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

2016 Annual Report

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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

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). 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-ill rotation. In 2016 both sites were planted to corn. Plot 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 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 1). 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 2. 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 1). 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 2) 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 $acre^{-1}$ P_2O_5). 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. 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 3 shows the plot layout for the Breathitt County site 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.

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 1) 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 ^{o}C, and weighed to estimate biomass. Normalized difference vegetative index (NDVI) was measured using active optical sensors (GreenSeeker^{TM}) 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. 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 response 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 subplots were included in each plot, allowing error estimation for the mean check yield in each plot (Equation 1). Using Equation 2, we calculated absolute yield response (ΔY) using the mean check yield ($\bar{Y}_0$) 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.

$$\bar{Y}_0 = \frac{Y_{0A} + Y_{0C}}{N} \text{ and } \sigma_{Y0} = \sum_{i=1}^{N} \sqrt{\frac{1}{N}(Y_{0i} - \bar{Y}_0)^2}$$

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_{Y0}). 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.

INITIAL RESULTS AND DISCUSSION

Soil phosphorus

Figure 4 shows the distribution of Mehlich 3 P concentrations for the subplots sampled at the Princeton and Quicksand sites. The mean soil test P concentrations at both sites fell well below Kentucky's critical level of 30 mg kg^{-1}, with a mean Mehlich 3 P concentration of 16.4 mg kg^{-1} at Princeton and 13.6 mg kg^{-1} at Quicksand. Nonetheless, 11.3% and 6.5% of subplots at Princeton and Quicksand, respectively, had Mehlich 3 P concentrations at or above Kentucky's critical level. The variability of soil test values reinforces the need for study into predicting phosphorus needs within a production field, but necessitate careful review of response data to ensure proper comparisons are made.

Yield response

Corn (*Zea mays*) yield for both sites exhibited an approximately normal distribution with a mean of 159 bu ac^{-1} for Princeton and 172 bu ac^{-1} for Quicksand (Figure 6). The raw yield data was cleaned by first removing values considered impossible to achieve either due to mechanical limits of the combine (ground speed) or the auger (high pressure readings on the impact plate). We defined outliers as points where the yield fell outside three standard deviations of the mean yield for that site (Figure 7). If any subplot yield was identified as an outlier the entire plot was removed from further analyses. Complete crop loss or low yield numbers due to factors other than soil fertility were the main causes of plots being identified as outliers. For example, the Quicksand site had instances of Diplodia ear rot, as well as unauthorized removal of complete plants, which resulted in inaccurate yield numbers. The Princeton site had issues with retarded germination due to sidewall compaction in some portions of the field due to high soil moisture at planting.

Since, the majority of the plots at the Princeton and Quicksand sites were below the Kentucky critical level we expected yield response to starter P fertilizer. The grain yield for the Quicksand site ranged from 109 to 230 bu ac^{-1} with an average of 171 bu ac^{-1} for the control subplots and from 111 to 217 bu ac^{-1} with an average of 173 bu ac^{-1} for the subplots that received P. The grain yield for the Princeton site ranged from 81 to 210 bu ac^{-1} with an average of 153 bu ac^{-1} for the control subplots and from 106 to 235 bu ac^{-1} with an average of 163 bu ac^{-1} for the subplots that received P. Figures 8 and 9 show ΔY response to soil Mehlich 3 P concentration (4" sample) at Princeton and Quicksand, respectively. Generally, we see no relationship between apparent response and soil P at either site. In addition, we see that overall a higher proportion of the plots at Princeton appeared to positively respond to P fertilizer than at Quicksand. We compared yield with P to yield without P at each site using a paired t-test. At Princeton the test found an average response of 10 bu a^{-1}, which was found to be significant at $P<0.0001$ (Figure 10). By comparison the paired t-test deemed the 3 bu a^{-1} difference at Quicksand to be nonsignificant (P-value = 0.25)

Figures 11 and 12 show the RY as a function of Mehlich 3 soil P concentration for the Princeton and Quicksand sites, respectively. In both figures there is a vertical line depicting the Kentucky critical level, above which we no longer expect response to fertilizer application. The horizontal line present in both figures is set at 95% RY to provide a guideline for response. For the Princeton site, the relative yield values are centered on the 95% relative yield line with a somewhat uniform spread across the soil test values. At Quicksand the mass of points is shifted

upwards further into the non-responsive region of the figure. The critical level for each site was calculated using the Cate-Nelson method, but neither one was significant at the 95% confidence level. Princeton's critical level was estimated to be 23.1 mg kg^{-1} with a p-value of 0.1947. The critical level at Quicksand was estimated to be 14.6 mg kg^{-1} with a p-value of 0.1298. The lack of significance is not surprising given that the method requires values in the higher, non-responsive soil test values in order to work properly

Early season biomass response

Biomass samples were taken from guess rows within subplots prior side dress of nitrogen. For Princeton only one of the three smaller fields was sampled at approximately V4. For Quicksand the entire field was sampled at approximately V6. The dry weight of each control sample relative to the adjacent treatment subplot was plotted versus the average soil test phosphorus shown in Figure 13. As expected, the majority of the subplots responded to phosphorus with increased biomass. For the Quicksand site, the response was visually apparent as the samples were taken. Phosphorus deficiency is usually more pronounced earlier in the plant's life, so additional early season measurements might prove more useful than relying on yield alone.

