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Phosphorus and Zinc Effects on Soybean [Glycine Max (L.) Merrill] Yield and Agronomic Efficiency

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PHOSPHORUS AND ZINC EFFECTS ON SOYBEAN [*GLYCINE MAX* (L.) MERRILL] YIELD AND AGRONOMIC EFFICIENCY

GODFRED ANKOMAH

44 Pages

Phosphorus (P) and zinc (Zn) are required for the growth and development of soybean (*Glycine max* (L.) Merrill). However, their interactions may affect the uptake of each nutrient and soybean growth, development, and yield. The objective of this research study was to identify possible interactions of P and Zn and determine the effect upon soybean nutrient status, yield, and agronomic efficiency. This study was conducted at the Illinois State University Farm at Lexington and Normal in 2020. The experimental design was a 4 × 4 factorial arranged in a randomized complete block design with four blocks at each location. Phosphorus was applied as mono-ammonium phosphate (MAP) at 0, 33, 67, 100 kg P ha^{-1} and zinc was applied as zinc sulfate ($ZnSO_4$) at 0, 5, 11, 16 kg Zn ha^{-1}. Fully developed trifoliate leaves from the top three nodes were collected at R2 and seed samples were taken at R6 for analysis of P and Zn. Phosphorus fertilization had no effect on the P concentration of leaves but increased the P concentration of the seeds. The P concentration of the seeds for the highest rate (100 kg P ha^{-1}) was greater than the control (0 kg P ha^{-1}) and the lowest P rate (33 kg P ha^{-1}). Zinc application had no effect on Zn concentration of soybean leaves and seeds. Neither P nor Zn affected soybean yield components and yield. Phosphorus and zinc agronomic efficiency did not improve with P and Zn application, respectively.

There was no soil P-Zn interaction that affected soybean growth and yield. However, there was a weak inverse correlation between leaf P concentration and seed Zn concentration (r = -0.43), and between leaf Zn concentration and seed P concentration (r = -0.30). This study revealed that soybean yield and agronomic efficiency do not respond positively to P and Zn application when the soil test indicates P and Zn are sufficient.

KEYWORDS: Nutrient Interaction, Synergism, Antagonism, Agronomic Efficiency, Phosphorus Agronomic Efficiency, Zinc Agronomic Efficiency, Monoammonium Phosphate (MAP), Zinc Sulphate ($ZnSO_4$), Yield

PHOSPHORUS AND ZINC EFFECTS ON SOYBEAN [*GLYCINE MAX* (L.) MERRILL] YIELD AND AGRONOMIC EFFICIENCY

GODFRED ANKOMAH

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GODFRED ANKOMAH

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G.A.

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CHAPTER I: INTRODUCTION

Phosphorus is essential nutrient for photosynthesis, respiration, energy storage and transfer, cell division and enlargement (Li et al., 1998). Phosphorus deficiency can severely limit biological nitrogen fixation (Weisany et al., 2013). Phosphorus deficiency can result in stunted growth and dark green or purple coloration of leaves.

Zinc is a key constituent of many enzymes and proteins, and it is needed in carbohydrate metabolism, protein metabolism, flowering, and seed production (Alloway, 2008). Zinc deficiency leads to interveinal chlorosis or browning which is initially observed on lower leaves, gradually results in leaf necrosis, and may reduce yields (Rao and Reddy, 2010)

Nutrient interaction occurs when the application of one nutrient affects the uptake and function of another nutrient in the plant (Rietra et al., 2017). Mineral nutrients can interact in three possible ways: zero (no) interaction, synergism, and antagonism (Aulakh and Malhi, 2005; Fageria, 2001; Sumner and Farina, 1986). There is no interaction when the yield increase from the addition of two nutrients is the same as the sum of the increase observed when either nutrient alone is added. Synergism occurs when the yield response of two nutrients applied together is greater than the sum of the yield response of the individual nutrients. Antagonism occurs when two nutrients applied together produce less yield compared to the sum of the individual nutrient response.

Antagonism of P and Zn is not uncommon in agronomic systems (Loneragan and Webb, 1993; Olsen, 1972; Payne et al., 1986). Phosphorus-zinc antagonism occurs when Zn concentration is low in the soil and P concentration in the soil is high or when excess phosphorus fertilizer is applied. (Murphy et al., 1981). Zinc deficiencies often occur at high soil P concentrations through formation of insoluble zinc phosphate compounds leading to Zn immobilization on root surfaces (Loneragan and Webb, 1993; Sarret et al., 2001). Excess P concentration can interfere with the metabolic function of Zn at certain sites within plant cells (Lindsay, 1972). Excess P also may decrease vesicular-arbuscular mycorrhizal infection, which can reduce the effective absorbing area of the roots (Olsen, 1972). To enhance P and Zn uptake and use efficiency, it is important that, both P and Zn be present in adequate amounts in the soil.

Illinois is characterized by regions of soils with low, medium, and high P-supplying power. Central Illinois falls in the medium P-supplying power region. To ensure soybean yield is not restricted by P availability, soil P test should be maintained at about 50 kg P ha^{-1} (22.5 mg kg^{-1}). However, when the soil test indicates that P is higher than 50 kg P ha^{-1} (22.5 mg kg^{-1}), there is no economic and agronomic advantage in applying additional P (Fernández and Hoeft, 2009). Excess P can be lost through runoff and lead to eutrophication of surface waterbodies. Zinc deficiency is rare in Illinois, so Zn application to soybean is not a common practice. The critical value for soil Zn (DTPA extraction) is 1.12 kg Zn ha^{-1} (0.5 mg kg^{-1}); levels less than that are deficient, while those greater than that are sufficient (Fernández and Hoeft, 2009).

Agronomic efficiency measures the increase in yield per unit of fertilizer applied (Fageria et al., 2008). Positive agronomic efficiency results when the yield from the fertilized plots is greater than the yields from the unfertilized plots. On the contrary, negative agronomic efficiency is observed when the yield from the unfertilized plots exceeds the yield from the fertilized plots.

Several researchers have studied P-Zn interaction in cotton (Marschner and Cakmak, 1987; Marschner and Cakmak, 1986), subterranean clover (Loneragan et al., 1979), wheat (Zhu et al., 2001) and corn (Mallarino and Webb, 1995), but research on the P-Zn interaction in soybean is limited. Phosphorus and zinc effects on soybean in Illinois, USA, is also limited. This indicates a need to study P and Zn effects on soybean. The objectives of the study were to: (1) identify possible P and Zn interactions, (2) determine the P and Zn effect on P and Zn concentrations of soybean leaves and seeds (3) determine P and Zn effect on yield, and P and Zn agronomic efficiency.

CHAPTER II: LITERATURE REVIEW

Effect of pH on P and Zn Availability

Phosphorus and Zn availability in the soil is strongly influenced by soil pH. In acid soils, P forms insoluble phosphate compounds with iron and aluminum; it reacts with calcium in alkaline soils to form calcium phosphate. These compounds are not available for uptake by plant roots. For optimal growth, most leguminous crops need neutral or slightly acidic soils (Brockwell et al.,1991). Zinc solubility decreases with increase in soil pH. When the pH is above 7, Zn forms precipitates with oxides, hydroxides, carbonates, or silicates which reduces Zn solubility and availability (Hafeez et al., 2013; Lindsay, 1972).

