

Introduction

Corn planted in two locations (Thrall & Snook, TX) was fertilized with banded liquid phosphorus (P) applied at different rates and depths to investigate the effect on rooting patterns. Banded P was applied 15 cm off the seed row. Root crowns were excavated along with all top plant matter at the V10 - V12 growth stage prior to tasseling. Photo-images of washed crowns were analyzed for spatial density of roots relative to the zone of concentrated P fertilizer. Images were processed using the free-ware application 'ImageJ' (image processing and analysis in Java). The analyses indicated decreased root densities associated with the volume of soil nearest the banded zone. However, there was positive relationship between both rate of phosphorus and depth of application to concentration of P in corn leaf tissue collected at the same time. Additionally, the highest yielding plots in both locations were associated with P fertilizer placed below the surface of the soil, demonstrating improved P-use efficiency in subsurface banded applications.

Objective

Investigate the physiological response of corn roots in the field to rates and depth of liquid P fertilizer placement and evaluate the suitability of the open-architecture program ImageJ for processing and analyzing images of excavated corn plant crown roots.

Materials & Methods

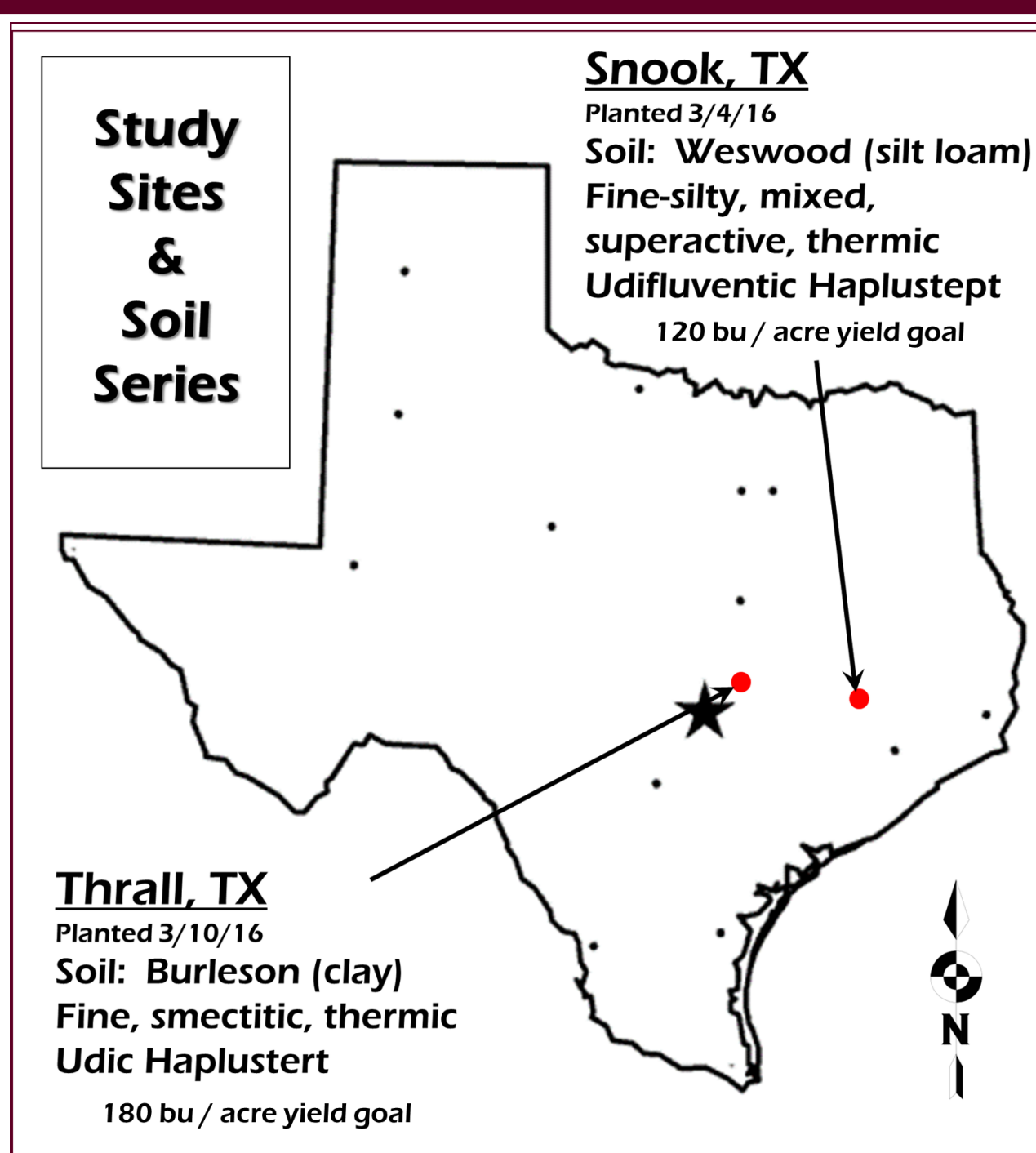
Sites: Two sites were planted with corn (*Zea mays*, var. Terral REV 25BHR26) seed-drilled into a default no till system. The site at Thrall, TX was preceded by a corn/fallow rotation. The site at Snook, TX was preceded by a corn/oat rotation. Liquid P fertilizer was applied in all plots except the control (trt 1) as ammonium polyphosphate (11-37-0). The remaining required nitrogen (N) was supplied as per soil test recommendation as liquid UAN (32-0-0) at the same depth as P, except in trt 1 and trt 2, where N was knifed into the soil to 15 cm. Regardless of depth, all liquid fertilizers were placed 15 cm off the seed row shortly after planting (V2 - V4).