Yield component response

Realistically, not enough plots were sampled at Princeton to have faith in the results. Nonetheless, we have presented the data for reference only. Conversely, we sampled all plots at Quicksand and have a much higher level of confidence in those data from that perspective, but wonder how much disease at that site interfered with mechanical harvest. For the Princeton site, the number of kernel rows ranged from 13.5 to 16.5 with a mean of 15.0 for the treatment subplot and ranged from 14.25 to 16.5 with a mean of 15.3 for the check subplots. Kernel rows at the Quicksand site ranged from 14.5 to 19 with a mean of 16.5 for the treatment subplot and from 14.25 to 17.5 with a mean of 15.9 for the check subplot. The paired t-test found the differences between treatment and check plot kernel row to be statistically significant at one site with an alpha of 0.05. The mean difference was -0.29 with P =0.0576 at Princeton and 0.60 with P <0.0001 at Quicksand, respectively. The number of kernels in an average row for the Princeton site ranged from 34 to 46 with a mean of 40 for the treatment subplot and from 36 to 43 with a mean of 40 for the check subplot. For Quicksand, number of kernels ranged from 30 to 41 with a mean of 37 for the check subplot and from 32 to 43 with a mean of 40 for the treatment subplot. The paired t-test found the differences in kernel number to be statistically significant at only the Quicksand site. The mean difference was 0.60 with P =0.2086 at Princeton and 2.46 with a P <0.0001 at Quicksand. The average weight per kernel at the Princeton site ranged from 0.29 to 0.36 g with a mean of 0.319 for the treatment subplot and from 0.26 to 0.36 g with a mean of 0.31 for the check subplot. For Quicksand, the average kernel weight ranged from 0.29 to 0.39 g with a mean of 0.34 for the treatment subplot and from 0.29 to 0.38 g with a mean of 0.34 for the check subplot. The paired t-test found the differences between treatment and check plot kernel weight to be statistically significant at each site. The mean difference was 0.011 with P = 0.0001 at Princeton and 0.005 g with P =0.01 at Quicksand, respectively.

FUTURE PLANS

The subset (described previously) of plots selected to represent the range of soil properties and degrees of response are currently undergoing additional analysis, including inorganic phosphorus fractionation, organic matter, P sorption isotherms, and particle size analysis (hydrometer method). We will use P response along with the additional soil characterizations to select plots where we will evaluate how microbial community structure responds to the spatial variability present in the field. This will be completed using high-throughput phospholipid fatty acid (PLFA) and Next Generation Sequencing (NGS). These techniques will allow us to assess total microbial and fungal biomass as well as the relative proportion and concentration of major microbial groups (G+, G-, arbuscular mycorrhizal fungi, etc.) to fully elucidate the specific organism present. In addition, this fall we will map soil electrical conductivity (EC) using a Veris unit. Final decisions on more advanced methods will be determined by project partners after collection of additional data from the initial year's soil samples for selected plots. Multivariate analysis will then be used to correlate shifts in variables such as microbial community structure and variation in P forms present to the changes in the various environmental variables measured.

The time to canopy closure and percent of plants showing silks when high P plots have reached approximately 90% silked were proposed measurements, but were not taken. The window of time when accurate silking measurements could be taken was very small in 2016 and therefore missed. In cooperation with the UK-BAE faculty, Dr. Michael Sama, we captured UAS multispectral images for Quicksand site (Figure 18). Going forward, we intend to explore using temporally dense UAS imagery to estimate time to early season biomass production, time to canopy closure, and P influence on proportion of plants silked. Capturing the image of an entire field can be completed quickly with post processing of the imagery making up the bulk of time spent. Scheduling time for flights with our UK-BAE colleague limited our ability to do these assessments in 2016, therefore we plan to purchase our own UAS this fall. We will continue to rely on Dr. Sama for his technical expertise, but will be able to collect the imagery ourselves from each of the project fields going forward.

CONCLUSIONS

Our most important and challenging finding in 2016 was our inability to precisely estimate yield with high spatial resolution. Ultimately, our ability to carry out the objectives of this project is dependent on our ability to collect precise yield estimates at very fine spatial resolution. Certainly there was natural noise in the data as a result of pest or disease pressure and soil property variability (e.g. compacted areas). However, there was also a tremendous amount of noise that was likely a direct result of the mechanics of harvesting and thrashing grain along with estimating yield during that operation. Two issues exist when harvesting with a plot combine. First, thrashing is not spatially discrete, meaning that as the combine travels across the field it is mixing grain collected from different locations before sending that grain to the impact plate where yield is estimated. However, testing of combine harvesting and thrashing performance will allow us to extend the treated plot length and place the harvest length within that distance so that we generate yield data confined to the treatment. The more challenging issue is the small scale noise found in each data point. A yield monitor estimates yield by measuring impulse in newton seconds (Ns) as grain is thrown from the clean grain elevator against a pressure plate. This information is then converted to a yield estimate based on header width, combine speed, and grain moisture content. In 2016, we logged yield every second while attempting to travel between 0.67 ms^{-1} (1.5 mph) and 1.56 ms^{-1} (3.5 mph), resulting in approximately 7.6 to 17.7 plants being harvested per yield point (assuming 30,000 plants per acre). We used the center 3 m of the 9 m application plot for yield (in order to account for imprecision in thrashing described above). This resulted in 1 – 10 yield points recorded per plot. At this scale the yield is highly dependent on each individual yield point and each individual plant within that point and therefore sensitive to factors beyond P fertilizer application. Further complicating matters, yield is calculated using speed from the RTK corrected GPS. The antennae for the GPS were mounted on the cab of the plot combine, which was subject to considerable pitch and yaw. Therefore, as the combine bounces across the field some data points are calculated using grossly inaccurate speeds, not to mention the influence of the bounce on the pressure plate readings. We took every effort to clean the data by looking at point to point variability in mass flow, speed, and moisture. We looked for extreme values in each of these measurements and the calculated yield. Nonetheless, all these factors combined, contribute to our lack of confidence in the precision of the yield data collected. Fundamentally data from a properly calibrated yield monitor is accurate, but imprecise. We weighed all harvested grain using a weigh wagon and this value was very close to the total mass recorded by the yield monitor, but each yield data point had the potential to be far from the true value for that point. Going forward the success of this project will be dependent on developing better methods to select responsive and non-responsive plots. We intend to use multiple measurements to select plots, including biomass, imagery, and hand sampling. However, we are also working with engineers to develop better mechanized methods of harvest that will afford better spatial performance. We hope to deploy revised methods in the 2018 harvest.

