

**DO CRITICAL SOIL PHOSPHORUS CONCENTRATIONS VARY IN
SPACE AND IF SO WHY?**

2017 Annual Report

Prepared by

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OBJECTIVES

Our overarching objective is to determine if variable rate P applications can be used to efficiently manage P in grain crop production. Our specific research objectives are to evaluate spatial and temporal variability in soil P critical concentrations for grain crop production. We will investigate variability in P supply as it relates to soil chemistry, soil P forms, and soil biology as well as rhizosphere interactions that influence crop P requirements. Furthermore, we hope to use this fellowship as a vehicle to initiate a regional, open source research group that shares samples and associated data for broader soil P research.

Our proposed methodology relies on having a large number of sample points covering not only the variation within fields, but also between fields and across time. In order to evaluate critical soil test concentration variability within fields this project requires high sample densities in each field. However, in order to determine what factors control critical concentration variability we also need a diverse sample set across multiple fields. Ultimately, we hope to collect a large, diverse data set that will allow us to evaluate parameters that influence yield response variation within narrow ranges of soil P concentration. To date variable rate P management relies on the relationship between covariance and distance, where it is assumed that the farther apart two points are the less similar their behavior or the lower their covariance. This is the basis for interpolation of grid sampled soil data. However, what we propose is to evaluate the covariance of yield response within ranges of soil P concentration, at both field and regional scales.

Research Questions

1. Do soil P critical concentrations vary in space and time?
2. Can traditional soil testing be used to estimate variable soil P critical concentrations and prescribe spatially variable P fertilizer rates?
3. What soil chemical and biological factors as well as rhizosphere processes control crop P requirement?

MATERIALS AND METHODS

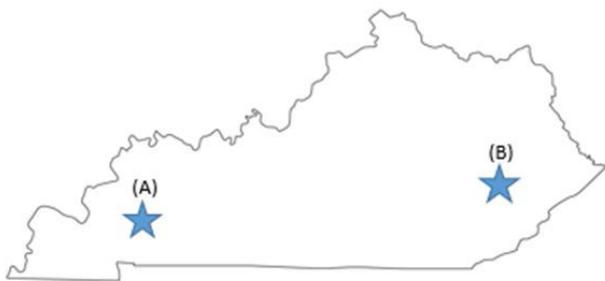


Figure 1. The two field sites established in 2016 are shown with Princeton and Quicksand labeled as A and B, respectively.

establishment, soil and plant sampling, fertilizer application, and yield monitoring were mapped using Real Time Kinetic corrected Global Positioning System (RTK-GPS).

Initially, we overlaid a 9 m (30 ft) grid following the planter path on each field using GIS

Our initial plan was to select one new site each year of the project. However, in 2016 we started the project with two sites in Kentucky, one in Breathitt County (2.55 ha) and one in Caldwell County (4.85 ha; Figure 1) referred to as Quicksand and Princeton, respectively. A corn-soybean, no-till rotation with a cereal grain cover crop between cash crops will be maintained at the Breathitt County site. The Caldwell County site will have a corn-wheat-double crop soybean, no-till rotation. In 2016 both sites were planted to corn. Plot

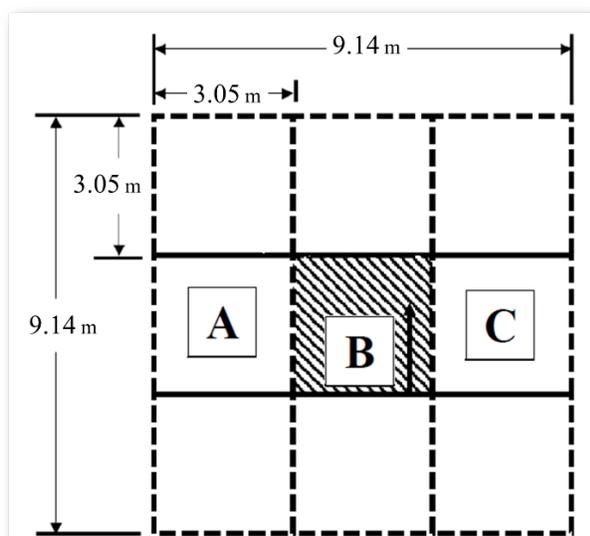


Figure 3. Original plot design with nine subplots. Soil samples in 2016 were collected from subplots A - C.

