Research Project Title: A tool for cheap and rapid tracking of soybean inoculant populations in field soil

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Research Overview:

Soybean crops in North Dakota (ND) can obtain all the nitrogen they need from symbiosis with rhizobia bacteria called *Bradyrhizobium japonicum* applied during planting as inoculants. As a result, nitrogen fertilizers need not be applied to soybean (assuming the inoculant is present) reducing input cost.. In a process called nodulation, inoculant rhizobia form structures called nodules on soybean roots. Inside root nodules, rhizobia take nitrogen directly from the air and supply it to the crop. After inoculating in the first year or two, current recommendations are that inoculants need not be reapplied when soybean is grown again in the same field. This is thanks to year-over-year persistence of the inoculant in the soil. However these practices are primarily based off studies and farmer experience growing soybeans in Eastern ND. As soybean acres expand to more challenging soil and climate conditions in Western ND it is unclear how well this agronomic advice holds up. Unnecessary inoculation wastes farmers' money and cuts into their bottom line. However choosing not to inoculate carries significant risks since if nodulation does not occur, soybean crops may not get enough nitrogen resulting in yield losses. This is especially an issue in Western ND where more challenging soil types (acid or saline) or drought may affect the survival of inoculant strains in the soil, and the more well-defined recommendations for inoculation in Eastern ND may not apply.

Over the last two years we have developed a molecular tool (NDSoy) that can be used to quickly measure rhizobia populations in farmer's soil at low cost, using the same samples that are already collected for chemical analysis. The long term goal of the tool is to provide a service to farmers that can guide decisions about whether to inoculate or not, and to accelerate agronomic research on rhizobium inoculants in the state.

Preliminary data from last years' work on this project indicated rhizobia populations are lower in Western ND than in Eastern ND (Table 1), suggesting that inoculation might continue to be important much sooner than five years after the previous inoculation as currently advised in Esatern ND. With an anticipated finalized tool in place following FY23, in this project in FY24, we plan to collaborate with Williston, Hettinger and Carrington Research Extension Centers to perform trials that evaluate the nodulation and yield response of soybean to different inoculation methods based on varying levels of residual rhizobia in the soil.

This study will
1) Establish guidelines for inoculant recommendations based on the levels of rhizobia in field soil by identifying thresholds of rhizobia where there is a nodulation or yield response.
2) Investigate whether more frequent inoculation of soybean crops is required in Western ND compared to Eastern ND.

3) Compare the efficacy of different inoculant options in achieving a positive response to inoculation (liquid, peat, granular and double inoculation) under varying soil rhizobia levels.

Objectives:

Objective 1: Estimate a quantitative threshold of rhizobia below which farmers are expected to see a response to inoculation.
Objective 2: Evaluate and compare the response to inoculation with different inoculant types in soybeans grown in Western and Eastern ND soils with different residual levels of rhizobia.

Materials and Methods:

Fields were selected based on agronomic history at each of the RECs as those that had had soybeans planted/inoculated recently (Previous year at Carrington and Hettinger, and 3 years ago in Williston) (high rhizobia plots), or not recently (Never for Williston, 8 years ago for Hettinger and 7 years ago for Carrington (low rhizobia plots). Soil samples were collected from each of the plots and sent to the Geddes lab at NDSU for characterization of *Bradyrhizobium* population. We applied the NDSoy2.0 qPCR assay to these samples to enumerate the number of *Bradyrhizobia*. Briefly, DNA was extracted from each soil sample, and used as template for a qPCR reaction that included two primers and a TaqMan probe that target the Bradyrhizobium symbiosis gene *nodZ*. The resulting reaction mix was run with a genomic DNA standard curve which was used to estimate the total number of *Bradyrhizobium* per gram of soil. Data were collected from three replicates and averaged.

Inoculant plot trials were then performed at each of the three research extension centers in the sites with high and low levels of *Bradyrhizobium*. The trials included five treatments: uninoculated, liquid inoculant (BYSI-N Soybean), granular inoculant (Lalfix Spherical Soybean), peat inoculant (N-CHARGE) and double inoculant (N-CHARGE + Lalfix Spherical Soybean). Each treatment was applied two 5 replicate plots arrayed in a random design. Nodulation was tested by counting the average nodule number on ten plants after six weeks following planting. Following plot harvest, yield (bu/ac), test weight (lb/bu), protein content (%) and oil content (%) was analyzed. These data were used for statistical analysis of effects from the inoculant treatment by two-way ANOVA using treatment and location as factors.

