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Received: 8 September 2023 | Accepted: 31 July 2024

DOI: 10.1002/agg2.20573

Agrosystems, Geosciences & Environment OPEN ACCESS

ORIGINAL ARTICLE

Agrosystems

Assessing the use of native rhizobia to improve nitrogen fixation under abiotic stress

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Assigned to Associate Editor Anil Somenahally.

Funding information
North Central SARE Research and Education, Grant/Award Number: LNC18-405; USDA Pulse Crop Health Initiative, Grant/Award Number: 58-3060-9-048

Abstract

Biological nitrogen fixation by rhizobia bacteria plays a pivotal role in sustainable agriculture by converting atmospheric nitrogen into a form that plants can assimilate, thereby reducing the need for synthetic fertilizers. This process can be dramatically reduced by various abiotic stressors. Native rhizobia strains, which are naturally occurring, may be better adapted to the local soil and climatic conditions, making them more resilient to stress factors such as drought, salinity, temperature extremes, and pH variations compared to commercial strains that may have been developed in and for different environments. This study aimed to compare the efficacy of native rhizobia species with a commercial inoculant and uninoculated controls in maintaining nitrogen fixation under induced stress by delayed planting in field peas over two growing seasons (2021 and 2022) in central South Dakota. Our findings indicate that native rhizobia, while not outperforming the commercial inoculant, demonstrated competitive nitrogen fixation capacities. Overall, total nitrogen fixation was not statistically different between a commercial inoculant and native rhizobia formulations. Planting date emerged as a significant factor influencing nitrogen fixation, with later planting substantially reducing overall effectiveness. These results highlight the potential of native rhizobia as an alternative to commercial inoculants and underscore the need for increased screening throughput and improved methods to assess rhizobia efficacy and nodule competition in field settings.

Abbreviations: BNF, biological nitrogen fixation; NDFA, nitrogen derived from the atmosphere (%); PT, planting timing; Rlv, *R. leguminosarum* bv viceae; YM, yeast mannitol.

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1 | INTRODUCTION

Against the backdrop of rising global food demand and concerns over climate change, calls for sustainable intensification of agricultural production are growing. Relatively low input, broadly adaptable crops, such as field peas (*Pisum sativum*, L.), serve a potentially critical role in this transition (Cusworth et al., 2021). Central to this paradigm is the process of biological nitrogen fixation (BNF), in which a plant-microbe symbiosis is established with rhizobia bacteria converting atmospheric nitrogen to a bioavailable form utilized by the plant in exchange for carbon energy. Globally, BNF adds 50–70 Tg N annually to agricultural systems, roughly equal to one-quarter of the global N applied as fertilizer, representing an enormous savings of external N inputs (Herridge et al., 2008).

While rhizobia are common in most soils, the host–symbiont relationship is species specific, initiated through biochemical signal exchange between plant and rhizobia (Oldroyd, 2013). In the case of field peas, *R. leguminosarum* bv. viceae (Rlv) is the most common bacteria found in the nodule. Previous work has demonstrated a broad range of BNF efficiency and competitiveness across Rlv strains in field pea production (Boivin et al., 2020; Laguerre et al., 2007; Mendoza-Suárez et al., 2020; Mutch & Young, 2004). Certain rhizobia may be very competitive in terms of establishing nodules with the crop host but are not effective and produce little nitrogen via fixation in exchange for the carbon they acquire (Mendoza-Suárez et al., 2021). The reverse may also be true with strains that are highly effective being unable to compete for nodule occupancy.

Moreover, the processes of nodule formation and BNF are susceptible to numerous abiotic stressors, including high salinity and drought (Sinclair & Serraj, 1995). In both high salinity and drought conditions, nodulation and BNF are generally limited by carbon and ATP acquisition, low nutrient uptake (i.e., inorganic phosphorus) through poor root development, and decreased nodule O_2 permeability (Serraj et al., 1999). Hence, the development of elite, competitive, and effective inoculant strains that can be applied across multiple environments, plant genotypes, and biotic stressors remains elusive (Bashan et al., 2014).

Despite the nuance that governs the establishment of this symbiosis and variation from strain to strain, much of the current inoculant market is dominated by just a few strains. In field peas, farmers in the United States have only a handful of inoculant options with the same strains being applied across the entire country. However, inoculant formulations comprised of native rhizobia strains with high BNF capacity may be superior to commercial inoculants because of their adaptivity to local conditions (Mendoza-Suárez et al., 2021). We hypothesize that native rhizobia, isolated and cultured from drought- and salinity-prone environments, will also confer greater tolerance to abiotic stessors over broadly applied commercial inoculants. This study was conducted to examine the ability of a select panel of native Rlv to maintain BNF under field conditions with induced abiotic stress when compared to a commercial strain.