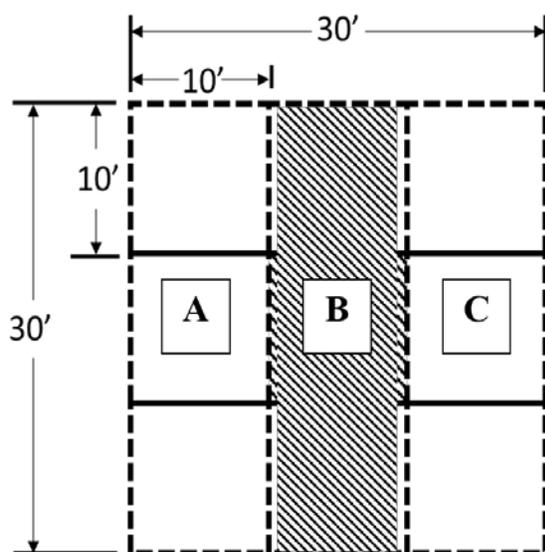


Figure 2. Plot design used in 2016. The center subplot (B) received phosphorus fertilizer at planting (indicated by shading). Subplots A and B received no phosphorus. Individual soil samples were collected from the center 10 ft in all three subplots.

sampled in 2016. Since this site was only 2.55 ha we were able to sample over 50% of the field. The center subplots that received P fertilizer are indicated with the shaded circle.

software. Then a 3 m (10 ft) grid, matching the planter width (four row – 30 in rows) was overlaid on the 9 m grid. As a result each plot had nine subplots measuring 3 m x 3 m (Figure 2). In 2016, 125 and 100 plots were randomly selected from the Breathitt and Caldwell county sites, respectively. We originally planned to randomly select three of the subplots from each selected plot to receive P in the starter fertilizer. We planned to estimate yield response to P fertilizer for each plot using the mean response across the nine subplots. However, during testing of our planter fertilizer system, we determined that a 3 m long plot was too short for precise fertilizer application. Therefore, we separated the 30 ft square plots into three subplots, measuring 3 m by 9 m (10 ft x 30 ft;) as shown in Figure 3. Prior to planting, two soil samples, one to a depth of 10 cm and one to a depth of 20 cm, were collected from each of the center three subplots (labeled A – C, Figure 3). Mehlich 3 P, K, Ca, Mg, and Zn concentrations, soil pH, and Sikora Buffer pH were determined on all samples by the University of Kentucky Soil Testing Laboratory.

During corn planting, the center subplot (B in Figure 3) received starter P fertilizer (polyphosphate) placed approximately 5 cm beside and 5 cm below (2 in by 2 in) the seed at a rate of 29 kg ha⁻¹ P (60 lb acre⁻¹ P₂O₅). The planter was modified to inject UAN into the starter stream to balance the amount of nitrogen (N) applied to each plot. In this way the entire field, whether receiving P or not, received 56 kg ha⁻¹ N (50 lb acre⁻¹ N) in the starter. For the soybean portion of the rotation no fertilizer additions were made. The randomized P treatments, and concomitant N balancing treatments, were programmed using dual product, variable rate software prior to going to the field. In subsequent years, when corn is rotated back to the fields, a subset of the plots not yet used will be randomly selected and P will be applied. Figure 4 shows the plot layout for the Breathitt County site

We harvested both project fields in their entirety (including non-plot area) using a two row plot combine (Massey Ferguson model 8). Yield was estimated from impulse and moisture measurements taken by an AgLeader sensor plate (Part#4000215) and moisture module (244), which were then logged to an AgLeader Insight display along with position and speed determined by RTK corrected GPS. We extracted yield data from the center two rows (planter rows two and three) and the middle 3 m of each subplot (indicated by A, B, and C in Figure 3) using GIS software. We expected this harvest method to allow adequate precision and accuracy to match yield response to P treatments and soil physical, chemical, and biological measurements. However, results from 2016, which are discussed below, indicated otherwise.