FIGURES

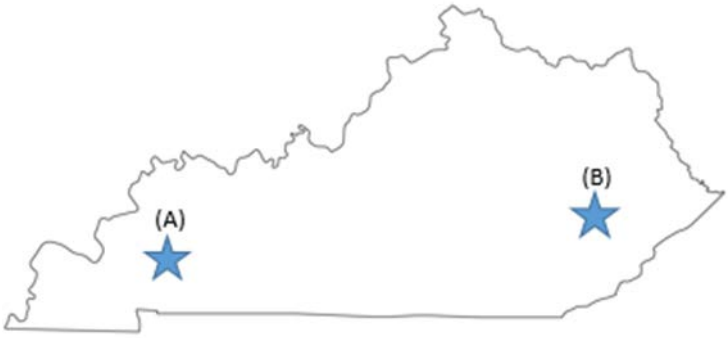

Figure 1. The two field sites established in 2016 are shown with Princeton and Quicksand labeled as A and B, respectively.

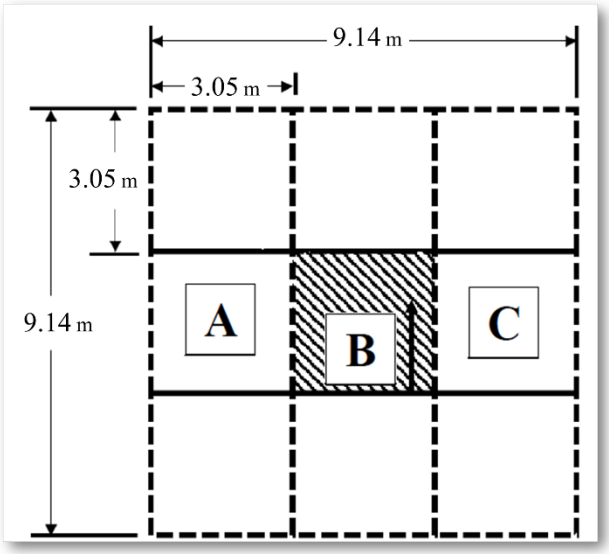

Figure 2. Original plot design with nine subplots. Soil samples in 2016 were collected from subplots A - C.

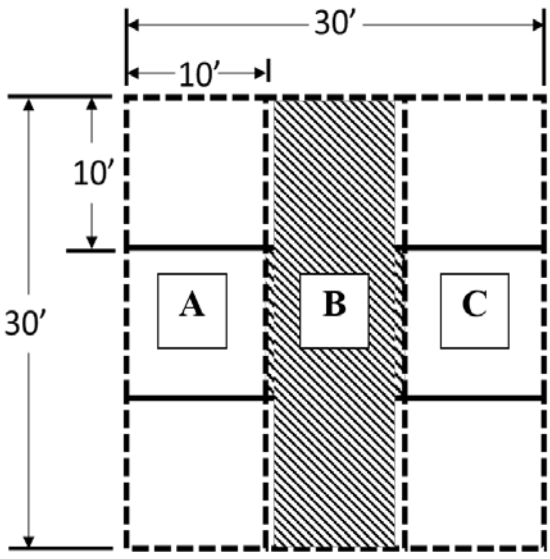

Figure 3. Plot design used in 2016. The center subplot (B) received phosphorus fertilizer at planting (indicated by shading). Subplots A and B received no phosphorus. Individual soil samples were collected from the center 10 ft in all three subplots.

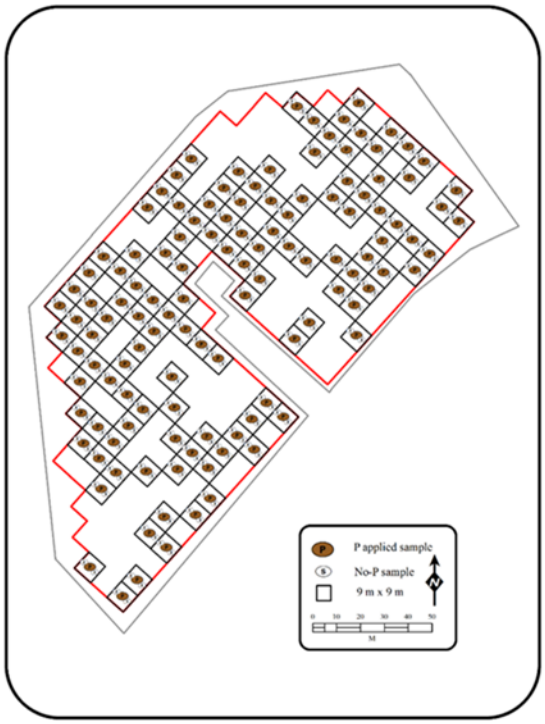

Figure 4. Breathitt County site showing the 30 ft plots and three subplots that were sampled.

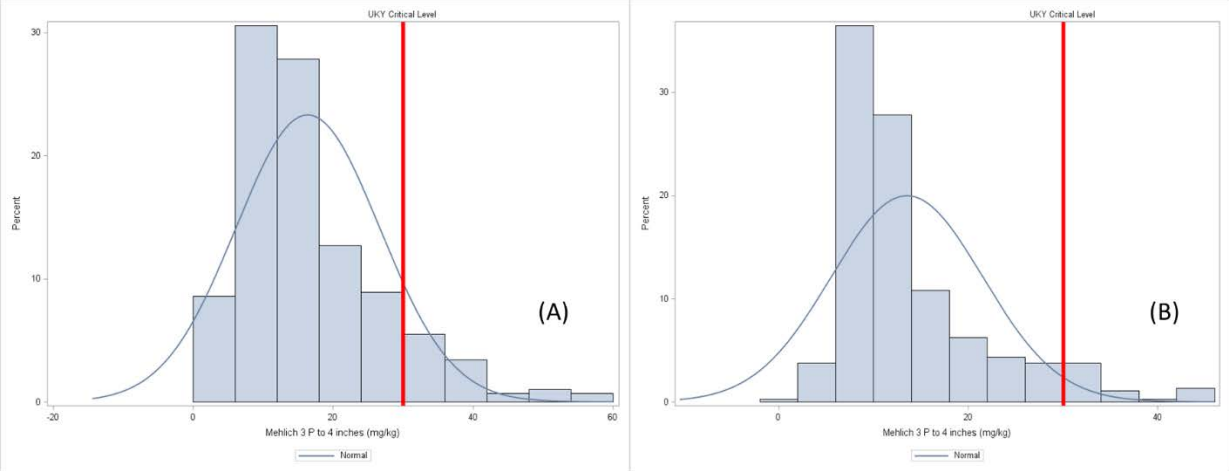

Figure 5. The distribution of Mehlich 3 phosphorus for soil samples taken from the 0-4in depth at the Princeton (A) and Quicksand (B) sites are shown with a normal curve overlaid. The current critical level used for phosphorus in Kentucky is shown by the vertical red line.

