

Phosphorus fertilizer placement and rate affect soybean root growth and nutrient uptake in soil with high fertility

Fernando Dubou Hansel^{1*}, Dorivar Ruiz Diaz¹, Alexandre Tonon Rosa² and Colby Moorberg¹

¹Department of Agronomy, Kansas State University, 2713 Throckmorton Hall, Manhattan, Kansas, United States, 66506-5501. ²Department of Agronomy, University of Nebraska, 202 Keim Hall, Lincoln, Nebraska, United States, 68583-0915.

*Corresponding author. Email: fernandodhansel@ksu.edu

ABSTRACT

Phosphorus (P) fertilizer management can alter soybean shoot and root growth promoting morphological imbalances in the plant. In order to assess soybean (*Glycine max* (L.)) morphological adjustments to different placements and rates of P fertilization in high soil test P, a greenhouse study was conducted with two primary objectives: 1) evaluate the effect of P fertilization on root and shoot biomass accumulation and the associated changes on root length; and, 2) estimate the effect of root growth changes on the macro and micronutrients uptake in the plant. Fertilizer treatments were: (1) broadcast P on soil surface (BR), (2) band-applied P 5x5 cm (B), and (3) deep band P at 20 cm depth (DB); using two rates: (1) 60 and (2) 120 kg P₂O₅ ha⁻¹ in soil with high fertility. Minirhizotron images and SPAD measurements were performed once a week until flowering stage. Root and shoot dry weight, as well as total macro and micronutrients uptake were evaluated at the same stage. The increase of P levels in the soil promoted by fertilization shows a negative effect on root dry weight at the rate of 60 kg P₂O₅ ha⁻¹, and very little stimulus to biomass allocation in the roots when P rate was increased to 120 kg P₂O₅ ha⁻¹ in B and DB treatments. The control treatment (no fertilizer) showed 108% higher root length than B-60 treatment. These changes also altered macro and micronutrients uptake and affected chlorophyll content in the soybean plants.

Key words: High soil test P, mineral fertilization, morphological imbalances, P fertilization strategy, root system growth, soybeans.

INTRODUCTION

Plants are part of a complex and competitive environment where all biological processes are in balance, saving energy and recycling nutrients to ensure vital functions (Marschner et al., 1996). Changes in the environment such as temperature, light intensity, water and nutrients availability, will require physiological and morphological adjustments in the plant for the efficient use of resources (Hermans et al., 2006). Thus, photosynthates and nutrients in the plant can be retranslocated to organs involved in acquiring resources as they become scarcer (Marschner et al., 1996). For example, plants will allocate more biomass to roots if the limiting factor for growth are nutrients or water (Poorter et al., 2012). This biomass balance in the plant is known as “functional equilibrium concept” of biomass allocation (Thornley 1972; Iwasa and Roughgarden 1984) or balanced growth hypothesis (Shipley and Meziane 2002). Increased availability of nutrients as a result of fertilization in annual cropping systems can promote changes in root growth (Williamson et al., 2001). With increased access to nutrients, plants could reallocate resources from root growth towards the growth of other organs in the shoot with greater nutrient demand, generating an imbalanced shoot-root ratio (Poorter et al., 2012).

Immobile nutrients such as phosphorus (P), when applied as mineral fertilizers, promote a local increase in soil test P levels (Nunes et al., 2011). Thus, P fertilizer management has the potential to significantly affect the root morphology and crop growth (Hansel et al., 2017b). Root system exposure to soil high phosphate concentration zones cause localized increases of initiation and subsequent extension of the primary and secondary roots when compared to the low concentration zone (Drew 1975; Salisbury and Ross 1992). However, root growth stimulation in treatments with low P concentration has also been reported (López-Bucio et al., 2003; Muller and Schmidt 2004) where physiological and hormonal signals are

involved in promoting root growth (Nacry 2005; Péret et al., 2011). The resulting increased root surface area improves P uptake. As a consequence of root architecture, variations in some levels of water and other nutrients uptake could be verified (López-Bucio et al., 2003).

The P fertilization, even in high soil test P, has become a common practice among farmers due to the increase of nutrient removal by modern soybean varieties, and also to the low P availability in the soil. However, inappropriate fertilization practice under these conditions can dangerously affect root growth and plant adaptability under extreme environmental conditions. Therefore, to better understand the effect of P placements and rates fertilization in the soybean morphology in high soil test P, a greenhouse study was conducted with two primary objectives: 1) evaluate the effect of P fertilization on root and shoot biomass accumulation, and the associated root length changes; and, 2) estimate the effect of root growth changes on the macro and micronutrients uptake in the plant.