In addition to yield, other response variables were collected in order to assess P response at the plot level. Whole plant samples were collected prior to side-dress (V4 at Princeton and V6 at Quicksand) to estimate early growth response to P. All subplots were sampled at Quicksand, but only a subset of plots were sampled at Princeton due to time constraints. Ten plants were collected from the guess rows (rows 1 and 4) in each subplot, dried at 65 °C, and weighed to estimate biomass. Normalized difference vegetative index (NDVI) was measured using active optical sensors (GreenSeeker™) at the time of sidedress N application. In order to estimate yield components we collected four representative corn ears by hand from the guess rows in each subplot at Quicksand and a subset of plots at Princeton at harvest time. The number of rows per ear, kernels per row, and average kernel mass were recorded. The number of rows and the number of kernels per row were counted on each of the four ears then averaged to obtain one value for each subplot. Kernel weight was determined using the mass of 100 kernels taken at random from the four shelled ears. We also partnered with the Biosystems and Agricultural Engineering Department at University of Kentucky (UK-BAE) to collect high resolution imagery of project fields to see if spectral measurements can aid in predicting P response and to quantify P response across time. An unmanned aerial system (UAS) equipped with a multispectral camera was deployed over one of the test sites two times in the 2016 season after silking. Visible and near-infrared images were stitched into a georectified orthomosaic and reflectance indices (e.g. NDVI) extracted over the individual subplots. For the soybean portion of the rotation yield was the only data collected. Weather data was acquired using Kentucky Mesonet weather stations present near the two sites.

We originally designed the experiment so that absolute response and relative to P fertilizer could be estimated for each plot along with an error term for response variability within the plot. However, as described previously we did not replicate the P-fertilized treatment within plots and therefore could not estimate error for response at the plot level. Conversely, two no-P control

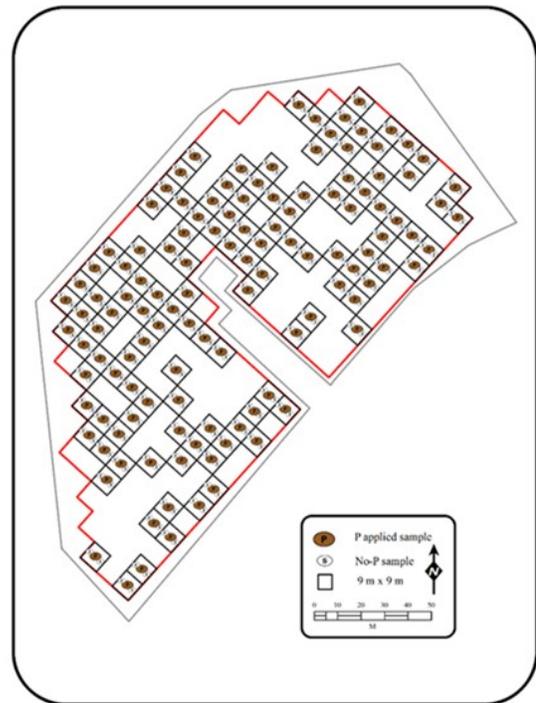


Figure 4. Breathitt County site (Quicksand) showing the 30 ft plots and three subplots that were sampled.

subplots were included in each plot, allowing error estimation (σ_{Y_0}) for the mean check yield (\bar{Y}_0) in each plot (Equation 1). Using Equation 2, we calculated absolute yield response (ΔY) using the mean check yield from the two subplots that received no P fertilizer (A and C in Figure 3) and the P fertilized subplot yield (B in Figure 3). Relative yield (RY) response was estimated using Equation 3 so that results from across sites or within sites, where absolute yield potential differed, could be compared. Relative and absolute response, along with mean and error terms for the no-P check, for other response variables (e.g. biomass, yield components, active optical sensor NDVI) were calculated in a similar manner to yield.

