

Soybean Response to Inoculation and Nitrogen Application Following Long-Term Grass Pasture

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ABSTRACT

Current demand for soybean grain [*Glycine max* (L.) Merrill] may lead to a conversion of pasture and Conservation Reserve Program fields into soybean or corn (*Zea mays* L.) production. Our objective was to determine the effect of soybean seed inoculation with *Bradyrhizobium* spp. and fertilizer N application rate on soybean productivity planted 1 and 2 yr after conversion to row crop production. Soybean followed the pasture the first year and followed an intervening corn crop the second year. Treatments consisted of a factorial arrangement of seed inoculation and fertilizer N rate. Application of rhizobia inoculum significantly increased soybean grain yield by an average of 130 kg ha⁻¹. Plant dry matter (DM), N concentration, N accumulation, and grain N were also increased with soybean seed inoculation; however, seed quality was not affected. Fertilizer N application increased plant DM but did not increase grain yield, with or without inoculation. Furthermore, N fertilizer did not increase plant N or improve seed quality. Results from this study indicate that growers should inoculate soybean seed when planted after long-term grass pasture. Application of N fertilizer is not suggested even if no rhizobia seed inoculation is used because fertilizer N did not improve grain yield.

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Abbreviations: DM, dry matter.

SPURRED BY GRAIN DEMAND for biofuel production, the conversion of grass pasture to corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merrill] production has increased dramatically in the United States in recent years. In Iowa, approximately 75% of the total area (145,743 km²) is used for crop production, approximately 14% is under grass pasture or Conservation Reserve Program (CRP), with the remaining 11% consisting of forest, developed land, and water areas (USDA, 2003). A major challenge for this conversion is that soybean productivity may be adversely affected by limited N due to poor symbiotic N fixation and low levels of soil inorganic N. Larson and Siemann (1998) completed a study of midwestern U.S. grass pasture fields and found that the ability of soybean to form nodules decreased with the length of time since the crop was last grown. Results of that study also suggested that less than half of the normal population of soybean-compatible rhizobia is left in the soil after 30 yr of grass production. Published research on use of inoculation when soybean has not been grown for many years is lacking for Iowa soils and production conditions. Based on work from other regions, it is currently recommended to use a rhizobia inoculant in Iowa if fields have never been planted to soybean or where nodulated soybean has not been grown in a field in the past 3 to 5 yr (Pedersen, 2004). Inoculation is also recommended if soil pH has not been maintained above 6.0, the soils are sandy, or fields have been flooded for more than a week (Pedersen, 2004). Research is needed to confirm these suggestions.

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Soybean response to N fertilization has been evaluated extensively (Salvagiotti et al., 2008). In an effort to improve soybean grain yield with N fertilization, two general timings of N application have been studied. One is N application at reproductive stages. Nitrogen-fixing capacity begins to decline after the R5 growth stage (Fehr and Caviness, 1977), which also coincides with the time of peak N demand (Shibles, 1998). However, studies with N applied during reproductive stages have generated inconsistent results. Nitrogen applied at R3 to R4 stages was suggested to significantly increase grain yield with high-yield-potential irrigated soybean (Gascho, 1993; Wesley et al., 1998). However, other rain-fed studies have shown no significant effect on grain yield from fertilizer N applied at those growth stages (Freeborn et al., 2001; Schmitt et al., 2001; Gutiérrez-Boem et al., 2004). Barker and Sawyer (2005) evaluated the effect of in-season (R3) N application to soybean in Iowa and showed that soybean grain yield was not affected by fertilizer N application. However, plant N concentration and plant dry matter (DM) were increased.

The other potential times for N application to soybean are either preplant or during early vegetative growth. Soybean yield increases have also been inconsistent with N application at those times (Oplinger and Bundy, 1998; Randall and Schmitt, 1998). Measured yield response has varied with location, soil type, inorganic soil N level, soybean variety, growing season, and disease presence. Generally, soybean yield response has not been of sufficient magnitude or consistency for early-season N application to be economical. Early-season N application can also result in temporary suppression of nodule formation due to increased nitrate in the soil (Hungria et al., 2005). Conversely, early-season N deficiency due to inadequate soil inorganic N or symbiotic fixation can delay crop growth and the development of an efficient nodulation system.

