# EVALUATION OF NITROGEN FERTILIZER AND RHIZOBIUM INOCULATION TO IMPROVE GRAIN YIELD AND QUALITY OF SOYBEAN AND DRY BEAN IN EASTERN MONTANA

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## ABSTRACT

Interest in production of irrigated soybean and dry bean in Eastern Montana's Lower Yellowstone River Valley increased substantially following the closure of a sugar beet processing facility in Sidney, MT resulting in the need for a replacement crop(s) on 30,000 to 40,000 acres annually. However, research-based information addressing appropriate agronomic practices for these crops is lacking, including nitrogen (N) fertility management.  N management for legumes is complicated by their symbiotic N fixation with rhizobia, which is determined by the presence of crop specific rhizobia in the soil or introduced via commercial inoculants. The success of commercial inoculants can vary based on product selection, crop type and field cropping history. Furthermore, the addition of fertilizer N can have variable effects on the establishment and productivity of symbiotic N fixation. Understanding when commercial rhizobium inoculants are needed and what levels of N fertilizer applied at planting are beneficial is critical for successful production and profitability of these crops. To address these issues, a two-year study evaluating soybean and dry bean's response to rhizobium inoculation and N fertilization was conducted. Two cultivars each of soybean and dry bean were subjected to N fertilizer rates of 0, 6 and 56 kg N ha$^{-1}$ applied at planting. Each fertilizer rate was planted with and without rhizobium seed inoculation for a total of six treatments. Soybean seed yield and protein increased significantly with inoculation. Fertilization of soybean with 6 kg N ha$^{-1}$ did not affect yield, but fertilization at the high N rate partially reduced the benefits derived from inoculation in soybean. The response of dry beans to the same set of treatments differed considerably from soybeans. No positive response to inoculation was observed and a small decrease in seed yield was observed with the high N rate in one of the two years. The results of this study indicate that the two crops respond differently to rhizobium inoculation and that the addition of small to moderate rates of N fertilizer at planting provides little benefit in Eastern Montana.

## INTRODUCTION

The N requirements of legumes can be met through uptake of mineral N from the soil or from fixation of atmospheric N derived from a symbiotic interaction with rhizobia bacteria. This symbiotic N fixation (SNF) is energy intensive requiring photosynthetically derived sugars from the plant. As a result, legumes are sensitive to soil mineral N levels and will limit SNF when N levels in the soil are high. The percentage of plant N derived from SNF varies depending upon plant species and location with estimates for soybean ranging from 44% to 78% and dry bean at 38% (8). N fertilization can reduce the percentage of SNF-derived N in the plant with estimates in soybean of 84% in unfertilized conditions and 34% following N fertilization at or above 84 kg N ha$^{-1}$ (5).

N fertilization recommendations for soybean and dry bean vary based on location and agricultural practices. Typically, N fertilization of soybeans is not recommended unless specific conditions exist that might result in a N deficiency in the crop. Studies evaluating the yield response of soybean to N fertilization observed yield increases of 8-32% following N application (7). However, variability in site- and year-specific environmental effects, management practices and N fertilization rates and methods generates inconsistent responses both within and across studies (7). As a result, it is difficult to draw broad conclusions regarding the response of soybean to N fertilizer. Dry bean N fertilization is a common practice in the Northern Plains with producers applying 56 kg N ha$^{-1}$ on average with recommendations for N application beginning when soil N levels fall below 56 kg ha$^{-1}$ (2). The dry bean response to N application varies across studies. Buetow et al. (2) reported no increase in seed yield at a rate of 56 kg N ha$^{-1}$ whereas Chekanai et al. (3) reported an increase in seed yield at 40 kg N ha$^{-1}$. Furthermore, Edje et al. (4) observed a seed yield response to N at 200 kg N ha$^{-1}$ but not at 40 to 160 kg N ha$^{-1}$. Finally, challenges associated with determining the need for N fertilization in soybean and dry bean are further confounded by the lack of consistent responses of each crop to rhizobium inoculation as well as a negative association between soil N levels and SNF (9,10). The objectives of this study were to 1) evaluate the response of soybean and dry bean to rhizobium inoculants in the Lower Yellowstone River Valley of Easten MT and 2) examine the need for the additional N fertilizer at planting and assess what N rates are appropriate.