Core Ideas

- Native *R. leguminosarum* inoculations performed similarly to an elite commercial strain under both stressed and non-stressed field conditions
- Native *R. leguminosarum* inoculation showed potential for reduced field pea reliance on soil-derived nitrogen
- Further research is needed to assess nodule competitiveness of native rhizobia and to increase field screening throughput.

2 | MATERIALS AND METHODS

2.1 | Rhizobia isolation

Soil was collected from 24 unique sites across Montana, North Dakota, and South Dakota in 2019 and 2020. The sites represent distinct (never cultivated) biomes, including high-altitude, mountainous regions, forest, undisturbed prairie, and shrub-steppe grassland. Soil was collected near native legume species that were likely to host Rlv. This included *Astralagus* spp., *Oxytropis* spp., *Dalea purpurea*, *Lathyrus ochroleucus, Pediomelum argophyllum, Psoralea esculenta*, and *Vicia americana*. Uninoculated field peas were grown in each of these soils as a 25:75 mixture with sterilized perlite and vermiculite media, replicated three times, under lab conditions, and harvested after 8 weeks to determine colonization by native rhizobia. To minimize the potential for lab contamination, the surface of each pot was covered with approximately 2.5 cm of sterilized vermiculite/perlite and all water added to the pots was pre-sterilized de-ionized, distilled water.

From the field peas that were successfully nodulated, five representative nodules from plants of each sample site-replicate were surface sterilized for 1 min with 70% ethanol, 1 min with 3% sodium hypochlorite, and then six changes of sterile DDI water. The nodules were then transferred to 15-mL Falcon tubes and crushed in 5 mL of yeast mannitol (YM) media (Vincent, 1970) with a sterile, disposable polypropylene pestle. The broth was then strained through a 70-µm nylon cell strainer (Corning Falcon) and 750 µL of filtrate was mixed with equal parts 50% glycerol solution and stored at −80 C° until further culturing of individual colonies (Howieson & Dilworth, 2016).

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TABLE 1 Nutrient and chemical analysis of soil collected from 0 to 60 cm soil depth. Values are averages of four replicated soil composites.

Year	Depth (cm)	pH	Organic matter (%)	Nitrate-N (ppm)	Ammonium-N (ppm)	Olsen-P (ppm)	Potassium (ppm)	Sulfate-S (ppm)
2021	0–15	6.7	3.9	4.7	6.8	13.1	484.5	13.9
	15–30	7.7	3.1	4.6	7.2	3.9	284.3	10.9
	30–60	8.1	2.6	5.5	6.4	3.7	225.5	11.4
2022	0–15	6.9	3.9	9	6.2	19.2	454.8	34.5
	15–30	7.6	3.1	6.8	7.1	4.4	276.3	21.2
	30–60	8.2	2.3	3.6	6.4	3.5	240.8	14.7

Next, single colony isolates were acquired by culturing the glycerol stocks in 10 mL of liquid YM medium in 50-mL centrifuge tubes and placed in a shaker (28° at 245 rpm) for 72 h. After successful growth, broth was streaked on 1.2% YM agar plates (Howieson & Dilworth, 2016). Five representative colonies were selected and transferred into nitrogen-free media. After 3–5 days of growth, isolates were harvested and subjected to further in vitro testing for stress tolerance.

At the cellular level, we postulate that similar to plants, microbes respond to early abiotic stress by regulating the osmotic gradient between the cell and its surrounding environment to maintain water flow (Bremer & Kramer, 2019; Kempf & Bremer, 1998; Ma et al., 2020; Numan et al., 2018). We screened rhizobia strain tolerance to increasing salinity, assuming a similar response to induced drought. To determine saline tolerance, isolates were screened in NaCl-supplemented liquid YM cultures (three replicates) at salinity concentrations of 0.0017, 0.25, and 0.5 M and measured the OD600 over a 5-day period. In previous salinity testing work by Dong et al. (2017), a tolerance range from control YM media to 0.5 M NaCl showed a steep decline in isolation of bacteria for high saline tolerance. Since a broader purpose of our work involves in finding halotolerant rhizobia that can survive abiotic stress conditions in the field, the selection criteria of 0.5 M NaCl was used as an upper limit, with the 0.25 M NaCl used as a midpoint to test under still stressful but less extreme conditions. Optical density was measured against a blank YM standard. USDA 110, a highly characterized rhizobia, was used as a control to determine the reliability of the experiment (Dong et al., 2017).

Those rhizobia exhibiting tolerance at the highest levels of salinity were advanced to further testing, including indole acetic acid production, acetylene reduction, phosphorus solubilization, antibiotic resistance, and genetic verification (manuscript in process). Eventually, the candidate list was pared to three rhizobia species for field testing—denoted as V23.3, V47, and V85. Each species identity was confirmed through 16S rRNA sequencing prior to field evaluation.