Equation 1. The mean no-P check yield and its standard deviation could be calculated for each plot using yields from subplots A and C, which received no P fertilizer.

$$\bar{Y}_0 = \frac{Y_{0A} + Y_{0C}}{N} \text{ and } \sigma_{Y_0} = \sum_{i=1}^N \sqrt{\frac{1}{N}(Y_{0i} - \bar{Y}_0)^2}$$

Equation 2. Yield response at the plot level was calculated using the mean No-P check yield and P-fertilized subplot yield.

$$\Delta Y = Y_P - \bar{Y}_0$$

Equation 3. Relative yield response to phosphorus fertilizer was estimated from the P-fertilized yield and mean no-P check yield for each plot.

$$RY = 100\% \times \frac{\bar{Y}_0}{Y_P}$$

Comparisons were made between response variables for the treatment subplots and no-P check utilizing SAS and Student's t test on the paired samples (Student, 1908; SAS Institute, 2011). The t test determines if there is a significant difference between the two means, which in our case would indicate a response to P on average. The paired t test is essentially the same as a two treatment analysis of variance (Clewer and Scarisbrick, 2013).

The initial sample density would be cost and time prohibitive for more advanced laboratory methods and multiple sample depths. Therefore, we used responsiveness to P application to select a subset of plots for more detailed analysis, using advanced analytical methods, across the rooting depth. In essence we wanted to select plots within discrete ranges of Mehlich 3 soil test P where there was clearly a response to P and clearly no response to P. In this way we would have sets of plots with the same soil test P, but different responses to P. In order to determine if a plot was actually responsive we used the standard deviation in control subplot yield (σ_{Y_0}). If yield of the P-fertilized subplot exceeded one standard deviation plus the mean no-P check yield for the plot then we considered that plot responsive to P application. This method provided a simple method to account for noise inherent in the data. This same method was employed for each type of response variable collected. A plot had to be consistently responsive or unresponsive across all variables to be included in the subset for further analysis.

In 2017, the Quicksand and Princeton sites rotated to no-till soybeans. No P fertilizer was

added, however, yield was measured to determine if there was carryover response from the 2016 application with corn planting. Also in 2017, an additional field site was added near Blacksburg, Virginia (0.65 ha) in cooperation with Drs. Wade Thomason and Rory Maguire. Thomason and Maguire modified the UKY plot design to accommodate the site and equipment available. The field was planted to no-till corn and starter P fertilizer was applied in a 2x2 band similar to Kentucky. However, instead of small plots the P was applied in field length, four-row strips alternating every other four-rows. We superimposed plots on this field measuring 12.2 m (40 ft) long by 6.1 m (20 ft) wide. Corn was planted on 0.76 m (2.5 ft) rows with using a 2-row planter. A 0-10cm and a 0-20cm soil sample was taken from each subplot and taken to Regulatory Services for the same routine testing discussed earlier in this report.

The 2018 we plan to add two additional field sites. One will be located in Texas in cooperation with Dr. Doug Smith (USDA-ARS, Temple TX) and one in Ohio with Dr. Steve Culman (OSU). Dr. Smith is currently reviewing our field study design and developing a design that accommodate the equipment and sites he has available. Dr. Culman is working to select a site in the southern half of Ohio and we intend to plant and sample using UKY equipment to maintain consistency with the two sites underway in KY. However, we plan to modify the KY layout based on lessons learned to date. Instead of the checkerboard plot layout used previously, a 12.2 m (40 ft) by 12.2 m square will be separated into four strips 3 m (10 ft) wide. The treatment of starter P will be randomized and applied to two of the four strips. We decided on this change to strengthen our statistical analysis of the yield by including a measure of variation in treatment yield within a plot.

INITIAL RESULTS AND DISCUSSION

The majority of the lab work associated with this project thus far was comprised of the initial testing of soils for nutrients and pH. After examining the initial year's yield data and removing outliers via an x-graph, plots were grouped by their response to P with plots which saw increased biomass midseason and increased yield were considered responsive. A Hedley fractionation was completed on the initial soil samples which quantified the inorganic fractions, as well as the organic P associated with the more labile inorganic fractions. We plan to examine the presence of an acid phosphatase enzyme in the rhizosphere soil of the corn plants. A colleague at the University of Kentucky completed a greenhouse experiment which showed promising results, but was limited to sampling at V6 or later due to the amount of soil needed. We plan to sample an earlier growth stage by increasing the number of plants sampled thereby increasing the amount of soil sampled.