Phosphorus Effect on Soybean Nutrient Concentration, Yield and Agronomic Efficiency

Soybean forms a symbiotic relationship with *Bradyrhizobium japonicum* that fixes nitrogen (N), thus N is usually not a limiting nutrient. Phosphorus is often the most limiting nutrient for growth and development of soybean in most productive soils. Soybean response to P depends mainly on the soil available P. Several studies indicate that soybean respond positively to P fertilization when soil test P is low. However, when the soil test P is high, P application may have no effect on soybean nutrient concentration, yield, and agronomic efficiency.

In a soil with low available P (5.1 mg P kg^{-1}), Tairo and Ndakidemi (2014) found P application significantly increased P content in the root, shoot, pods, and the whole plant of soybean relative to the control. Antonangelo et al. (2019) found soybean leaf P was higher for treatments that received P relative to the control, however, there was no difference among P rates. Slaton et al. (2010) on the contrary, reported P application had no significant effect on P content of soybean leaves when the soil available P was high (35 mg P kg^{-1}).

Phosphorus effect on soybean yield has been documented across the world. In a soil with low P concentration (9 mg P kg^{-1}), Shahid et al. (2009) reported a significant increase in soybean grain yield with P application at these rates; 0, 25, 50, 75 and 100 kg P_2O_5 ha^{-1}. The maximum fertilizer rate (100 kg P_2O_5 ha^{-1}) increased yield by 61 %. In a soil with low soil available P (3.2 mg P kg^{-1}), Devi et al. (2012) also found grain and stover yield of soybean increased with increasing rate of phosphorus fertilizer. They discovered that an application rate of 80 kg P_2O_5 ha^{-1} produced the maximum grain yield but was not significantly different from that of 60 kg P_2O_5 ha^{-1}. A study by Appiah et al. (2014) in South Dakota showed P did not significantly affect soybean yield. The initial soil P concentration at the study locations were closer to the P recommended rate of 15 mg P kg^{-1} in South Dakota. Slaton et al. (2010) also reported that soybean yield was not significantly affected by P application because the soil-test value of 35 mg P kg^{-1} was near optimum (36 to 50 mg P kg^{-1}). Increasing P rate above soybean P requirement may not translate into increase in yield. Antonangelo et al. (2019) found no significant difference in soybean yield when P was applied at 20 kg P_2O_5 ha^{-1}, 40 kg P_2O_5 ha^{-1}, 50 kg P_2O_5 ha^{-1}. Mallarino and Blackmer (1992), Webb et al. (1992) and Mallarino (1997) estimated the critical P concentration for soybean in Iowa to be 12 to 20 mg kg^{-1} by Bray-P_1 or Mehlich-3 tests. When P is applied above this critical concentration limit, there are no yield responses or economic benefits (Dodd and Mallarino, 2005). Research in Iowa (Mallarino et al., 1991; Webb et al., 1992) indicated soybean need to be cultivated for 8 to 9 years without P fertilization in high-testing soils (30-40 mg P kg^{-1}, Bray-P_1) before significant yield responses would be observed.

Common nutrient use efficiency indices are partial factor productivity, partial nutrient balance, agronomic efficiency, apparent recovery efficiency, internal utilization efficiency and physiological efficiency. Partial factor productivity and partial nutrient balance are long-term indicators of the productivity of nutrients applied while agronomic efficiency, apparent recovery efficiency, internal utilization efficiency and physiological efficiency are short-term indicators of the productivity of the nutrients applied. Agronomic efficiency (AE) measures the units of yield increase per unit of the fertilizer applied. Yield from plots without fertilizer input, yield produced from plots with fertilizer input and the amount of fertilizer applied are required in determining AE (Fageria et al., 2008; Dobermann, 2007). Phosphorus agronomic efficiency in soybean is mostly influenced by the P rate applied or the soil available P. Devi et al. (2012) reported maximum agronomic efficiency of phosphorus was observed from the application of 60 kg P_2O_5 ha^{-1}. Munthali et al. (2017) found AE to decrease with increasing P levels (7.5, 15, and 22.5 kg P ha^{-1}). The average available soil P at the study locations was 10.5 mg P kg^{-1}. As P was applied at 7.5 kg P ha^{-1}, soybean yield responded positively but increasing the rate to 15 and 22.5 kg P ha^{-1} did not result in significant yield increase because P was not a limiting factor. Phosphorus is an important nutrient required for soybean production, however, when soil test indicates P is adequate, there is no need to apply P.

Zinc Effect on Soybean Nutrient Concentration, Yield and Agronomic Efficiency

Recent studies in the Midwest indicated that soybean is less sensitive to Zn fertilizer than other crops. A three-year field experiment conducted in Minnesota showed Zn application did not have a significant effect on Zn concentration on soybean leaf and seed (Sutradhar et al., 2017). Although soybean is less sensitive to Zn fertilizer, research in Iowa indicated that foliar application of Zn increased Zn concentration in soybean trifoliate leaf and seed (Enderson et al., 2015). Demeterio et al. (1972) reported similar findings. They found Zn fertilization increased the Zn concentrations in soybean leaves, stems, and roots.

Applying micronutrients in addition to macronutrients, is often recommended to realize increase in crop yields. Although Zn is required in smaller amounts, soils deficient in Zn may cause a decrease in soybean grain yield. However, supplemental Zn application to soybean grown in soils with high Zn levels may not result in yield increase. Sutradhar et al. (2017) reported that Zn application did not increase grain yield. Zinc levels in the soil ranged from 0.5 to 4.6 mg kg^{-1}. Enderson et al. (2015) reported similar findings; foliar application of Zn did not increase soybean grain yield. The soil-test Zn ranged from 1.2 to 11 mg kg^{-1} (Mehlich-3 test).

Zinc agronomic efficiency is influenced mainly by soil available Zn. However, increasing the concentration of soil available Zn or Zn application rates do not necessarily result in higher agronomic efficiency. Accumulation of Zn in soybean leaf and seeds does not always correlate with an increase in grain yield. Tiwari et al. (2006) reported that a lower zinc application rate (1.8 kg ha^{-1}) resulted in higher agronomic efficiency compared to higher application rates (3.6 and 5 kg ha^{-1}). This trend was seen possibly because the initial soil available Zn was low (0.55 kg ha^{-1}) and as a result, soybean yield increased with initial Zn application. However, applying Zn above 1.8 kg ha^{-1} did not lead to any significant increase in yield.

Research has revealed that, Zn seldom has significant effect on soybean yield, although Zn application may increase the Zn concentration in the leaves and seeds. It suggests that, if the Zn concentration in the soil is sufficient, there is no need to apply Zn.

Phosphorus-Zinc Interaction in Soybean

P-Zn interaction is usually described as ‘P-induced-Zn deficiency, a mechanism where P affects Zn utilization in plants. The possible reasons stated for this interaction effect are: P and Zn reaction to form insoluble compounds, dilution of Zn concentration with P application, slower uptake, and translocation of Zn from the roots to the shoot, and metabolic disorder within plant cells (Olsen, 1972).

Varying P and Zn rates influence the uptake and concentration of either P or Zn in soybean leaf and seed (Rani et al., 2000). Increasing P rates in combination with no Zn application resulted in 60% increase in total P uptake in soybean stover, root and seed, but P uptake decreased with increasing the Zn levels. There was a reduction in Zn uptake at higher rates of P application. Increasing Zn levels in combination with no P application on the contrary resulted in 34% increase in total Zn uptake in soybean stover, root and seed (Rani et al., 2000). Application of higher levels of P with low levels of Zn usually leads to P and Zn antagonism, thereby affecting Zn uptake. Research by Payne et al. (1986) confirmed P-Zn antagonistic effect on Zn concentration in soybean leaf. They found application of P reduced Zn concentration when no Zn was applied. However, there was a significant increase in the leaf Zn concentration when both P and Zn were applied. There is limited research on P-Zn interaction effect on soybean yield and agronomic efficiency. Research on P and Zn interaction in soybean is needed to bridge this gap.