2.2 | Field methods

2.2.1 | Study site

Field studies were conducted in central South Dakota at the Dakota Lakes Research Farm (44.2929°N, −100.0066°W), on a Pierre clay loam soil (Fine, smectitic, mesic Torrertic Haplustepts) over two growing seasons from 2021 to 2022. The soil nutrient profile and chemistry is shown in Table 1. Growing season precipitation and temperature are depicted in Figure 1.

2.2.2 | Trial establishment

Two planting dates were established with an "on-time" planting occurring within the traditional planting window (mid-April) and a "late" planting, which occurred approximately 3 weeks later. Bueckert et al. (2015) found a strong negative correlation between mean maximum daily temperature and the length of reproductive growth. Concomitant seed yield was significantly decreased for later planting dates, corresponding to a greater exposure to higher temperatures and a shortened reproductive phase. Assuming anthesis occurs approximately 40–50 days after emergence, the second planting timing (PT) in this study was designed to push the flowering date later into the summer where the probability of high heat was greater, hence inducing greater abiotic stress for this treatment. Temperature and precipitation for the study are depicted in Figure 1, which demonstrates that temperatures began to exceed the stress threshold for field peas in early to mid-June

Additionally, in 2021, precipitation was below normal with cumulative precipitation roughly 30% below the precipitation in 2022. The majority of the 2021 growing season was in likely water stress with most of the growing season precipitation arriving after the reproductive stage began. In 2022, the growing season precipitation was more conducive for growth of the on-time planting as hypothesized in the study design.

Sterilized field peas (cv. Carver) were planted on 25-cm row spacing with a no-till grain drill (Model 750, John Deere

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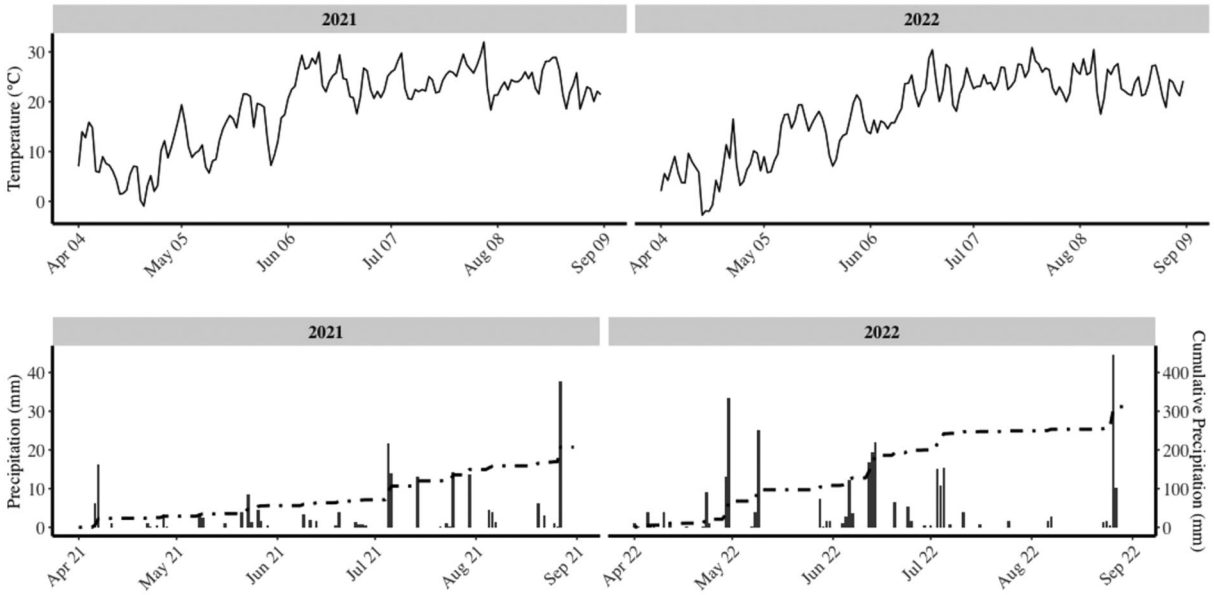


FIGURE 1 Temperature (°C) and precipitation (mm) for each site and year of the study. For the precipitation, the dotted line depicts cumulative precipitation on the secondary *y*-axis.

Co.) at a population of 86 pure live seed m^{-2}. Prior to planting, the grain drill seed tubes were sterilized by rinsing with 70% ethanol, and all drill parts were wiped down with paper towel and 70% ethanol. To minimize contamination at planting, the drill closing wheels were tied up, which left the furrow open and the uninoculated seed exposed. This allowed hand application of rhizobia inoculants on a per-plot basis. In 2021, the on-time and late planting dates were April 27, 2021 and May 19, 2021. In 2022, these planting dates were April 27, 2022 and May, 22, 2022, respectively.

