LENTIL NITROGEN FIXATION RESPONSE TO FERTILIZER AND INOCULANT IN THE NORTHERN GREAT PLAINS

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ABSTRACT

Lentil production in the semi-arid northern Great Plains has increased dramatically over the past two decades, providing agroecosystem benefits of efficient water use, pest cycle disruption, and biological nitrogen (N) fixation. Through N fixation, lentil may help alleviate soil acidification and groundwater contamination by reducing N fertilizer needs. Despite widespread farmer adoption of lentil in the region, little is known about the benefits of fertilizer or inoculant type on N fixation. The aim of this study was to determine how different nutrients (potassium, sulfur, and foliar-applied micronutrients), as well as *Rhizobia* inoculant types (seed-coat and granular), influence N fixation of lentil. The study was conducted at two field sites in Montana from 2019 to 2021. Fixed N amounts were calculated using both an N difference approach and the ^{15}N natural abundance method. N fixation proved to be highly responsive to climatic conditions and soil characteristics. The amount of N fixed did not respond to potassium fertilization. In a moderately dry year at a site with low soil sulfate levels, sulfur fertilization increased N fixed by 40 percent. No differences in N fixed between inoculant types were found, and inoculated lentil fixed more N than uninoculated lentil in two site-years. The study's findings reveal that sulfur fertilization and rhizobial inoculation have potential to significantly increase lentil N fixation amounts in the northern Great Plains.

INTRODUCTION

The prevalence of cereal crop monoculture and summer fallow in the northern Great Plains (NGP) presents several challenges to the region's agricultural industry. Reliance upon N fertilizers causes soil acidification (Brown et al., 2008; Engel unpub data) and elevated nitrate concentrations in groundwater (John et al., 2017). Summer fallow is known to be destructive to soil quality (Carlyle, 1997). Pulse crops, including lentil, a common summer fallow replacement, can reduce N fertilizer inputs through N fixation. As a result, lentil has experienced rapid growth over the past 20 years and was planted on 368,000 ac in 2020 in Montana (USDA-NASS, 2020). While phosphorus is understood to limit N fixation, there has been no research on how other nutrients, such as potassium (K), sulfur (S), or micronutrients impact lentil N fixation in the NGP. Additionally, the effect of inoculant type on lentil N fixation is unknown despite granular inoculant being 3 to 4-fold more costly than seed-coat inoculant per acre. The objective of this study was to evaluate if fertilizer (K, S, and foliar-applied micronutrients) and inoculant type (seed-coat or granular) influence N fixation amounts of lentil.

MATERIALS AND METHODS

Field Descriptions and Experimental Design

Field experiments were conducted at Montana State University's Arthur H. Post Agronomy Research Farm (PF) near Bozeman, MT and at Northern Agricultural Research Center (NARC) near Havre, MT from 2019 to 2021. Soils at PF were Amsterdam silt loams (Typic Haplustolls), and soils at NARC were Telstad and Kenilworth loams and sandy loams (Aridic Argiustolls). Site selection at PF and NARC was based on soil sampling for a range of parameters including nitrate-N, sulfate-S, and exchangeable K to identify locations within suitable fields that had relatively similar nutrient levels.

Treatments included the following eight combinations of inoculant and fertilizer formulations.

1. Control
2. Granular inoculant w/o K or S
3. Seed-coat inoculant w/o K or S
4. Granular inoculant w/ K (15 lb K_2O/ac)
5. Seed-coat inoculant w/ K
6. Granular inoculant w/ K + S (5 lb S/ac)
7. Seed-coat inoculant w/ K + S
8. #6 w/ foliar micronutrients (Micro1000, by AgroLiquid, at 0.25 gal/ac)

The foliar micronutrient treatment was not included in 2019 trials. The experiment was conducted in a randomized complete block design with five replicates.

Plot Management

Avondale lentil, a medium green variety, was seeded at a rate of 11 plants/ft^2 in 6x25 ft plots. Seeds were inoculated with commercial granular and seed-coat inoculants following manufacturer labels. A non N-fixing reference crop of flax was seeded in a 2-m perpendicular strip adjacent to the lentil plots. Monoammonium phosphate (MAP, 11-52-0) was applied in furrow at a rate of 45 lb/ac to provide 5 lb/ac of starter N and 23 lb P_2O_5/ac of starter P. Appropriate pest management practices were conducted throughout the growing season.

