Evaluating the effects of seeding rates and inoculant performance on nodulation, weed suppression, and relative yields of different lentil varieties grown in the Northern Great Plains

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Abstract

Lentils are important for diversifying wheat-based cropping systems and are also beneficial in enhancing soil health. These benefits have contributed to the exponential growth in pulse crop acreage in The Northern Great Plains (NGP). There are several challenges facing organic lentil production. Lack of approved herbicide for use in organic pulse crop production provides a challenge to weed management. Little is known about the optimum seeding and appropriate inoculation rates to improve; crop growth, nutrient acquisition, weed management, and yield potential for lentils in organic systems. It is hypothesized that growing lentils that tolerate high population densities could suppress weeds while maintaining and/or achieving higher yields and better economic returns to the grower. The goals of this project are to evaluate effects of seeding rates on lentil yields and weed competition. The effect of inoculation rates on nodulation and the soil community profile will also be determined. A multi-site replicated trial was established on grower's fields in three different lentil growing areas of Montana. Three lentil varieties were selected based on seed sizes; large, medium, and small, and were planted at four different rates. An inoculant containing both rhizobia and mycorrhizal fungi was applied at the recommended rate and twice the recommended rate. The results showed that increased seeding rates 100 to 500 plants m^{-2} led to significant yield increases and reduction in weed densities regardless of lentil variety or geographic location. Furthermore, at the highest seeding rate the yield and weed density response had not plateaued which suggests that even higher seeding rates could lead to additional yield gains and weed suppression.

Rationale and Literature review

In the USA, large acreages of lentils are found in the low-rainfall areas of Montana, North Dakota, South Dakota, eastern Washington, and eastern Idaho where they are grown in rotation with wheat, barley and other legumes including peas and chickpeas [5,15]. Pulse crop acreage has increased in Montana and North Dakota by 128% and 274%, respectively [2]. Lentils are important for diversifying wheat-based cropping systems and are also beneficial in enhancing soil health when grown either as a cash crop or cover crop. Research has shown that the presence of endophytic rhizobia associated with pulses can have a positive impact on many soil processes and provide benefits to subsequent non-legume crops [11].

Most organic lentil growers in the Northern Great Plains (NGP) base the seeding rates of all lentil varieties on a target population of 130 plants m^{-2} which is borrowed from conventional farming systems ([1] and personal conversation with Jim Barngrover, Timeless Seeds Inc.). However, crop management strategies and inputs used in organic system differ from those used in conventional cropping systems [1]. For example, while conventional lentil growers can use a number of approved synthetic seed treatment and weed management products, these are not approved for use in organic systems. Organic growers are often encouraged to seed densely to account for the loss of stand due to seed and soil pathogens and achieve desired plant density in the field. More research is needed given the wide range of seeding rates in lentil growing regions around the world [1, 16] and there is a need for better seeding rate recommendations.

In addition to improving yield, optimal seeding rates can also enhance weed control. Lentil plants are short (averaging 30-35cm high) in stature and are poorly competitive with weeds. Conventional lentil growers have access to products that they can use to manage weeds in their fields. To our knowledge, there are no certified products approved for use in weed management in organic lentil fields. Therefore, organic lentil growers rely heavily on cultural practices to manage weeds in their fields. Strategic practices employed to control weeds in organic farms include use of cover crops in place of summer fallow and repeated cultivation pre-seeding. The drawback of repeated cultivation in the spring is that soil moisture is lost, and the crops will often have to contend with insufficient moisture throughout the growing season. An alternative method that can be employed to help control weeds in organic lentil fields is increasing the planting density.

Research has shown that planting density can influence weed pressure. Baird *et al.*, [1] found that weed biomass was reduced by 59% when lentil planting density was increased from 100 to 229 plants m^{-2}. They suggested that organic farmers could suppress weeds and increase yield and profitability in their lentil production by increasing the seeding rate from the recommended target population of 130 plants m^{-2} to 229 plants m^{-2} in Saskatchewan, Canada. This is above the current recommended seeding rates of 130 plants m^{-2} for the NGP. Seed size can also affect emergence patterns and ability of a crop to compete with weeds. Crawford [6] evaluated the effect of cultivar and seed size on emergence and competitiveness of edamame with weeds. The results have shown that smaller edamame seeds attained 50% emergence 0.7 days earlier than large seeds. Also, cultivar choice and seed size had significant effect on weed suppressive ability, with larger seed sizes having higher biomass and surface area covered, and greater weed tolerance 8 weeks after emergence. Place *et al.*, [17] reported that soybean plants from larger seed size (~6.75 mm diameter) had more canopy coverage, more biomass, and improved competitiveness with weeds at 3-7 weeks after emergence. They suggested that using large-sized soybean seeds may be used as a better weed control strategy in organic soybean production. More research is needed to determine if similar responses will be seen with lentils and to identify cultivars that are weed suppressive or tolerant while maintaining yields.