The same randomized complete block design was established each growing season with six replications of each treatment, which includes native species V23.2, V47, and V85 plus a commercial liquid rhizobia inoculant (single strain—proprietary) as a positive control and an uninoculated negative control to control for background soil rhizobia. At the time of application, each native rhizobia was grown in a secondary fermentation for 3–5 days. At this time, each inoculant was centrifuged and re-suspended in phosphate buffer solution as the carrier to OD600 = 0.08 (Balatti & Pueppke, 1992; Begum et al., 2001). The commercial liquid inoculant was applied according to the manufacturer's specifications.

2.2.3 | Sampling and harvest

Biomass sampling for nitrogen fixation and seed harvest was taken from the center two rows, leaving 0.5 m on the front and back of each plot and the outer two rows acting as buffer areas. The harvested area consisted of four planted rows of 3.8 m^{-2}. Grain yield was determined by conducting a stand count for each plot then harvesting 15 representative plants. Total biomass and yield were calculated on a dry weight basis (kg ha^{-1}). Nitrogen content in the seed and shoot (%) was determined using a dry combustion C:N analyzer (TruSpec CHN, Leco Corporation).

2.3 | Quantifying N fixation and N uptake

After verification that nodulation was present, biomass samples for quantifying N fixation were taken at full bloom for all treatments and planting dates. Biomass samples consisted of 1 m^2 from the center two rows of the plot with the whole shoot being used for further analysis. All samples were oven-dried at 60°C until a constant weight was achieved, then ground and analyzed for total N and $\delta^{15}N$ at the University of Wyoming Stable Isotope Facility. Nitrogen derived from the atmosphere (NDFA) and total N-fixation were based on the natural abundance method calculated according to Equations (1)–(3) (Unkovich et al., 1994):

$$\%\text{NDFA} = \frac{(\delta^{15}\text{N of reference plant} - \delta^{15}\text{N of } N_2 \text{ field peas})}{(\delta^{15}\text{N of reference plant} - B)} \times 100 \quad (1)$$

where $\delta^{15}N$ was calculated by subtracting the atom% ^{15}N natural abundance of the atmosphere (0.3663 atom%) from the corresponding atom%^{15}N excess in field pea and the reference crop. For the latter, spring wheat (*Triticum aestivum*, L) and flax (*Linum usitatissimum*, L) were established within the study area to ensure similar access to $\delta^{15}N$ in soil N pools as

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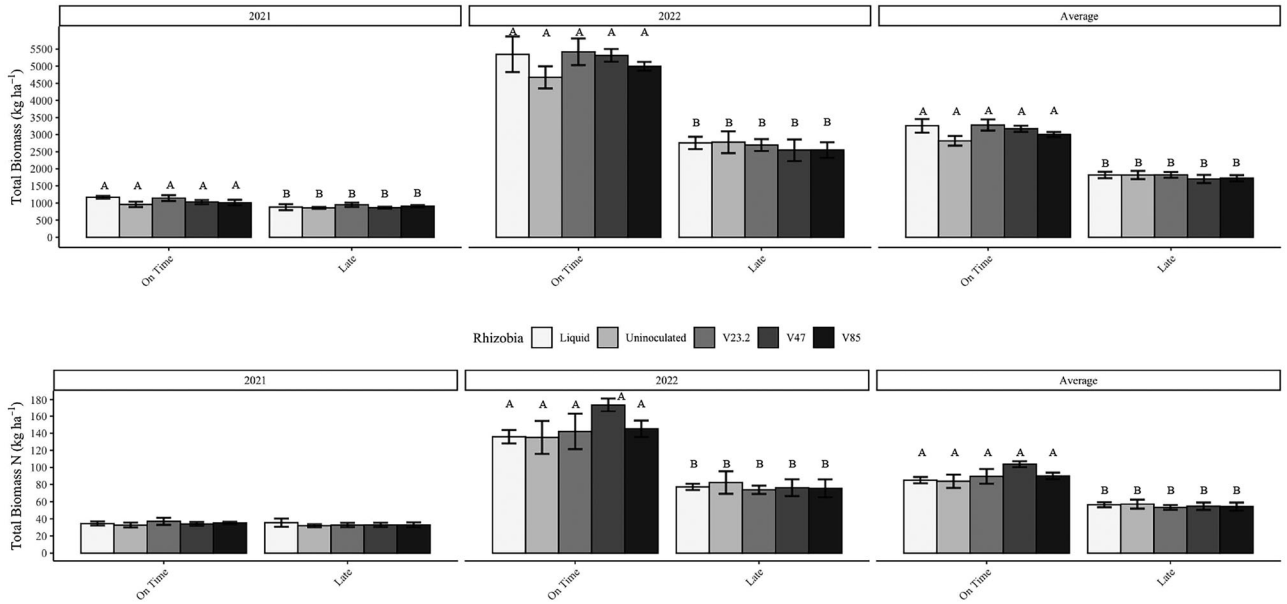