Our objective was to determine if rhizobia inoculation and/or early-season N fertilization would increase soybean productivity when planted after long-term grass pasture.

MATERIALS AND METHODS

This study was completed during two successive years at two sites within a central Iowa field that had been in permanent grass pasture for at least 20 yr (based on grower knowledge). The soil both years was a Canisteo clay loam (fine-loamy, mixed, superactive, calcareous, mesic Typic Endoaquolls). In the first year soybean followed the grass pasture, while in the second year it followed a non-N-fertilized corn crop that had been planted after the grass pasture. The experimental design was a factorial treatment arrangement in a randomized complete block design with four replications. Plot size was six 76-cm rows wide by 9 m in length. Treatment factors included seed inoculation with or without *Bradyrhizobium* spp. (Nitragin at 3.4 g kg⁻¹ seed; EMD

Crop BioScience, Milwaukee, WI) and N fertilizer application rate. Fertilizer N as ammonium nitrate was surface applied after planting at six rates, ranging from 0 to 280 kg N ha⁻¹ in increments of 56 kg N ha⁻¹. Inoculum containing *Bradyrhizobium* spp. was applied to the soybean seed before planting. Soybean ('Prairie Brand PB2352RR') was planted on 2 May 2003 and 6 May 2004 at 290,000 seeds ha⁻¹. Weeds and insects were managed using cultural practices appropriate for the geographic region. Glyphosate [N-(phosphonomethyl)glycine] was applied twice during the season at a rate of 865 g acid equivalents ha⁻¹. To control soybean aphids (*Aphis glycines* Matsumura) in 2003, the pyrethroid insecticide lambda-cyhalothrin (Warrior; Syngenta Crop Protection, Greensborough, NC) was applied at a rate of 27.6 g a.i. ha⁻¹. Tillage was moldboard plowing in the fall of 2002, with secondary disk and field cultivation in the spring before planting. In 2004, the corn stalks were disked once before planting.

Soil samples were taken from the 0- to 15-cm depth across each study site-year in the spring before planting. Collected soil samples were air-dried, ground to pass a 2-mm sieve, and analyzed for P and K using the Mehlich-3 extraction (Warncke and Brown, 1998), soil pH in a 1:1 water suspension, organic matter by dry combustion using a LECO CHN-2000 analyzer (LECO Corp., St. Joseph, MI) (Nelson and Sommers, 1982), and soil NO₃⁻-N by extraction with 2 M KCl and analyzed using a Lachat flow injection analyzer (Lachat Instruments, Milwaukee, WI) (Gelderman and Beegle, 1998). During 20 yr of pasture, neither site had a history of manure application. However, the grower indicated that an old farmstead was located in the area and the very high soil test P and K (Table 1) indicates manure likely had been applied many years ago. Soils had very high soil test P and K (Table 1) as interpreted for agronomic crop production in Iowa (Sawyer et al., 2002). Therefore, no P or K fertilizers were applied.

Whole aboveground soybean plants were sampled for total plant DM at the point of maximum biomass accumulation (R5.5) (Fehr and Caviness, 1977). Plants were cut at the soil surface from two random 50-cm sections of row plot⁻¹. The plants were weighed immediately after collection. Subsamples consisting of five plants were dried at 65°C in a forced-air oven, ground to pass through a 2-mm sieve, and analyzed for total N by dry combustion using a LECO CHN-2000 analyzer (LECO Corp.).

Grain samples were collected at harvest and analyzed for protein and oil using near-infrared spectroscopy by the Iowa State Univ. Grain Quality Laboratory (Rippke et al., 1995). Plant and grain analyses are reported on a dry-weight basis. Plant and grain N were calculated from the N concentration

Table 1. Selected site characteristics, with 0- to 15-cm depth soil samples collected before planting each year in central Iowa.