The Virginia site layout was altered due to space constraints as well as equipment availability at

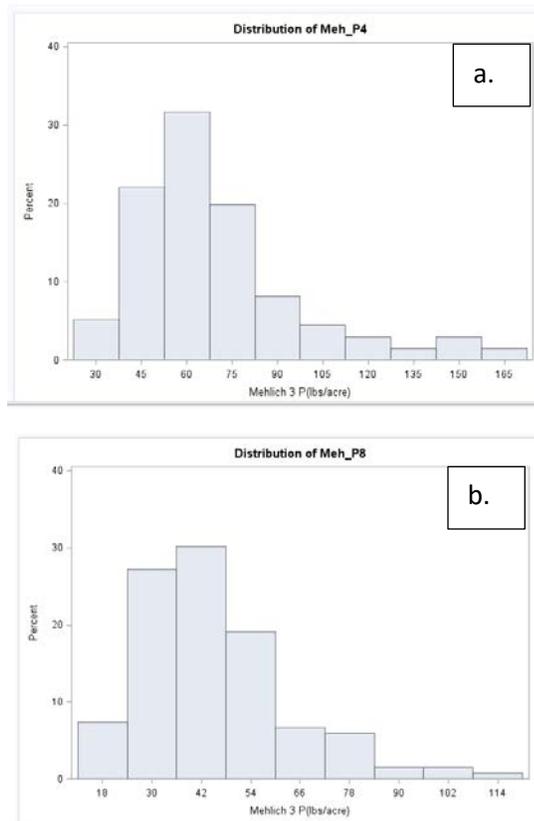


Figure 5 (a-b). Mehlich 3 phosphorus (lbs/acre) distribution at the Virginia field site for the 0-10cm (a) and 0-20cm (b) depths.

that location. The soil sampling was altered as well with four samples being taken from each plot. A sample was taken from the control rows in the center of the 12.2 m (40ft) strip as well as the treatment rows at the depth of 0-10cm and 0-20cm resulting in a total of eight samples per plot. The distributions of Mehlich 3 P for the 0-10cm depth and 0-20cm depth are shown in the Figure 5. The 0-10cm depth ranged from 33 to 168 lbs acre⁻¹ with a mean of 69.7 compared with the 0-20 cm which ranged from 16 to 110 lbs acre⁻¹ with a mean of 44.9. The two depths show a similarly shaped distribution. The apparent P stratification with depth is expected given a no-till management system and its lack of mixing within the surface. The pH exhibited similar trends for both depths with the majority of the values falling around neutral, so liming was not needed and there was no reason to be concerned with root growth or zinc deficiency.

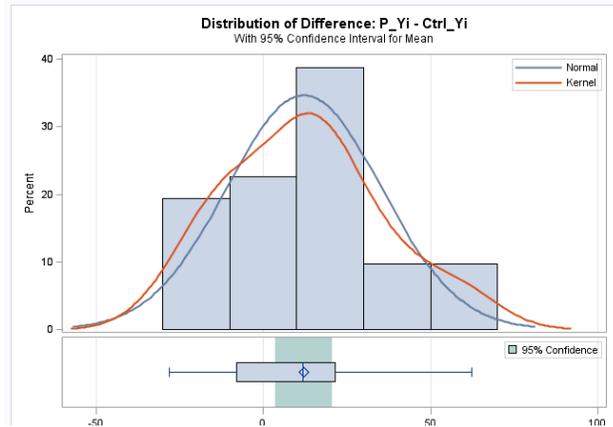


Figure 7. Paired *t* test comparing corn yield from the control (Ctrl_Yi) and phosphorus treatments (P_Yi) at Virginia site are shown overlain with lines indicating normal distribution and kernel density.

