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# Soybean Yield and Nodulation Response to Crop History and Inoculation

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# Soybean Yield and Nodulation Response to Crop History and Inoculation

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## ABSTRACT

Soybean [*Glycine max* (L.) Merr.] inoculation was imposed on a long-term continuous grain sorghum [*Sorghum bicolor* (L.) Moench] and soybean cropping systems study with and without manure application at Mead, NE. The objective was to determine the influence of 28-yr history of continuous grain sorghum and soybean production, inoculation, and manure on soybean yield and nodulation. Average 2-yr soybean grain yield was 0.5 Mg ha$^{-1}$ greater on plots with continuous grain sorghum crop history rather than soybean history, even after this history was broken by 2-yr crop rotation with grain sorghum in the 2 yr previous to the evaluation. Continuous production of soybean in the same field is undesirable, and besides short-term loss of yield from lack of crop rotation, also leads to long-term soybean yield reductions. Manure and inoculation application had no influence on soybean grain yield or number of nodules. Soybean in plots with 28-yr previous grain sorghum crop history had equal number of nodules to those in plots with soybean crop history and/or *Bradyrhizobium japonicum* inoculant. It is probable that this lack of response was due to *B. japonicum* movement among adjacent plots by wind erosion, water erosion, and/or soil transfer from contaminated tillage equipment used to till adjacent plots. Although the basis for long-term soybean crop history on yield is unclear, continuous soybean production should be avoided to prevent soybean yield depression.

Long-term continuous soybean cropping has received limited study, but is a common production system in Argentina and Brazil (Meriles et al., 2009; Salado-Navarro and Sinclair, 2009). Crop rotation with maize (*Zea mays* L.) and sorghum have been documented to increase succeeding soybean crop yields by 5 to 16% (Roder et al., 1989; Peterson and Varvel, 1989).

Soybean and *Bradyrhizobium japonicum* form a symbiotic relationship to fix atmospheric nitrogen ($N_2$) in nodules located on soybean roots. *Bradyrhizobium japonicum* is not native to soils in the United States, but has been introduced through inoculation of soybean crops and can persist in soils for long periods. Typically soybean yields increase with inoculation in fields with no soybean history (Larson, 2013; Albareda et al., 2009; Schulz and Thelen, 2008; Elkins et al., 1976) or when the soil *B. japonicum* population is low (Furseth et al., 2012). Larson and Siemann (1998) found that approximately half of the normal population of soybean-compatible rhizobia survived after 30 yr of forage grass production on a field. Similarly, Ruiz Diaz et al. (2009) found only a 3% yield increase from *B. japonicum* inoculation following 20 plus years of grass pasture. Elkins et al. (1976) found no response to *B. japonicum* inoculation for 11 yr following a well-nodulated soybean crop. Several studies have shown no soybean yield response to inoculation when a well-nodulated soybean crop was produced within 3 to 5 yr (De Bruin et al., 2010; Schulz and Thelen, 2008; Abendroth and Elmore, 2006). However, exceptions exist when nodulation does occur in fields without a soybean crop history (Larson, 2013; van Kessel and Hartley, 2000). *Bradyrhizobium japonicum* transfer by wind, equipment, and livestock has been suggested, with Lowther and Patrick (1993) reporting that rhizobia can move 4 m yr$^{-1}$ downslope.

Manure application increases soil nutrient and organic matter levels, water and nutrient holding capacities, and depending on the soil nutrient status and fertilizer application, can contribute to increased crop yields (Wortman et al., 2012; Kaye et al., 2007). Manure application can indirectly influence soybean nodulation by reducing soybean plant and nodule water stress through increased soil water storage; improving soil oxygen concentration by increasing soil porosity; and contributing $NO_3$–N directly or indirectly through mineralization (Kaye et al., 2007; Bagayoko et al., 1992). Manure application has been shown to have both a positive (Ganeshamurthy and Reddy, 2000; Tagoe et al., 2008; Johnson and Hume, 1972) and no effect (Javaid and Mahmood, 2010) on soybean nodule

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numbers and/or quantity of N fixation depending on soil and climatic conditions.

The objective of this research was to determine the influence of crop history, inoculation, and manure on soybean nodulation and yield.