CHAPTER III: MATERIALS AND METHODS

Study Location and Experimental Design

The study was conducted at Illinois State University research farms in Lexington, IL (40.670851 N -88782640 W), and Normal, IL (40.519917 N, -89.012059 W) in 2020. General characteristics of the locations are presented in Table 1 and Table 2. The experimental design was a 4 × 4 factorial arranged in randomized complete block design (RCBD) with four replications; blocks were used for replication. Phosphorus was applied as monoammonium phosphate (MAP, 11-52-0) at four rates (0, 33, 67, 100 kg P ha^{-1}). Zinc was applied as zinc sulfate monohydrate ($ZnSO_4$, 35.5 % Zn-16.5% S) at four rates (0, 5, 11, 16 kg Zn ha^{-1}).

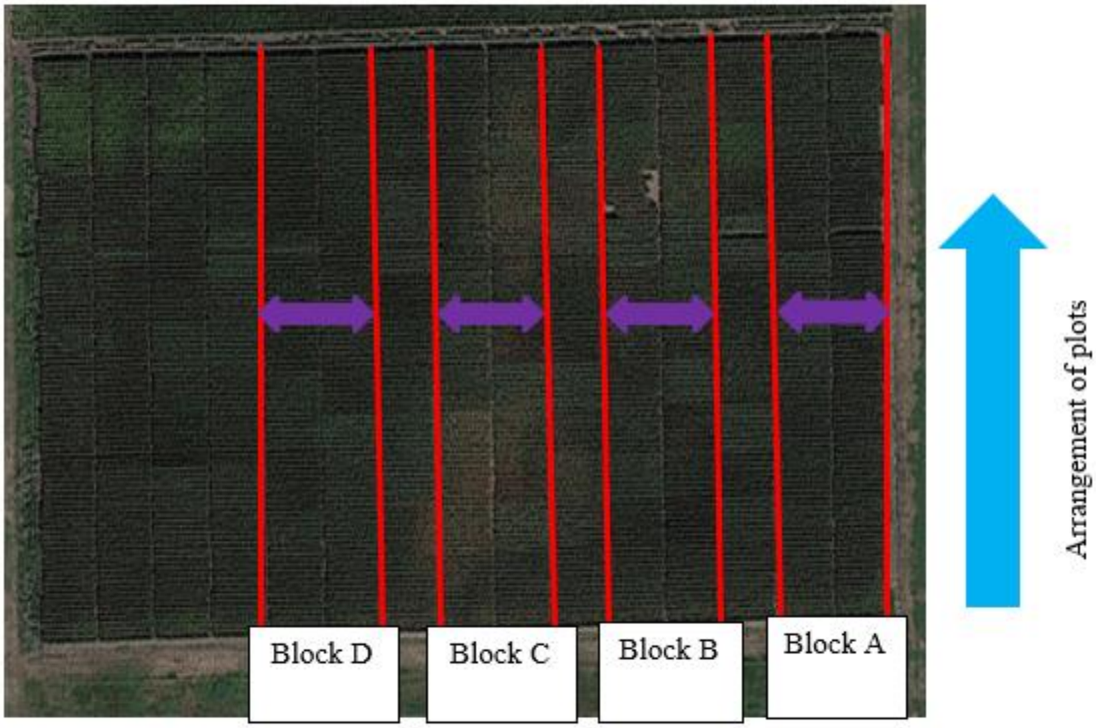

Figure 1. Blocks and plots layout at Lexington. Red lines with purple arrows represent blocks. Blocks were used as replication. Red lines with no purple arrows represent the space between blocks. Blue arrow shows the arrangement of plots in each block.

Figure 2. Blocks and plots layout at Normal. Red lines represent blocks. Blocks were used as replication. Blue arrow shows the arrangement of plots in each block. Block D was not uniform, so half of the of plots were arranged same as the other three blocks and the remaining half were arranged at the lateral of the other blocks.

Table 1:

General Characteristics at Lexington and Normal in 2020.

Site Characteristics	Lexington	Normal
Temperature (℃)		
Annual High Temperature	16.6	16.8
Annual Low Temperature	4.7	4.5
Annual precipitation (mm)	973	1074
Soil Type	Drummer silty clay loam (Fine-silty, mixed, superactive, mesic Typic Endoaquolls)	Catlin silt loam (Fine-silty, mixed, superactive, mesic Oxyaquic Argiudolls)

Table 2:

Monthly Precipitation at Lexington and Normal During the 2020 Growing Season.

Growing Season Precipitation		
Month	Lexington	Normal
	mm	
April	50.8	52.3
May	101.6	127.0
June	127.0	152.4
July	101.6	103.2
August	177.8	174.0
September	25.4	28.1
October	152.4	156.3

Initial Soil Analysis

Soil samples were taken with a 1.9 cm-diameter probe at a depth of 0-15 cm before treatments were applied. Four composite samples were taken from each block (16 samples from each location). Soil samples were air-dried and sent to United Soils Lab (Fairbury, IL) for the analysis. The analysis performed were pH (1:1 soil: water), organic carbon by loss on ignition method (Combs and Nathan, 1998), available P and Zn by Mehlich-3 extraction (Mehlich, 1984). The initial soil chemical properties at the two study sites are shown in Table 3.

Table 3:

Initial Soil Chemical Properties at Lexington and Normal in 2020.

Location	Block	pH	OC†	P	Zn
			(g kg^{-1})	-------- mg kg^{-1} --------	
Lexington	A	6.8	31.8	107.9	12.4
Lexington	B	6.9	29.8	81.0	10.0
Lexington	C	6.7	28.2	78.8	10.3
Lexington	D	6.8	29.6	82.5	10.5
Mean		6.8	29.9	87.6	10.8
Normal	A	5.5	30.3	19.5	4.0
Normal	B	5.8	29.9	13.6	3.7
Normal	C	5.7	29.5	16.0	3.5
Normal	D	6.1	30.5	30.6	5.4
Mean		5.8	30.1	19.9	4.2

† OC, organic carbon

Cultural Practices

Each plot size was 6.1 m × 6.1 m, comprised of 16 38-cm rows. Both fields were managed in a no-till system with a cereal rye cover crop. Prior to planting, cereal rye was terminated by spraying with glyphosate. Soybean seeds were planted at a depth of 2.5 cm and at a seeding rate of 345,800 seeds ha^{-1} at both locations. At Lexington, soybean variety planted was 31N06E while GH3582E3 was planted at Normal. Fertilizer was broadcast using a hand-spreader when soybeans were almost at the V1 stage (Iowa State University Extension and Outreach, 2014). The cultural practices and data collection dates are presented in Table 4.

Leaf Tissue Analysis

Twenty Fully developed trifoliate leaves from the top three nodes were randomly selected at R3 stage from the eight center rows of each plot,1 m from either end of the rows. Samples were oven dried at 80 °C for 48 hrs, then ground to pass a 2 mm-sieve and sent to United Soils Lab for the analysis. Tissue samples were digested with nitric acid. Phosphorus and zinc were then analyzed using inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Havlin and Soltanpour, 1980).

Table 4:

Cultural Practices and Data Collection for the Two Study Sites in 2020.