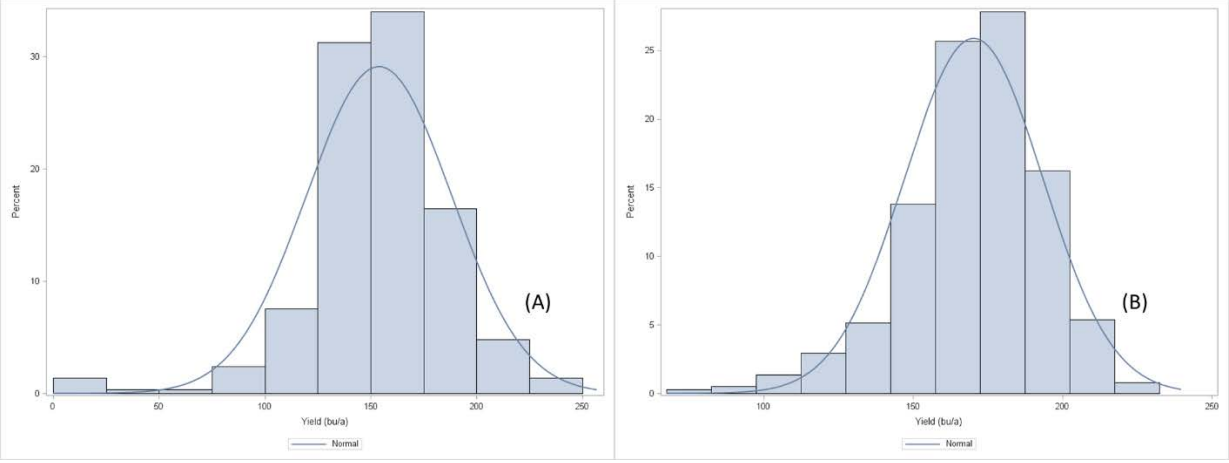

Figure 6. The distribution of corn (Zea mays) yield for the Princeton (A) and Quicksand (B) sites are shown with a normal curve overlaid.

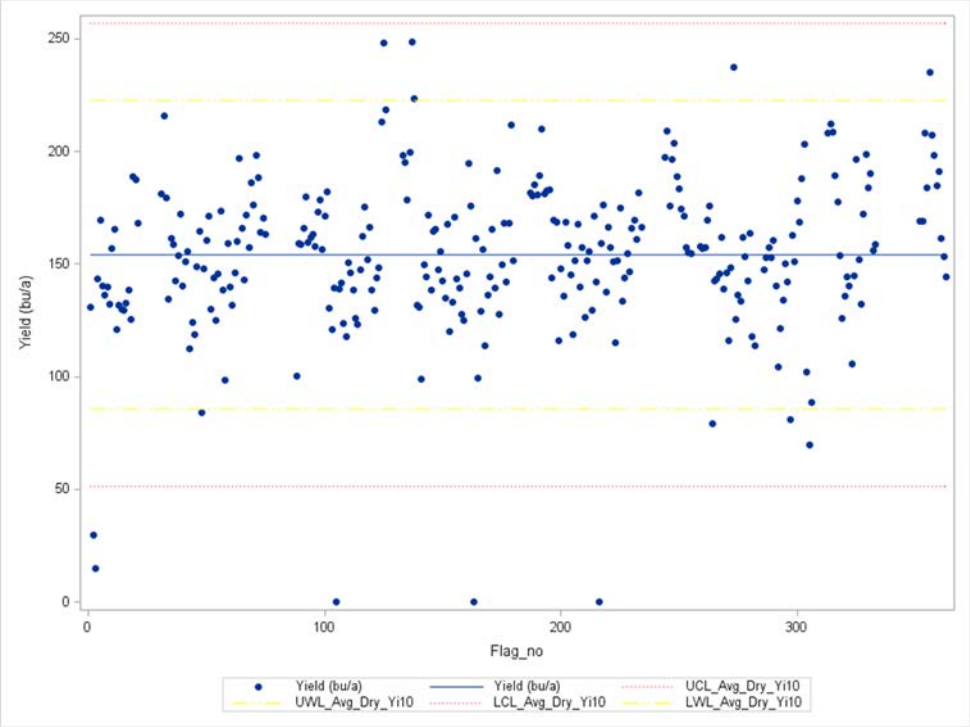

Figure 7. Princeton yield data X-graph used to identify outliers. Plots where any single subplot fell outside the upper or lower caution limit (three times the standard deviation) were removed from subsequent analyses.