## MATERIALS AND METHODS

A two-year study was conducted under sprinkler irrigation at the Eastern Agricultural Research Center in Sidney, MT in 2024 and 2025. The study site had no recent history (>10 years) of soybean or dry bean crops. The trials were proceeded by spring wheat in both years with background soil nitrate levels (0-61 cm) of 39 kg ha$^{-1}$ in 2024 and 20 kg ha$^{-1}$ in 2025. Two cultivars of soybean (CP0337X and R0422XF), one pinto bean (Cowboy) and one red bean (Viper) were grown at three different fertilizer levels including 1) None (Control), 2) 6 kg N ha$^{-1}$ and 29 kg P$_2$O$_5$ ha$^{-1}$ (6N) and 3) 56 kg N ha$^{-1}$ and 29 kg P$_2$O$_5$ ha$^{-1}$ (56N). Each fertilizer level was planted with and without rhizobium seed inoculation (PrimoGX2 for soybean and N-Charge for dry bean, Verdesian Life Sciences, Cary, NC) for a total of six treatments. Soybean and dry bean were planted at 43 and 21 seeds per square meter with a row spacing of 46 cm. At the R3 development stage, plants from one row meter were dug from each plot. Nodulation scores were derived from three categories including plant growth and vigor, nodule color and number, and nodule position with scores of 0, 1, 3, or 5 assigned for each category and summed to give a total nodulation score. Above ground biomass was collected, dried and weighed following evaluation of nodulation. Grain was harvested at maturity and yields were adjusted to 13 percent moisture content. Soybean protein and oil were determined by near-infrared spectroscopy. Data were analyzed using R statistical software version 4.2.2 (R Foundation for Statistical Computing, Vienna, Austria). Linear mixed effects models were fit using the lmer function in the *lme4* R package (version 1.1-35.3) (1) treating year, genotype and treatment as fixed effects and replicate as a random effect. Pairwise comparisons were performed in the *emmeans* (version 1.10.4) (6) R package using the Benjamini-Hochberg procedure for *P*-value adjustment.

## RESULTS

The results of an analysis of variance (ANOVA) are presented in Table 1. Due to the presence of significant year interactions with all parameters except nodule score and seed oil, ANOVA were performed by each year for soybean genotype and treatment main effects. Soybean seed yield was significantly affected by genotype in 2024 but not 2025 with R0422XF outyielding CP0337X in 2024 (Fig. 1A and 1C). Treatment significantly affected soybean yield in both years (Fig. 1B and 1D) with the inoculated (Inoc) and inoculated plus 6 kg N ha$^{-1}$ (Inoc + 6N) treatments producing the highest yields. Yields for the three uninoculated treatments were lower than the inoculated treatments in both years. Soybean nodule scores were significantly affected by year and treatment but not genotype (Table 1). Nodule scores were higher in 2025 than 2024 (Fig. 1E). The Inoc and Inoc + 6N treatments also produced the highest nodulation scores (Fig. 1F). Nodulation scores for the inoculation plus 56 kg N ha$^{-1}$ (Inoc + 56N) treatment did not differ from the Control treatment (Fig. 1F).

**Table 1.** ANOVA table mean squares showing the effects of year, genotype, treatment and their interactions on seed yield, nodule score, biomass, seed protein and seed oil.

| Source of Variation[a] | Seed Yield | Nodule Score | Biomass | Seed Protein | Seed Oil |
|---|---|---|---|---|---|
| **Soybean** | | | | | |
| Y | 16535158*** | 160.2*** | 830.6** | 18900*** | 125.6* |
| G | 11446 | 2.7 | 41 | 5813*** | 71.8* |
| T | 3236488*** | 169.8*** | 75.3* | 3822.2*** | 424.3*** |
| Y x G | 786792** | 11.1 | 15.7 | 438* | 41.3 |
| Y x T | 231790* | 1.4 | 265.4*** | 115.2 | 18.7 |
| G x T | 36926 | 0.7 | 27.6 | 56 | 11 |
| Y x G x T | 85105 | 1.8 | 22 | 85.8 | 8.5 |
| **Dry bean** | | | | | |
| Y | 37373677*** | 90.1** | 574.1 ** | | |
| G | 1109 | 73.5*** | 51.9 | | |
| T | 185168* | 21.8*** | 7.3 | | |
| Y x G | 529409** | 73.5*** | 0.4 | | |
| Y x T | 181548* | 3.9 | 34.6 | | |
| G x T | 59550 | 15* | 33.5 | | |
| Y x G x T | 73488 | 3.6 | 25.2 | | |