MATERIALS AND METHODS

Experimental design

The study was carried out during the 2015/16 winter season under a controlled greenhouse environment at Kansas State University, Manhattan, Kansas. The experimental design was completely randomized with four replications. Treatments consisted of three P fertilizer placements: (1) broadcast on the soil surface (BR), (2) band-applied 5 cm deep and 5 cm to the side of the seed (5 × 5) (B), and (3) deep band at 20 cm depth (DB). Triple superphosphate [(0-46-0), (N-P₂O₅-K₂O)] was applied using two rates: (1) 60 and (2) 120 kg P₂O₅ ha⁻¹. There was an additional treatment with 0 kg P₂O₅ ha⁻¹ (control). Large polyvinyl chloride (PVC) columns were used to grow soybean. Columns were 100 cm high and 20 cm in diameter to allow root growth without physical impedance. Each column received approximately 20 kg of blended soil, being 50% Eudora silt loam soil (fine-silty, mixed, superactive, hyperthermic Fluventic Hapludolls) from Rossville, KS (39°08'10"N; 95°57'06"W), 25% the growing media Metro-Mix® (Sungro Horticulture, Agawam, MA, USA), and 25% sand (<2 mm). The soil was blended to achieve a homogeneous material using a large Davis precision horizontal batch mixer for 5 minutes (model SD-5, Bonner Springs, KS, USA). Four blended samples were analyzed for extractable P determined by the Mehlich-3 method (Frank et al., 1998), and analyzed using inductively coupled plasma (ICP) spectrometer (720-ES ICP; Varian Australia Pty Ltd, Mulgrave, Victoria, Australia). Extractable potassium (K) was determined by ammonium acetate method (Warncke and Brown 1998). Soil pH was measured using a 1:1 soil-water ratio (Watson and Brown 1998), and soil organic matter (OM) was determined by loss on ignition (Hoskins 2002). The soil blend had 47 mg kg⁻¹ P, 243 mg kg⁻¹ K, pH 7.1 and 55.1 g kg⁻¹ organic matter. The fertilizer rate was calculated considering a surface area of 0.10 m² column⁻¹. This area corresponds to the row length (15 cm) and the distance between plants (70 cm) under field conditions according to usual practice.

Sampling and analyses

A transparent, acrylic minirhizotron tube was placed vertically inside each growing column to facilitate root imaging with the CI-600 In-Situ Root Imager (CID Bio-Science, Inc., Washington, USA) minirhizotron camera throughout the soybean development (Figure 1). Water was used to promote densification of the blend before planting. Final soil bulk density was approximately 1.4 g cm⁻³. After the soil blend volume stabilized, fertilizer was applied using a stake to open the row and locate the fertilizer. Soybean was sown with three seeds per column and thinned to one seedling after germination. The soybean genotype used was NK S45-V8 (Syngenta Seeds, Minneapolis, MN, USA) maturity group 4.5. Irrigation was provided with 400 mL per column at intervals of two days based on crop demand. The temperature was set to be 18.3°C at night and 26.7°C during the day.

The photoperiod was controlled to induce flowering 50 days after planting, starting at 16 hours of daylight and dropping every 2 weeks to reach 13 hours (Figure 2). Roots were imaged once a week at depth intervals of 0 to 22 cm, 22 to 44 cm, and 44 to 66 cm. The images collected were analyzed using the software Root Snap!™ version 1.2.8.23 (CID Bio-Science Inc., Camas, WA, USA) provided by the camera manufacturer, which requires manual digitization of roots.

Root length and other characteristics were summarized by the software at depth interval as well as imaging session, once the images were digitized. SPAD chlorophyll meter readings were collected on the 3rd

to the 4th trifoliolate from the top, with five repetitions at the time of the weekly root imaging sessions.

Soybean plants were sampled at the R2 growth stage (Pedersen 2003) and partitioned into shoot and root parts. Root samples were pre-cleaned in the greenhouse to remove most of the soil, and later tap water was used to separate the remaining soil. Shoots and roots parts were dried at 65°C for six days and



Figure 1. Transparent acrylic minirhizotron tube placed vertically inside each growing column to facilitate root imaging. Manhattan, Kansas, United States.

weighed to get the total shoot and root dry weight. The roots and shoots were ground to pass through a 2 mm-sieve and analyzed for total nutrient content. Total N, P, and K were analyzed by using the sulfuric peroxide digest as described by (Lindner and Harley 1942). Nitrogen digest was analyzed by an indophenol blue colorimetric procedure using the Rapid Flow Analyzer (Model RFA-300; Alpkem Corporation, Clackamas, Oregon, USA). Total P and K were determined using a coupled plasma (ICP) spectrometer (720-ES ICP) inductively; Varian Australia Pty Ltd, Mulgrave, Victoria, Australia). Analysis of sulfate (SO₄), Manganese (Mn) and Zinc (Zn) was done using the perchloric digest following the method of (Gieseking et al., 1935) and analyzed by using an inductively coupled plasma (ICP) spectrometer (720-ES ICP; Varian Australia Pty Ltd, Mulgrave, Victoria, Australia).

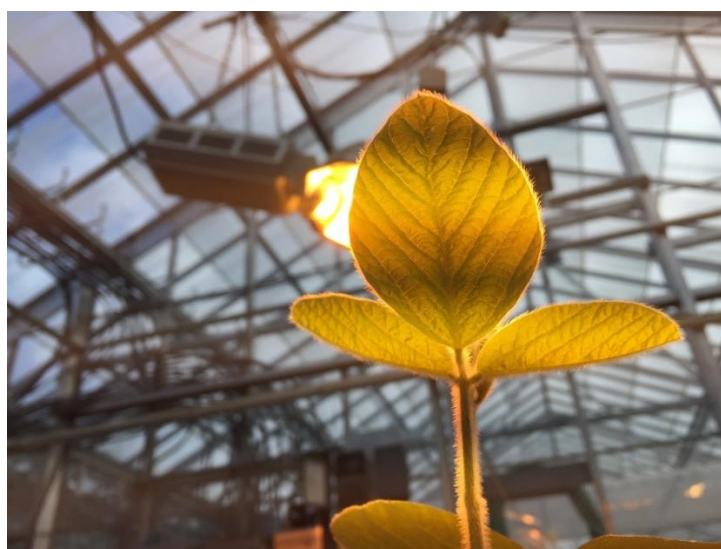


Figure 2. Controlled greenhouse environment at Kansas State University. The photoperiod was controlled to induce flowering 50 days after planting. Manhattan, Kansas, United States.