In addition to seeding rates, inoculants can also impact the performance of pulse crops. The microorganisms found in inoculants can provide a variety of plant benefits. In addition to rhizobia for N-fixation, microorganisms play a role in crop health and influence nutrient availability, provide protection against environmental stressors, and can provide protection against pathogens. Early studies with pure rhizobia isolates applied directly to the seed indicated a relationship between inoculant concentration and successful nodulation [14]. More recent studies have shown that increasing the inoculation rate to 2-4X the standard rate can result in increased nitrogen uptake and crop yield in cowpea and beans [10]. These results demonstrate the need for additional testing to determine optimum inoculation rates.

Finally, there is a need to better understand the impact of these agronomic practices on soil health in the context of the soil microbial community. Early work with rhizobia inoculants indicated that the inoculants were equally competitive with indigenous organisms and were persistent in the soil after 19 months [14]. Selection of appropriate inoculants could facilitate restoration of critical functions to degraded agricultural soils. However, more work is needed to better understand the dynamics of microbial interactions in these systems.

To our knowledge, there are no published data on the optimal seeding and inoculant rates or the performance of lentil varieties in organic systems across the different microclimates of the NGP. There is need for on-farm research to fill this knowledge gap to enhance the long-term success of organic lentil production in the region. Results from this work will help agronomists and growers understand effects of cultivar choice and seeding and inoculant rates on crop performance and will help growers make informed decisions on integrated weed management strategies for organic lentil production. By incorporating better crop management strategies, lentil growers can improve the profitability and sustainability of their operations.

Research Objectives

The objectives of the study were to;

1. Evaluate the effect of seeding rate on yields of three different lentil varieties based on seed size.
2. Determine yield stability of three different lentil varieties across environments in Montana.
3. Evaluate the effects of lentils seeding rates on weed suppression.
4. Evaluate the effect of inoculant application rate on the crop performance.
5. Evaluate the effect of seeding and inoculant rates on the composition of the soil microbial community.

Methods

Studies at Chester and Great Falls were planted 3 May, 2019 and the Geraldine location was planted 13 May 2019. The plot size for each treatment was 1.5m x 5m (5’ x 15’) and all treatments were planted with 3 replicate plots. Large Green (LGL), French Green (FGL), and Black Beluga (BBL) lentils were planted at seeding rates of 100, 200, 300, and 500 plants m^{-2} on a 6-inch row spacing. Agtiv granular pulse inoculant containing rhizobia and mycorrhizal fungi was applied at the label rate of 4 lbs/acre and at a 2X rate. Weed counts were performed by selecting three random quadrants measuring 0.33 m^2 in each plot. Nodule counts were performed on 2 July 2019 at Chester and 15 July 2019 at both Geraldine and Great Falls when plants were at early flowering. Lentil plants from three random quadrants in each plot and were dug out using a shovel and the soils gently cleaned off to preserve the integrity of the roots and nodules. Plants were gently shaken to remove bulk soil and transported in a cooler to the lab for nodule counts. Following nodule counts, a subset of the root samples were submerged in a saline solution and sonicated 5 minutes to dislodge the remaining soil which was subsequently archived in a freezer for microbial community analysis. Additional funding has been obtained to cover the costs of sequencing and samples have been submitted for sequencing, but the data were not yet available at the time of this report.

Given wet field conditions at Great Falls and Geraldine, post flowering measurements of plant height, primary and secondary branches, number of pods per branch and number of seeds per pod were not determined in the field. Plant heights were subsequently collected at harvest on all sites. Collection of primary and secondary branches, number of pods per branch and number of seeds per pod was attempted post-harvest, but because the plants were dry and brittle, accurate measurements could not be obtained.