[a] Year (Y), Genotype (G), Treatment (T)
*, **, *** Significance at the 0.05, 0.01 and 0.0001 probability levels

Soybean biomass was significantly affected by treatment (Table 1). The 56 kg N ha$^{-1}$ (56N) and Inoc + 56N treatments produced the least biomass in 2024 and the most in 2025 (Table 2). Soybean seed protein was significantly affected by both genotype

and treatment with R0422XF higher than CP0337X (Table 2).  The Inoc and Inoc + 6N treatments produced the highest seed protein and the Control, 6N and 56N treatments the lowest.  Soybean oil contents varied by year, genotype and treatment (Table 1). Year and genotype effects, although significant, were small and likely not biologically relevant (Table 2). Treatment effects on seed oil content were opposite that of protein with the three uninoculated treatments generating the highest oil contents (Table 2).
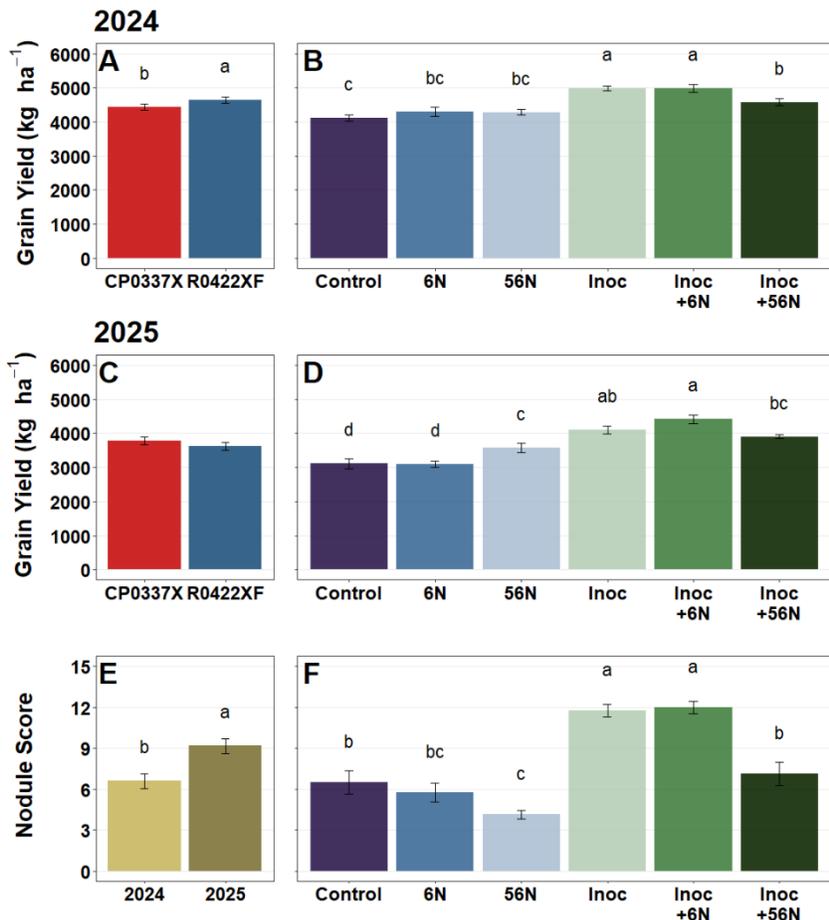


**Figure 1.** Effect of genotype (A and C) and treatment (B and D) on soybean seed yield in 2024 and 2025 and effect of year (E) and treatment (F) on soybean nodule score. Treatments include none (Control), 6 kg N ha$^{-1}$ (6N), 56 kg N ha$^{-1}$ (56N), rhizobium inoculation (Inoc), rhizobium inoculation with 6 kg N ha$^{-1}$ (Inoc + 6N) and rhizobium inoculation with 56 kg N ha$^{-1}$ (Inoc + 56N). Error bars are standard errors of the means and bars labelled with the same letter within a panel are not statistically different at $P \leq 0.05$.

Dry bean seed yield was significantly affected by year and treatment as well as year by genotype and year by treatment interactions (Table 1). Viper produced higher seed yields than Cowboy in 2024 but not 2025 (Fig. 2A and 2C). Treatment effects were observed only in 2024 with the 6N treatment producing the highest yields (Fig. 2B). Dry bean biomass was significantly affected by year but not genotype or treatment (Table 1) with more biomass in 2025 (4990 kg ha$^{-1}$) than 2024 (3864 kg ha$^{-1}$).