FIGURE 2 Total biomass and total N uptake (kg ha^{-1}) at the on-time and late planting dates averaged across both years of study. Error bars represent the standard error of the mean. Letters above each bar represent the statistical means separation with different letters denoting statistically significant differences between treatments determined at a significance level of $p < 0.05$.

the field peas. Both reference plant plots were replicated six times and the final δ^{15}N value for the reference plant was the average δ^{15}N of both crops within each season and planting date. However, in practice, these two reference plants generally yield very similar results. Additionally, the *B*-value used for all rhizobia inoculants was −0.66‰ based on average values obtained from Unkovich et al. (2008).

$$\text{Total N fixation (NFix)} \left(\text{kg N ha}^{-1}\right) = \frac{(\%\text{NDFA})\,(\text{Total shoot N})}{100\%} \quad (2)$$

$$\text{Total shoot (or seed) N} \left(\text{kg N ha}^{-1}\right) = \frac{(\text{DW})\,(\%\text{N})}{100\%} \quad (3)$$

where %N is the percentage of N in the crop and DW is the shoot dry matter weight (kg ha^{-1}).

2.4 | Statistical analysis

Data were analyzed as a linear mixed-effects analysis of variance model with the Kenward–Roger approximation for denominator degrees of freedom using the lme4 and lmerTest libraries (Bates et al., 2015; Kuznetsova et al., 2017) in the R statistical package (v4.2.2; R Core Development Team, 2022). When analyzing across the full study period, Rlv and PT were the primary treatment factors of interest with the goal of generalizing the results across the range of weather outcomes. Therefore, year was analyzed as a random effect. Replications were also analyzed as a random effect. For individual year analysis, R and PT were analyzed as fixed effects with replication as a random effect.

Boxplots were used to assess the data structure and potential outliers. Statistical analysis of outliers was confirmed using Rosner's Test in the EnvStats package (Millard, 2013). From this analysis, two samples were discarded based on total biomass. Residual *Q*–*Q* plots, plots of residual error versus fitted values, density distribution, and the Shapiro–Wilk test were applied to examine the normality of residuals and any departure from homogenous variance and skedasticity. The most common departure from normality was as a negative skewness. In these cases, Tukey's Ladder of Powers transformation was utilized to transform the data to a more normal distribution (Tukey, 1977). Significance was determined at $p \leq 0.05$ (unless otherwise stated) with means separation determined using the Tukey method in the lsmeans module (Lenth, 2022). Pearson's correlation coefficient (r) was used to determine linear correlation between any two variables. All correlations reported were statistically significant at $p \leq 0.05$.

3 | RESULTS

3.1 | Total biomass, C:N, and total N uptake

Total biomass production varied dramatically between growing seasons and PT (Figure 2). For differences between PT under prevailing drought conditions (2021 season), biomass

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TABLE 2 Analysis of variance (ANOVA) table for C:N, total biomass (kg ha^{-1}) and total N uptake (kg ha^{-1}).

Source	DDF	Total biomass	C:N	Total N uptake
Rhizobia (Rlv)	4	NS	NS	NS
Planting timing (PT)	1	<0.001	0.02	<0.001
Rlv × PT	4	NS	NS	NS

Note: Statistical results (Pr > *F*) represent the combined dataset from 2021 and 2022 determined at a significance level of $p < 0.05$.
Abbreviation: DDF, denominator degrees of freedom.

TABLE 3 Analysis of variance (ANOVA) table for N derived from the atmosphere (NDFA, %) and total nitrogen fixation (Nfix, kg ha^{-1}).

Source	DDF	NDFA	Nfix	Seed N uptake	Yield
Rhizobia (Rlv)	4	0.02	NS	NS	NS
Planting timing (PT)	1	<0.001	<0.001	<0.001	<0.001
Rlv × PT	4	NS	NS	NS	NS

Note: Statistical results (Pr > *F*) represent the combined dataset from 2021 and 2022 determined at a significance level of $p < 0.05$.
Abbreviation: DDF, denominator degrees of freedom.

production dropped by ~20% on average across all rhizobia treatments. In 2022, under more favorable growing conditions, total biomass production was reduced by 48% with a late planting. As Table 2 denotes, the planting date differences were statistically significant. However, no statistical differences were observed between rhizobia inoculants.