## MATERIAL AND METHODS

Research was conducted at the University of Nebraska Agricultural Research Development Center near Mead, NE, on a Yutan silty clay loam (fine-silty, mixed, superactive, mesic Mollic Hapludalf) with 2 to 4% slopes in 2011 and 2012. The experiment was imposed on plots contained in a long-term rotation study established in 1980 (Roder et al., 1988, 1989). The experimental factors studied were crop history, soybean inoculation, and manure application. The crop history was continuous grain sorghum or soybean followed by 2 yr grain sorghum as shown in Table 1. Plots either received no manure or annual application of fresh beef feedlot manure at 17.3 Mg $ha^{-1}$ (dry matter) until 2008, applied in spring and incorporated by disking. No manure was applied in 2009 and 2010, but manure was applied to experimental plots at 16.6 Mg $ha^{-1}$ (dry matter) in 2011 and 12.5 Mg $ha^{-1}$ (dry matter) in 2012. The manure application goal was 15 to 20 Mg $ha^{-1}$ (dry matter) each year, but differences in annual manure application rate varied due to differences in water content of the fresh manure. The N supplied by manure in the 2011 was estimated to be 54 kg $ha^{-1}$ in 2011 and 28 kg $ha^{-1}$ in 2012 (Wortmann and Shapiro, 2012).

The experiment was conducted in a randomized complete block design with a split-plot treatment arrangement and three replications. The whole plot was a factorial combination of grain sorghum or soybean crop history and with and without manure. Subplots were inoculant treatments with and without Novozyme Optimize 400 (selected *B. japonicum* inoculant plus lipochitoligosaccharides) applied on seed immediately before planting at a rate of 0.4 µg $seed^{-1}$. The subplots were 3 m wide and 7.9 m long. Weeds were controlled by using herbicides and hand weeding as needed. Due to extreme drought in 2012 (Table 2), supplemental 60 mm $ha^{-1}$ irrigation was furrow applied on 23 July to reduce drought stress and approximate average growing conditions.

The soybean variety Asgrow 3332 was planted following disk tillage in both years. Soybean was planted on 30 May 2011 and 3 June 2012 using a six-row John Deere 7100 maxi-merge planter (John Deere, Moline, IL) in 76 cm row spacing at the rate of 445,000 seeds $ha^{-1}$.

### Soybean Nodule Counts and Grain Yield Determination

Soils were sampled to depth increments of 0 to 20 and 20 to 60 cm before planting and analyzed for organic matter, and extractable P, K, and Zn using a Mehlich-3 extraction in both years (Combs et al., 1998). Soils were sampled at a depth of 0 to 20 cm at the V4 (approximately 26 d after emergence) and R5 soybean stages (approximately 70 d after emergence) (Pedersen, 2009), and analyzed for $NO_3$–N using the automated cadmium reduction method analyzed by flow injection (Gelderman and Beegle, 1998) following calcium phosphate extraction (Combs et al., 1998). Five soybean plants were dug up; carefully washed; and nodules counted at the V4 and R5 growth stages. Grain yield was machine-harvested from the middle two rows of each four-row (3 by 9 m) plot; machine threshed; weighed; and corrected to 130 g $kg^{-1}$ water content. Subsamples were hand harvested from a 30.5-cm row section before machine harvesting, and then the number of pods (per plant); seeds

Table 1. Cropping history between 1980 and 2011 and 2012 at Mead, NE.

| | 2011 Experiment | | | | |
|---|---|---|---|---|---|
| Crop history | 1980–2008 | 2009 | 2010 | 2011 | |
| Grain sorghum | Grain sorghum | Grain sorghum | Grain sorghum | Inoculated or non-inoculated soybean | |
| Soybean | Soybean | Grain sorghum | Grain sorghum | Inoculated or non-inoculated soybean | |
| | 2012 Experiment | | | | |
| Crop history | 1980– 2008 | 2009 | 2010 | 2011 | 2012 |
| Grain sorghum | Grain sorghum | Grain sorghum | Grain sorghum | Grain sorghum | Inoculated or non-inoculated soybean |
| Soybean | Soybean | Grain sorghum | Grain sorghum | Grain sorghum | Inoculated or non-inoculated soybean |

Table 2. Monthly temperature and precipitation at the Agricultural Research and Development Agronomy Farm, Mead, NE, in 2011 and 2012.