Lentils were harvested in Chester on 2 August 2019 while Geraldine and Great Falls were harvested on 8 August 2019. Plant biomass was determined by collecting whole lentil plants above crown from three random quadrants each measuring 0.33 m^2 in each prior to harvest. Total biomass and yield were measured and the harvest index (HI) determined by dividing yield by the total biomass. The differences in yields among treatments was determined using analysis of variance (ANOVA) performed using R statistical software (R Core Team, 2013). Where treatment effects were significant, a post hock analysis was conducted using Fishers, LSD to determine the differences among significant mean values.

Results

Early in the growing season differences could be observed between different varieties and seeding rates. The LGL and FGL clearly had a growth advantage early in the season compared to the smaller BBL. This led to earlier canopy closure (Figures 1 & 2) but did not contribute significantly to better weed suppression relative to the smaller BBL (Figures 6). Total precipitation from April – August 2019 was 7.88 inches at Great Falls, 7.64 inches at Geraldine, and 4.33 inches at Chester. At Chester, there was no recorded rainfall in the month of June which is a critical period of early season crop growth prior to flowering. During flowering, which occurred in early July, only 0.57 inches of rain was recorded at Chester. When compared to the 30 year precipitation average, the precipitation from April – August was 16% below average at Great Falls, 24% below average at Geraldine, and 42% below average at Chester. While precipitation was also lower than average at Great Falls and Geraldine the impact was minimal since the lower precipitation levels occurred later in the growing season and early season precipitation was average or above average.

Figure 1: Lentil study at Great Falls in early July. Differences in weed density could already be observed between seeding rates and lentil varieties. Red box in the background marks a FGL plot seeded at 300 seeds m^{-2} and the white box in the foreground marks a FGL plot planted at 100 plants m^{-2}.

Figure 2: Close up view of differences in weed density between high (right) and low (left) seeding rates. Red line indicates border between the two plots.

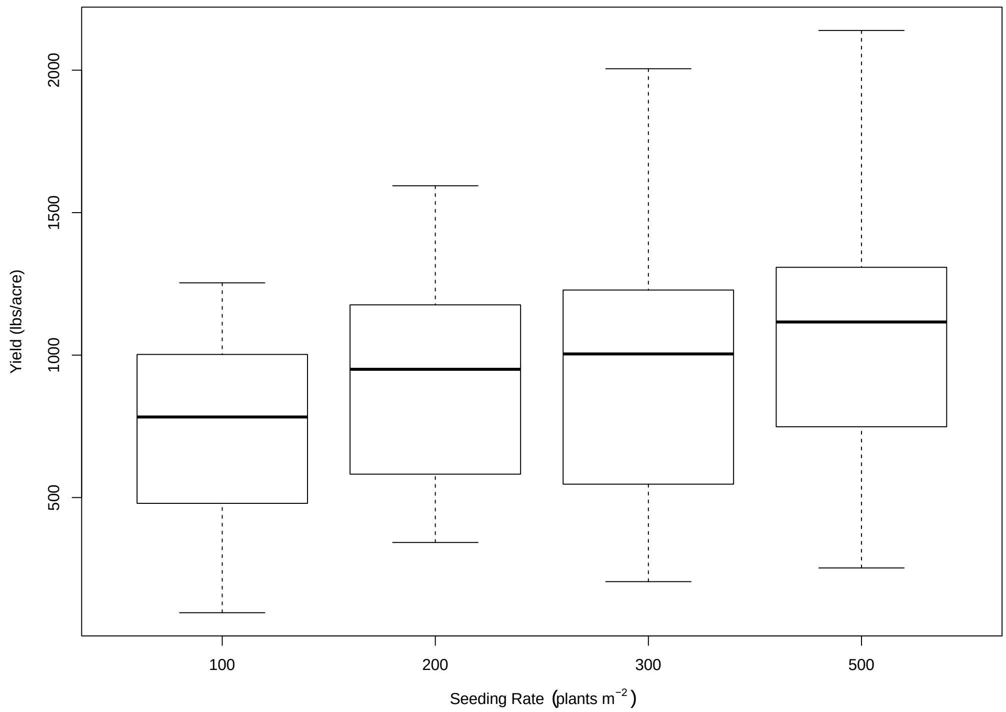

Figure 3: Box plots of the average yield across all varieties and locations for each seeding rate. The dark line represents the mean, boxes represent the interquartile range, and whiskers represent the minimum and maximum values in the data set.