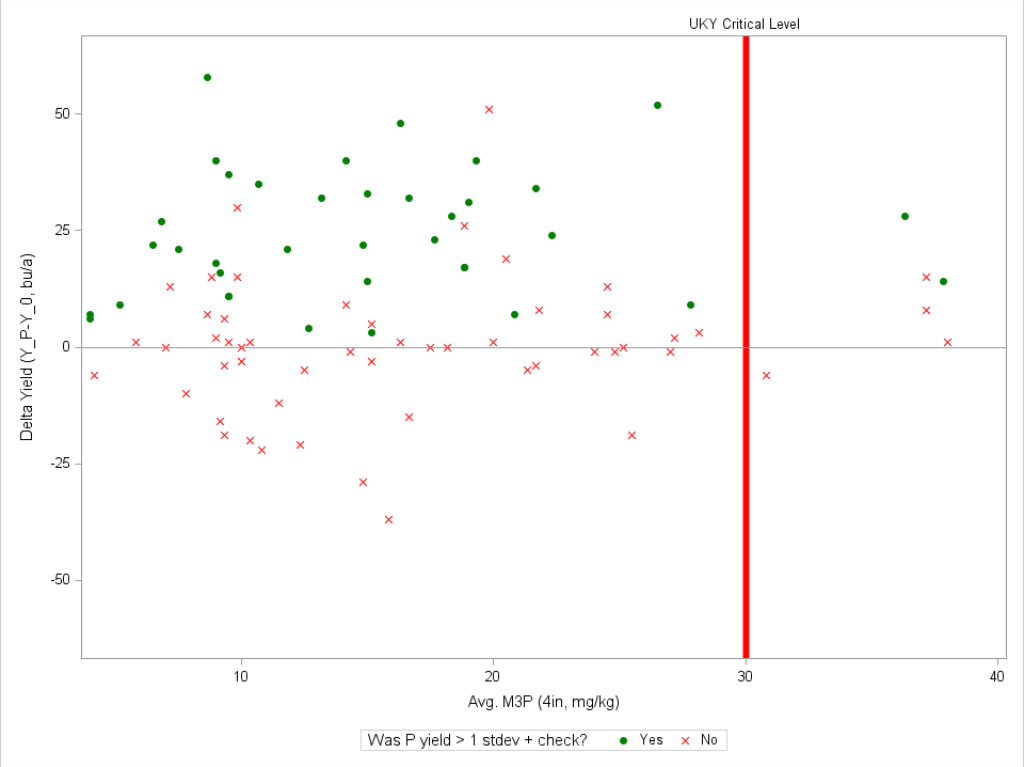

Figure 8. Delta yield as a function of Mehlich 3 soil P concentration at the Princeton site. The horizontal line indicates a difference of zero between fertilized and unfertilized yields and the vertical red line indicates the critical phosphorus level according to University of Kentucky guidance. Plots where the P-fertilized yield exceeded the mean no-P check yield plus one standard deviation are represented by a solid circle.

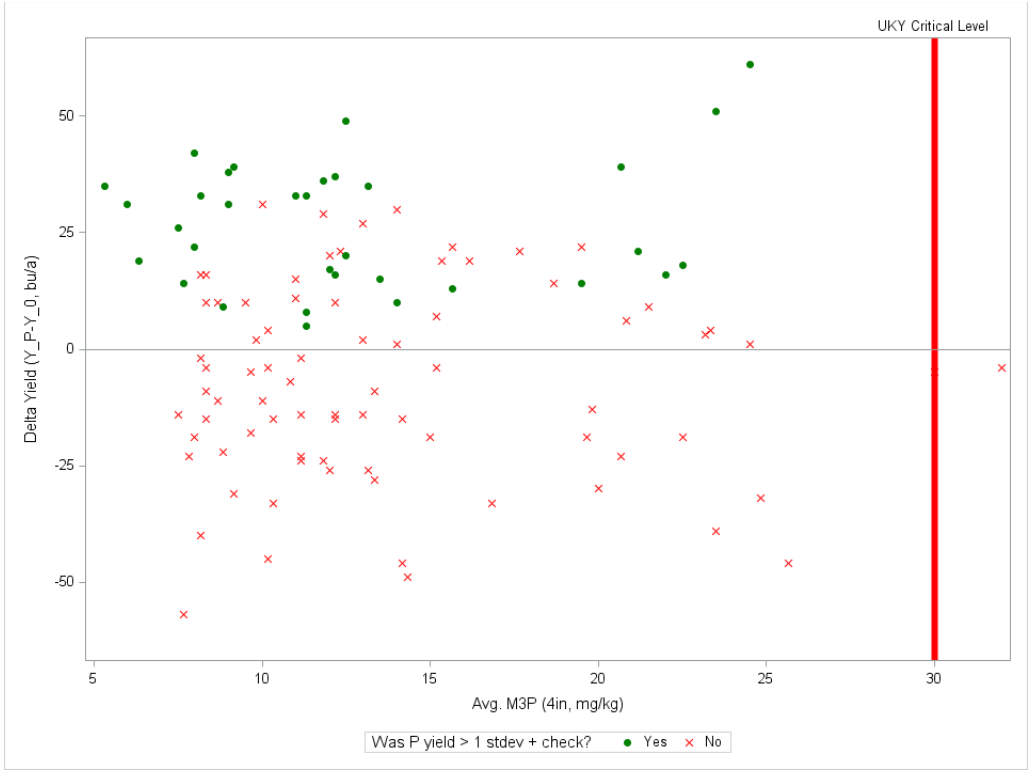

Figure 9. Delta yield as a function of Mehlich 3 soil P concentration at the Quicksand site. The horizontal line indicates a difference of zero between fertilized and unfertilized yields and the vertical red line indicates the critical phosphorus level according to University of Kentucky guidance. Plots where the P-fertilized yield exceeded the mean no-P check yield plus one standard deviation are represented by a solid circle.