Characteristic [†]	Year	
	2003	2004
Soil pH	6.9	7.4
M3P (mg kg ⁻¹)	390	365
M3K (mg kg ⁻¹)	690	740
OM (g kg ⁻¹)	42	40
NO ₃ ⁻ -N (mg kg ⁻¹)	4.3	4.0
May precipitation (mm)	122	178

[†]M3P and M3K, Mehlich-3 extractable P and K, respectively; OM, organic matter. Historical precipitation for May is 115 mm (51-yr average).

and amount of plant or grain material. Soybean grain was harvested with a plot combine from the center two rows. Grain yields are reported on a 130 g kg⁻¹ moisture basis.

Analysis of variance was completed using PROC MIXED of SAS (SAS Institute, 2003). Years and blocks (nested within each year) were considered as random in the model and therefore results are presented across years. Treatment effects were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Soybean production after the long-term pasture approached 4000 kg ha⁻¹. Inoculating soybean seed with rhizobia bacterium resulted in a statistically significant grain yield increase of approximately 130 kg ha⁻¹, averaged across all N rates and years (Tables 2 and 3). The yield response was consistent across all N rates except for the 112 kg N ha⁻¹ rate, which had a higher grain yield when not inoculated. Imsande (1989, 1998) suggested that soybean deriving N from N₂ fixation (inoculated soybean) may have an advantage over a soybean attaining N solely from the soil. This would be due to rapid and large N₂ fixation during the seed filling period, which provides the N for seed protein and grain yield. The yield increase measured with inoculation indicates the soil did not have sufficient rhizobia or competitive rhizobia for needed N₂ fixation. While the yield increase was not large, the response to inoculation shows that when soybean follows long-term pasture, that is, no history of soybean production, rhizobia inoculation should be added before planting.

There was no significant effect of N fertilizer application or response to N rate, including no interaction of inoculation or no inoculation with N rate on soybean yield (Tables 2 and 3). This lack of yield response to N application found in our study agrees with that of several other research studies (Freeborn et al., 2001; Schmitt et al., 2001; Gan et al., 2002; Barker and Sawyer, 2005). Despite the soil NO₃⁻-N concentration being low at planting (Table 1), apparently there was adequate inorganic N supplied from soil mineralization to meet soil-derived N needs for soybean growth. With the pasture being aggressively tilled (fall moldboard plow and disking), it is likely that considerable mineralization occurred. The initial soil test showing 40 to 42 g kg⁻¹ soil organic matter (Table 1) supports this conclusion.

Table 2. Simple effects of inoculation (Yes or No) and N rate on soybean grain yield and seed weight, protein, and oil, across 2003 and 2004 in central Iowa.

N rate	Grain yield	Seed		
		Weight	Protein	Oil
kg N ha ⁻¹	kg ha ⁻¹	g 100 seed ⁻¹	g kg ⁻¹	g kg ⁻¹
0	3910	15.2	355	187
56	3860	15.4	353	189
112	3890	15.3	353	189
168	3880	15.5	352	188
224	3940	15.6	354	187
280	3840	15.3	352	188
Inoculation				
Yes	3950a [†]	15.4	353	188
No	3820b	15.4	353	188

[†]Mean comparisons with different letters are significantly different at the 0.05 probability level. Complete treatment and interaction statistical significance is given in Table 3.

Grain quality parameters, including 100 seed weight, protein, and oil concentration were not improved with either inoculation or N fertilization (Tables 2 and 4). The interaction of inoculation and N rate was not significant for these parameters. Barker and Sawyer (2005) found similar results in Iowa with late-season fertilizer N application.