Yield

Average yield across all varieties and locations were 746, 915, 951, and 1089 lbs/acre for seeding rates of 100, 200, 300, and 500 plants m^{-2}, respectively (Figure 3). This represents a 46% yield increase at 500 plants m^{-2} relative to the lowest seeding rate, regardless of variety or location. Yields increased with each successive seeding rate indicating that the maximum possible yield response was not achieved. Analysis of yield by location and variety showed a similar trend of increased yield with increasing seeding rate, for each variety at each location (Figure 4). Great Falls had the highest average yields which is reflective of the higher precipitation and greater yield potential for that location. The Chester location experienced drought conditions during grain fill which resulted in lower yields. However, a positive yield response was still observed with increasing seeding rates.

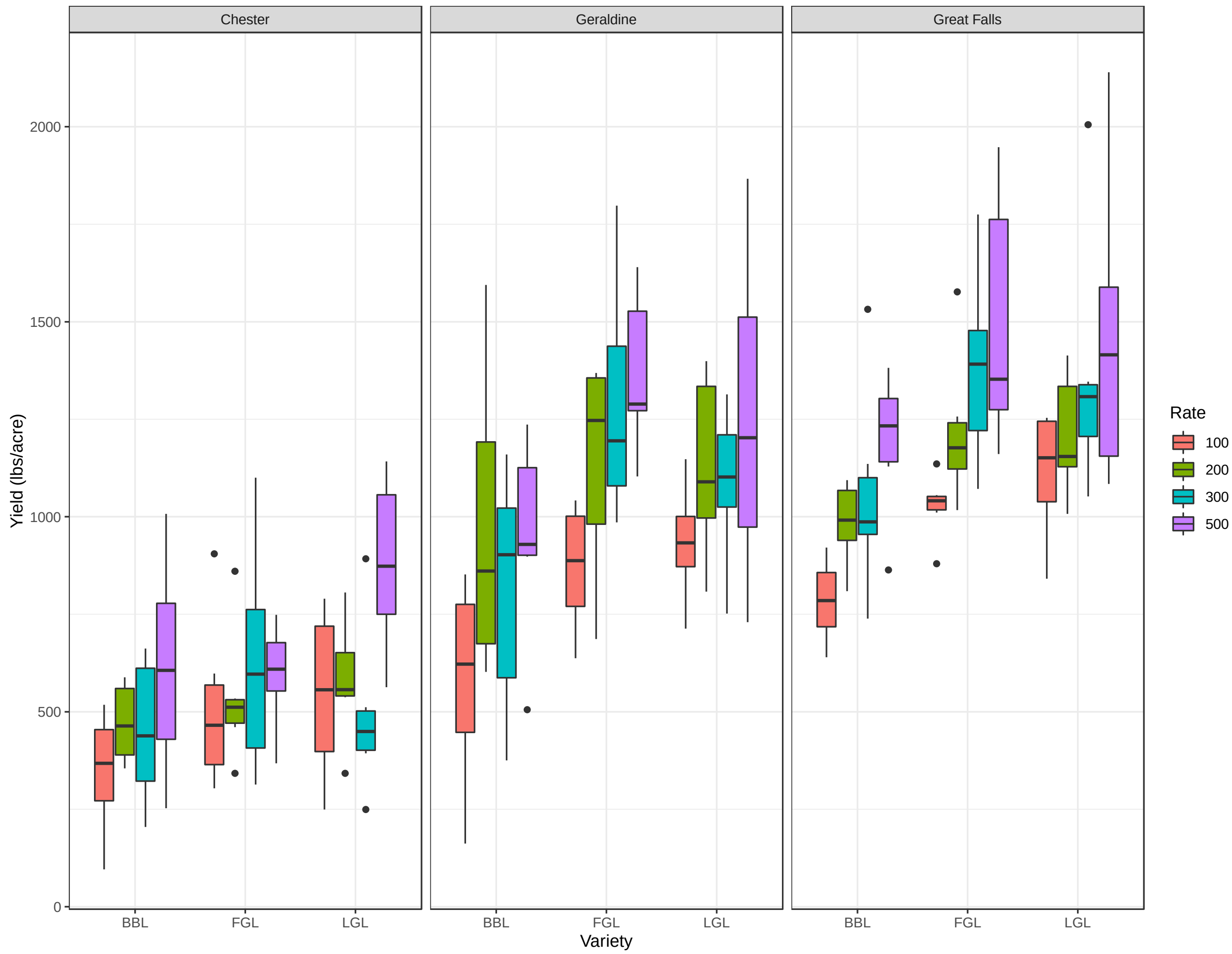

Figure 4: Lentil yield as a function of variety, seeding rate, and location.

