Agriculture MREDI Grant
Quarter 7 Sub-project Reports

Research Center/MAES subproject of the Agriculture MREDI Grant

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Executive Summary

This past quarter we administratively focused on detailing project accomplishments with PIs and closely monitoring budgets to achieve maximum impact from the project. While funding for this project ends June 30, it is clear from most subprojects that work will continue either from grant funds acquired because of this funding or from grants already applied for. Examples are the precision agriculture OFPE project which has garnered funding to address 9 new fields and research involving hyperspectral sensors (PIs Shaw and Jha) to identify weed infestations in standing crops and to identify herbicide resistant weeds. In addition because of this funding and reallocation of funds amongst projects such as the pulse soil microbiology/nutrient project (sub project 41W220 - Peters and Chen: reallocated $27,615 from other projects) were able to expand the microbial DNA profiles and nutrient data to a full set of statewide samples taken spring and summer of 2016. These data will greatly enrich the data from analysis of 2015 samples and will help understand more deeply the impacts of pulse crop agriculture (rotation, genotype, and environment) on the microbiological ecosystems in a range of Montana soils. These initial studies have set up the groundwork to address the best approach to microbiome sustainability in the soil and will help focus on microbial ecosystems and nutrients associated with both high and low yields. Such data will guide future research and will be the genesis of hypothesis driven research proposals and projects well into the future. It is likely that this work will someday lead to new microbial based seed treatment products that enhance yields and fix atmospheric nitrogen on both legume and non-legume crops. Other projects, such as the Weaver project involving pulse and cover crops and wheat sawfly parasites, have provided unique information on sawfly parasite biology and how changes to a more complex agroecology (from grassland-wheat to wheat- pulse or wheat-cover crop) might provide unforeseen changes in ecosystem services for management of a major pest. This project and the one coordinated by Darrin Boss have served as the basis for new federal grant proposals. These proposals along with proposals generated by the hyperspectral sensor and pulse soil microbiology project will be uniquely highly competitive at the federal level (USDA, NSF, and DOE) because of the base data developed through MREDI funding.

The work on factors involving production of high protein peas by the Miller and Chen groups will provide Montana with a competitive advantage in siting a pea protein fractionation plant and have identified the importance of environment, applied fertilizers, type of rhizobial inoculant and genotype in production of high protein pea crops. Also, research by both the Miller and Chen groups have contributed to development of reliable tests that can be used by buyers who choose to buy peas based on protein content. This work should enhance the economic return to MT growers who will sell into such markets.

The cover crop research reported by Boss and Glunk demonstrate the potential for cover crops and annual forages to produce high quality forage and increase income where cover crops replace fallow acres. The work by Boss clearly demonstrated that winter wheat yield were not negatively affected by replacing fallow with cover crops terminated in early July and instead of a cost of chemical fallow of ~$60/ acre this could be replaced with a crop generating ~$100/acre. It must be noted that during this 5 year study rainfall was in the normal 12-14”/year range but that rainfall was timely in terms of

establishing and growing the winter wheat crop. We have not been able to study the effects of these cropping systems under severe drought conditions.

In this quarter we started to receive the first economic return information from Dr. Bekkerman. Included in this report is information on the economic effect of pulse crops replacing fallow acres. This research has addressed average reported Montana pea and lentil yields. Based on research from a broad range of locations we have shown that yields can be 50-100% higher by selection of optimal variety and cultural techniques for specific locations based on research done in this project. In the final report there will be reports on impacts of cover crops replacing fall acres and impacts on the application of precision agriculture form the OFPE study and on weed control economics.

