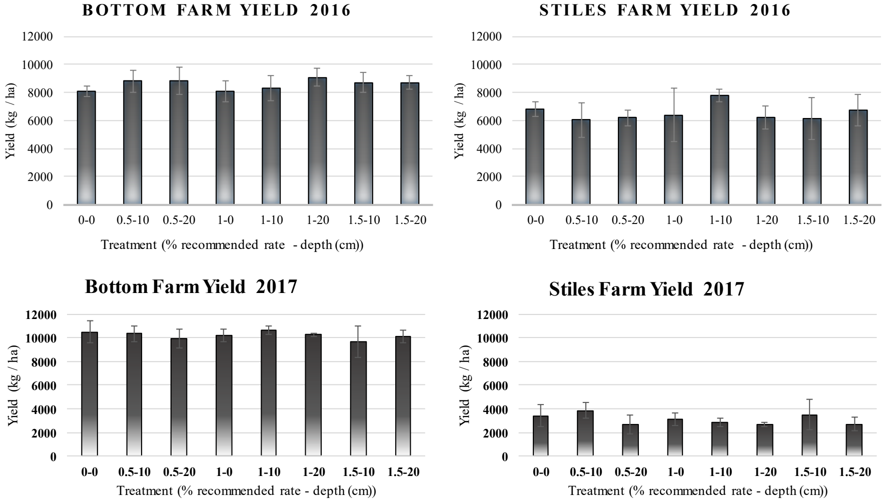


Figure 3. Corn grain yields were not responsive to rate or depth of P fertilizer applications in any of the 4 site years of the study. Recommended rates of P application varied from 56 to 67 kg / ha (50 to 60 lbs / acre) P_2O_5. Yields were near or above goals in 3 site years. Stiles 2017 harvest was below yield goal.

Root Architecture

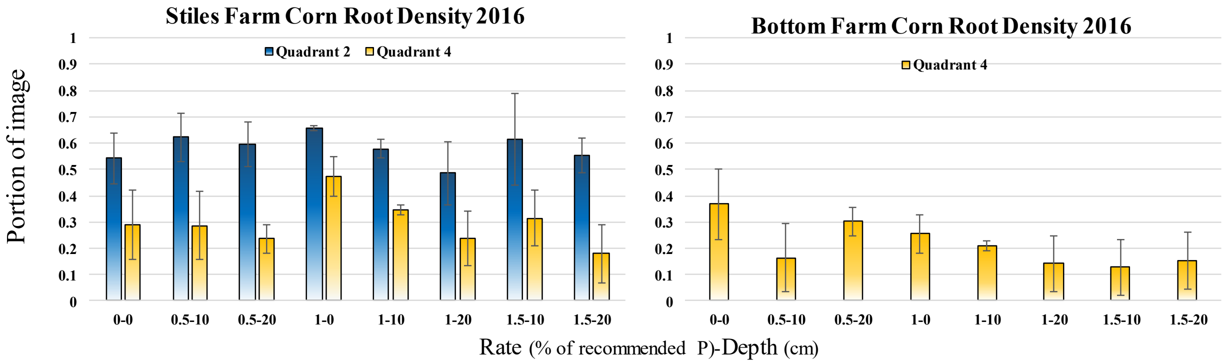


Figure 4. Root density in quadrants from image analysis indicate a significant effect of depth on density in the 2nd and 4th quadrants at the Stiles Farm in 2016 and in the 4th quadrant at the Bottom farm in 2016. The 2nd and 4th quadrants were adjacent to the band. The 1st and 3rd quadrants were unaffected by rate or depth of P fertilizer placement in all site years. In both 2016 and 2017 at the Bottom Farm, the control treatment exhibited the greatest root mass in quadrant 4.

Corn Root / AMF Association

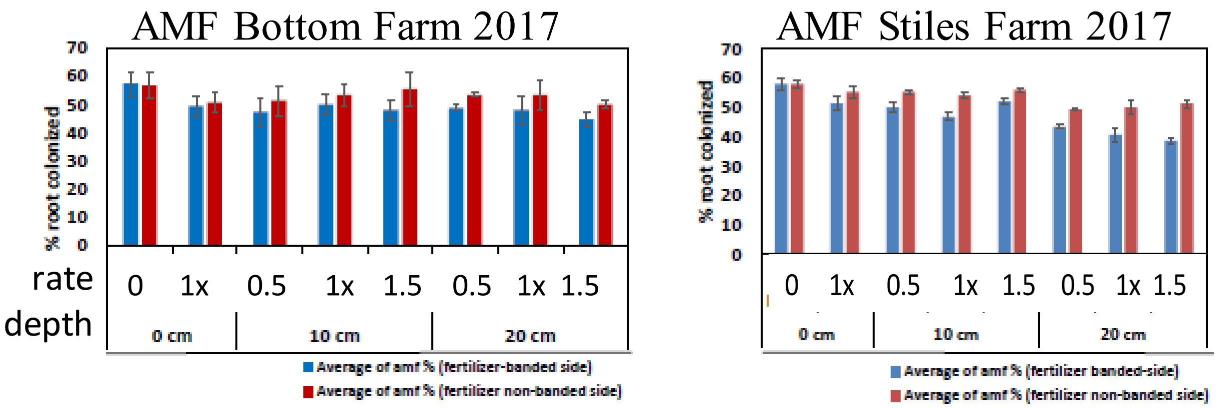


Figure 5. AMF association with corn roots was in every case greater on the side of the seed row away from the band placement. This indicates an inhibitory effect of P fertilizer on AMF association in the field. In the control treatment, no difference was seen in AMF density between sides of the seed row. The control treatment also either significantly the greatest or grouped with the greatest treatments in terms of amf density in any single quadrant. This indicates that *any* application of P fertilizer can inhibit amf / root association.

The need to increase P fertilizer use efficiency has stimulated suggestions for strategies towards achieving that goal. However, as this study indicates, there are potential negative interactions between some of these strategies. In this case, the use of an improved P fertilizer source inhibited the effect of other strategies, such as the encouragement of rooting architecture that is more prolific and capable of accessing immobile P from a greater soil volume. Mutualism between corn and AMF was also depressed by both rate and depth of placement of APP. This research raises questions about the accuracy of soil test recommendations for no-till crops. Correlation and calibration work in the U.S. has historically and almost exclusively been performed on conventionally tilled soils. The established relationships between soil disturbance (and now P fertilizer application) and rooting proliferation and AMF association suggest that a new examination of P fertilizer recommendations in conservation tillage is an appropriate and timely area for research updates.

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