Weed density

Weed density showed a significant decrease with increased seeding rates. The average weed density across all varieties and locations was 21.4, 19.4, 15.1, and 12.7 weeds m-2 for seeding rates of 100, 200, 300, and 500 plants m-2, respectively (Figure 5). This translates to a 41% reduction in weed density when the seeding rates were increased from 100 to 500 plants m-2. Weed density decreased with each successive seeding rate increase, indicating that the maximum possible weed density reduction was not achieved. Analysis of weed density by location and variety showed a similar trend of decreased weed density with increasing seeding rate, regardless of the variety or location (Figure 6). Overall, weed densities were lowest at Chester and decreases in weed densities were not significant. This was likely due to drought conditions that occurred in that region.

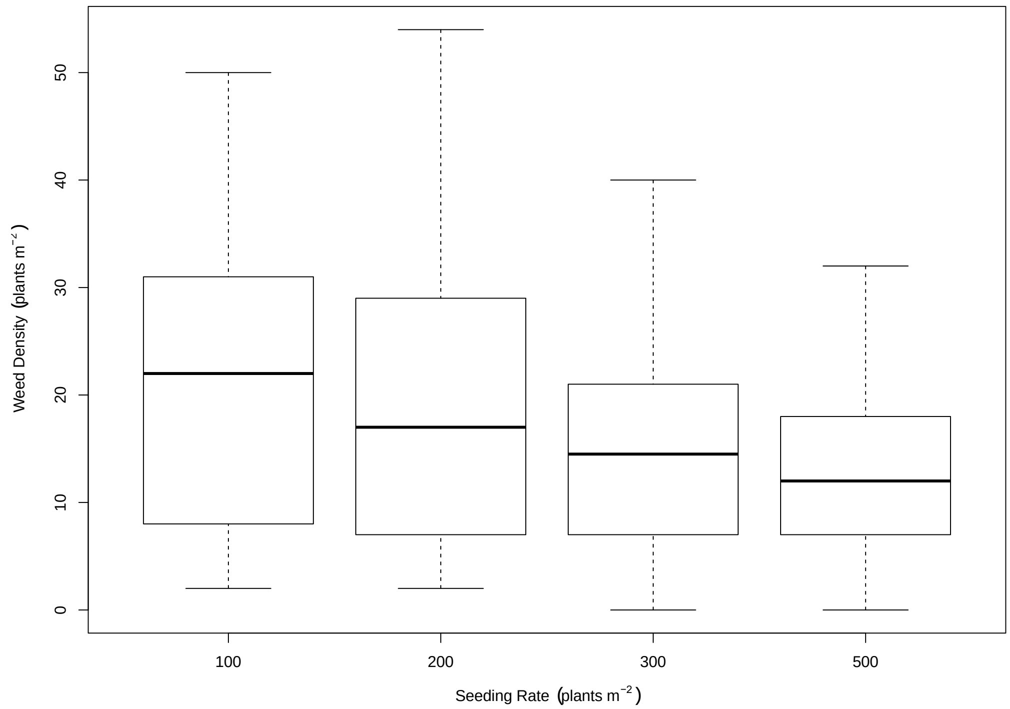

Figure 5: Weed density averaged across all locations and varieties for each seeding rate.