Experimental Design →: Individual plots were 4 rows wide (3 m) x 21 meters long. Plots were arranged in randomized complete block design with P fertilizer treatments as described below. STR = soil test recommended rate.

Crown Root Collection was performed in three replicates per site by excavating a 30 cm diameter by 35 cm deep cylinder surrounding 1 plant from the center two rows in each plot (Image 1). Wooden skewers were inserted through the base of the stalk and perpendicular to the band and seed row to maintain orientation for imaging. Excavated soil and crown roots were allowed to soak for 2-5 days in 18.9 L plastic buckets saturated with tap water to soften soil for removal. Roots were gently washed free of soil using a garden hose shower attachment and immediately imaged.

Imaging and Processing were managed by placing intact corn plants on a black felt sheet with the 'fertilizer band' side to the right (Image 2). Images were captured with a Nikon digital camera with a ruler and sample ID tag in the frame. In the ImageJ software, the procedure was as follows:

- Open and choose image from file
- Draw a line 1 cm in length with the 'Straight Line Tool'
- Go to Analyze > Set Scale > and enter 1.0 in 'Known Distance' and enter units as 'cm'
- Using the 10 cm scale bar, draw a 10 cm² square. Drag the square so that one side bisects the vertical axis of the corn stalk and place the top at the soil surface. Set line thickness to 1 pt.
- Construct four squares using Edit > Draw to construct four numbered quadrants within a 20 cm² square (image 3).
- Each quadrant was then analyzed for percent root coverage by selecting Image > Adjust > Color Threshold and manually adjusted to highlight all of the roots.
- Percent root coverage in each quadrant was calculated individually by selecting Analyze > Measure. Results (as %) were stored in a Microsoft excel spreadsheet for statistical analysis.



- Treatments**
- 1) Control (no P fertilizer)
 - 2) P at STR - surface applied
 - 3) P at 50% STR 15 cm depth
 - 4) P at 100% STR 15 cm
 - 5) P at 150% STR 15 cm
 - 6) P at 50% STR 21 cm
 - 7) P at 100% STR 21 cm
 - 8) P at 150% STR 21 cm

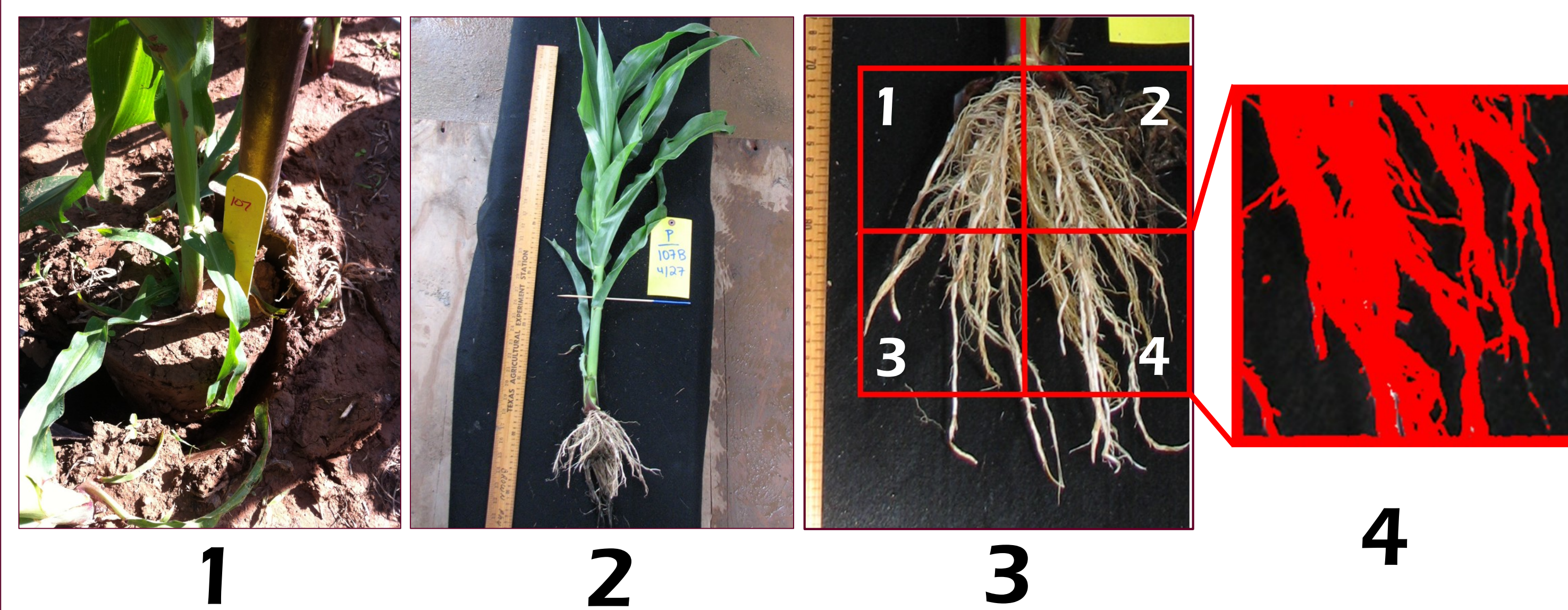
Mat. & Meth. Cont. .