Data Collection

A representative area of each lentil plot was sampled for aboveground biomass around early to late pod fill. A corresponding flax sample was taken for every three lentil plots, except in 2021 when a flax sample was taken for each lentil plot. All biomass samples were dried (120°F, 72 h), weighed, and ground (<0.5 mm). Tissue subsamples were analyzed for total N via combustion (LECO, St. Joseph, MI, USA). Isotope ^{15}N was analyzed with a continuous flow isotope mass spectrometer (Stable Isotope Facility, UC Davis, USA). After lentil was harvested, one 3 ft soil sample was taken from each lentil plot. Soil was also sampled from each location where flax biomass was harvested. The soil samples were split in the field into three 1-ft subsamples. Soils were dried (120°F, 72 h), weighed, and bulk density was calculated. Soils were ground (<2 mm), extracted using 1M KCl, and analyzed for nitrate-N. Total N pool for each 3 ft sample was calculated.

Measuring N Fixation

The amount of N fixed by lentil was calculated using two methods: an N difference approach (ND) and the ^{15}N natural abundance (NA) method. The ND approach used the following equation as reported by Unkovich et al. (2008):

$$N\ fixed = \left(Biomass\ N_{lentil} - Biomass\ N_{flax}\right) + \left(Soil\ N_{lentil} - Soil\ N_{flax}\right) \quad [1]$$

Biomass N is the product of dry aboveground biomass and biomass N concentration. Soil N is the total nitrate-N in the 90 cm soil sample taken shortly after lentil harvest.

For the NA method, the fraction of N derived from the atmosphere (fNdfa) was calculated using the following equation from Shearer and Kohl (1986):

$$fNdfa = \left(\frac{\delta^{15}N_{reference} - \delta^{15}N_{legume}}{\delta^{15}N_{reference} - B}\right) \quad [2]$$

Where $\delta^{15}N$ is:

$$\delta^{15}N\ (‰) = \left(\frac{atom\%N_{sample} - atom\%N_{atmosphere}}{atom\%N_{atmosphere}}\right) * 1000 \quad [3]$$

B in Eq. 2 refers to the $\delta^{15}N$ of a pulse crop grown in the absence of soil N, meaning it reflects conditions in which all plant N is fixed from air. A B value of -0.99 was determined by growing Avondale lentil in a greenhouse using a perlite/vermiculite growing medium and N-free nutrient solution. In the NA method, total N fixed is the product of pulse biomass N (lb/ac) and fNdfa.

Statistical Analyses

All statistical procedures were conducted using R (R Core Team, 2021). Analysis of variance (ANOVA) was performed for response variables of biomass N, fNdfa, post-harvest soil nitrate-N, and N fixed (calculated using both the ND and NA methods). Treatment was treated as a fixed effect and block was treated as a random effect in a linear mixed model. Site-years were analyzed independently for treatment effects. Treatment differences were further evaluated using pre-planned orthogonal contrasts and Tukey-Kramer familywise comparisons of means with a 90% confidence level.

RESULTS AND DISCUSSION

There was substantial year-to-year variation in precipitation over the course of this study. Weather data, along with notable N fixation findings discussed below, are summarized in Table 1. The 2019 growing season (April to July) was wetter and cooler than normal, and N fixation at PF was high (90-140 lb N/ac). At both sites, precipitation over the growing season in 2020 and 2021 was low, and N fixation amounts were lower as well. It was particularly hot and dry in June 2021. At PF, N fixed amounts calculated with the ND method ranged from ca. 75 to 115 lb N/ac in 2020 and 35 to 70 lb N/ac in 2021. Overall, N fixation was lower at NARC than at PF, reflecting its drier and hotter climate. The field at NARC in 2020 had high and variable nitrate-N and N fixation was not able to be calculated with confidence; therefore that site year was excluded here.

Table 1. Summary of climate and major N fixation findings for PF and NARC, 2019-2021.