Significant genotype interactions with year and treatment were identified for dry bean nodule scores (Table 1). Therefore, the two beans were analyzed separately. No year effect was observed for Cowboy (Fig. 2E). However, nodule scores were higher in 2025 than 2024 for Viper (Fig. 2G). Treatment effects on nodule scores were significant. The Inoc treatment produced the highest nodulation score for Cowboy (Fig. 2F) and the lowest for Viper (Fig. 2H). The Control treatment produced the highest nodulation score for Viper (Fig. 2H) and the second highest score for Cowboy (Fig. 2F). The addition of

56 kg N ha$^{-1}$ in the 56N and Inoc + 56N treatments resulted in a significant decrease in nodulation scores relative to the control treatment for both beans.

**Table 2.** Mean separation of soybean biomass, seed protein and seed oil as affected by year (seed oil only), genotype and treatment in 2024 and 2025.

| | Biomass | Biomass | Seed Protein | Seed Protein | Seed Oil |
|---|---|---|---|---|---|
| | kg ha$^{-1}$ | kg ha$^{-1}$ | g kg$^{-1}$ | g kg$^{-1}$ | g kg$^{-1}$ |
| | 2024 | 2025 | 2024 | 2025 | |
| **Year** | | | | | |
| 2024 | - | - | - | - | 196.9 b |
| 2025 | - | - | - | - | 199.6 a |
| **Variety** | | | | | |
| CP0337X | 3834 | 5372 | 291.3 b | 267.5 b | 197.4 b |
| R0422XF | 3768 | 5088 | 311.1 a | 278.8 a | 199.1 a |
| **Treatment** | | | | | |
| Control | 3745 ab | 4257 b | 280.8 c | 261.4 c | 204.1 a |
| 6N | 3783 ab | 4766 b | 290.1 c | 258.1 c | 202.8 ab |
| 56N | 3414 ab | 6065 a | 292.1 c | 262.3 c | 201.2 b |
| Inoc | 4297 a | 4837 b | 321.3 a | 289.8 a | 192.4 d |
| Inoc + 6N | 4228 ab | 4984 b | 316.9 ab | 293.4 a | 192.7 d |
| Inoc + 56N | 3350 b | 6473 a | 306.1 b | 273.9 b | 196.3 c |

Mean values within a column that share the same letter are not significantly different at $P$ ≤ 0.05.

## DISCUSSION

In this study, both genotypic selection and agronomic practices influenced soybean seed yield and quality. Genotype effects on seed yield varied by growing season but the pattern of effects on seed protein was consistent between years indicating that genotype by environment interactions impact seed yield more than seed protein. The lower N fertilization rate of 6 kg ha$^{-1}$ failed to generate a yield response and the addition of N at 56 kg ha$^{-1}$, even when combined with inoculation, suppressed the yield gains observed by inoculation alone and reduced the effectiveness of inoculation. This is evident in the reduction of nodulation scores in the Inoc + 56N treatments relative to the other inoculated treatments (Fig. 1 E and F). These results indicate that successful rhizobium inoculation is more critical than N fertilization to achieve maximum soybean seed yield and seed protein in fields with no recent history of soybean as utilized in this study. In addition, N fertilization of soybean at rates less than required to attain full yield potential may have negative impacts on yield.

The impact of rhizobium inoculation on dry bean was very different from that observed for soybean. Inoculation of both dry beans resulted in no improvement in seed yield or nodulation scores (Fig. 2 E,F,G and H). Poor nodulation was observed in Control treatments for soybean, whereas dry bean nodulation patterns were similar between the Control and Inoc treatments. This suggests that indigenous soil rhizobium