Cultural practices and Data Collection	Lexington	Normal
Planting Date	5/13/2020	6/2/2020
Soil Sampling Date	6/2/2020	6/15/2020
Fertilizer Application Date	6/2/2020	6/15/2020
Leaf Sampling Date	8/4/2020	8/12/2020
Seed Sampling Date	9/22/2020	09/29/2020
Harvest Date	10/20/2020	10/24/2020

Seed Analysis

At R6 growth stage of the soybean, five plants were taken from the eight center rows of each plot:1 m from either end. All pods were detached from the plants and shelled to collect the seeds. A composite seed sample was taken for each plot and dried at 80 °C for 48 hrs.

The seed samples were ground to pass a 2-mm sieve and sent to the United Soils Lab for the analysis. Seed samples were digested with nitric acid. P and Zn were analyzed using ICP-OES.

Yield Components and Yield

Plant population was determined for each plot by randomly counting plants from four rows (2.7 m long for each row) and converted to plants per ha. Plots were hand-harvested at soybean physiological maturity. Eight plants were harvested from the four center rows. The number of pods and seeds were counted, and the seed weight was measured in kg. The number of pods, seeds, and seed weight from the eight plants were converted to number of pods per plant, number of seeds per pod and thousand seed weight (yield components). The yield was measured in kg ha^{-1}.

Equation 1.

$$\text{Yield (kg ha}^{-1}\text{)} = \frac{seed\ weight\ (kg)\ /plot}{plot\ size\ (0.00372\ ha)}$$

Agronomic Efficiency

Phosphorus agronomic efficiency and zinc agronomic efficiency were determined using the following equation:

Equation 2.

$$\text{AE (kg kg}^{-1}\text{)} = \frac{G_f - G_u}{N_a} \quad \text{(Fageria et al., 2008)}$$

where G_f is the grain yield of the fertilized plot (kg), G_u is the grain yield of the unfertilized plot (kg), and N_a is the quantity of nutrient applied (kg).

Statistical Analysis

The fixed factors were P and Zn rates, and location and block were random factors. The dependent variables evaluated were P and Zn concentration of the soybean leaves and seeds, yield components, yield, phosphorus, and zinc agronomic efficiency. Data were analyzed using PROC MIXED and PROC CORR procedures of SAS 9.4, with a significance level of $\alpha \leq 0.05$, trends were noted when $\alpha < 0.10$. (SAS Institute, 2010). Analysis of variance (ANOVA) was performed using PROC MIXED considering P and Zn rates as fixed factors and location and block as random factors. When fixed factors were significant, means were compared using Tukey-Kramer test. Correlation analysis was performed among the P and Zn concentration of leaves and seeds using the PROC CORR. There was no significant interaction among locations and treatments for all the dependent variables, except phosphorus agronomic efficiency. Phosphorus agronomic efficiency data from Lexington and Normal were separated for analysis. All other data from Lexington and Normal were combined for analysis.

The linear model for the block, location, treatments, and treatments × location effects is shown below:

Equation 3.

$$y_{ijkl} = \mu + L_i + B_{i(j)} + P_k + Zn_l + PZn_{kl} + LP_{ik} + LZn_{il} + LPZn_{ikl} + \varepsilon_{ijkl}$$

Where;

y_{ijkl} is the observed value

μ is the population mean

L_i is the location effect

$B_{i(j)}$ is the block effect nested within location

P_k is the treatment effect (P)

Zn_l is the treatment effect (Zn)

PZn_{kl} is the P × Zn interaction effect

LP_{ik} is the location × P interaction effect

LZn_{il} is the location × Zn interaction effect

$LPZn_{ikl}$ is the location × P × Zn interaction effect

ε_{ijkl} is the random error or residual

CHAPTER IV: RESULTS AND DISCUSSION

Environmental Conditions at Lexington and Normal

The average high and low temperatures at Lexington and Normal were similar, however the annual accumulated precipitation at Normal was about 100 mm higher than Lexington (Table 1). The growing season precipitation at Lexington and Normal are shown in Table 2. The amount of rainfall at Normal was higher than Lexington. The amount of rainfall for the other months were similar at Lexington and Normal.

Soil Chemical Properties at Lexington and Normal

The initial soil tests revealed pH, P, and Zn were lower at Normal than at Lexington, though the organic carbon content was similar (Table 3). Overall, Lexington soil mean P concentration was 300% more than those at Normal. Soil P concentrations at Lexington were greater than 22.5 mg kg^{-1}, the critical P required for soybean production in Central Illinois (Fernández and Hoeft, 2009), whereas at Normal, the concentrations were below the critical level except in Block D. The mean zinc concentration at Lexington was 157% greater than that of Normal, however, the concentration at Lexington and Normal were greater than 0.5 mg kg^{-1}, indicating Zn is sufficient (Fernández and Hoeft, 2009). The observed higher P and Zn concentrations at Lexington were probably due to excessive application of livestock manure on the field in the past. Organic phosphorus and zinc in the manure may have mineralized and increased the inorganic pool of P and Zn. Lime application possibly accounts for the higher pH at Lexington compared to Normal (Whalen et al., 2000), as the Lexington farm has been managed more intensively than the Normal farm.

Location and Treatment Interaction Effects on Soybean Yield Components and Yield

The location and treatment interaction had no effect on soybean yield components and yield (Table 5; $P > 0.05$). Location had a significant effect on the number of seeds per pod ($P < 0.05$), thousand seed weight ($P < 0.05$), and possibly influenced the plant population ($P < 0.10$) and pods per plant ($P < 0.10$). The plant population at Normal was greater than Lexington, although the pods per plant and seeds per pod at Lexington exceeded Normal (Table 6). Normal received more precipitation than Lexington in May and June, which possibly explains the difference in the plant population. Soybeans at Lexington produced more pods per plant, and seeds per pod than Normal, however, the thousand seed weight at Normal was greater than Lexington. The differences in the yield parameters accounted for the similarity of yields between the locations, which is related to the physiological ability of soybean to compensate for the yield at different plant populations (Stivers and Swearingin, 1980). This may be explained by a difference in the light interception and net assimilation rate is greater at low plant population than at high plant population, so there was no difference in yield Jim Board (2000). When soybean is planted at a low population, there is less competition for sunlight, moisture, and nutrients.

Phosphorus and Zinc Effects on Soybean Yield Productivity

Phosphorus and zinc fertilizer rates had no effect on the soybean yield components and yield (Table 5 and Table 7), probably because the initial soil P and Zn concentrations were sufficient (Appiah et al., 2014; Slaton et al., 2010; Sutradhar et al., 2017; Enderson et al., 2015). The results indicate soybean do not respond to P and Zn application when the soil available P and Zn is sufficient or high.

Table 5:

ANOVA Table for Yield Components and Yield

Source of Variation	df	Yield Components				Yield
		Plant Population	Pods/Plant	Seeds/Pod	Thousand Seed Weight	
				$P > F$		
P	3	0.5987	0.5708	0.9254	0.4557	0.9109
Zn	3	0.3109	0.2141	0.5000	0.2612	0.7678
P × Zn	9	0.9962	0.2124	0.3519	0.2043	0.1384
Location	1	0.0566	0.0703	0.0235	0.0073	0.4043
Block	3	0.0366	0.8006	0.3955	0.4967	0.6053
Location × P	3	0.6447	0.6077	0.3057	0.2214	0.1747
Location × Zn	3	0.2397	0.3955	0.1312	0.5520	0.0617
Location × P × Zn	9	0.3116	0.5617	0.5205	0.9586	0.7896
Error	93					

Table 6:

Means of Yield Components and Yield by Location.