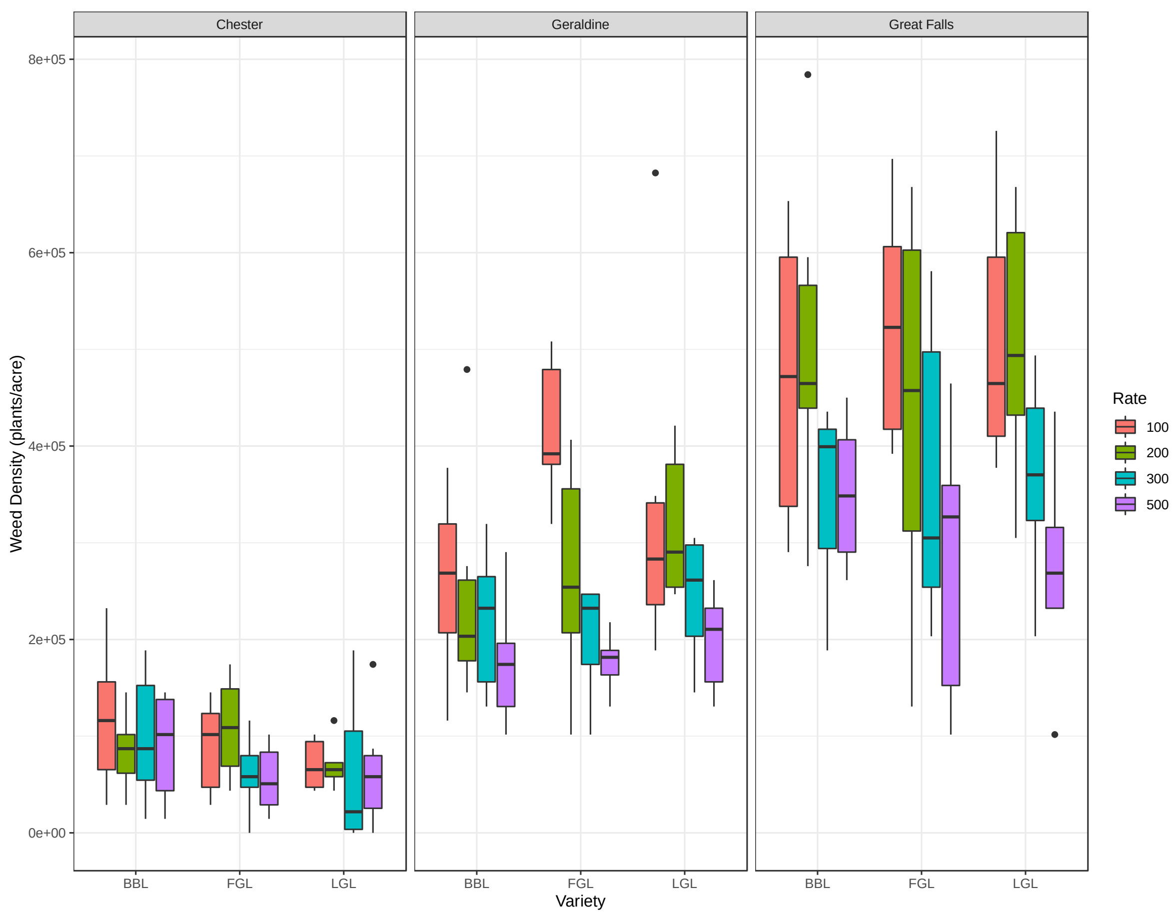

Figure 6: Weed densities as a function of variety, seeding rate, and location.

Other factors

Analysis of variance (ANOVA) was performed to identify factors and interactions between factors that were significant with respect to yield components. Factors and associated p-values are shown in Table 1. Total biomass, yield, weed count, and HI were significantly different ($p < 0.001$) between seeding rates. Total biomass, protein, yield, and HI were also significantly different ($p < 0.001$) between lentil varieties. Inoculant rate had a significant effect ($p < 0.001$) on the number of nodules per plant with an average of 23 nodules per plant at a 2X inoculant rate compared to only 15 nodules per plant at the standard rate. Seeding rate x variety and variety x location interaction terms were also a significant factor ($p = 0.020$ and $p < 0.001$, respectively) for the HI. Weed density was also significantly influenced by seeding rate x location interactions. Additional interaction terms including Rate x Inoculant x Variety x Location were also tested but were not significant. Average values for each of these factors is shown grouped by seeding rate (Table 2) and variety (Table 3).

Average protein content was 31.6%, 28.7%, and 28.3% for BBL, FGL, and LGL varieties, respectively. Protein content did not vary with seeding or inoculant rate. Average 1000 seed weight was 17.5 g, 23.4 g, and 51.1 g for BBL, FGL, and LGL varieties, respectively. 1000 seed weight was significantly different ($p < 0.001$) between varieties but did not differ by seeding or inoculant rate.

Table 1: Analysis of variance (ANOVA) results showing p-values for factors that were significant with respect to yield components. Factors were tested individually and for interaction effects.