Statistical analysis

The effects of P placement and rate on soybean root length, shoot and root growth, as well as nutrients

uptake were determined by Anova in the R statistical environment (R Development Core Team 2009). When interactions or main effects were significant, multiple means comparisons among treatments including the control treatment were conducted by performing post-hoc Tukey's test, using the Agricolae package (Mendiburu 2010). Significant differences were established at $P < 0.05$.

RESULTS AND DISCUSSION

Shoot and root dry weight

The P placement and fertilization rates significantly affected soybean root length at different growth stages, as well as shoot and root nutrients uptake and dry weight at the R2 growth stage (Table 1).

The increase of P levels in the soil after fertilizer application showed a negative effect on root dry weight (RDW) at 60 kg P_2O_5 ha^{-1} rate and a very little stimulus to biomass allocation in the roots when P rate was increased to 120 kg P_2O_5 ha^{-1} in the B and DB treatments (Figure 3). More drastic effects in RDW were found for B and DB, using 60 kg P_2O_5 ha^{-1} with a reduction of 41 and 31% compared to the control, respectively, due to the proximity of the roots to the fertilized area.

Soybean shoot-root dry weight ratio showed an imbalance in biomass allocation promoted by P fertilization strategy, which could be associated with changes in root growth. In the R with 60 kg P_2O_5 ha^{-1} treatment, the lower root dry weight is likely to be the main factor responsible for the greater shoot-root ratio among treatments (Figure 3). However, when the rate was increased to 120 kg P_2O_5 ha^{-1} there was a rebalance of biomass.

Root system growth has a high build-up and maintenance costs (Eissenstat and Yanai 1997). Reduction in root biomass under higher STP levels might occur because the plant does not need to invest in energy to build up root biomass when there are enough roots to supply the plant nutrient (Hansel et al., 2017b). On the other hand, broadcast P did not affect root dry weight (Figure 3), since most of root system was not in contact with the fertilized area of "high availability", effect that was observed in the band treatments (Nacry 2005). As a consequence, changes in the shoot-root ratio are expected. Signals for root biomass allocation can be sent by the increase of carbohydrates concentration in the shoots (Hermans et al., 2006). According to Poorter et al. (2012), due to the balance concept, even for plants being supplied with unlimited nutrients or photosynthates, it is necessary to maintain a balance among functions and organ functions to the same degree. And the total shoot activity is proportional to the total root activity (Thornley 1972). Therefore, more biomass is located in the roots to compensate the imbalance promoted by the shoots.

Root length

Soybean root changes occurred throughout the growing season, suggesting different stimulus for root growth by P placement and rate strategies. However, the effect of P management was observed only at the beginning of the V5 development stage in soybean root length (Table 1). The control treatment (0 kg P_2O_5 ha^{-1} rate) showed increased root length, 108% higher than the B-60 treatment (lower root length) in the R2 soybean growth stage. However, the increase of P rate to 120 kg P_2O_5 ha^{-1} in B promoted a stimulus for root development observed in the beginning of the V5 growth stage. There was no difference between the control and B with 120 kg P_2O_5 ha^{-1} in R2 growth stage (Table 2).

In unfertilized soils, plants increase root growth adjusting the root system architecture to maximize interception of nutrient (Lynch and Brown 2001). Higher P concentration promoted by P fertilization, in general, resulted in a reduction of root growth. This behavior, in a soil with initial high P level represents the plant capacity to adopt a cost-effective model and save energy for another use (Sun et al., 2017). The drastic reduction observed in the B-60 treatment can be related to the close contact between fertilizer placement and plant root which increased P availability in the plant (Borkert and Barber 1985). According to Thibaud et al. (2010) 70% of P-responsive genes (genes with roles in P availability sensing) are locally controlled by external P availability. Thus, root tips are sensitive to P availability and adjust the root apical meristem activity accordingly (Abel 2011).

The effect of high P concentrations promoting stimulus to root growth had been reported in previous studies (Drew 1975; Anghinoni and Barber 1980). The antagonism of root growth behavior in our study led to the hypothesis that root growth stimulus can be dependent on the P application rate. Clearer effects are

observed when the fertilized zone is close to the root zone. However, it is still not possible to predict the magnitude of changes based on the P rates.

Table 1. F value significance for P placement and rate on soybean root length listed according to growth stages. Shoot and root nutrients uptake and dry weight at R2 growth stage.