Location	Yield Components				Yield
	Population	Pods/Plant	Seeds/Pod	Thousand Seed Weight	
	Plants/ha			kg	kg ha^{-1}
Lexington	251800b†	56a	2.5a	0.14b	4890a
Normal	304900a	42b	2.2b	0.16a	4620a
SE	6800	0.8	<0.1	<0.01	110

† Means with different letters in a column are significantly different according to Tukey-Kramer test ($P < 0.05$).

Table 7:

Aggregated Means (Lexington and Normal) of Yield Components and Yield for Soybean Fertilized with P and Zn.

Fertilizer rate	Yield Components				Yield
	Plant Population	Pods/Plant	Seeds/Pod	Thousand Seed Weight	
kg ha^{-1}	Plants/ha			kg	kg ha^{-1}
Phosphorus					
0	280100	48	2	0.16	4800
33	283400	50	2	0.15	4830
67	271900	49	2	0.15	4650
100	278000	50	2	0.16	4750
	ns†	ns	ns	ns	ns
SE	27400	7	0.3	0.01	210
Zinc					
0	283200	49	2	0.16	4820
5	276000	47	2	0.15	4560
11	292800	49	2	0.15	4930
16	261400	52	2	0.15	4720
SE	28268	7	0.3	0.01	248
	ns	ns	ns	ns	ns

† ns, not significant at $P = 0.05$.

Phosphorus and Zinc Concentration of Soybean Leaves and Seeds

Location and treatments interaction had no effect on P and Zn concentration of soybean leaves and seeds, however, location did alter the P and Zn concentration of soybean leaves and seeds (Table 8). The initial P and Zn concentration at Lexington was greater than Normal, which probably accounts for the observed location effect.

There was no phosphorus and zinc interaction effect on P and Zn concentration of soybean leaves and seeds (Table 8). Phosphorus concentration of soybean leaves was not influenced by P application. No differences were observed between the P rates for the P concentration of the leaves (Table 8 and Table 9). This result is comparable to a study by Slaton et al. (2010), who reported P application had no effect on P concentration of soybean leaves when the initial soil P concentration was high. Phosphorus application influenced the P concentration of soybean seeds (Table 8 and Table 9). The P concentration of seeds for the 100 kg P ha^{-1} rate was higher than the control and the 33 kg P ha^{-1}, but was not different from that of the 67 kg P ha^{-1}. The P concentration of the seeds for the 100 kg P ha^{-1} was 7.6 % higher than the control. Helget (2016) found similar results, where soybean seed P concentration increased with P application but there was no consistent yield increase.

No significant differences were observed between Zn rates for the Zn concentration of soybean leaves and seeds (Table 8 and Table 9). The initial soil Zn concentration was sufficient and as a result soybean did not respond to additional Zn application. This result is similar to the findings of Sutradhar et al. (2017), who reported Zn application had no significant effect on Zn concentration of soybean leaves and seeds.

Table 8:

ANOVA Table for Impact of P and Zn Fertilization on Soybean Leaves and Seeds.

Source of Variation	df	Leaf Concentration		Seed Concentration	
		P	Zn	P	Zn
		$P > F$			
P	3	0.6337	0.2777	0.0185	0.5103
Zn	3	0.9903	0.1645	0.9289	0.0547
P × Zn	9	0.6126	0.5141	0.8277	0.7979
Location	1	0.0275	.	0.0386	0.0119
Block	3	0.0886	0.0431	0.0002	0.0001
Location × P	3	0.2100	0.7283	0.7847	0.1219
Location × Zn	3	0.7295	0.9105	0.0779	0.4601
Location × P × Zn	9	0.7749	0.8818	0.3018	0.5316
Error	92				

Table 9:

Aggregated Means (Lexington and Normal, IL) for Phosphorus and Zinc Concentration of Soybean Leaves at R3 and Seeds at R6.

Fertilizer rate	Leaf Concentration		Seed Concentration	
	P	Zn	P	Zn
(kg ha^{-1})	mg kg^{-1}			
P				
0	6383	43.5	5828c†	46.9
33	6259	43.0	5955bc	47.3
67	6243	42.6	6169ab	48.5
100	6374	43.1	6272a	48.9
	ns‡	ns		ns
SE	510	2.0	349	3.5
Zn				
0	6324	42.2	5987	45.9
5	6305	42.6	6093	47.4
11	6310	43.7	6105	48.6
16	6319	43.7	6038	49.8
SE	505	2.0	366	3.4
	ns	ns	ns	ns

† Means with different letters in a column are significantly different according to Tukey-Kramer test ($P < 0.05$). ‡ ns, not significant at $P = 0.05$.

Phosphorus and Zinc Agronomic Efficiency

The zinc and location interaction affected phosphorus agronomic efficiency, as a result, data for phosphorus agronomic efficiency was separated by location to determine Zn effect on phosphorus agronomic efficiency at Lexington and Normal (Table 10). When phosphorus agronomic efficiency was analyzed by location, the Tukey-Kramer test indicated there were no observed differences in phosphorus agronomic efficiency for the zinc rates at Lexington and Normal (Table 11). The interaction existed because the trend in agronomic efficiency was opposite between the locations. There was no phosphorus and zinc interaction effect on phosphorus and zinc agronomic efficiency (Table 10). There were no observed differences between P rates for the phosphorus agronomic efficiency (Table 10 and Table 12). The phosphorus agronomic efficiency was negative for all the P rates. Negative phosphorus agronomic efficiency was observed because the yield from the control plots were higher than the other P rates. For phosphorus agronomic efficiency to increase, the yield from the other P rates should exceed the yield from the control plots. In this study, the initial P and Zn were sufficient and as a result soybean yield did not respond to P and Zn fertilization.

There were observed differences between Zn rates for the zinc agronomic efficiency, however, zinc agronomic efficiency was negative for all the Zn rates (Table 13). This trend was observed because the yield from the control plots were higher than the other Zn rates. This result indicates there is no benefit in applying Zn since Zn application did not improve the agronomic efficiency.

Table 10:

ANOVA table for Phosphorus and Zinc Agronomic Efficiency.

Source of Variation	df	Agronomic Efficiency	
		Phosphorus	Zinc
		$P > F$	
P	3	0.0608	0.6020
Zn	2	0.9751	0.0412
P × Zn	6	0.0681	0.2100
Location	1	0.5846	0.6568
Block	3	0.1751	0.4945
Location × P	3	0.1257	0.3524
Location × Zn	2	0.0159	0.2785
Location × P × Zn	6	0.9899	0.8596
Error	69		

Table 11:

Zinc Effect on Phosphorus Agronomic Efficiency Means by Location (Lexington and Normal).

Zinc Rate (kg ha^{-1})	Phosphorus Agronomic Efficiency kg kg^{-1}	
	Lexington	Normal
0	-21	-19
5	-17	-19
11	-19	-15
16	-12	-27
SE	7	6
	ns†	ns

† ns, not significant at $P = 0.05$.

Table 12:

Aggregated Means (Lexington and Normal) for Phosphorus Agronomic Efficiency.

Phosphorus Rate	Phosphorus Agronomic Efficiency
(kg ha^{-1})	(kg kg^{-1})
33	-29
67	-17
100	-10
SE	
	ns†

† ns, not significant at $P = 0.05$.

Table 13:

Aggregated Means (Lexington and Normal) for Zinc Agronomic Efficiency.

Fertilizer rate	Zinc Agronomic Efficiency
(kg ha^{-1})	(kg kg^{-1})
P	
Zn	
5	-244b†
11	-77a
16	-66a
SE	17

† Means with different letters in a column are significantly different according to Tukey-Kramer test ($P < 0.05$).