Factor	DF	Biomass (lbs/ac)	Protein (%)	Yield (lbs/ac)	Weed Count (plants/ac)	Nodules (#/plant)	Harvest index (HI)
Rate (seeding)	3	**<0.001*** **	0.133	**<0.001*** **	**<0.001*** **		**0.002** **
Inoculant Rate	1	0.497	0.106	0.175	0.130	**<0.001*** **	0.159
Variety	2	**<0.001*** **	**<0.001*** **	**<0.001*** **	0.971	0.771	**<0.001*** **
Rate:Inoculant	3	0.298	0.900	0.294	0.807		0.580
Rate:Variety	6	0.696	0.542	0.565	0.181		**0.020***
Inoculant:Variety	2	0.351	0.457	0.352	0.300	0.318	0.660
Rate:Location	6	0.213	0.326	0.858	**<0.001*** **		0.093
Inoculant:Location	2	0.943	0.393	0.722	0.848	0.256	0.676
Variety:Location	4	0.247	0.200	0.586	0.337	0.869	**<0.001*** **

Table 2: Summary of yield components by seeding rate showing average for each rate. Bolded numbers were significantly different between treatments based on the ANOVA results shown in Table 1.

Seeding Rate (plants/m-2)	Biomass (lbs/ac)	Protein (%)	Nodules (#/plant)	1000 seed wt (g)	Harvest Index (HI)
100	**2564.5**	29.1	16.9	**31.5**	28.3
200	3196.9	29.8	20.7	30.6	28.3
300	3506.5	29.4	13.4	30.2	**26.4**
500	**4070.0**	29.8	19.0	30.3	26.5

Table 3: Summary of yield components by variety showing average for each variety. Bolded numbers were significantly different between treatments based on the ANOVA results shown in Table 1.

Variety	Biomass (lbs/ac)	Protein (%)	Nodules (#/plant)	1000 seed wt (g)	Harvest Index (HI)
BBL	**2980.3**	**31.6**	18.0	**17.5**	25.5
FGL	**3326.4**	28.7	18.0	**23.4**	**29.7**
LGL	**3696.7**	28.3	19.8	**51.1**	27.0

Discussion

The results of this work suggest that the standard seeding rate of 130 plants m-2 used in conventional systems is less than optimum for organic systems. Yield increased and weed density decreased with increasing seeding rate across all locations and varieties. Yield and weed density showed a linear increasing trend that did not plateau or begin to decline at the higher seeding rates. This suggests that the range of seeding rates was not sufficiently high enough and that additional increases in seeding rate could further increase yield and decrease weed pressure. Higher seeding rates are needed to determine if further yield gains or weed reductions are possible.

The results of this work suggest that by increasing planting rates, organic growers will benefit from not only better yields but also greater weed suppression. A robust crop growth that results in early canopy closure will minimize available moisture, nutrients, and light for weed growth. In addition to weed suppression, identification of appropriate lentil varieties and seeding rates for improved ground-cover characteristics, i.e. early branching, foliage development, and early canopy cover can potentially lead to reduced lodging. This will make it easier to harvest the crop and minimize dirt-tag which could adversely affect quality and price.

Another outcome of this work is a better understanding of the effect of different inoculation rates on crop agronomic performance, seed protein, and test weight. Although some growers have reported better growth with higher inoculant rates, our results did not indicate any yield or protein benefit. While a 2X inoculant rate resulted in a 55.6% increase in the number of nodules per plant, this did not translate to a measurable increase in any of the yield components.

Although the results suggested even higher seeding rates could lead to additional yield gains, this work did not address the economic aspects and a cost-benefit analysis is needed to determine at what point the increased yield from higher seeding rates no longer provides an economic benefit due to the cost of the seed. Future work is planned to address this question.

Research outreach and potential impact

Research findings have been presented in at four meetings attended by pulse growers, University research scientists, and representatives from different pulse industries and seed organizations from Montana and the surrounding locations in the Pacific North West and the Northern Great Plains. The meetings where the findings of this research have been presented are shown in Table 4. The Q and A at the end of each of these sessions was very positive and is indicative that the project is attracting the interest and attention of the target audience. This work is expected to have a direct impact on grower practices and will result in growers using higher seeding rates for organic lentils in the Northern Great Plains.