The C:N of the shoot biomass only varied statistically by PT (Table 2). Across site-years, the C:N in 2021 was lower on average ($p < 0.05$) when compared to 2022 (12.4 vs. 15.7). A similar overall effect was observed when averaged across years between PT with the C:N decreasing from 14.6 to 13.5 between the first and second planting (Table 2). This is likely due to a dilution effect with higher biomass leading to a wider C:N. Pearson's correlation between C:N and total biomass was statistically significant ($p < 0.001$, $r = 0.53$).

Total N uptake varied statistically only with PT (Table 3). This difference resulted largely under more favorable growing conditions with a 47% decrease in total N uptake during the late planting (Figure 2).

3.2 | NDFA and total nitrogen fixation

When averaged across all treatments for on-time plantings, NDFA varied from a high of 72% in 2022 to a low of 35% under severe drought conditions in 2021. NDFA decreased significantly with the late planting (Table 3). This decline was especially acute for the uninoculated control, which decreased by 35%, while the average of the inoculants fell by 15% (Figure 3). Hence, irrespective of total biomass N uptake, as the planting date is pushed later into the spring, uninoculated field peas decreased their reliance on rhizobia symbiosis (i.e., %NDFA decreases) for N nutrition and shifted towards soil-derived N.

All rhizobia inoculants had statistically higher NDFA than the uninoculated control when averaged across both planting dates. On average, the inoculated treatments had an NDFA of 56.5% compared to 47.8% in the untreated control. However, when analyzed by PT, only V23.2 was statistically higher than the uninoculated control at both PTs (Figure 3). Despite differences in NDFA between rhizobia inoculants, total N fixation was not statistically different among inoculants (Table 3). However, total N fixation decreased significantly with delayed planting (Table 3). Nitrogen fixation is based on the combination of NDFA and total biomass, which both had high variability in this study, regardless of PT. Inoculation by any species generally produced greater N fixation compared to the uninoculated control, and the difference was exacerbated, particularly by the native species with adequate moisture and on-time planting (Figure 3).

3.3 | Seed N uptake and yield

Seed N content was not affected by Rlv but was significantly lower in the late PT (Table 3). Seed N uptake was decreased from an average of 98 to 46 kg ha^{-1} in the late planting. (Figure 4). Additionally, there was a significant correlation between total N in the biomass at anthesis and total N uptake in the seed ($p < 0.001$, $r = 0.71$).

Yield by inoculant showed a similar trend to N fixation. Overall, there was a strong statistical correlation between N fixation and yield ($p < 0.001$, $r = 0.77$). However, this appears to be related more strongly to total biomass production ($p < 0.001$, $r = 0.82$) rather than absolute N fixation. The correlation between seed yield and NDFA was also statistically significant but less strongly correlated ($p < 0.001$, $r = 0.53$). Statistical differences between the inoculants and the uninoculated control were not observed (Figure 4). PT significantly affected seed yield (Table 3). When averaged across all treatments, yield by PT decreased by 41% during the dry year (2021) and 64% in wetter year (2022).

4 | DISCUSSION

Overall, we found that delayed planting of field peas results in significant reductions in plant productivity as measured by biomass production and seed yield. Presumably, this is a result of increased temperatures affecting soil moisture availability and seed set (Sinclair & Serraj, 1995). The aim of the current study was to examine if native rhizobia, selected for stress

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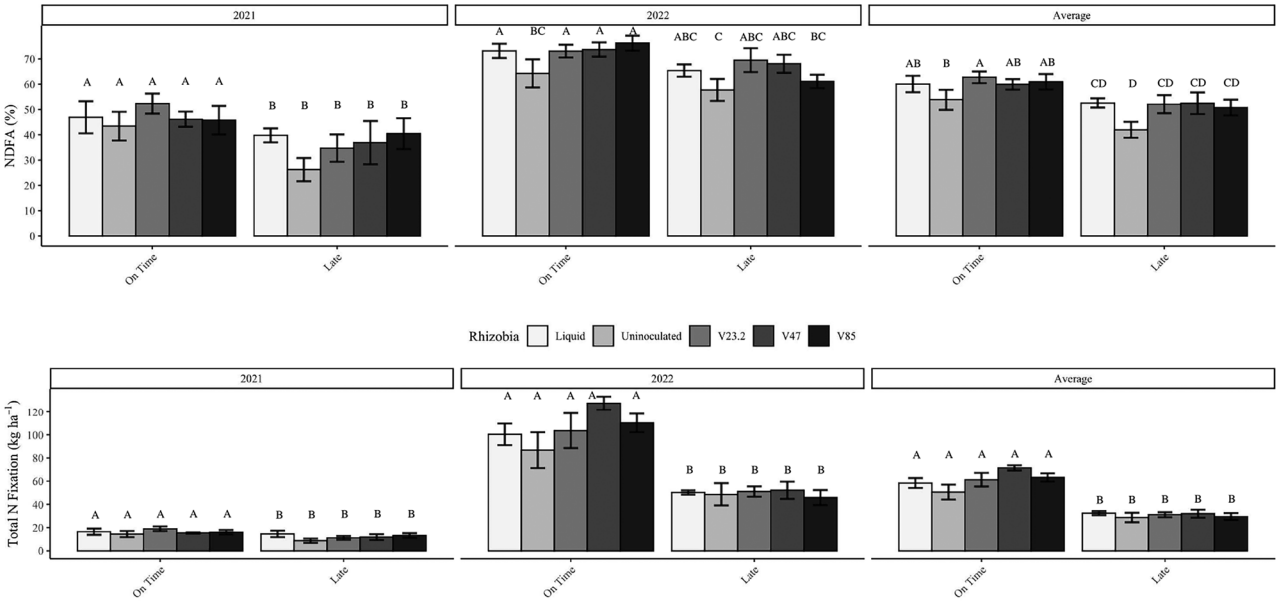