Correlation Analysis between P and Zn Concentration of Soybean Leaves and Seeds

The correlation of P and Zn concentration of leaves and seeds were examined in this study. There was no correlation between P concentration of the leaves and Zn concentration of the leaves (Figure 3; r = 0.04). The correlation analysis between P concentration of the seeds and zinc concentration of the seeds indicated zero interaction (Figure 4; r = 0.04). There was a significant antagonism between the leaf P concentration and the seed Zn concentration. As the P concentration of the leaves increased, the Zn concentration of the seeds decreased (Figure 5; r = -0.43). A similar phenomenon was observed between leaf Zn and seed P (Figure 6; r = -0.30). The phosphorus concentration of the seeds increased as the zinc concentration of the leaves decreased.

The antagonism between P and Zn was observed in soybean leaves and seeds although both initial soil P and Zn concentration were sufficient. This is contrary to previous studies where P and Zn antagonism occurred when the P concentration in the soil was high and there was low Zn concentration (Rani et al., 2000; Payne et al., 1986). The mechanism of P and Zn assimilation during photosynthate production in leaves that result in differences in P and Zn stored in seeds not clear.

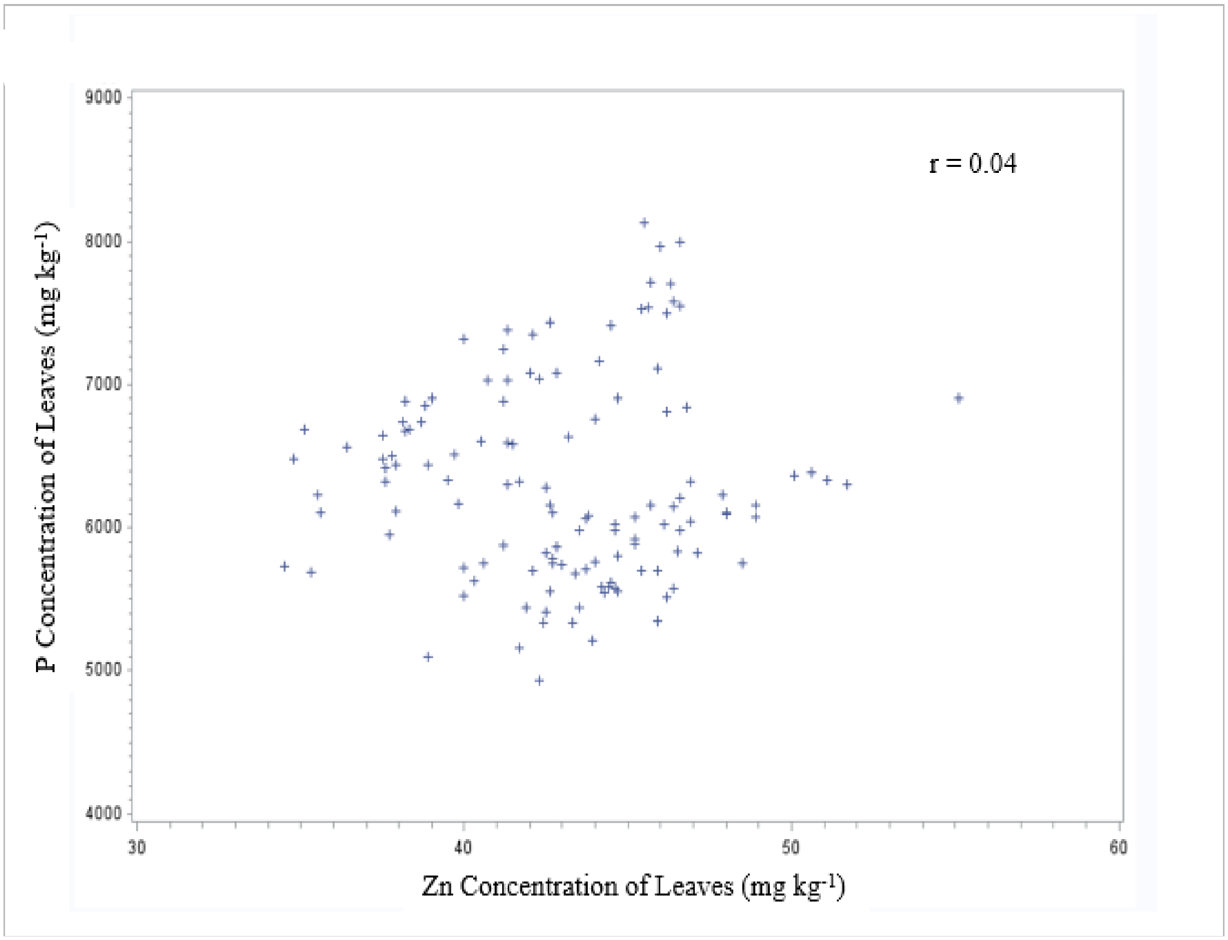

Figure 3. Correlation between phosphorus (P) concentration of soybean leaves and zinc (Zn) concentration of soybean leaves (r = Pearson Correlation Coefficient).