Research Results and Outcomes:

For each of the three "low" rhizobia plots (without previous soybean planting history), no significant amounts of Bradyrhizobia were detected; their populations were below the detectable limit of the assay (<1000 cells per gram), a result that would prompt us to recommend inoculation. For the "high rhizobia" plots at each REC, we detected 130,396 cells/gram at Williston, 4,551,348 cells per gram at Hettinger, and 8,555 cells per gram at Carrington. These results indicated the plot selection would serve as a useful test for response to inoculant with different rhizobia levels and soybean planting histories at the RECs.

Plot trials were performed at each REC in high and low rhizobia plots. The treatments included uninoculated, liquid inoculant, peat inoculant, granular inoculant and double inoculation. Nodule counts were collected for each replicant from each treatment at all of the RECs at approximately the flowering stage of the soybeans (Figure 1). Overall, the most significant increases in nodulation were observed in both Hettinger and Williston in the "Low" rhizobia plots. In both cases, a substantial increased nodulation response to liquid inoculum was observed. Response to other inoculant products was much more limited and was not statistically significant. While a similar response was not observed in Carrington "Low", this plot was compromised early on due to overrun with kochia multiple times. Overall, these data indicate farmers could expect a nodulation response to inoculation when A) there is no history of soybeans, or B) our qPCR assay shows a "not detectable" number of *Bradyrhizobia* present in the field. Regarding product selection, of the products and inoculation strategies tested we observed a clear superior performance of the liquid product (Lallemand BYSI-N Soybean), compared to granular or peat products either separately or combined in a double inoculation approach.