Tissue Concentration (early & late): upper most unfolded leaf (10 per plot) collected at V10-V14 & ear-leaf collected at grain-fill P content was determined using microwave assisted acid digestion and analysis by ICP-AES. Results are expressed on a dry-weight basis.

Yield was determined by hand-harvesting the center two rows of each plot at Thrall, TX and by yield monitor-equipped combine harvesting the center two rows of each plot at Snook, TX. Results are expressed on a 15.5 % moisture basis.

Statistical Analysis was performed using SAS software. Multiple linear regression using PROC REG and comparison of means (Fisher's protected LSD adjustment) was performed using PROC GLM at the $\alpha = 0.05$ significance level.

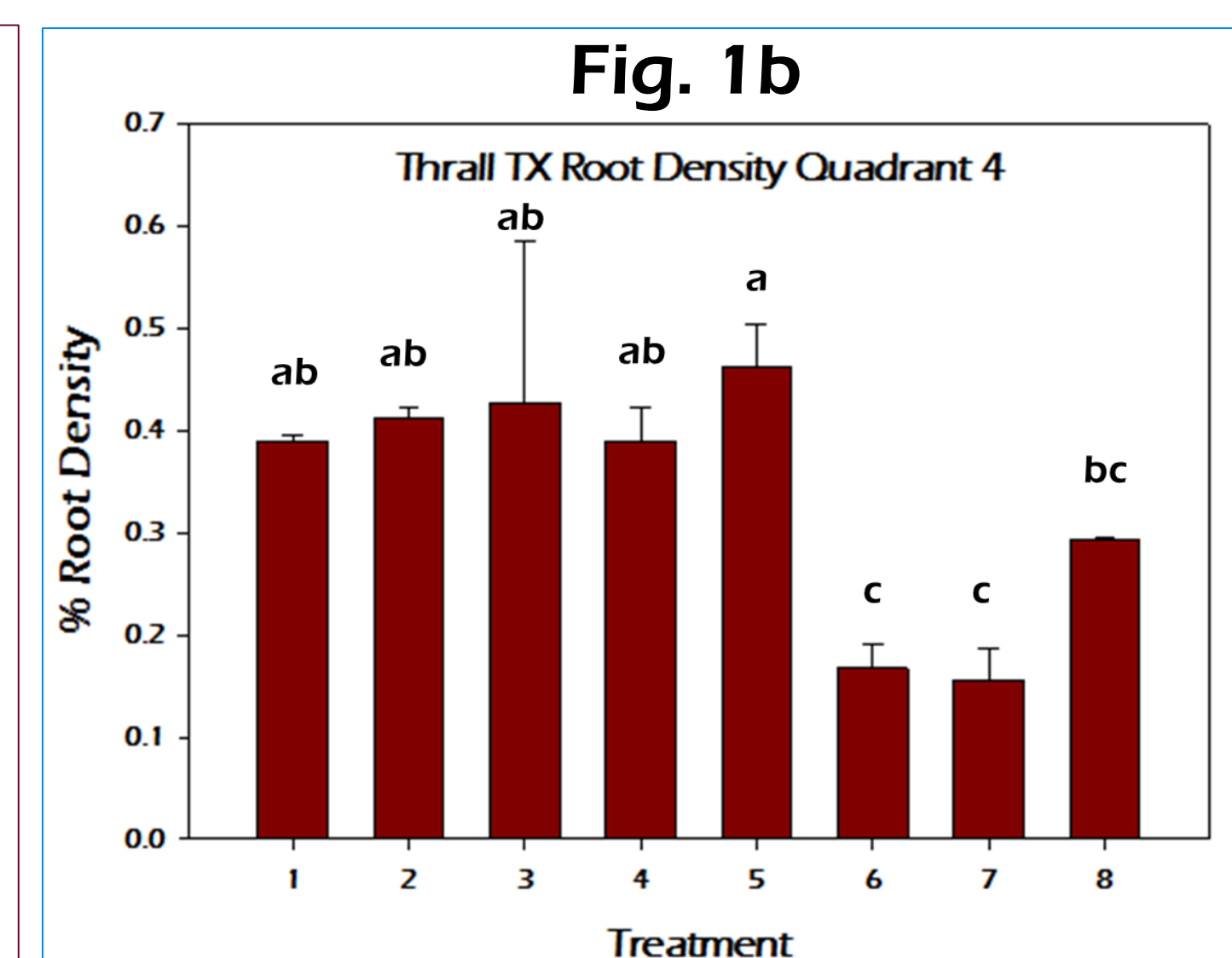
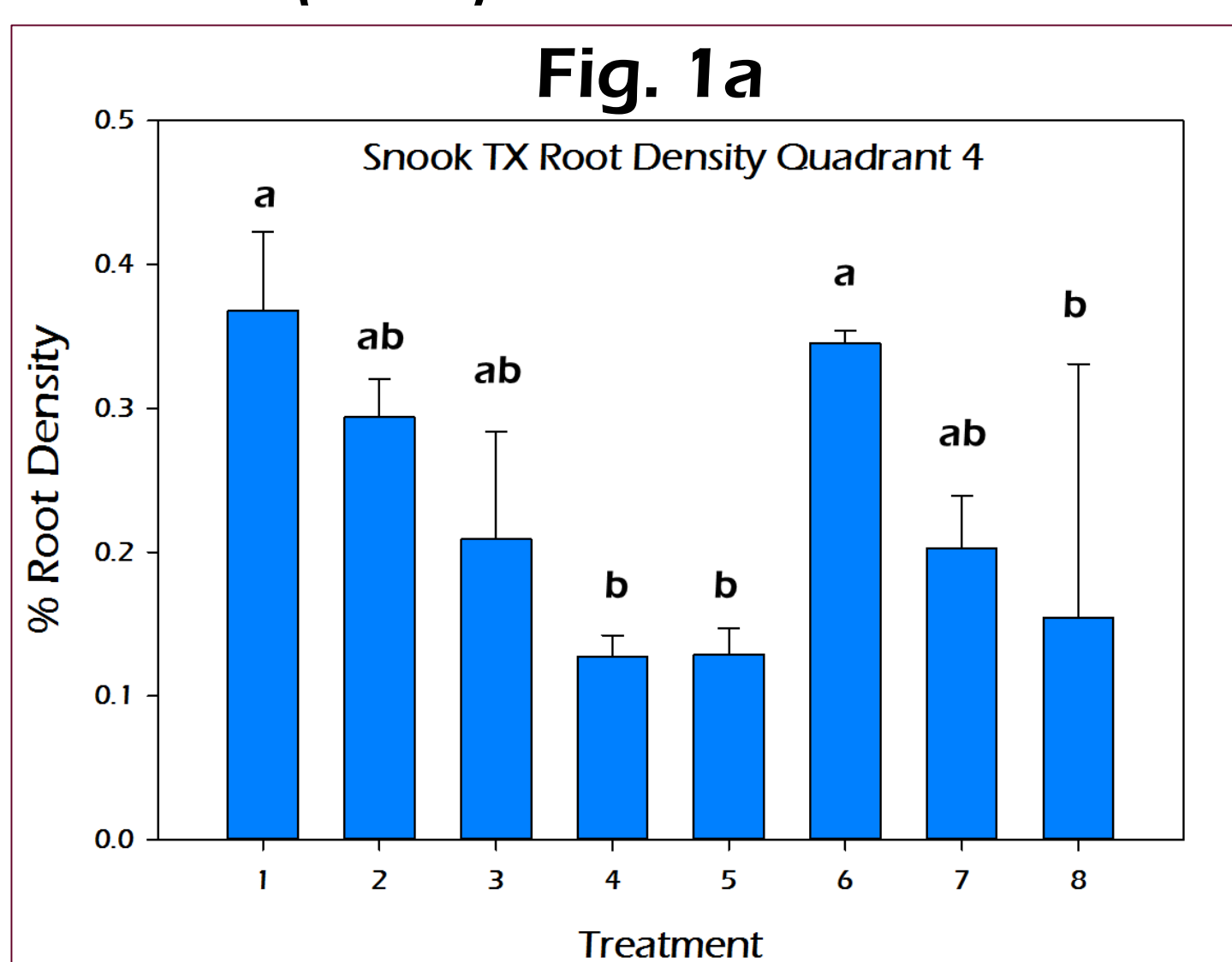
Root Imaging