Parameters	Fixed Effect		
	Placement (P)	Rate (R)	P × R
	----- p>F -----		
	<u>Root length</u>		
Growth stage			
V2	0.336	0.453	0.365
V3	0.297	0.565	0.064
V5	0.014	0.789	0.007
V7	0.008	0.050	0.006
V8	0.005	0.099	0.001
R2	0.305	0.003	0.012
	<u>Shoot biomass</u>		
Uptake			
N	0.092	0.230	0.025
P	0.246	<0.001	0.452
K	0.010	0.099	0.437
S	0.106	0.010	0.198
Mn	0.015	0.015	0.432
Zn	0.264	0.168	0.825
Dry weight	0.037	0.129	0.229
	<u>Root biomass</u>		
Uptake			
N	0.070	0.460	0.001
P	0.600	0.002	0.027
K	0.195	0.802	0.007
S	0.147	0.451	0.007
Mn	0.551	0.476	0.477
Zn	0.369	0.687	0.357
Dry weight	0.083	0.172	0.001

Macro and micronutrients uptake

Phosphorus placement and rates affected shoot and root P content in the tissue as well as nitrogen, potassium and sulfur in the plant (Figure 4 and 5). Phosphorus content in the shoot tissue went up with corresponding increases in P fertilization rate. Also, P placements, that promoted soil zones with high concentration in contact with roots, showed greater P uptake. This increase might be expected due to the fertilizer proximity to the roots and the increase of P fertilizer rates, promoting an increase in P availability to plants (Borkert and Barber 1985), resulting in greater P uptake. The DB with 120 kg P₂O₅ ha⁻¹ treatment showed the greatest P uptake among treatments, which is in consonance with the results found by Hansel et al. (2017a).

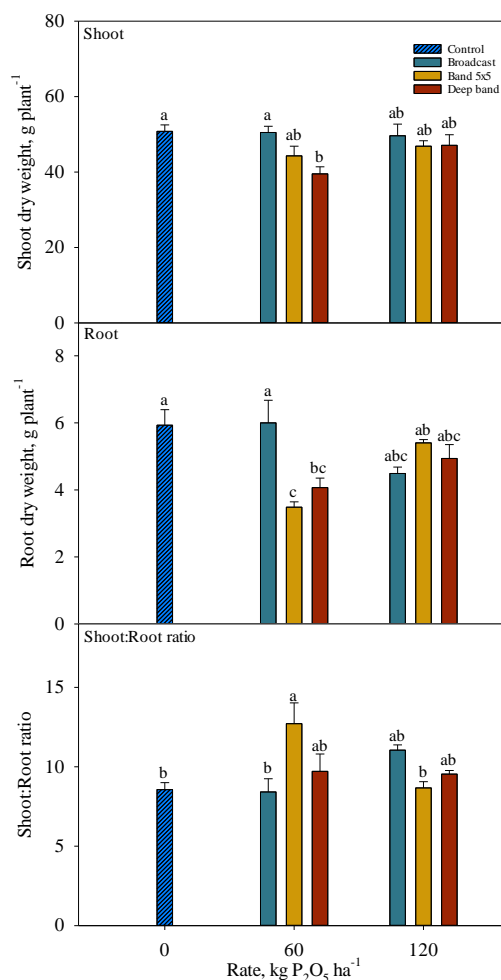


Figure 3. Soybean shoot and root dry weight and shoot-root ratio submitted to different P placement and rate in high soil test P environment. Values followed by same letter indicate no significant difference at the $p \leq 0.05$ probability level.

Table 2. Soybean root length from V2 to R2 growth stages submitted to different P placement and rate strategies in high soil test P environment.

Placement	Rate (kg ha ⁻¹)	Growth stage					
		V2	V3	V5	V7	V8	R2
-----Root length (cm)-----							
Broadcast	60	666	1073	1845 a ¹	2983 a	4081 a	5865 a
Band	60	471	612	819 b	1216 b	1993 c	3914 b
Deep band	60	443	826	1195 ab	1820 ab	2748 abc	4722 ab
Broadcast	120	582	897	1386 ab	2627 a	3650 ab	5703 ab
Band	120	680	1060	1528 ab	2833 a	4298 a	6572 a
Deep band	120	506	739	1048 b	1944 ab	2318 bc	5704 ab
Control	-	625	936	1616 a	3178 a	4178 a	8134 a
Broadcast	-	625	985	1616 a	2805 a	3866 a	5784 b
Band	-	576	836	1174 b	2025 b	3146 b	5244 b
Deep band	-	475	783	1122 b	1882 b	2534 b	5214 b
-	0	625	936	1616	3178	4178	8134 a
-	60	527	837	1286	2007	2941	4834 c
-	120	590	899	1321	2468	3422	5993 b

¹Values followed by the same letter indicate no significant difference at the $p \leq 0.05$ probability level.