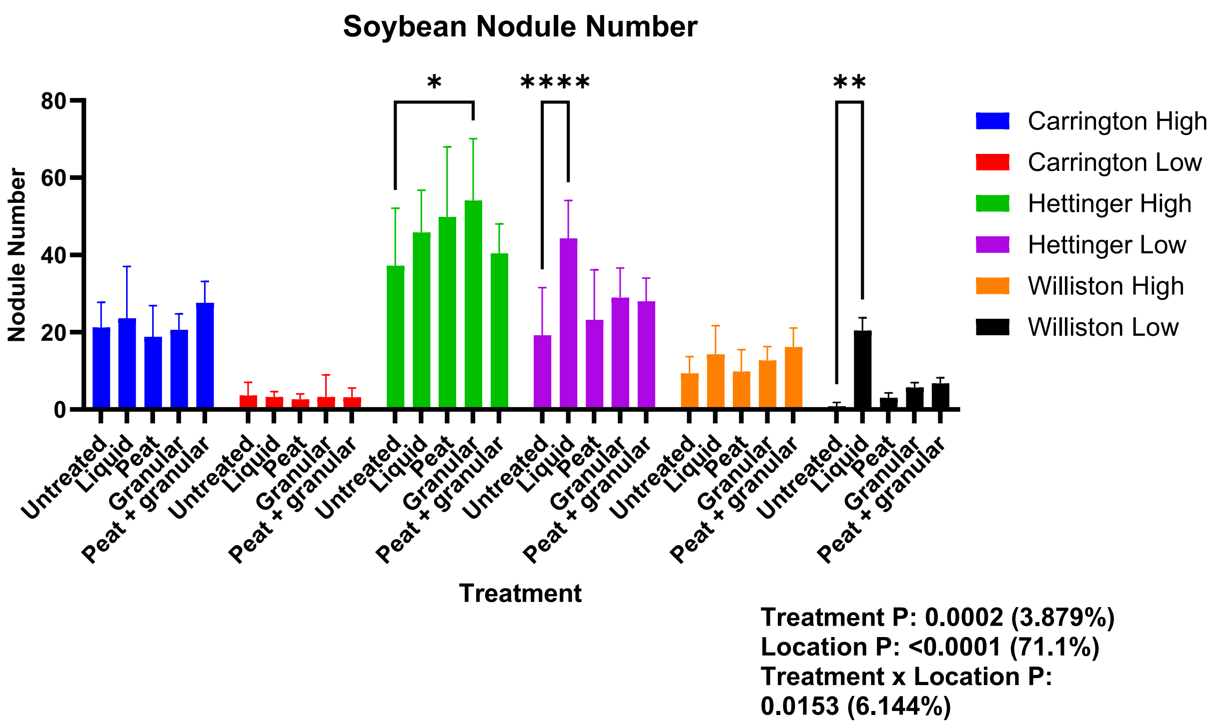
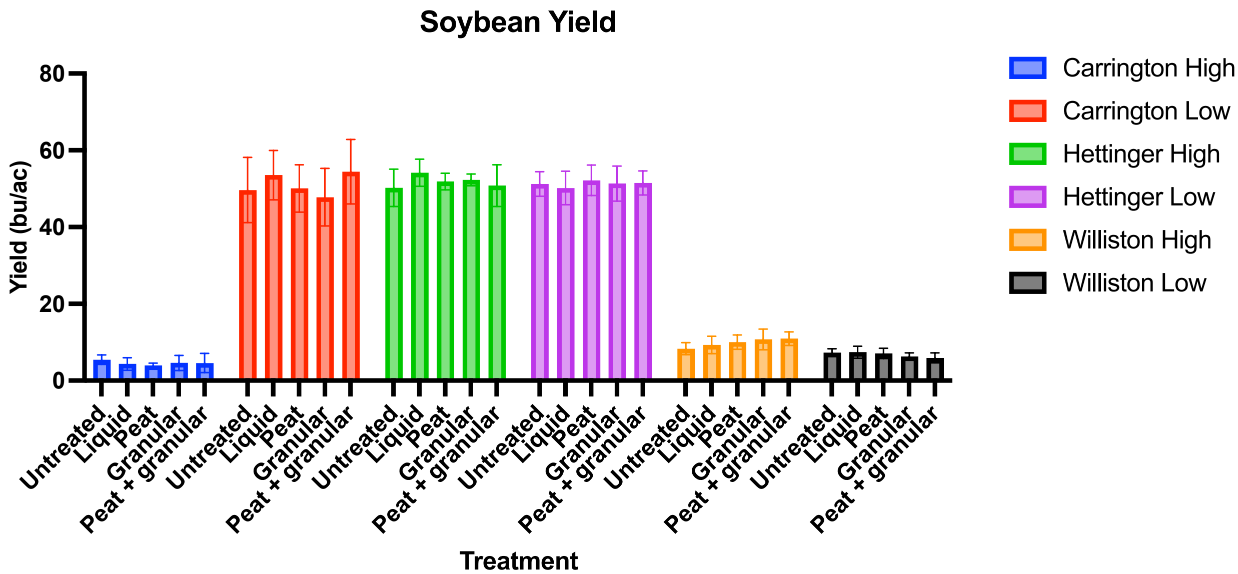


Figure 1. Nodulation data from plot trials with varying levels of rhizobia and soybean planting history.

Each of the plots above was harvested and yield data was collected to test if the response to inoculant (increased nodulation) resulted in increases in yield. However, no significant differences in yield were detected in any of the plots with inoculum treatment, including those that increased nodulation (Figure 2). Three of the six plots were compromised for yield due to hail in both Williston plots, and Kochia overrun in the Carrington "Low" plot. Therefore sufficient grain was only present in three plots for further analysis. However in these three, test weight, protein % and oil % of seeds was further analyzed (Figure 3-5). No significant difference was found according to inoculation treatment for any of these seed characteristics.



Treatment P: 0.7630 (0.038%)
Location P: <0.0001 (97.03%)
Treatment x Location P: 0.8190 (0.2926%)

Figure 2. Yield data from plot trials with varying levels of rhizobia and soybean planting history.