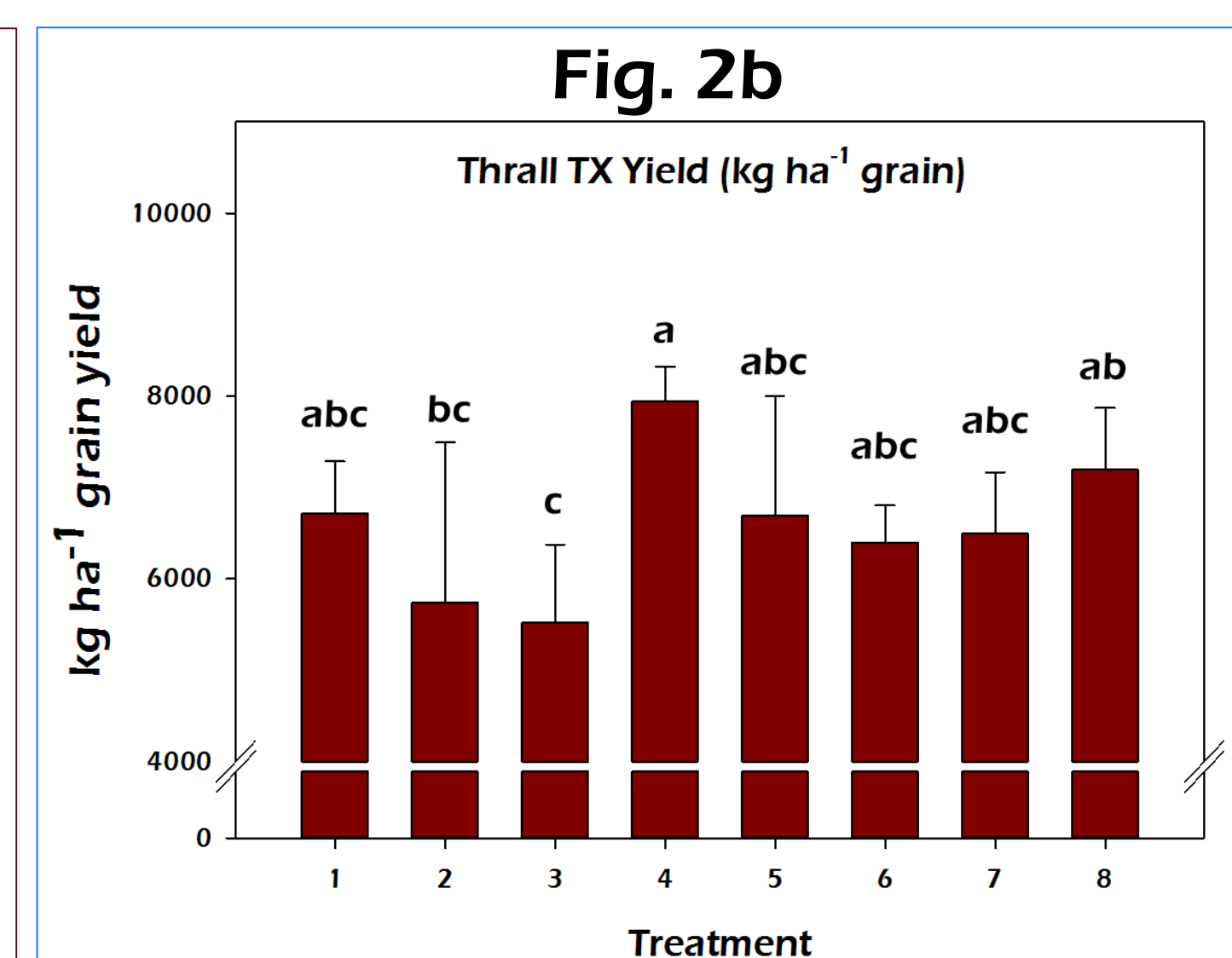