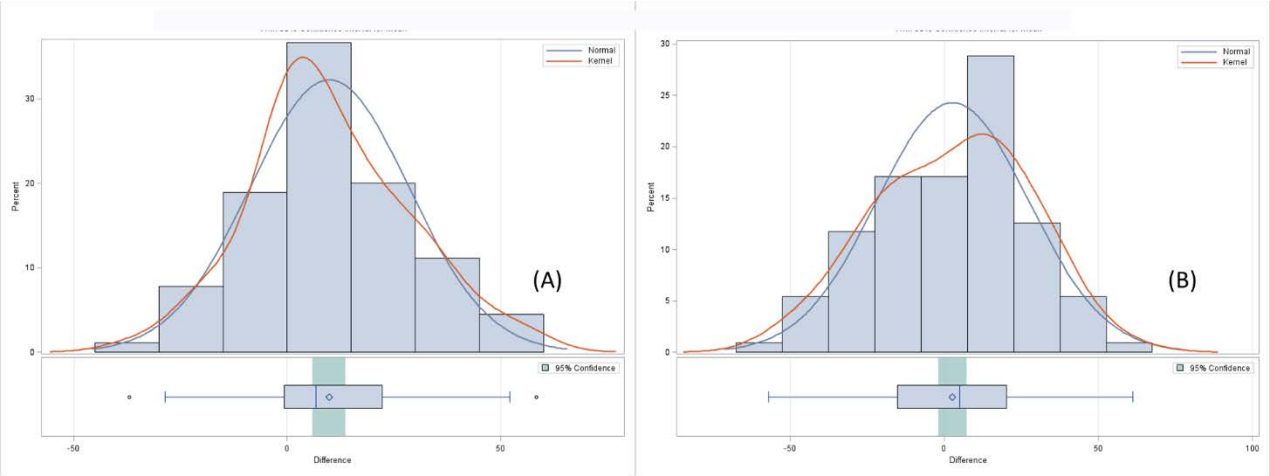

Figure 10. The results of a paired t-test comparing the treatment yield (Y_P) with the control yield (Y_0) are shown for the Princeton (A) and Quicksand (B) sites. The distribution of the difference between the two yields is shown as a histogram with both the normal curve and the smoothed kernel shown imposed upon the histogram.

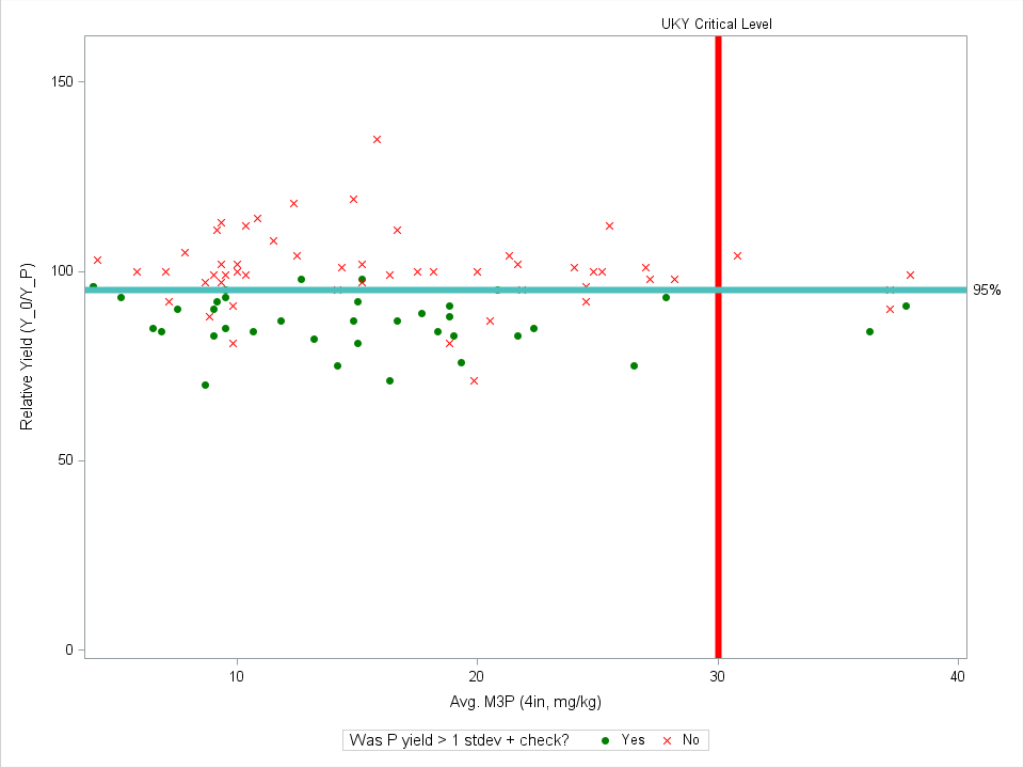

Figure 11. Relative yield (%) as a function of Mehlich 3 soil P concentration at the Princeton site. The horizontal blue line indicates 95% relative yield and the vertical red line indicates the critical phosphorus level according to University of Kentucky guidance. Relative yields from plots where the P-fertilized yield exceeded the mean no-P check yield plus one standard deviation are represented by a solid circle.