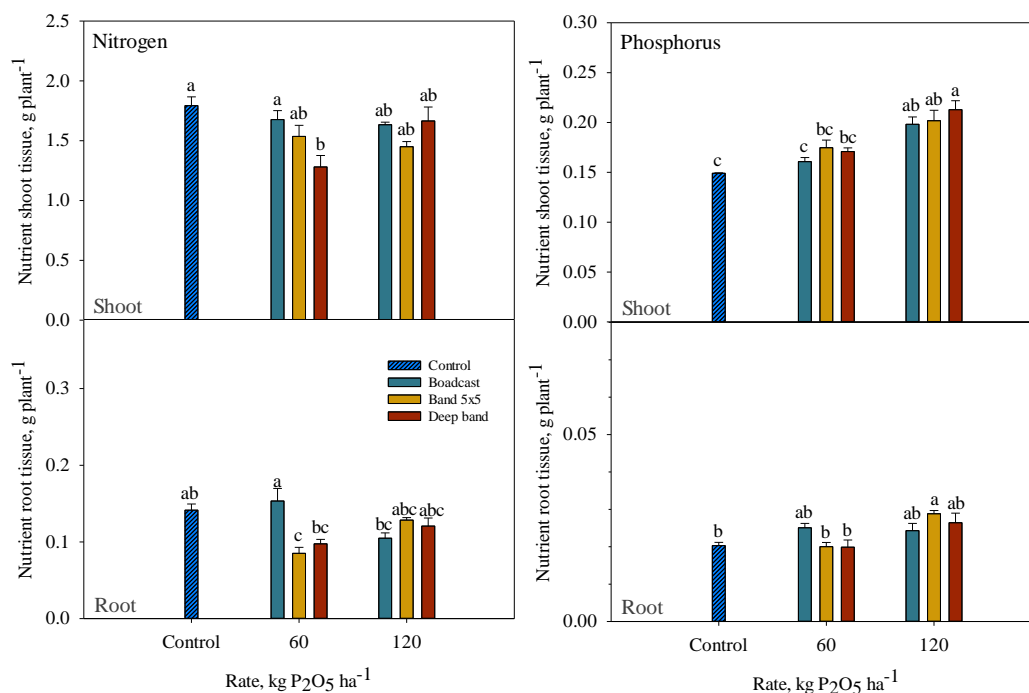


Figure 4. Nitrogen and phosphorus uptake influenced by different P placement and rate in high soil test P environment. No fertilization (Control). Values followed by same letter indicate no significant difference at the $p \leq 0.05$ probability level. ^{ns} not significant.

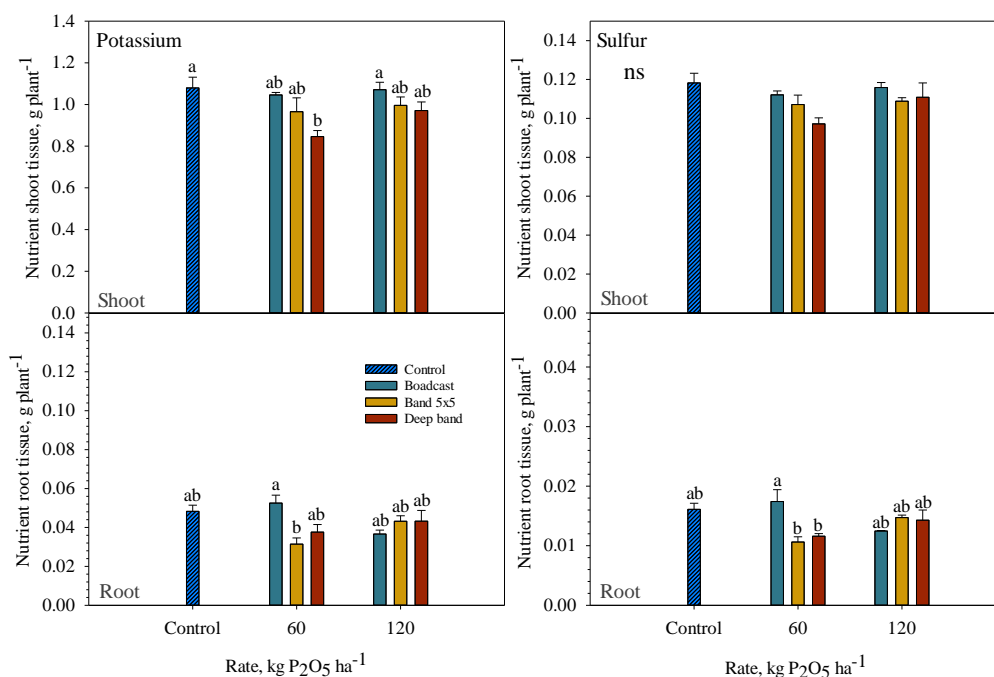


Figure 5. Potassium and sulfur uptake influenced by different P placement and rate in high soil test P environment. No fertilization (Control). Values followed by same letter indicate no significant difference at the $p \leq 0.05$ probability level. ^{ns} not significant.

The N and K content in the tissue were in agreement with differences observed in SDW and RDW (Figure 3), in which higher nutrient content was found in greater plant dry weight values. Total crop biomass is considered the driving force which provides structure for nutrient accumulation (Bender et al., 2015). However, lower root growth exhibited by the 60 kg P₂O₅ ha⁻¹ rate in B and DB treatments likely reduced plant capacity to explore the soil, and thus take up nutrients (Barber and Silverbush 1984). Sulfur tissue differences were found in root tissue content between placements at the 60 kg P₂O₅ ha⁻¹ rate (Figure 5). Under these conditions, it was observed a 54.5% greater S content in BR compared to the other banded treatments (B and DB). The control treatment showed 38 and 37% greater N and K in the shoot tissue

compared to DB with 60 kg P₂O₅ ha⁻¹, respectively (Figure 4 and 5).

The effect of P placement and rate in the analyzed micronutrients were observed only for Mn in the shoot plant tissue (Figure 6). There was an increase of 11.8% in the Mn shoot content with the rise in P rate from 60 to 120 kg P₂O₅ ha⁻¹. Greater P fertilizer rates could have contributed to the increase in Mn uptake. The solubilization reaction of the superphosphate granule can result in a local soil acidification (Hansel et al., 2014), which could have led to an increase in Mn availability in the soil. In this study, no changes were observed in tissue Zn content as an effect of P treatments.