Table 4: Research dissemination

Event	Date	Location
Timeless Seeds Inc. winter growers meeting	01/31/20	Great Falls, MT
12th Montana Organic Association Conference	01/23/20-01/25/20	Bozeman, MT
10th Organic Seed Alliance Conference, Oregon State University	02/12/20–02/15/20	Corvallis, OR
MSU Plant Science and Plant Pathology Departmental seminar	02/25/2020	Bozeman, MT

Budget

The following table details budget expenditures for the project.

Date	Vendor	obj code	reconciled	amt	Operations Balance	Labor Balance
	Beginning Balance			Supplies $121.00 Travel $1,679.00 Contract Serv $3,600	$ 5,400.00	$ 14,600.00
5/6/19	Joyce Feed & Fuel - Fuel for travel to field sites	62405	x	$ 50.15	$ 5,349.85	
5/28/19	Jed Eberly, travel expense	62407	x	$ 24.00	$ 5,325.85	
9/9/19	Payroll-Joseph Kibiwott	61127	x	$ 2,370.00		$ 12,230.00
9/9/19	Payroll-Joseph Kibiwott - GRA additional payment	61127A	x	$ 130.00		$ 12,100.00
9/9/19	benefits		x	$ 8.67		$ 12,091.33
10/4/19	Pcard Rebates FY20		x	$ 0.75	$ 5,326.60	
10/9/19	Payroll-Joseph Kibiwott	61127	x	$ 2,370.00		$ 9,721.33
10/9/19	Payroll-Joseph Kibiwott - GRA additional payment	61127A	x	$ 130.00		$ 9,591.33
10/9/19	benefits		x	$ 6.88		$ 9,584.45
11/6/19	Payroll-Joseph Kibiwott	61127	x	$ 2,370.00		$ 7,214.45
11/6/19	Payroll-Joseph Kibiwott - GRA additional payment	61127A	x	$ 130.00		$ 7,084.45
11/6/19	benefits		x	$ 6.88		$ 7,077.57
12/9/19	Payroll-Joseph Kibiwott	61127	x	$ 2,370.00		$ 4,707.57
12/9/19	Payroll-Joseph Kibiwott - GRA additional payment	61127A	x	$ 130.00		$ 4,577.57
12/9/19	benefits		x	$ 6.88		$ 4,570.69
1/21/19	MSU-LRES, contract services for sample analysis	62198	x	$ 913.00	$ 4,413.60	
1/22/20	Diversified Biotech In., supplies	62208	x	$ 204.00	$ 4,209.60	
1/8/20	Payroll-Joseph Kibiwott	61127	x	$ 2,370.00		$ 2,200.69
1/8/20	Payroll-Joseph Kibiwott - GRA additional payment	61127A	x	$ 130.00		$ 2,070.69
1/8/20	benefits		x	$ 6.88		$ 2,063.81
3/4/20	Amazon, supplies	62208	x	$ 39.99	$ 4,169.61	
3/7/20	UPS, sample shipping	62304	x	$ 19.14	$ 4,150.47	
3/7/20	UPS, sample shipping	62304	x	$ 19.14	$ 4,131.33	
3/7/20	UPS, sample shipping	62304	x	$ 5.80	$ 4,125.53	
3/9/20	Payroll-Joseph Kibiwott	61127	x	$ 1,927.00		$ 136.81
3/9/20	Payroll-Joseph Kibiwott - GRA additional payment	61127A	x	$ 130.00		$ 6.81
3/9/20	benefits		x	$ 5.66		$ 1.15
3/15/20	UPS, sample shipping	62304	x	$ 15.42	$ 4,110.11	
3/15/20	UPS, sample shipping	62304	x	$ 5.80	$ 4,104.31	
3/26/20	FEC Woodhall Dist from 923512, fuel expenses for travel and field work	62216	x	$ 527.00	$ 3,577.31	
3/26/20	FEC Woodhall Dist to 923512, fuel expenses for travel and field work	62242	x	$ 236.00	$ 3,341.31	
3/30/20	Woodhall Distributing, fuel expenses for travel and field work	62216	x	$ 377.00	$ 2,964.31	
3/31/20	Fisher Ag LTD, farmer cooperator support	62199	x	$ 1,000.00	$ 1,964.31	
4/2/20	Carl Mehmke, farmer cooperator support	62199	x	$ 1,000.00	$ 964.31	
	Ending balance				$ 964.31	$ 1.15

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