| | Maximum temperature | | | Minimum temperature | | | Precipitation | | |
|---|---|---|---|---|---|---|---|---|---|
| Month | 2011 | 2012 | 30-yr avg. | 2011 | 2012 | 30-yr avg. | 2011 | 2012 | 30-yr avg. |
| | °C | | | | | | mm | | |
| May | 22.5 | 26.5 | 23.1 | 10.6 | 12.0 | 9.7 | 161 | 80 | 75 |
| June | 27.9 | 30.2 | 28.6 | 16.4 | 16.4 | 15.2 | 128 | 93 | 98 |
| July | 31.9 | 35.7 | 30.8 | 20.9 | 19.9 | 17.6 | 73 | 2 | 79 |
| Aug. | 28.8 | 31.9 | 29.5 | 17.3 | 14.5 | 16.2 | 88 | 14 | 96 |
| Sept. | 23.1 | 27.6 | 25.4 | 8.1 | 8.4 | 10.9 | 20 | 34 | 78 |
| Avg./Total | 26.8 | 30.4 | 27.5 | 14.7 | 14.2 | 13.9 | 470 | 223 | 426 |

per pod; and seed weight were determined (not reported). Seed weights were corrected to 130 g kg$^{-1}$ water content and added back to the grain yield.

### Statistical Analysis

Data were analyzed by using SAS Proc Mixed procedures, version 9.3 (SAS Institute, 2014) for analysis of variance. Crop and manure history, inoculation and year were considered to be fixed effect while replication and interactions with replication were considered to be random. Differences of $P \le 0.05$ were declared significant.

## RESULTS AND DISCUSSION

### Climate

On average, the maximum and minimum temperatures were below average in 2011 and above average in 2012 (Table 2). In 2011, the average maximum temperature was higher than the 30-yr average only in the month of July, while in 2012 it was consistently 2 to 5°C higher than average. Minimum temperatures were higher than average in both years except for the month of August in 2011, and September in both years. Precipitation in 2012 was very low, especially near the critical pod-elongation growth stage (Pedersen, 2009), when low precipitation combined with the high maximum temperatures, resulted in a stressful environment for soybean growth, thus supplemental furrow irrigation of 60 mm was applied to reduce drought stress and approximate average growing conditions.

### Soil Nutrient Levels

Manure application from 1980 through 2008 increased soil pH (from 6.7 to 7.0); organic matter (from 31 to 33 g kg$^{-1}$); $NO_3$–N (from 1.9 to 2.9 g kg$^{-1}$); P (from 43 to 165 g kg$^{-1}$); K (from 425 to 634 g kg$^{-1}$); and Zn (from 1.2 to 3.0 g kg$^{-1}$) compared to non-manured plots in the top 20 cm. Crop history had no influence on soil organic matter or nutrient levels. Soil levels for all nutrients were well above the sufficiency levels for soybean production in all plots (Ferguson et al., 2006), thus differences in soybean yield would be not be expected based on soil nutrient levels (Wortman et al., 2012; Kaye et al., 2007, Bagayoko et al., 1992).

### Grain Yield

Soybean grain yield differences were caused by crop history. Plots with grain sorghum history yielded 0.5 Mg ha$^{-1}$ more than those with soybean crop history (Table 3). Since all plots were planted to grain sorghum in 2009 and 2010, a similar yield increase of 5 to 16% would be present for both crop histories (Roder et al., 1089; Peterson and Varvel, 1989). Therefore, the soybean yield difference was the consequence of continuous grain sorghum and soybean production between 1980 and 2008 even though soil organic matter and nutrient levels of plots were all well above sufficiency levels (Ferguson et al., 2006) and in spite of the continuous soybean crop history being interrupted for 2 yr. Clearly continuous soybean production not only has a negative consequence on the succeeding soybean crop yield (Roder et al., 1089; Peterson and Varvel, 1989) but also has longer-term negative yield effects. This is an important consideration in countries where soybean is commonly grown without rotational crops.