Root growth related to SPAD measurements

Plant root growth was sensitive to conditions imposed by the fertilizer treatment applications altering root length growth throughout soybean plant development (Table 2). The SPAD measurements were collected keeping the same timing of when root images were generated and revealed a narrow correlation between these two parameters (Figure 7). At the V2 soybean grow stage the correlation between root length and SPAD measure were 72% ($p < 0.001$). At the V5 the correlation was about 83% and at the V7 of 79% (both $p < 0.001$).

The SPAD measurements showed a strong correlation with leaf photosynthesis in soybeans (Ma et al., 1994). The availability of mineral nutrients, particularly N and P, can be a limiting factor for photosynthetic activity (BassiriRad et al., 2001), where greater nutrient uptake usually is correlated with greater root growth (Barber and Silverbush 1984). Also, SPAD measurements have high correlation with N content in the tissue (Chapman and Barreto 1997). A greater root length observed in treatments with higher SPAD values could indicate a more aggressive soil exploration and consequently greater N uptake. However, is not possible to corroborate from this study if the N in the plant is primarily uptake from the soil pool or from an increased rhizobium symbiosis promoted by nutrient availability and higher interaction between roots and soil.

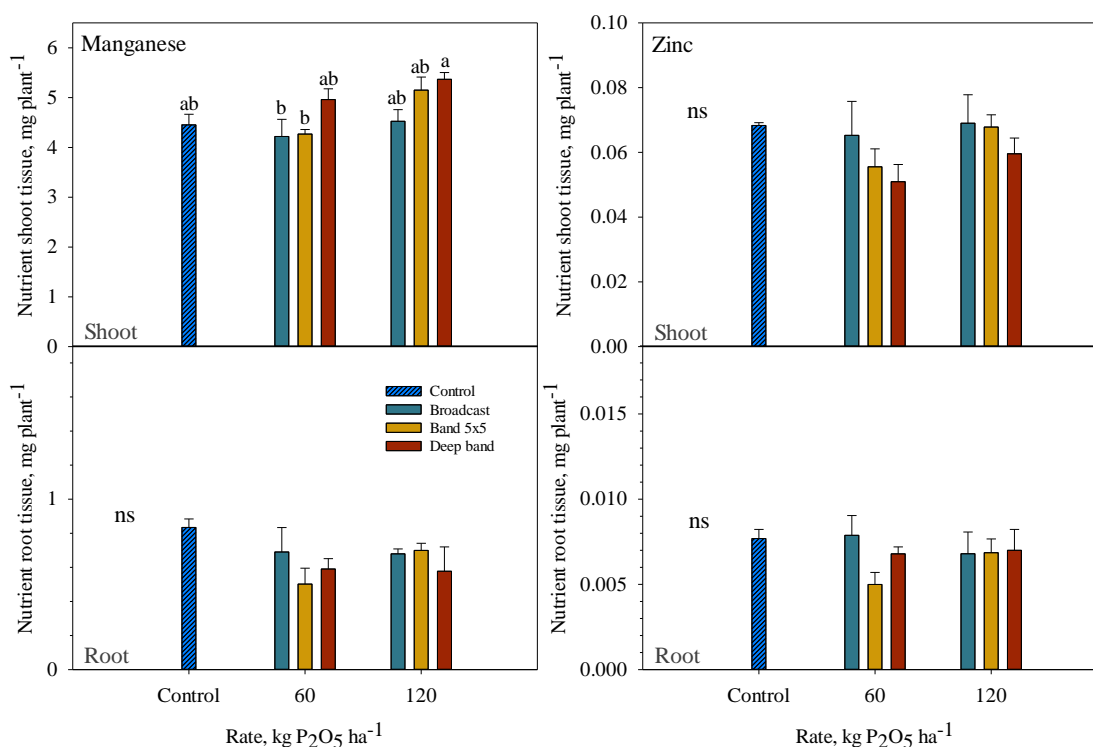


Figure 6. Manganese and zinc uptake influenced by different P placement and rate in high soil test P environment. No fertilization (Control). Values followed by same letter indicate no significant difference at the $p \leq 0.05$ probability level. ^{ns} not significant.

In our study, all reported changes in RDW and root length promoted by P fertilizer management, which also affected nutrient uptake, showed indirect effect on chlorophyll meter readings in the soybean plants (Figure 7). Thus, direct and indirect observed effects can be associated with plant health (Hendry and Price 1993), and might alter the plant resilience to environmental stresses in the field.