The corn yield at the Virginia site has not had any outliers removed using the methods

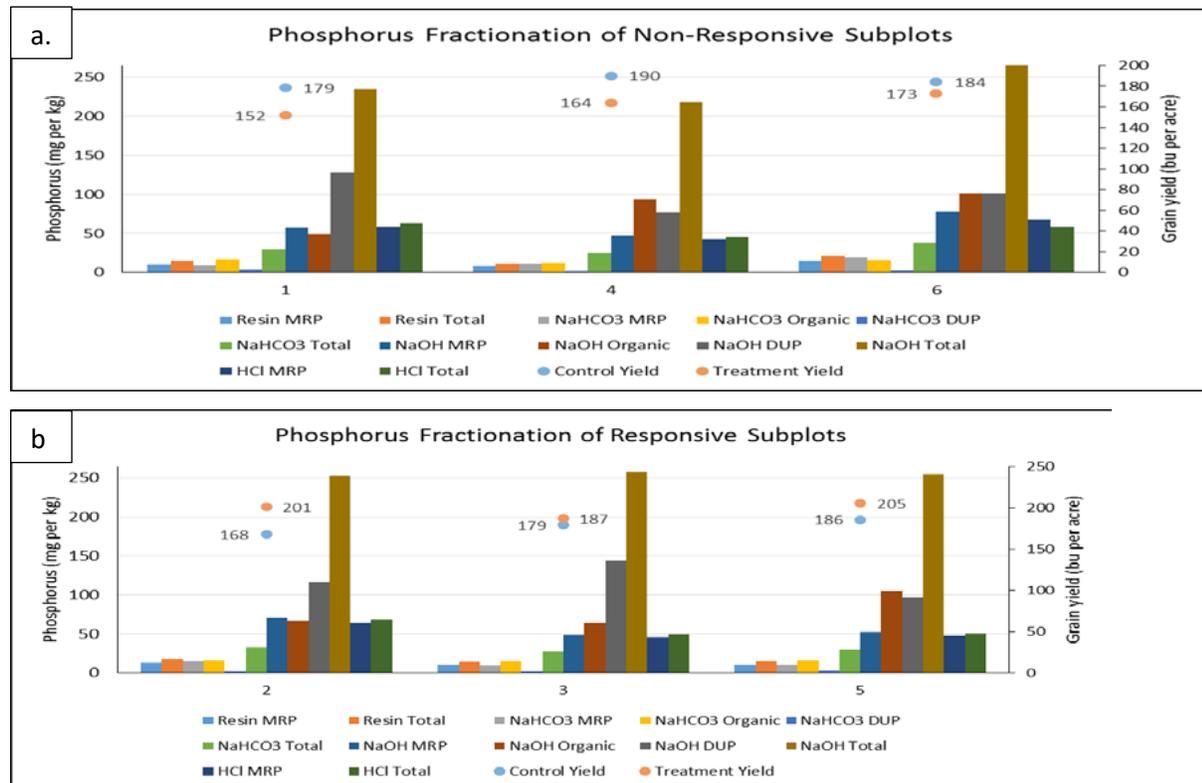


Figure 6 (a-b). The results from the Hedley fractionation are shown for three non-responsive treatment subplots (a) and three responsive treatment subplots. The yield for both the treatment and adjacent control subplot are also shown. (MRP molybdate reactive phosphorus DUP digestible unreactive phosphorus).

utilized for the 2016 corn yields in Quicksand and Princeton. Of the original 68 plots, 36 were lost due to poor corn affected by the proximity of the tree line and damage from bears. The yield for the control plots ranged from 73 to 172 bushels per acre with a mean of 116.6 compared to the treatment plots which ranged from 78 to 176 bushels per acre with a mean of 126.9. The comparison of the two yields using a paired t test is shown in Figure 6. The paired t-test showed a statistically significant response to P, however, these results deserve closer scrutiny after the data has been cleaned.