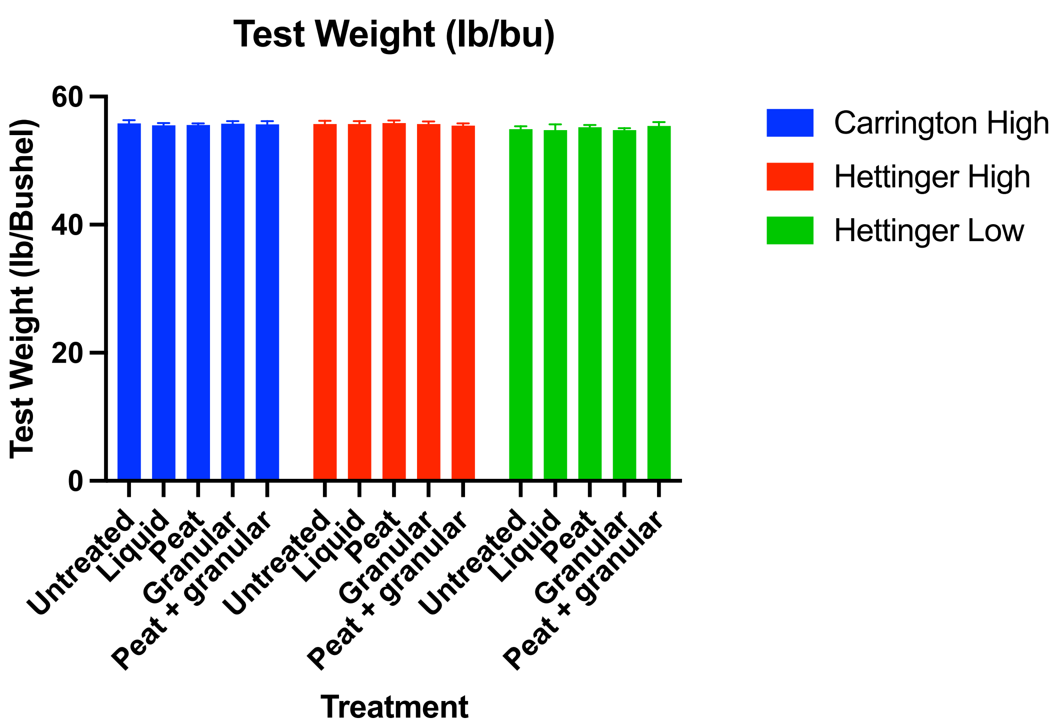


Figure 3. Test weight data from plot trials with varying levels of rhizobia and soybean planting history.

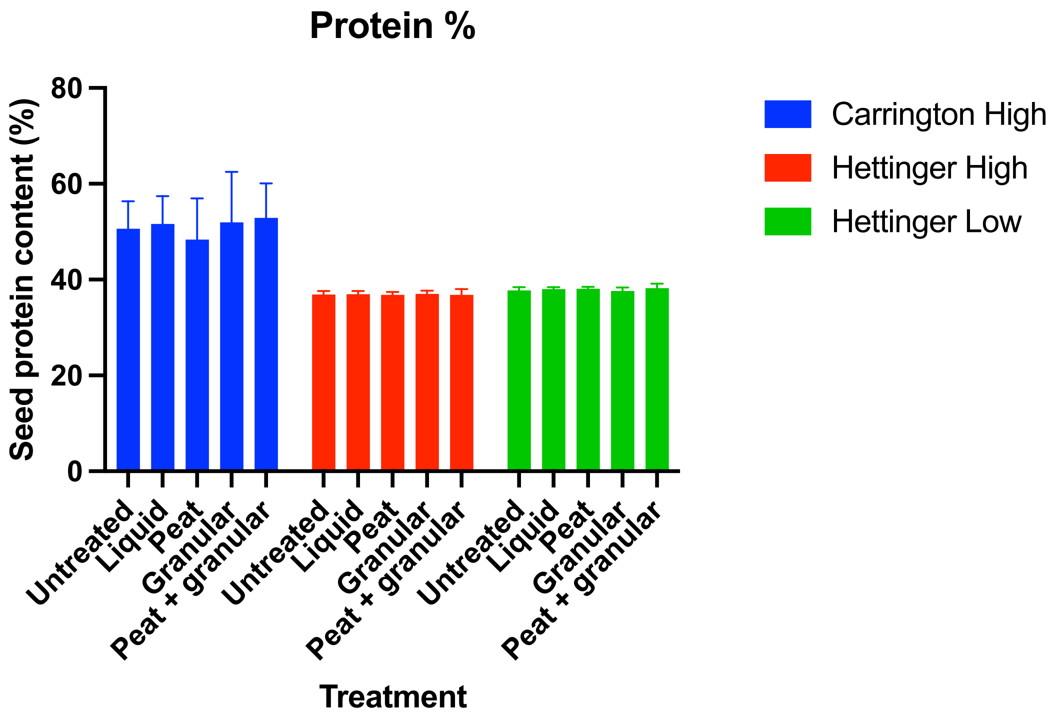


Figure 4. Seed protein content data from plot trials with varying levels of rhizobia and soybean planting history.