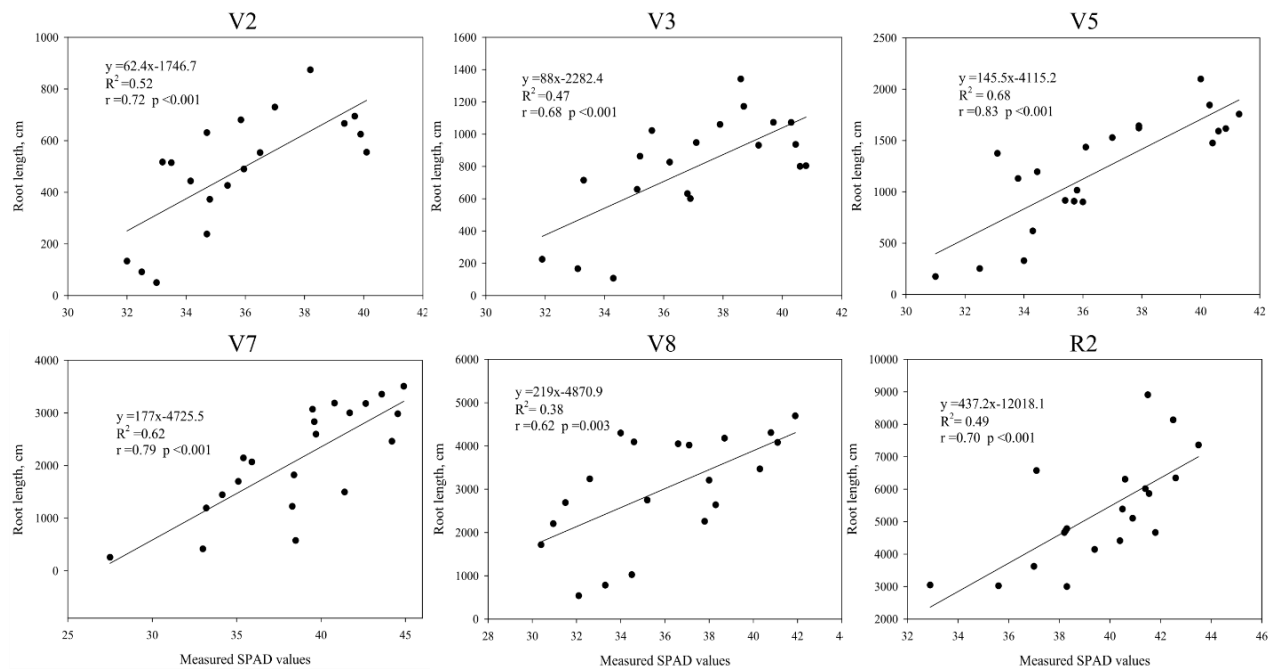


Figure 7. Pearson's correlation between soybean measured SPAD values and root length at different soybean vegetative growth stages submitted to different P placement and rate in high soil test P environment.

CONCLUSIONS

Phosphorus placement and application rates promoted changes in the soybean shoot and root growth, in macro and micronutrients uptake, as well as in chlorophyll meter readings in the plants. The increase of P levels in the soil promoted by fertilization showed a negative effect on RDW at the rate of 60 kg P₂O₅ ha⁻¹ and a slight stimulus to biomass allocation in the roots when the P rate was increased to 120 kg P₂O₅ ha⁻¹ for the B and DB treatments. These changes generated a shift in the balance of shoot-root ratio in the plants. The control treatment (no fertilizer) showed 108% higher root length than the B with 60 kg P₂O₅ ha⁻¹ treatment. These morphological changes altered the plant capacity for macro and micronutrients uptake. There was a rise in P uptake with the increase of P fertilizer application rate. Nitrogen and K content in the tissue were greater and equivalent to the differences observed in SDW and RDW. Significant correlations were found between root length growth and leaf chlorophyll meter readings, suggesting that P managements also affected overall soybean plant health. Therefore, the direct and indirect effects promoted by P fertilization strategies can be particularly relevant under extreme environmental conditions in the field.

ACKNOWLEDGMENTS

The authors thank Dr. S.V. Krishna Jagadish, associate professor at Kansas State University, and members of the plant physiology lab for the help and support on this project. Contribution no. 19-179-J from the Kansas Agricultural Experiment Station.

REFERENCES

- Abel S (2011) Phosphate sensing in root development. *Current Opinion Plant Biology* 14: 303-309.
- Anghinoni I and Barber SA (1980) Phosphorus influx and growth characteristics of corn roots as influenced by phosphorus supply. *Agronomy Journal* 72: 685-688.
- Barber SA and Silverbush M (1984) Plant root morphology and nutrient uptake. In: Barber SA, Bouldin DR, Kral DM and Hawkins SL (eds.) *Roots, Nutrient and Water Influx, and Plant Growth*. ASA Special American Society of Agronomy, Madison, WI. p. 65-88.