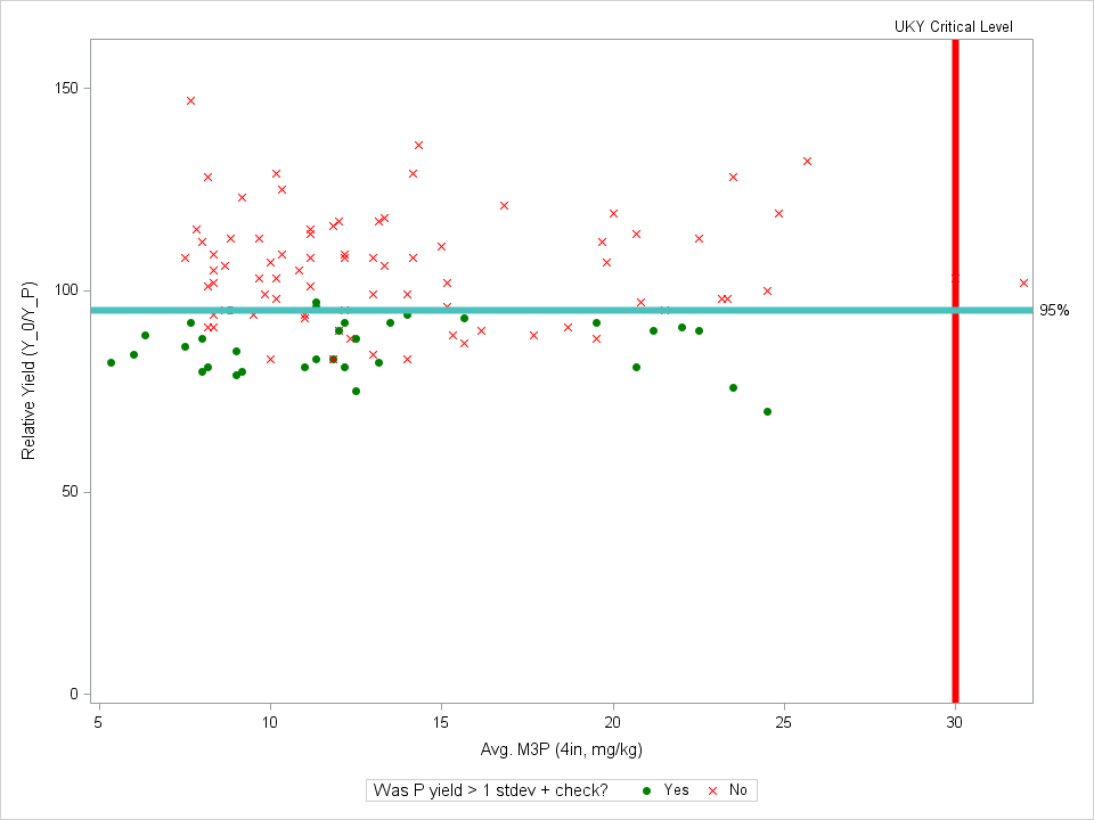

Figure 12. Relative yield (%) as a function of Mehlich 3 soil P concentration at the Quicksand site. The horizontal blue line indicates 95% relative yield and the vertical red line indicates the critical phosphorus level according to University of Kentucky guidance. Relative yields from plots where the P-fertilized yield exceeded the mean no-P check yield plus one standard deviation are represented by a solid circle.

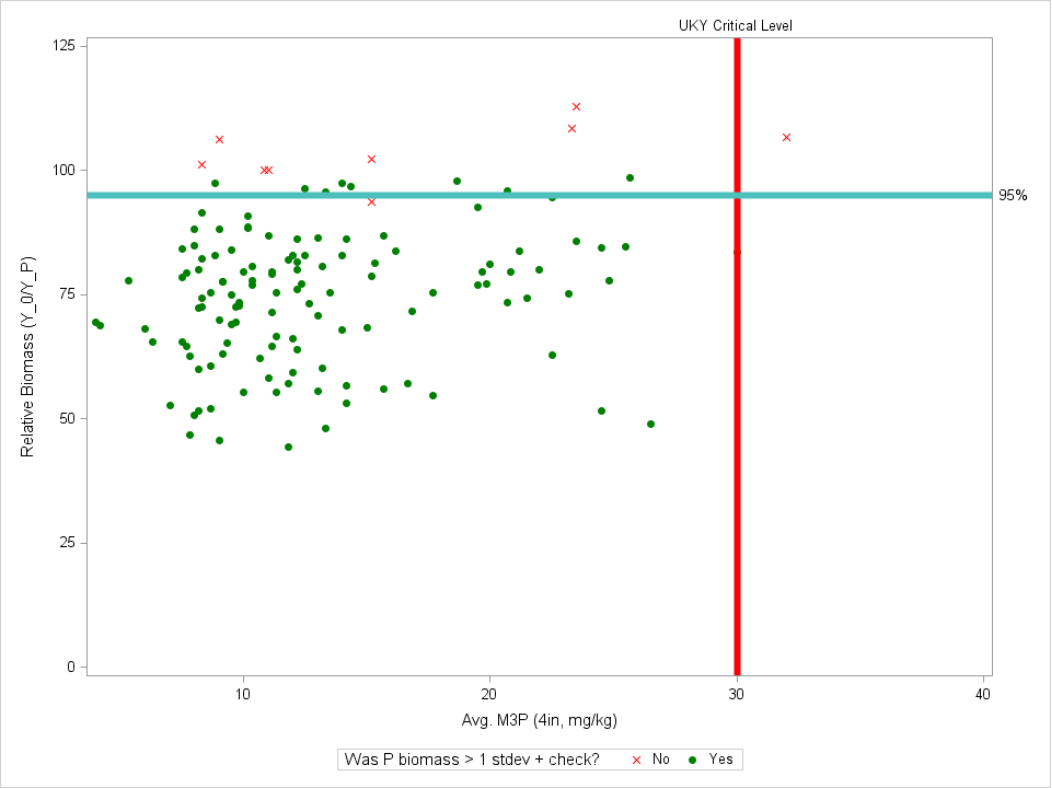

Figure 13. The biomass response to phosphorus for both sites is shown as the relative biomass of the control to the treatment subplot for each of the two control subplots. Relative biomass is plotted versus the average soil test phosphorus average of the treatment and control subplot.

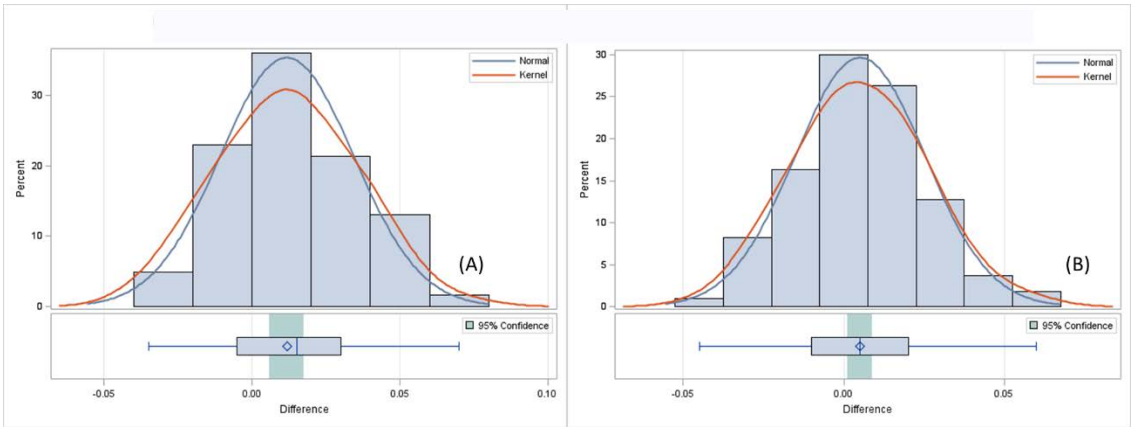

Figure 14. The results of a paired t-test comparing the kernel weight with the control kernel weight are shown for the Princeton (A) and Quicksand (B) sites. The distribution of the difference between the two kernel weights is shown as a histogram with both the normal curve and the smoothed kernel shown imposed upon the histogram. PR(Pr>\|t\| 0.0001) QS(Pr>\|t\| 0.010).