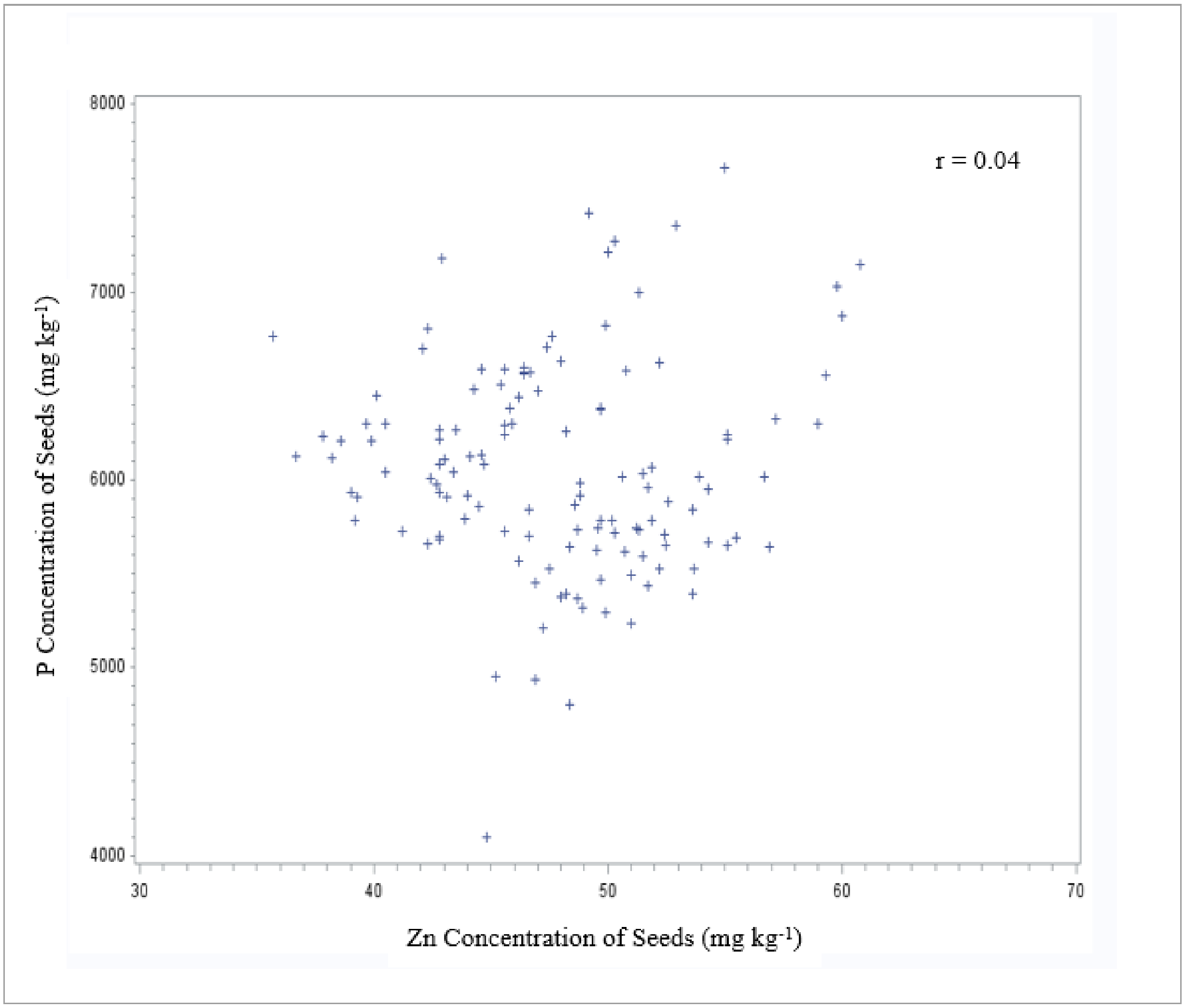

Figure 4. Correlation between phosphorus (P) concentration of soybean seeds and zinc (P) concentration of soybean seeds (r = Pearson Correlation Coefficient.

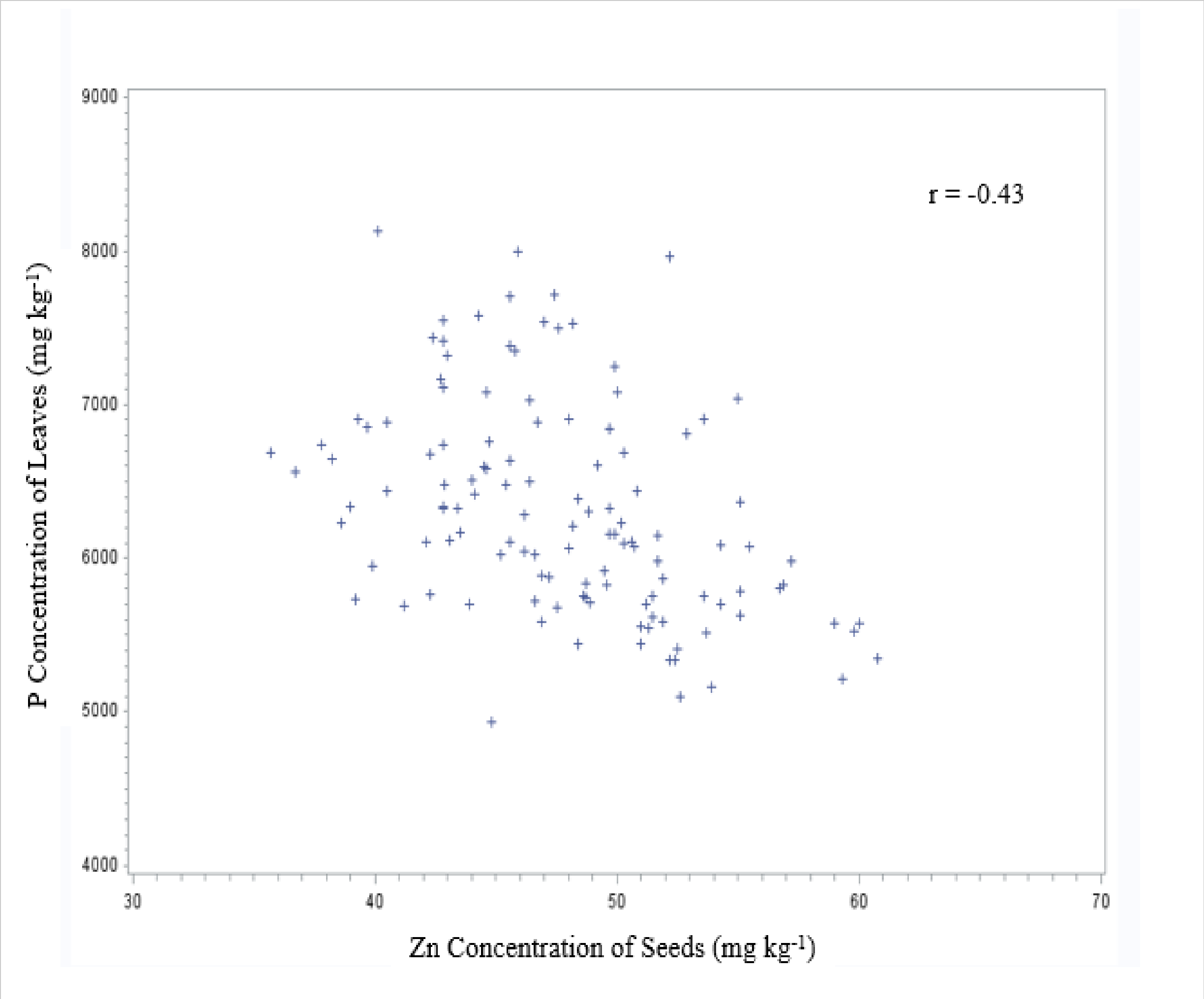

Figure 5. Correlation between phosphorus (P) concentration of soybean leaves and zinc (Zn) concentration of soybean seeds (r = Pearson Correlation Coefficient).