- BassiriRad H, Gutschick VP and Lussenhop J (2001) Root system adjustments: Regulation of plant nutrient uptake and growth responses to elevated CO₂. *Acta Oecologia* 126: 305-320.
- Bender RR, Haegele JW and Below FE (2015) Nutrient uptake, partitioning, and remobilization in modern soybean varieties. *Agronomy Journal* 107: 563-573.
- Borkert CM and Barber SA (1985) Soybean Shoot and Root Growth and Phosphorus Concentration as Affected by Phosphorus Placement. *Soil Science Society of America Journal* 49: 152-155.
- Drew MC (1975) Comparison of the effects of a localized supply of phosphate, nitrate, ammonium and potassium on the growth of the seminal root system, and the shoot, in barley. *New Phytologist* 75: 479-490.
- Chapman SC and Barreto HJ (1997) Using a Chlorophyll Meter to Estimate Specific Leaf Nitrogen of Tropical Maize during Vegetative Growth. *Agronomy Journal* 89:557-562.
- Eissenstat DM and Yanai RD (1997) The Ecology of Root Lifespan. *Advances in Ecological Research* 27: 1-60.
- Frank K, Beegle D and Denning J (1998) Phosphorus. In: Brown JR (ed.), *Recommended chemical soil test procedures for the North Central Region*. North Cent. Missouri Agric. Exp. Stn, Columbia, Missouri. p. 21-30.
- Gieseking JE, Snider HJ and Getz CA (1935) Destruction of organic matter in plant material by the use of nitric and perchloric acid. *Industrial & Engineering Chemistry Analytical* 7: 185-186.
- Hansel FD, Amado TJC, Bortolotto RP, Trindade BS and Hansel DSS (2014) Influence of different phosphorus sources on fertilization efficiency. *Applied Research & Agrotecnology* 7: 103-111.
- Hansel FD, Ruiz Diaz DA, Amado TJC and Rosso LHM (2017a). Deep Banding Increases Phosphorus Removal by Soybean Grown under No-Tillage Production Systems. *Agronomy Journal* 109: 1-8.
- Hansel FD, Amado TJC, Ruiz Diaz DA, Rosso LHM, Nicoloso FT and Schorr M (2017b) Phosphorus fertilizer placement and tillage affect soybean root growth and drought tolerance. *Agronomy Journal* 109: 1091-1099.
- Hendry GAF and Price AH (1993) Stress indicators: chlorophylls and carotenoids. In: Hendry GAF and Grime JP (eds.) *Methods in Comparative Plant Ecology*. Chapman & Hall, London. p. 148-152.
- Hermans C, Hammond JP, White PJ and Verbruggen N (2006) How do plants respond to nutrient shortage by biomass allocation? *Trends in Plant Science* 11: 610-617.
- Hoskins B (2002) *Organic Matter by Loss on Ignition*. University of Maine.
- Iwasa Y and Roughgarden J (1984) Shoot/root balance of plants: Optimal growth of a system with many vegetative organs. *Theoretical Population Biology* 25: 78-105.
- Lindner RC and Harley CP (1942) A Rapid Method for the Determination of Nitrogen in Plant Tissue. *Science* 96: 565-566.
- López-Bucio J, Cruz-Ramírez A and Herrera-Estrella L (2003) The role of nutrient availability in regulating root architecture. *Current Opinion Plant Biology* 6: 280-287.
- Lynch JP and Brown KM (2001) Topsoil foraging - An architectural adaptation of plants to low phosphorus availability. In *Plant and Soil*. p. 225-237.

- Ma BL, Morrison MJ and Voldeng HD (1994) Leaf Greenness and Photosynthetic Rates in Soybean. *Crop Science Society of America* 35: 1411-1414.
- Marschner H, Kirkby E and Cakmak I (1996) Effect of mineral nutritional status on shoot-root partitioning of photoassimilates and cycling of mineral nutrients. *Journal Experimental Botany* 47: 1255-1263.
- Mendiburu F (2010) *Agricolae: Statistical Procedures for Agricultural Research*. R Package Version 1.0-9. Accessed December 18, 2016. <http://CRAN.R-project.org/package=agricolae>
- Muller M and Schmidt W (2004) Environmentally induced plasticity of root hair development in *Arabidopsis*. *Plant Physiology* 134: 409-419.
- Nacry P (2005) A Role for Auxin Redistribution in the Responses of the Root System Architecture to Phosphate Starvation in *Arabidopsis*. *Plant Physiology* 138: 2061-2074.
- Nunes RS, Sousa DMG, Goedert WJ and Vivaldi LJ (2011) Distribuição de fósforo no solo em razão do sistema de cultivo e manejo da adubação fosfatada. *Revista Brasileira de Ciencia do Solo* 35: 877-888.
- Pedersen P (2003) *Soybean Growth and Development*. Iowa State University Extension Publication. Iowa State University, Ames, IA.
- Péret B, Clément M, Nussaume L and Desnos T (2011) Root developmental adaptation to phosphate starvation: Better safe than sorry. *Trends in Plant Science* 16: 442-450.
- Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P and Mommer L (2011) Biomass allocation to leaves, stems and roots: meta-analysis of interspecific variation and environmental control. *Phytologist* 193: 30-50.
- R Development Core Team (2009) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Salisbury FB and Ross CW (1992) *Plant physiology*. 4th ed. Belmont, CA. Wadsworth.
- Shipley B and Meziane D (2002) The balance-growth hypothesis and the allometry of leaf and root biomass allocation. *Functional Ecology* 16: 326-331.
- Sun C-H, Yu J-Q and Hu D-G (2017) Nitrate: A Crucial Signal during Lateral Roots Development. *Frontiers in Plant Science* 8: 485.
- Thibaud MC, Arrighi JF, Bayle V, Chiarenza S, Creff A, Bustos R, Paz-Ares J, Poirier Y and Nussaume L (2010) Dissection of local and systemic transcriptional responses to phosphate starvation in *Arabidopsis*. *Plant Journal* 64: 775-789.
- Thornley JHM (1972) A Balanced Quantitative Model for Root: Shoot Ratios in Vegetative Plants. *Annals of Botany* 36: 431-441.
- Warncke D and Brown JR (1998) Potassium and other basic cations. In: Brown JR (ed.) *Recommended chemical soil test procedures for the North Central Region*. North Cent. Missouri Agric. Exp. Stn., Columbia, Missouri, p. 31-34.
- Watson ME and Brown JR (1998) pH and lime requirement. In: Brown JR (ed.) *Recommended chemical soil test procedures for the North Central Region*. North Cent. Missouri Agric. Exp. Stn, Columbia, Missouri, p. 13-16.
- Williamson LC, Ribrioux S, Fitter AH and Leyser HMO (2001) Phosphate availability regulates root system architecture in *Arabidopsis*. *Plant Physiology* 126: 875-882.

Received: March 22, 2019.

Accepted: May 7, 2019.

Published: June 28, 2019.