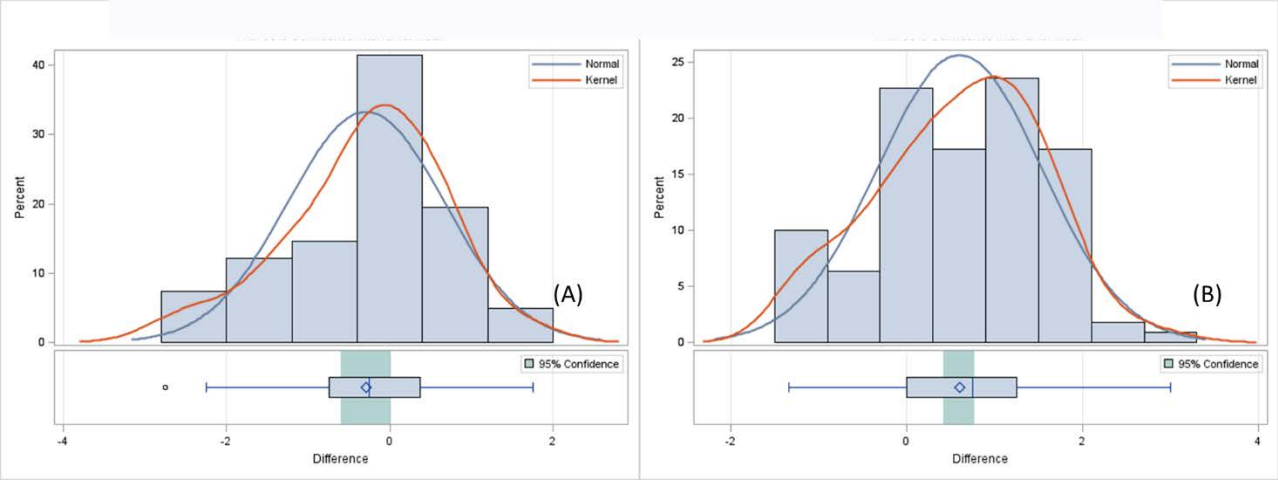

Figure 15. The results of a paired t-test comparing the kernel row with the control kernel row are shown for the Princeton (A) and Quicksand (B) sites. The distribution of the difference between the two kernel row values is shown as a histogram with both the normal curve and the smoothed kernel shown imposed upon the histogram. PR(Pr>\|t\| 0.0576) QS(Pr>\|t\| <0.0001).

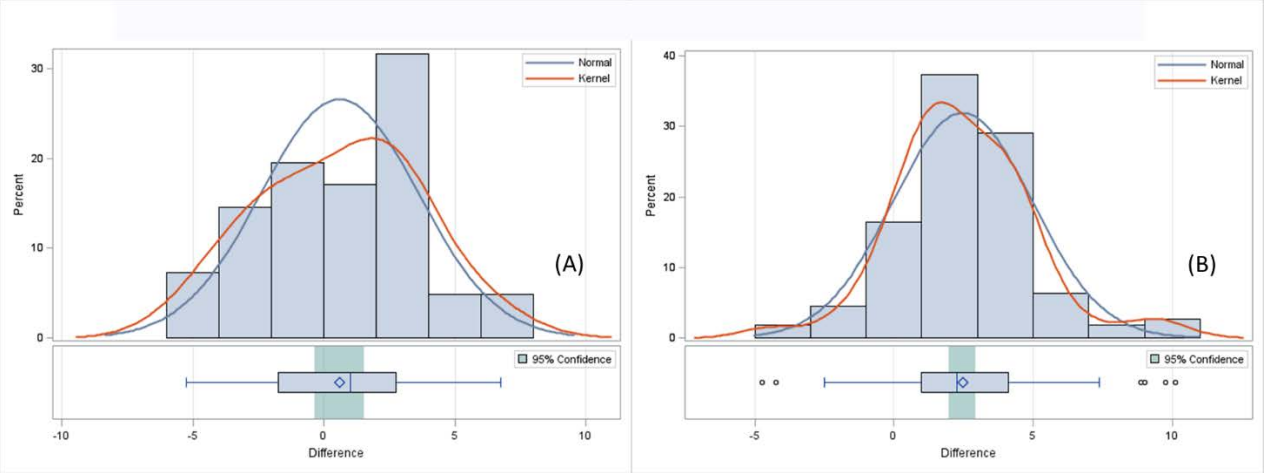

Figure 16. The results of a paired t-test comparing the kernel number with the control kernel number are shown for the Princeton (A) and Quicksand (B) sites. The distribution of the difference between the two kernel numbers shown as a histogram with both the normal curve and the smoothed kernel shown imposed upon the histogram. PR(Pr>\|t\| 0.2086) QS(Pr>\|t\| <0.0001.

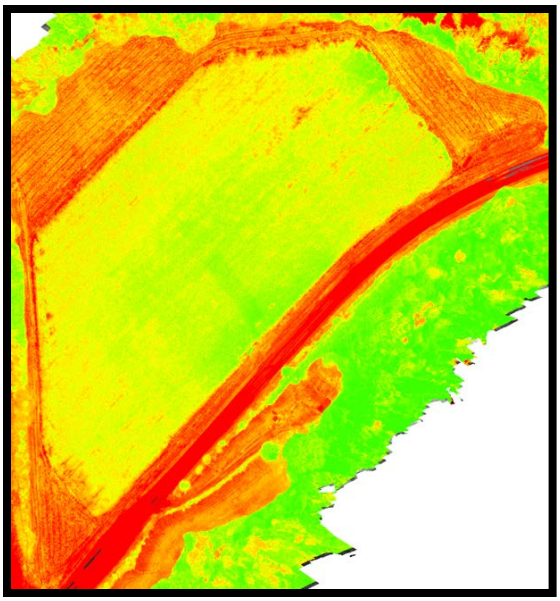

Figure 17. An image of the normalized difference vegetative index (NDVI) of corn after silk emergence at the Quicksand site is shown.

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