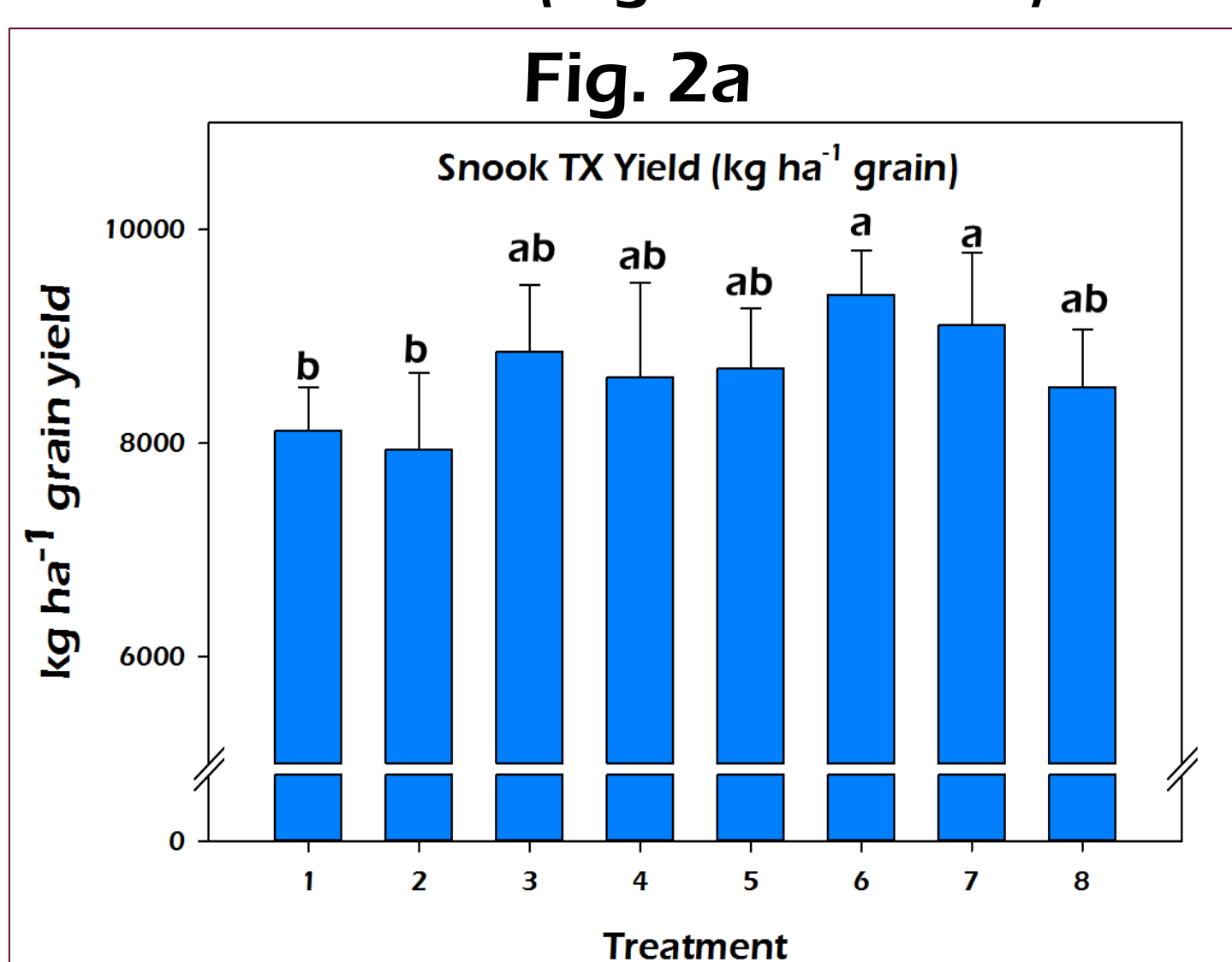
Results