populations exist, even in the absence of a field history of dry bean, that compete with the inoculant strain for nodule occupancy or are capable of nodulation and N fixation at levels that are indistinguishable from the inoculant strain.
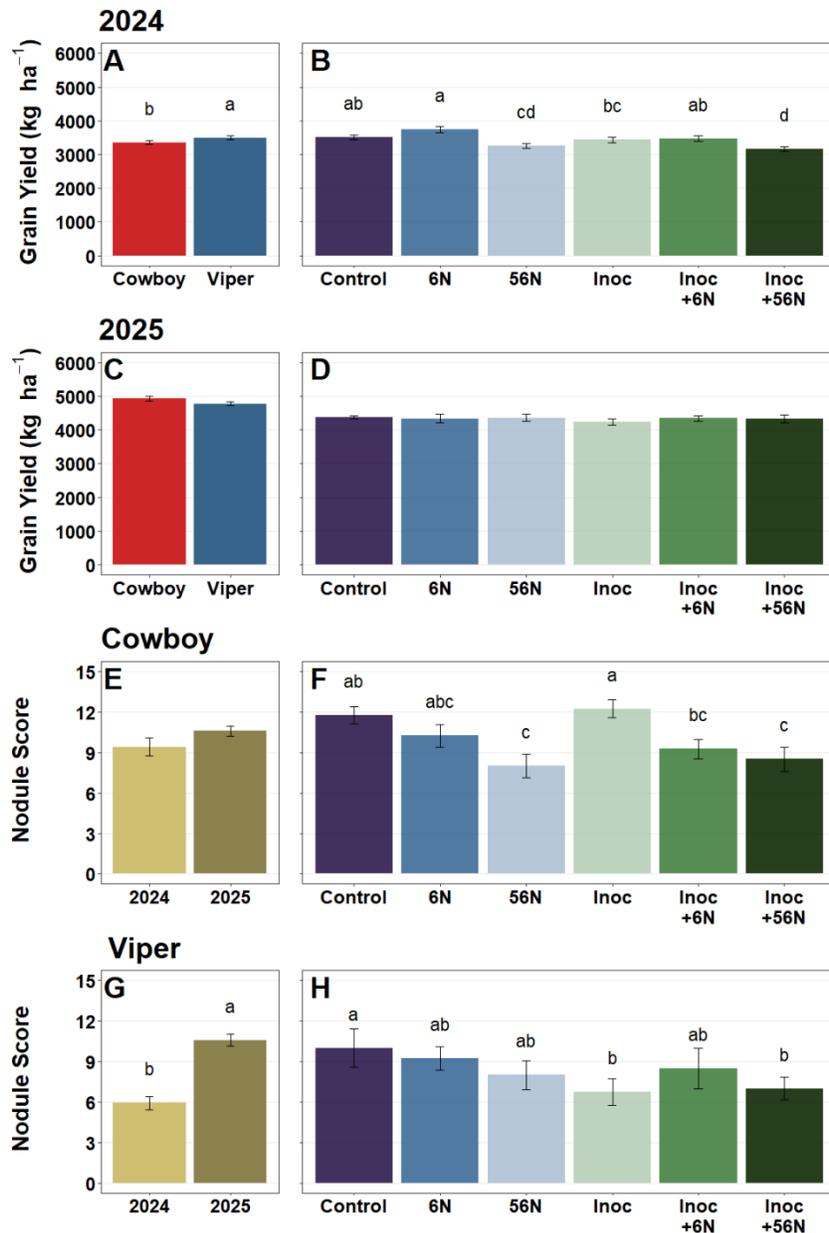


**Figure 2.** Effect of genotype (A and C) and treatment (B and D) on dry bean seed yield in 2024 and 2025 and effect of year (E and G) and treatment (F and H) on Cowboy and Viper nodule scores. Treatments include none (Control), 6 kg N ha$^{-1}$ (6N), 56 kg N ha$^{-1}$ (56N), rhizobium inoculation (Inoc), rhizobium inoculation with 6 kg N ha$^{-1}$ (Inoc + 6N) and rhizobium inoculation with 56 kg N ha$^{-1}$ (Inoc + 56N). Error bars are standard errors of the means and bars labelled with the same letter within a panel are not statistically different at $P \leq 0.05$.

Either of which would explain the lack of response to the commercial inoculant. Furthermore, the negative impacts of N fertilization on seed yield and nodulation scores observed with soybean were less pronounced for dry bean with reduction in nodulation scores being much smaller to absent and a small yield reduction observed in only one of the two study years.

The results of this study indicate that inoculation of soybeans in Eastern Montana fields with little or no history of the crop is critical to maximize yield, and the yield benefits are more than adequate to generate a net positive return on the input. On the other hand, rhizobium inoculation of dry beans appears to offer little benefit even in the

absence of a dry bean field history. Finally, N fertilization of both soybean and dry bean at levels below that required for full N fertility should be undertaken with caution as the negative impacts on nodulation can outweigh the benefits of partial N supplementation.

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