### Soybean Nodule Numbers and Soil Nitrate-Nitrogen Concentration

Neither manure, crop history nor inoculation had an effect on nodule numbers on soybean plants (Table 3). Averaged across years, manured plots had 8.9 mg kg$^{-1}$ soil $NO_3$–N concentration vs. 5.5 mg kg$^{-1}$ for non-manured plots at the V4 growth stage, and 5.0 mg kg$^{-1}$ vs. 3.0 mg kg$^{-1}$ at the R5 growth stage. Soybean crop history increased the soil $NO_3$–N concentration compared with grain sorghum crop history. However, the soil $NO_3$–N concentration was higher and crop history difference greater in 2012 than 2011 (9.7 mg kg$^{-1}$ vs. 6.8 mg kg$^{-1}$ in 2012, and 2.3 vs. 2.1 mg kg$^{-1}$ in 2011). The higher soil $NO_3$–N concentration in 2012 may have been due to greater soil mineralization resulting from higher temperatures (Table 2) as observed by MacDonald et al. (1995). Soybean crop history increased soil $NO_3$–N concentration in the soil at both the V4 and R5 growth stages (Table 3). Soil $NO_3$–N concentration has been shown to influence soybean nodule formation (Salvagiotti et al., 2008), but the range of soil $NO_3$–N concentrations present in this study had no influence on soybean nodulation (data not presented).

Differences for the number of nodules among treatments at the V4 and R5 growth stages were greater in 2011 than in 2012, and increased between the V4 and R5 growth stages (Table 3). The difference in number of nodules was likely due to the cooler temperatures, higher rainfall (Table 2), and lower water stress in 2011 than in 2012 (Serraj et al., 1999; Purcell et al., 2004).

It was expected that the nodule number per soybean plant would be similar in plots with a soybean crop history and in inoculated plots with grain sorghum history, while non-inoculated plots with grain sorghum history would have no or few nodules present (Larson, 2013; Schulz and Thelen, 2008; Elkins et al., 1976). However, no differences were found for the number of nodules between continuous grain sorghum and other crop systems. While this was unexpected, it has been previously documented in the literature (Larson, 2013; van Kessel

Table 3. Influence of crop history, year, manure and inoculation on soybean yield and number of nodules (no significant interaction effects were present).

| Treatments | Grain yield | Soybean nodules V4 | Soybean nodules R5 |
|---|---|---|---|
| | Mg ha$^{-1}$ | no. plant$^{-1}$ | |
| Crop history | | | |
| Soybean | 3.6 | 24.7 | 36.3 |
| Sorghum | 4.1 | 24.4 | 34.8 |
| Probability | <0.01 | 0.88 | 0.61 |
| Year | | | |
| 2011 | 4.0 | 28.0 | 38.0 |
| 2012 | 3.8 | 21.5 | 33.1 |
| Probability | 0.50 | 0.04 | 0.09 |
| Manure | | | |
| Manured | 3.9 | 25.6 | 36.2 |
| Non-manured | 3.9 | 23.5 | 35.0 |
| Probability | 0.98 | 0.25 | 0.67 |
| Inoculation | | | |
| Inoculated | 3.8 | 25.2 | 36.2 |
| Non-inoculation | 3.9 | 24.0 | 35.0 |
| Probability | 0.98 | 0.77 | 0.62 |

and Hartley, 2000). The presence of nodules in non-inoculated soybean plots was likely due to movement of *B. japonicum* with soil from adjacent inoculated and/or well nodulated plots by wind and/or water, or from un-cleaned tillage equipment used in adjacent plots during the long-term study (Lowther and Patrick (1993).

## CONCLUSION

Soybean grain yield was 0.5 Mg ha$^{-1}$ greater on plots with grain sorghum crop history than soybean history, even though this history was broken by 2 or 3 yr of grain sorghum production and no significant differences in soil organic matter or nutrient levels were found. Continuous production of soybean in the same field produces lower yields, due to lack of short-term crop rotation yield enhancement and long-term crop history effects. This result is logical, but this long-term effect has not been previously reported. Surprisingly, soybean planted into plots after continuous grain sorghum production for more than 30 yr had similar number of nodules to plots with soybean history and/or plots inoculated with *B. japonicum* at planting. This is likely due to movement over time of *B. japonicum* among adjacent plots by wind and/or water erosion, and/or contaminated tillage equipment, but also confirms other reports of nodule formation in fields without application of inoculant. Although the exact reason for long-term soybean crop history on yield is unclear, continuous soybean production should be avoided if possible and further study on *B. japonicum* inoculation of fields without soybean history is merited.

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