Root Imaging: ImageJ was found to be highly suitable for the simple approach of estimating 2-dimensional root density following separation of the area into four quadrants. Manual manipulation was required for processing. In the future it may be possible to enable more rapid processing via more careful preparation in the image collection phase. Quadrant 4 is the focus of results below.

Rooting Density: There were significant differences in rooting density in quadrant 4 nearest the 15cm and 21 cm P fertilizer placement depths (Figures 1a & 1b). At the Snook TX site, treatments 4, 5, & 8 resulting in decreased rooting densities nearest the band. At the Thrall TX site, treatments 6 & 7 resulted in decreased rooting densities as compared to the control and to surface applied P fertilizer (Trt. 2).



Grain Yield: Significantly higher yields were observed at the Snook TX site associated with the 21 cm placement of liquid P fertilizer as compared to the control but were not significantly separable any of the other treatments. At the Thrall TX site, no single treatment receiving fertilizer was significantly different from the control (Figures 2a & 2b).



Results Cont. .

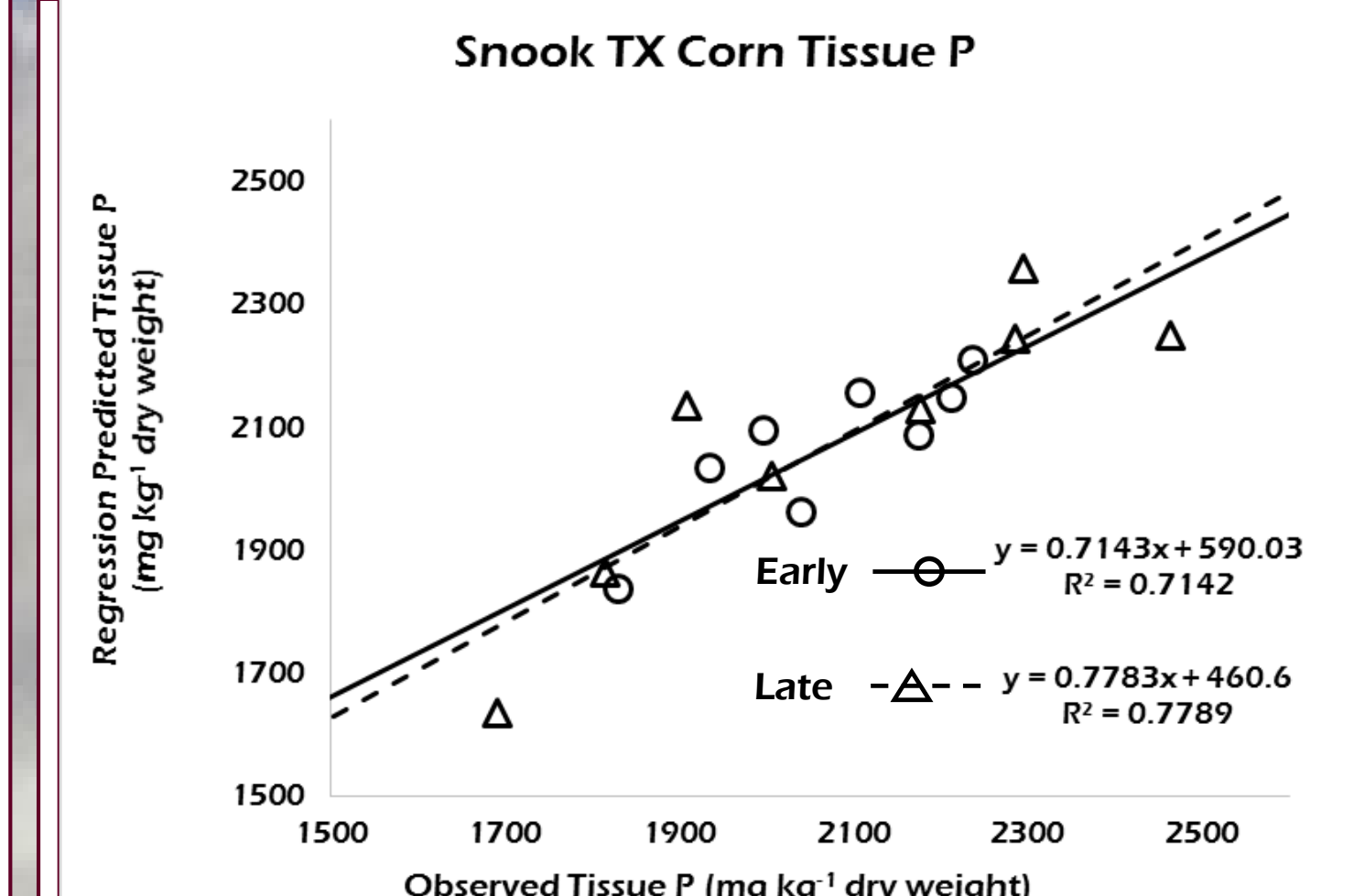


Figure 3a. Early and late tissue P contents in corn taken from the Snook TX study site. Both sampling times significantly ($\alpha \leq 0.1$) and positively correlated with both rate and depth of P fertilizer placement