Site	Year	Growing Season (Apr-Jul) Total Precipitation (in)	Growing Season (Apr-Jul) Mean Temperature (°F)	Major Findings: ND method	Major Findings: NA method
PF	LTA*	8.7	54.9		
	2019	9.8	54.0	No effects	No effects
	2020	5.9	54.9	S fertilizer increased N fixed by 34 lb N/ac	S fertilizer increased N fixed by 29 lb N/ac
	2021	5.8	57.7	No effects	No effects
NARC	LTA	7.4	58.3		
	2019	6.2	55.8	Inoculation increased N fixed by 33 lb N/ac	Inoculation increased N fixed by 21 lb N/ac
	2021	4.1	58.5	Inoculation increased N fixed by 12 lb N/ac	Inoculation increased N fixed by 8 lb N/ac

*Long-term average, 1981-2010 from the Western Regional Climate Center, Desert Research Institute, Reno, NV

Inoculant Effects on N Fixation

Inoculated lentil fixed more N than non-inoculated lentil in two out of five site-years. At NARC in 2019, inoculated lentil fixed on average 33 lb N/ac (ND method) and 21 lb N/ac (NA method) more than non-inoculated lentil (Table 1). Inoculating lentil increased N fixed by 12 lb N/ac (ND method) and 8 lb N/ac (NA method) at NARC in 2021. Early in the 2021 growing season, non-inoculated lentils were visibly less green than inoculated lentils at NARC, but this became less obvious as the season progressed. The N fixed differences were more modest in 2021 due to drought, but they are still notable given that drought can limit N fixation. Inoculation did not influence N fixation in any year at PF, suggesting adequate *Rhizobia* populations in the soil. This is especially interesting in 2020, because that field to our knowledge had no history of pulse crops. The 2019 field at NARC, where a large inoculant effect was observed, had a recent history of pulse crops. This could suggest that *Rhizobia* persistence is lower at NARC, where soil organic matter is lower and climate is drier, or that there are more native *Rhizobia* at PF. There were not significant differences in N fixed amounts between inoculant types (granular and seed-coat) for any site-year, but Miller et al. (2022; in these Proceedings) found an inconsistent effect of inoculant type on lentil yield.

Fertilizer Effects on N Fixation

In 2020 at PF, we observed much darker green and more robust plants in all the +S treatments. Tissue concentrations of both N and S were generally higher in the K+S treatments than in the +K only treatments, implying that the darker green foliage was due not only to more S, but also greater N content presumably from increased N fixation. Treatments with K+S fertilizer fixed approximately 34 lb N/ac (ND method) and 29 lb N/ac (NA method) more N than corresponding +K only treatments. Sulfur is directly needed in the N fixation process, but given smaller S needs of *Rhizobia* than of plants, it's likely that the S effect was indirect, meaning S fertilization increased plant growth, which allowed the plants to send more carbon to the root nodules, boosting N fixation. This is supported by a study on peas in which S-deficient plants had low carbohydrate levels and fixed less N than S sufficient plants, likely due to high carbohydrate demand of N fixation (Scherer et al., 2006). Given that only a small minority of Montana producers fertilize lentil (Warne et al., 2019), it's likely that insufficient S is limiting lentil N fixation in certain Montana regions that are prone to S deficiency.

At PF, the 2019 field had similar sulfate-S levels (around 4 ppm in the top 6 in) to the 2020 field, yet an N fixation response to S fertilizer was not observed in 2019. There was far more precipitation in 2019, and more S may have mineralized with the extra moisture. At NARC, fields in 2019 and 2020 were high in sulfate-S (>14 ppm) and no S response was observed. For both sites in 2021, sulfate-S was low, but drought substantially limited lentil growth and consequently N and S demand. Soil exchangeable K levels were high across site-years, and no N fixation response to K fertilizer was observed. Foliar micronutrients did not influence N fixation.

CONCLUSION

This study found that S fertilization and inoculation have the potential to increase N fixation of lentil in the NGP. This was the first study to investigate lentil N fixation response to S and inoculant in the region. Given the considerable growth in lentil production and current lack of research in the NGP, the findings are valuable for lentil producers to better manage their crops. More research would help us better understand lentil S response, because our results were inconsistent as climate and sulfate-S levels varied. Further research is also needed on inoculant types, as lentil yield and N fixation responses were not similar nor consistent across site-years.

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