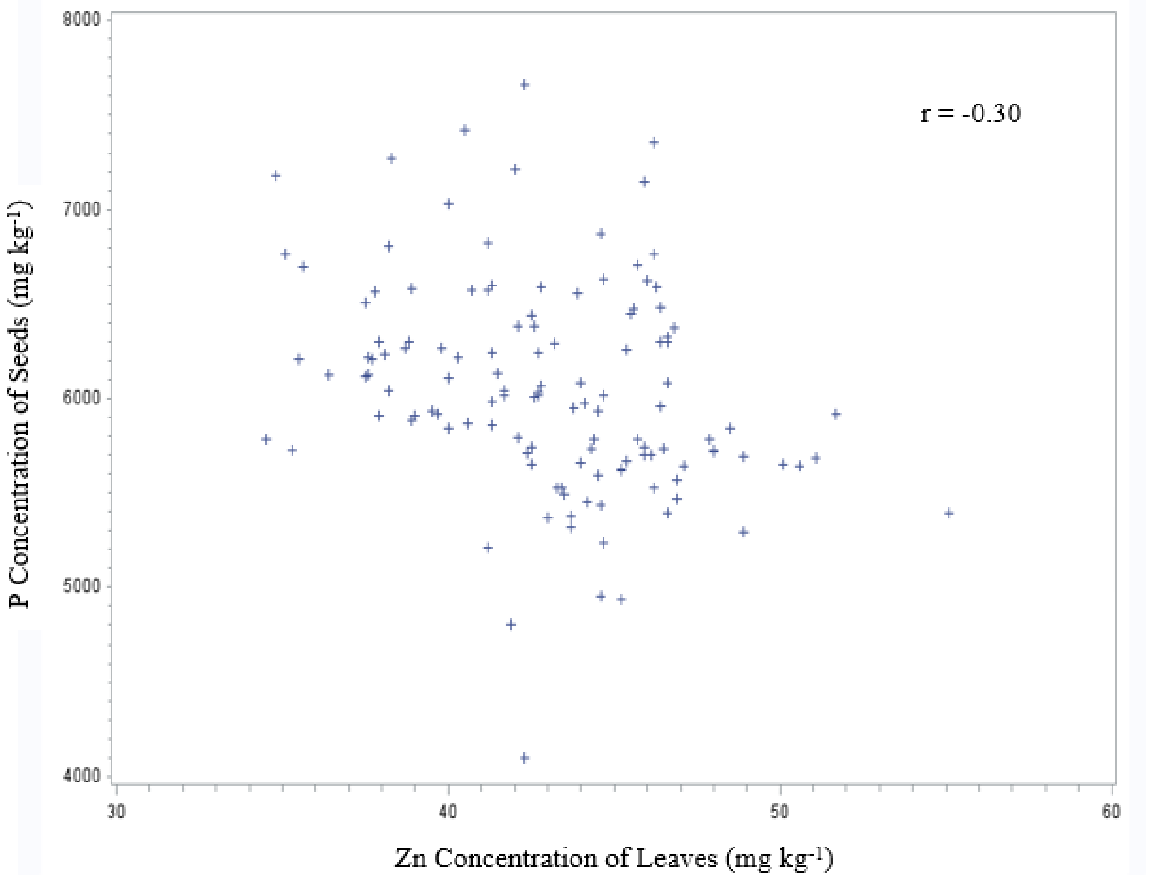

Figure 6. Correlation between phosphorus (P) concentration of soybean seeds and zinc (Zn) concentration of soybean leaves (r = Pearson Correlation Coefficient).

CHAPTER V: CONCLUSION AND RECOMMENDATIONS

This study indicates soybean does not respond positively to phosphorus and zinc application when the initial soil P and Zn concentration is adequate or high. In addition, neither P nor Zn affected the soybean yield. Phosphorus application did not affect leaf P concentration but affected seed P concentration. Phosphorus agronomic efficiency was not improved with phosphorus application. Zinc application did not affect leaf or seed Zn concentration and did not improve the zinc agronomic efficiency. The suggests that, when the soil test indicates P and Zn are adequate or high, there is no agronomic advantage in applying P and Zn fertilizer. Antagonistic interaction of P and Zn occurred in soybean leaves and seeds.

Future studies and enhancements should include:

- A field experiment using soils which have low P and Zn concentrations, and the P and Zn rate should be reduced.
- Soil samples taken for each plot before planting and after harvesting to analyze for the effect of initial soil P and Zn concentration.
- Phosphorus and zinc fertilizer application before planting to enhance uptake of P and Zn.
- A greenhouse component, where initial P and Zn concentration could be controlled in soil or in all concentration controlled directly in nutrient solutions in a hydroponic system, so that phosphorus and zinc and their interaction could be observed without interference from external factors.
- Examination of the nature of the P and Zn assimilation into photosynthates in leaves to understand the antagonism between leaf concentration of one and seed concentration of the other.

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APPENDIX : SAS CODES

PROC MIXED PROCEDURE

Plant population:

```
proc mixed data=PZn method=type3;
class block loc MAP Zn;
model Pop =MAP Zn MAP*Zn/ ddfm=kr outpred=CheckMe;
random Loc block Loc*MAP Loc*Zn Loc*MAP*Zn;
lsmeans MAP Zn/pdiff adjust=tukey;
run;
```

Number of pods per plant:

```
proc mixed data=PZn method=type3;
class block loc MAP Zn;
model Pods =MAP Zn MAP*Zn/ ddfm=kr outpred=CheckMe;
random Loc block Loc*MAP Loc*Zn Loc*MAP*Zn;
lsmeans MAP Zn/pdiff adjust=tukey;
run;
```

Number of seeds per pod:

```
proc mixed data=PZn method=type3;
class block loc MAP Zn;
model seeds=MAP Zn MAP*Zn/ ddfm=kr outpred=CheckMe;
random Loc block Loc*MAP Loc*Zn Loc*MAP*Zn;
lsmeans MAP Zn/pdiff adjust=tukey;
run;
```

Thousand Seed Weight:

```
proc mixed data=PZn method=type3;
class block loc MAP Zn;
model Seedwt =MAP Zn MAP*Zn/ ddfm=kr outpred=CheckMe;
random Loc block Loc*MAP Loc*Zn Loc*MAP*Zn;
lsmeans MAP Zn/pdiff adjust=tukey;
run;
```

Yield:

```
proc mixed data=PZn method=type3;
class block loc MAP Zn;
model yield =MAP Zn MAP*Zn/ ddfm=kr outpred=CheckMe;
random Loc block Loc*MAP Loc*Zn Loc*MAP*Zn;
lsmeans MAP Zn/pdiff adjust=tukey;
run;
```

Phosphorus agronomic efficiency by location

Lexington:

```
proc mixed data=Lexington;
class block MAP Zn;
model AEP=MAP Zn MAP*Zn;
random block;
lsmeans MAP Zn/pdiff adjust=tukey;
run;
```

Normal:

```
proc mixed data=Normal;
class block MAP Zn;
model AEP=MAP Zn MAP*Zn;
random block;
lsmeans MAP Zn/pdiff adjust=tukey;
run;
```

Zinc agronomic efficiency:

```
proc mixed data=PZn method=type3;
class block loc MAP Zn;
model AEZn =MAP Zn MAP*Zn/ ddfm=kr outpred=CheckMe;
random Loc block Loc*MAP Loc*Zn Loc*MAP*Zn;
lsmeans MAP Zn /pdiff adjust=tukey;
run;
```

Phosphorus concentration of leaves:

```
proc mixed data=PZn method=type3;
class block loc MAP Zn;
model Pleaf =MAP Zn MAP*Zn/ ddfm=kr outpred=CheckMe;
random Loc block Loc*MAP Loc*Zn Loc*MAP*Zn;
lsmeans MAP Zn /pdiff adjust=tukey;
run;
```

Phosphorus concentration of seeds:

```
proc mixed data=PZn method=type3;
class block loc MAP Zn;
model Pseed=MAP Zn MAP*Zn/ ddfm=kr outpred=CheckMe;
random Loc block Loc*MAP Loc*Zn Loc*MAP*Zn;
lsmeans MAP Zn /pdiff adjust=tukey;
run;
```

Zinc concentration of leaves:

```
proc mixed data=PZn method=type3;
class block loc MAP Zn;
model Znleaf =MAP Zn MAP*Zn/ ddfm=kr outpred=CheckMe;
random Loc block Loc*MAP Loc*Zn Loc*MAP*Zn;
lsmeans MAP Zn /pdiff adjust=tukey;
run;
```

Zinc concentration of seeds:

```
proc mixed data=PZn method=type3;
class block loc MAP Zn;
model Znseed=MAP Zn MAP*Zn/ ddfm=kr outpred=CheckMe;
random Loc block Loc*MAP Loc*Zn Loc*MAP*Zn;
lsmeans MAP Zn /pdiff adjust=tukey;
run;
```

PROC CORR PROCEDURE

Correlation between P and Zn concentration of leaves:

```
proc corr data=PZn plot;
var Pleaf Znleaf;
run;
proc gplot;
plot Pleaf* Znleaf;
run;
```

Correlation between P concentration of leaves and Zn concentration of seeds:

```
proc corr data=PZn plot;
var Pleaf Znseed;
run;
proc gplot;
plot Pleaf* Znseed;
run;
```

Correlation between P concentration of seeds and Zn concentration of leaves:

```
proc corr data=PZn plot;
var Pseed Znleaf;
run;
proc gplot;
plot Pseed* Znleaf;
run;
```

Correlation between P concentration of seeds and Zn concentration of seeds:

```
proc corr data=PZn plot;
var Pseed Znseed;
run;
proc gplot;
plot Pseed* Znseed;
run;
```