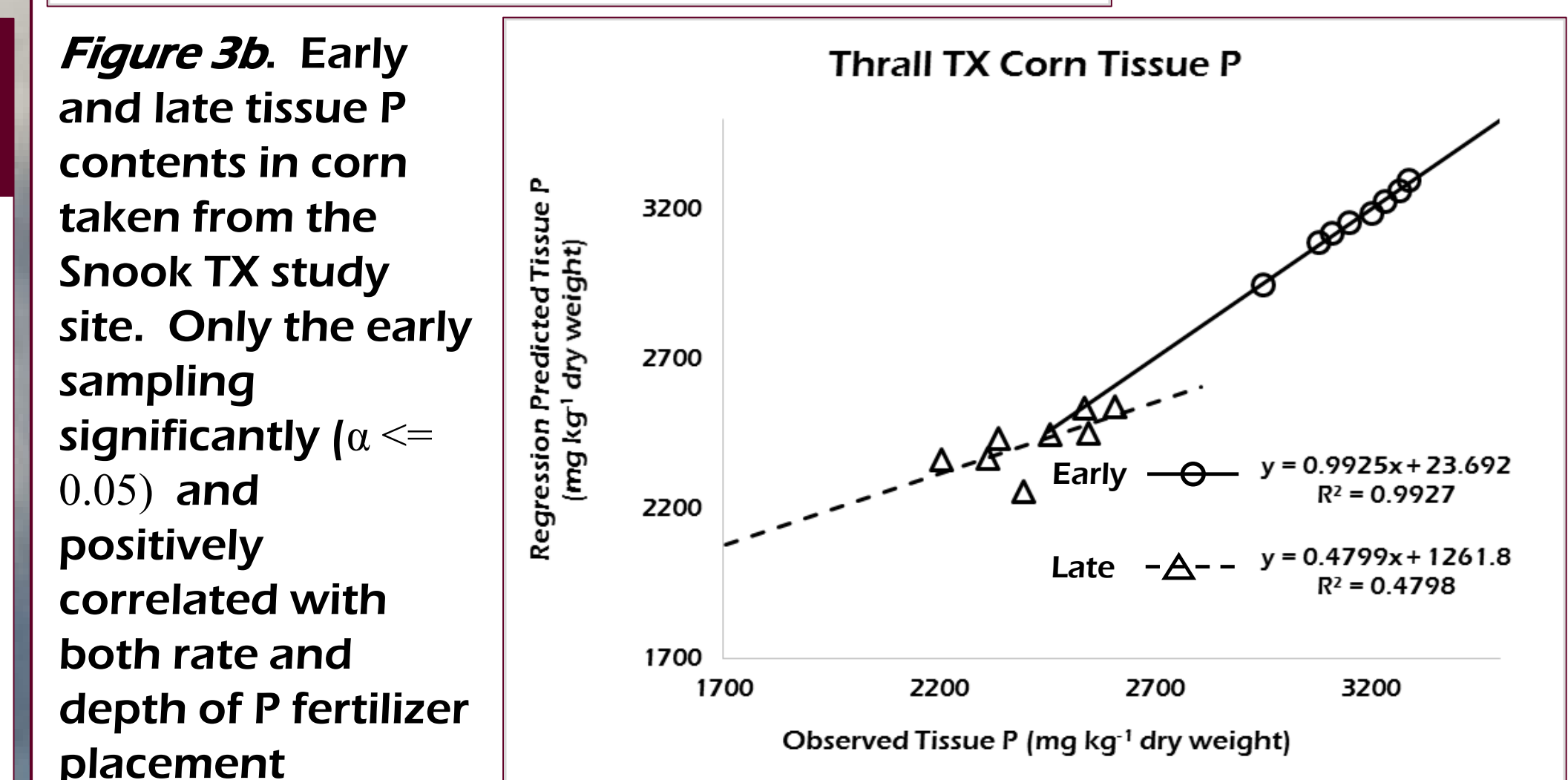


Figure 3b. Early and late tissue P contents in corn taken from the Snook TX study site. Only the early sampling significantly ($\alpha \leq 0.05$) and positively correlated with both rate and depth of P fertilizer placement

Regression Analysis of Leaf Tissue Concentration revealed significant relationships between P uptake in corn at both sites as a function of both rate and depth of placement of liquid P fertilizer (Figures 3a & 3b). In the equation:

$$P_{\text{conc}} = a * \text{rate} + b * \text{depth} + c$$

Where a, b, & c are constant parameters fit to the observed data resulted in equations where all fit parameters were significant at the $\alpha = 0.10$ level except for the case of the late tissue sampling at the Thrall TX study site. Parameters for the early tissue sampling event at the same site were significant at the $\alpha = 0.05$, however. The Thrall TX site is on a poorly drained soil and experienced several severe rainfall events between early and late sampling events that left the soil ponded and/or saturated for prolonged periods of time. This condition resulted in increased variability within treatments across blocks that can explain the poor relationship in the late tissue sampling regression results as well as the mixed results in grain yield from the same site (Figure 2b).

Conclusions

- Open source software such as ImageJ can be used effectively to evaluate differences in root growth patterns
- Lower root densities observed near the concentrated zone of banded P fertilizer can be explained as a 'rhizo-economic' reaction, where less energy is necessary to extract localized nutrients
- An inhibition of root growth due to the salt effect can be rejected due to the significant and positive effects of both rate and placement on corn P uptake, as indicated by both early and late season tissue analysis

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