FIGURE 3 Nitrogen derived from the atmosphere (NDFA, %) and total N fixation (kg ha^{-1}) at the on-time and late planting dates averaged across both years of study. Error bars represent the standard error of the mean. Letters above each bar represent the statistical means separation with different letters denoting statistically significant differences between treatments determined at a significance level of $p < 0.05$.

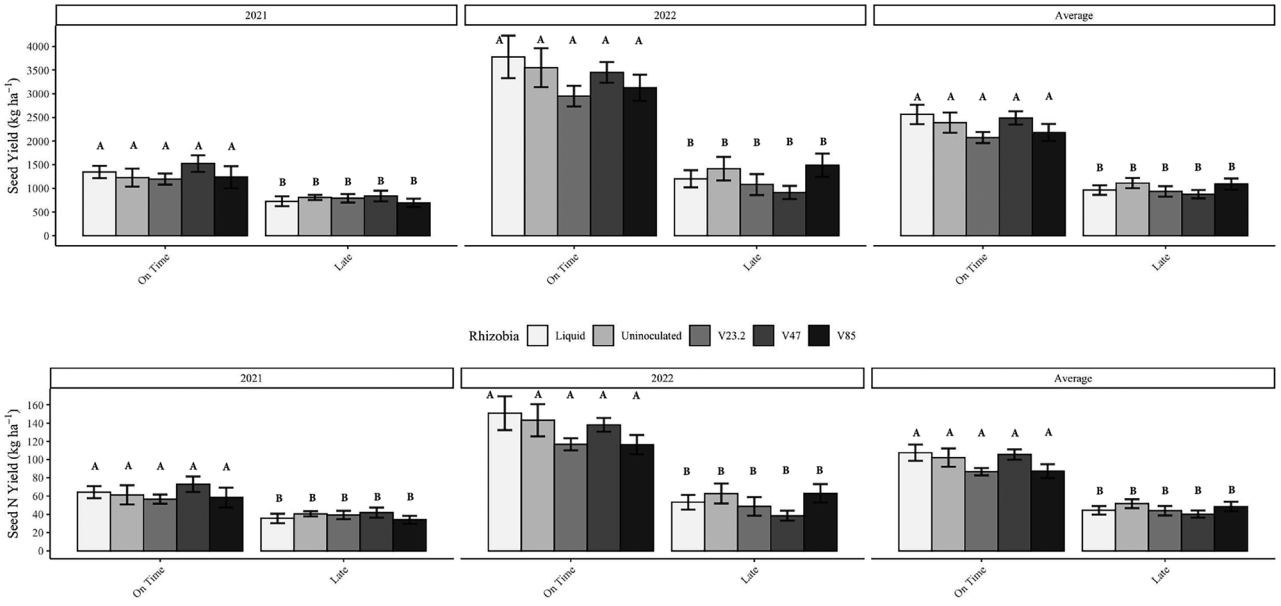


FIGURE 4 Seed yield (kg ha^{-1}) and seed N yield (kg ha^{-1}) for each rhizobia treatment at on-time and late planting timings across both years of study. Error bars represent the standard error of the mean. Letters above each bar represent the statistical means separation with different letters denoting statistically significant differences between treatments determined at a significance level of $p < 0.05$.

tolerance in lab conditions, could maintain N fixation and yield over uninoculated and/or commercial inoculants under the stress conditions experienced in a field setting. This was borne out for NDFA to some extent when compared to the uninoculated control but not compared to the commercial inoculant. The yield was unaffected by inoculant (Figure 4).