Aboveground plant DM at the R5.5 growth stage was significantly affected by inoculation and N rate (Tables 3 and 4) but not the interaction of the two factors. Across N rates, the inoculated soybean produced an additional DM of 580 kg ha⁻¹ compared with the noninoculated soybean (Table 4). Plant DM increased with increasing N rate. With the highest two N rates, the plant DM was equivalent to that with rhizobia inoculation. An increase in plant DM as a result of inoculation and N application has been observed in some studies (Sinclair, 1998; Gan et al., 2002), but not always with N application (Barker and Sawyer, 2005). Plant height at maturity was not affected by either inoculation or N application (Table 3).

Significantly greater plant N concentration and plant total N were found with the inoculated soybean compared with the noninoculated soybean (Tables 3 and 4). Copeland and Crookston (1992) found significantly lower plant N concentration for soybean grown for the first time in a field compared with soybean grown in rotations that had included soybean. The authors suggested that the positive rotation effect was due to presence of rhizobia in the soil where soybean had been previously grown. Fertilizer N application had no effect on plant N concentration or total plant N (Tables 3 and 4). Other research has shown an increase in plant N with fertilizer N application (Barker and Sawyer, 2005; Tewari et al., 2007), but none was measured in our study.

Grain N concentration was not affected by inoculation or fertilizer N application (Tables 3 and 4). However, total N in harvested grain was greater for the inoculated soybean, which would be attributed to the higher grain yield with the inoculated treatment and not to a protein increase (Table 2). Also, the concentration and amount of N in harvested grain was not affected by fertilizer N rate, which is consistent with the plant N found at growth stage R5.5.

CONCLUSIONS

Soybean productivity was quite good after conversion of pasture to row crop production and was significantly increased with addition of rhizobia seed inoculation. Grain yield, plant DM, plant N, and grain N were greater with inoculated soybean. However, grain quality parameters were not affected. There was a significant increase in plant DM with fertilizer N application. However, this increase in plant DM did not result in an increase in grain yield. Furthermore, grain quality and total N uptake were not significantly affected by fertilizer N application. Results from this study suggest that growers should inoculate soybean during the years after long-term pasture until there is a history of soybean production in the field. A similar suggestion could help improve soybean production following long-term CRP where soybean may not have been grown for many years. Since early-season N application produced no benefit for soybean yield or grain quality, N

Table 3. Probability values from the analysis of variance for each of the measured soybean crop parameters, across 2003 and 2004 in central Iowa.

Source of variation	Grain yield	Seed			Plant			Grain N conc.	Plant N	Grain N
		Weight	Protein	Oil	Dry matter	Height	N conc.			
<i>P > F</i>										
Inoculation (I)	0.037	0.607	0.945	0.751	0.001	0.711	0.003	0.960	<0.001	0.028
N rate (NR)	0.938	0.639	0.662	0.766	0.037	0.846	0.123	0.667	0.125	0.907
I × NR	0.106	0.659	0.940	0.410	0.210	0.093	0.643	0.941	0.430	0.184

Table 4. Simple effects of inoculation (Yes or No) and N rate on soybean plant dry matter (DM), height, and N at maturity and grain N, across 2003 and 2004 in central Iowa.

N rate	Plant DM	Plant height [†]	Plant N conc.	Grain N conc.	Plant N	Grain N
kg N ha ⁻¹	kg ha ⁻¹	cm	g kg ⁻¹		kg ha ⁻¹	
0	5220	96.0	29.6	56.8	159	220
56	5590	97.2	29.6	56.4	167	215
112	5730	97.8	28.1	56.5	160	218
168	5880	97.8	29.3	56.4	176	217
224	5980	97.2	30.7	56.7	176	220
280	6030	97.6	31.2	56.3	187	214
Inoculation						
Yes	5960a [‡]	97.4	30.6a	56.5	186a	221a
No	5380b	97.1	28.9b	56.5	156b	213b

[†]Plant height at maturity.

[‡]Mean comparisons with different letters are significantly different at the 0.05 probability level. Complete treatment and interaction statistical significance is given in Table 3.

fertilization is not suggested for soybean following long-term pasture.

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