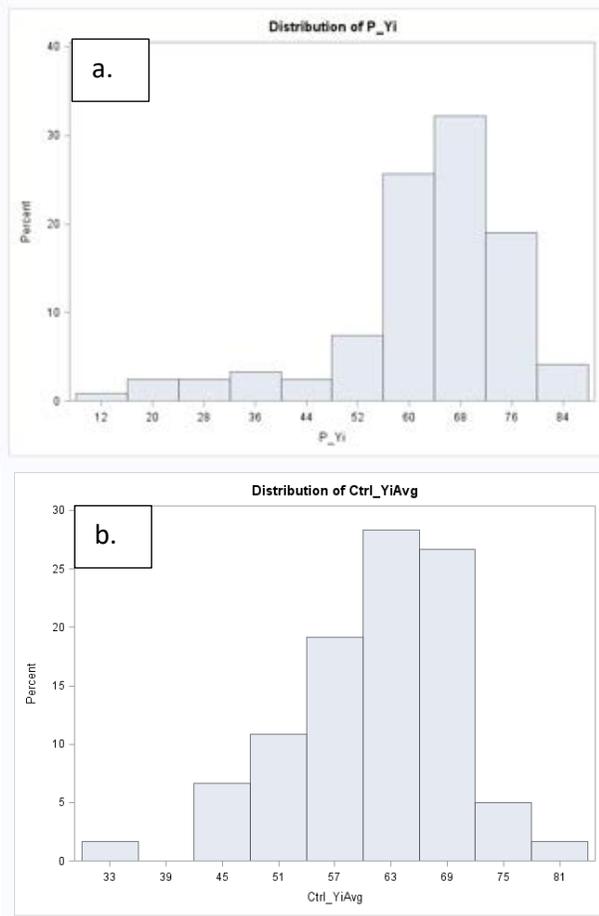


Figure 8. Distribution of 2017 Quicksand soybean yields (bu acre^{-1}) for the phosphorus treatment (a) and control average (b).

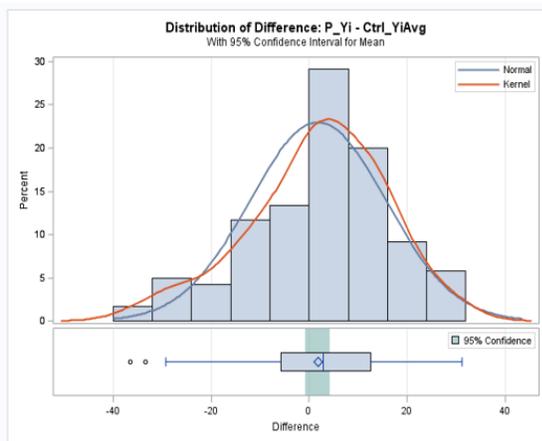


Figure 9. Results of the paired t test of the control and phosphorus treatment soybean yield at Quicksand in 2017.

A subset of the plots which were determined to have responded in the 2016 season were subjected to further testing. In order to be considered responsive both the midseason biomass sample and the grain yield had to exceed the control average and noise within a plot. Thirty six samples were tested consisting of the 0-10cm and 0-20cm depths from the treatment and one control of 9 plots totaling 12 samples from Princeton and 24 samples from Quicksand. A Hedley fractionation was completed on the samples. The fractionation was modified slightly with the initial extraction of water being replaced with an anion resin strip instead and centrifuging coupled with decanting replacing vacuum filtration. The organic fraction was also determined for the sodium bicarbonate and sodium hydroxide fractions via digestion in UV light.

The control and treatment subplots were found not to be significantly different for any of the fractions. The fractions for the treatment subplots for the non-responsive and responsive plots are shown in Figure 7.

For the Quicksand and Princeton sites 2017 was the soybean portion of their rotation. The data from Princeton is in process so is not included in this report. The Quicksand soybean yield for the treatment plots ranged from 15 to 85 bushels per acre with a mean of 63.0 compared to the average control which ranged from 32 to 79.5 bushels per acre with a mean of 61.1. The distributions for yield are shown in Figure 8. The control and treatment were

compared using a paired t test which found their difference non-significant with a p-value of 0.1635 (Figure 9).

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