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

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b. **Research Overview and Objectives**

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 costs (1). 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 (Andres 2019). 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 (Graham 1992, Howieson and Dilworth 2016), 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.

c. Completed Work:

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). We applied the NDSoy2.0 assay to these samples to enumerate the number of rhizobia. For each of the three low rhizobia plots, 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.

d. Progress of Work and Results to Date:

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).

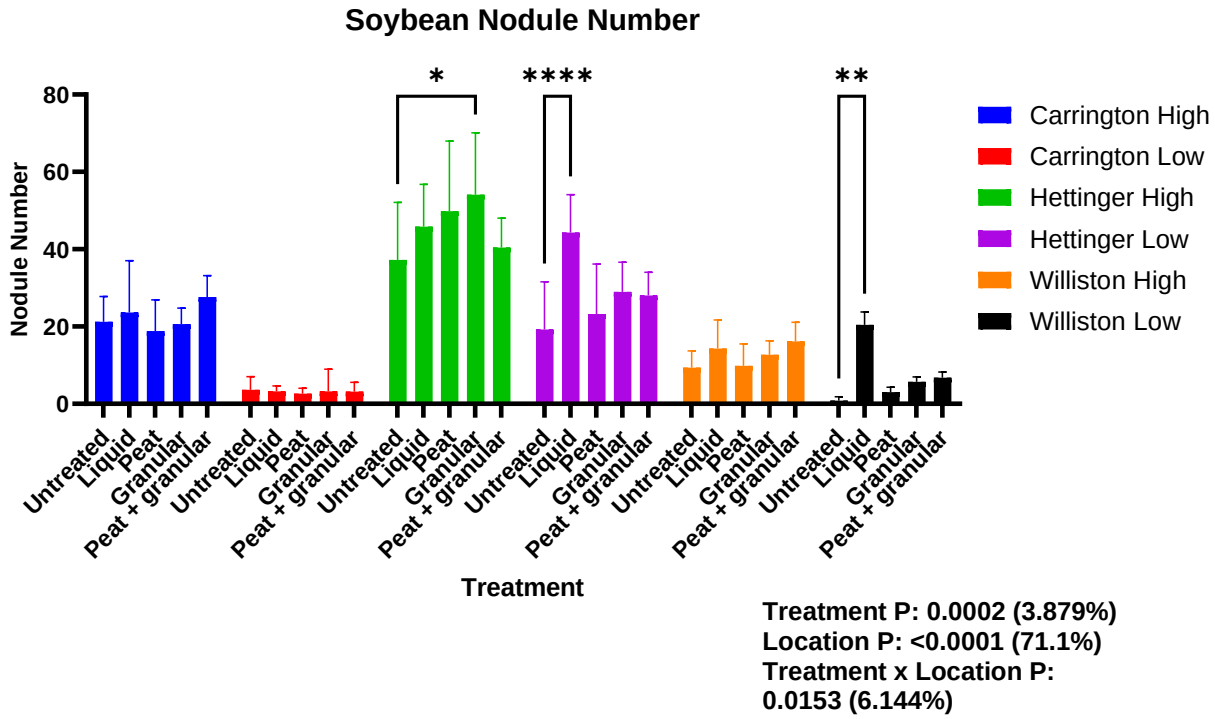

Figure 1. Nodulation data from plot trials with varying levels of rhizobia and soybean planting history.

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, perhaps indicating nitrogen levels that may be inhibiting nodulation. When response to inoculation was observed, liquid inoculant far outperformed the other inoculation approaches. These data are an added benefit to this study, perhaps guiding inoculant selection for ND farmers. Each inoculant was from the same company and with the same inoculant strain, so this was a robust test of inoculant technology. 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.

e. Work to be Completed:

The yield analysis from field trials and the connection of these finalized results to rhizobia levels from the fields to inform our assay is still to be performed.

f. Other Relevant Information:

None to add to above progress.

g. Summary:

Overall, these data supported that our molecular tool can guide farmers inoculant decisions, though soybean planting history (>5 years ago) can also guide that selection as is currently suggested in the NDSU field guide. Liquid inoculant proved to be by far the most effective product at producing nodules when inoculation was necessary.