When averaged over both years, total NDFA trended higher in treatments inoculated with each strain of native rhizobia, with V23.2 being the only inoculant (including the commercial strain) statistically higher than the uninoculated control at both planting dates (Figure 3). However, total N fixation was not statistically different (Table 3). When PT was

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delayed, all treatments tended to merge to a similar range. The discrepancy between NDFA and total N fixation may be due to either competitiveness or fixation efficiency. Multiple rhizobia strains may inhabit the same nodule with varying efficiency in nitrogen fixation (Checcucci et al., 2017; Mendoza-Suárez et al., 2020).

Additionally, the field pea host has the ability to sanction poor-performing rhizobia by limiting carbon assimilates to the nodule (Kiers et al., 2003; Westhoek et al., 2017). While a higher NDFA with inoculation suggests more reliance from the plant on atmospherically derived nitrogen. The plant also has the ability to invest more energy in root development and supplemental soil N uptake to continue plant growth and biomass accumulation. A statistical difference in NDFA and a lack of statistical difference in yields between inoculants suggests that soil N was adequate to offset any efficiency gains between species (Table 1). These differences may be exacerbated where residual soil N is limited or where the local rhizobia population is deficient (Thies et al., 1991; Vargas et al., 2000).

On the other hand, a lack of statistical difference for both total N fixation and yield parameters between the native rhizobia inoculants and the commercial inoculant suggests that there is potential to mine native rhizobia populations in the search for elite field pea inoculants. In contrast to our hypothesis, the native rhizobia performed particularly well under more conducive growing conditions (2022; Figure 3) rather than under stress conditions. For example, V47 fixed roughly 30 kg ha^{-1} more than the commercial inoculant in the on-time planting in 2022. This represents a significant amount of N addition to the soil. However, due to the lack of significant differences with the uninoculated control on most measures, significant efforts should be placed not just on growth parameters in the lab but also on competitiveness and carbon use efficiency in the field.

These results are similar to Bourion et al. (2018), who found no correlation between competitiveness and nitrogen fixation efficiency of introduced rhizobia against high background rhizobia populations despite favorable nitrogen fixation capacity under controlled, sterile environments. This issue is exacerbated as plants cannot distinguish efficiency differences between two species of rhizobia in the early stages of nodule formation (Westhoek et al., 2021). In environments where high background rhizobia persist, as in the present study, differences between rhizobia species in a mixture are harder to distinguish.

Further, given the many interacting variables across diverse landscapes that influence the N fixation process, a consortium approach combining both commercial strains with rhizobia native to a given environment may provide significant benefits (Mitter et al., 2021). However, screening promising rhizobia strains in the field remains extremely difficult due to high variability in baseline levels of N fixation. Figure 3 suggests that contamination was limited based on the statistically lower NDFA in the uninoculated treatment, however, we did not confirm this experimentally. Improved planter design with the ability to independently deliver inoculated seed through replaceable seed tubes would allow for vastly increased throughput.

5 | CONCLUSION

This study demonstrates that native rhizobia, screened under laboratory conditions for increased abiotic stress, can perform similar to commercial inoculants under field conditions. With respect to N fixation parameters, both native rhizobia inoculants and the commercial control inoculant performed similarly under both stressed and non-stressed conditions. The percent of NDFA was generally higher than in the uninoculated control. This suggests that native rhizobia strains have the potential to serve as either alternatives to current elite strains or in consortia to improve the resilience of the field-rhizobia complex under abiotic stress. Moreover, future work may build on this evidence to promote the development of a more distributed inoculant production system where inoculants are tailored to specific environments. Further work is necessary to determine nodule occupancy and competitiveness of promising native rhizobia species and to increase the throughput of screening potential candidates under field conditions.

AUTHOR CONTRIBUTIONS

Jesus Loya: Conceptualization; data curation; formal analysis; investigation; methodology; writing—original draft; writing—review and editing. **Christopher Graham**: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; supervision; visualization; writing—original draft. **Audrey Kalil**: Conceptualization; funding acquisition; investigation; methodology; writing—review and editing. **Jed Eberly**: Investigation; methodology; writing—review and editing.

ACKNOWLEDGMENTS

The authors would like to thank all the workers involved with this project and in particular the staff at the SDSU West River Research Farm including Bruce Swan, Travis Iverson, Logan Hoon, and the Dakota Lakes Research Farm including Dr. Dwayne Beck and Sam Ireland. Funding for this research was through a SARE Research and Education Grant (LNC18-405) and the USDA Pulse Crop Initiative (3060-21650-002-006-S) as well as the South Dakota State University Experiment Station.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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How to cite this article: Loya, J., Subramanian, S., Kalil, A., Keene, C., Sanyal, D., Eberly, J., & Graham, C. (2024). Assessing the use of native rhizobia to improve nitrogen fixation under abiotic stress. *Agrosystems, Geosciences & Environment*, *7*, e20573. https://doi.org/10.1002/agg2.20573

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