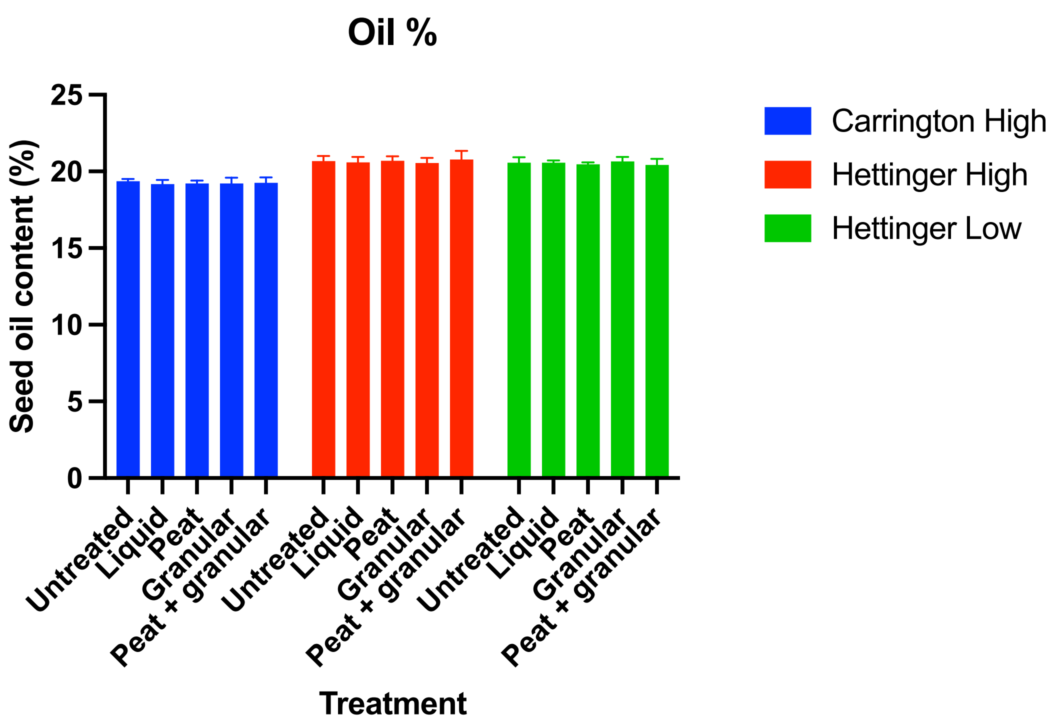


Figure 5. Seed oil content data from plot trials with varying levels of rhizobia and soybean planting history.

Discussion

Overall the nodulation data show significant responses to inoculant in both the Williston and Hettinger Low sites (where the assay indicated inoculation was necessary. No response to inoculant was detected at Carrington Low site, however the overall nodulation was very low at that site which was overrun by kochia outbreaks several times. These data are an added benefit to this study, perhaps guiding inoculant selection for ND farmers.. None of the high sites showed a strong response to inoculation, indicating when rhizobia are detected with our assay (even at low levels ~8000 cells per gram of soil), inoculation may not be beneficial. Two of the three sites with low levels of rhizobia (from lack of prior inoculation history) showed significant response in terms of nodulation to inoculation. However notably, only the liquid inoculant showed highly significant differences in nodulation outcome. Each inoculant was from the same company (Lallemand), so this was a robust test of inoculant technology and our data suggests liquid inoculant may be the best option for soybeans. When considering yield, test weight, protein content and oil content however, no significant differences were observed, even when inoculation proved beneficial in improving soybean nodulation. Presumably, the soybeans could derive sufficient nitrogen from the residual nitrogen in the soil to support optimal yield even when symbiotic nitrogen fixation was not optimal.

Conclusions/Benefits to Soybean Farmers.

Overall, these data supported that our molecular tool can guide farmers inoculant decisions. If the tool indicates rhizobia are not detectable, these data would indicate farmers could expect to see outcomes from inoculation (such as improved nodulation. It should also be noted that soybean planting history (>5 years ago) can also guide that selection as is currently suggested in the NDSU field guide. When comparing inoculation treatments, liquid inoculant proved to be by far the most effective product at producing nodules when inoculation was necessary.