When this project was written Montana had 700-800 thousand acres of pulse crops and in 2016 had more than 1.1 million acres with this trend continuing upwards in 2017. Montana is now the largest pulse crop producer in the USA. Between 2013 and 2016 Montana producers converted nearly 250,000 acres (~7.5% reduction) from summer fallow to cropping to primarily pulse crops and some to cover crops. In the prior 3 year period (2010-2013) fallow acres decreased by only~ 0.7%. Based on reports of seed sales, insurance records, planting intentions and observations this trend has continued to increase. The research in this project has provided Montana growers with confidence that they can profitably grow these crops in annual cropping systems while negating the effects of fallow on soil organic matter and other characteristics of healthy soils. One of the key barriers to adoption of annual cropping systems has been the complexity of weed control and negative effects of herbicide carry over in wheat-pulse cropping systems. Excellent work by Dr. Jha has provided answers such that this barrier to adoption should no longer exist. This confidence was achieved by the many outreach efforts of project participants including field days and workshops at MAES Research Centers, news articles, reports at grower meetings, radio and television. It should be noted that much of this conversion from fallow to annual cropping is happening in the "Golden Triangle" region where wheat-fallow was the predominant cropping system (see pages 66 and 68 in Bekkerman project report). In the final report on this project next month we will document known economic impacts, predicted economic impacts, and potential impacts from this project and how barriers to producer adoption of these new technologies have been addressed. We will address the new computer deep learning technologies developed to provide optimal prescriptions for application of precision agriculture (variable rate fertilizer and weed control strategies) to wheat production in an easy to use producer interface and value-added by developing durum wheats adapted to Montana. In addition we will document, publications, grants in dollars received related to this project and the dollar value of grants submitted based on this project. Besides the final written report we are developing a booklet that documents the impacts of this project in lay terms. This booklet should be available in mid-July.

Hiring

- No additional hires in Quarter 7.

Expenditures

- Total Personnel Services: $234,854.90
- Payroll Encumbrance: $5,133.00
- Total Operations: $31,596.12

Pulse Crop Research subproject of the Agriculture MREDI Grant
41W211 – Principal Investigator: Chengci Chen; Email: cchen@montana.edu
Co-investigators: Yesuf Mohammed, Maninder Walia, Perry Miller, Peggy Lamb, Jessica Torrion, Zachariah Miller, Kent McVay, Patrick Carr

Progress towards milestones

During the 7th quarter of the project we:

1) Analyzed the pea yield data from 11 locations.
2) Analyzed pea protein contents for the samples collected from 11 locations.
3) Published 2016 Montana Spring Pulse Variety evaluation report, 500 copies of the reports have been distributed to Montana, North Dakota, and Washington growers.
4) Continued to process NIR data and develop NIR model for pea protein estimation.
5) Coordinated with Dr. John Peters to analyze soil samples from seven locations across Montana for chemical and biological properties.
6) Performed data analysis to relate soil chemical properties to pea yield and protein.
7) Coordinated with scientists at 7 research centers to repeat the statewide variety evaluation project in 2017. Six varieties of peas were packaged and sent to 7 research centers and planted at 11 locations.

Hiring

- No additional hires in Quarter 7.

Expenditures

- Total Personnel Services: $82,944.09
- Total Operations: $19,848.62

Soil Microbiology and Pea Protein subproject of the Agriculture MREDI Grant
1) 41W212 – Principal Investigator: Perry Miller, Email: pmiller@montana.edu

Progress towards milestones

The project objectives were to: a) determine standard management and environmental effects on yellow pea protein, and identify if management variation impacted yellow pea protein content; and b) evaluate the precision of NIR for yellow pea protein predictions. In order to achieve our first objective, over 74 Montana producers provided us with 138 independent yellow pea samples and associated management details from the 2013-2016 growing season. (Thank You!) Observed standard practices for yellow pea growers include, 1) no-till seeding in wheat stubble in April, 2) use of rhizobial inoculant, and 3) use of synthetic fertilizers and pesticides. Protein concentration ranged from 18 to 29%, with the 22-23% category representing the median, and with an overall mean of 23.6%. Yellow pea management varied importantly for rhizobial inoculant type (granular vs. powder), variety, and nutrient management (application of N, P, K, or S fertilizers). Our results showed that severe drought environments increased pea seed protein by ~1.5%-units over wet environments, and that granular inoculant boosted protein by ~1.6%-units relative to powder seed coatings in severe drought environments. The top two yellow pea varieties (CDC Meadow and CDC Treasure) accounted for 52% of all samples collected, and come from the University of Saskatchewan Crop Development Center. The remaining 48% of samples came from more than 18 different pea varieties. Application of N, P, K, or S fertilizers did not have a detectable effect on yellow pea protein.

Our second objective, validating NIR predictions with protein measurements, was accomplished by first setting up a controlled experiment to assess laboratory measurement uncertainty in yellow pea. Specifically, we tested how sample size (1, 2, or 3 Tbsp.) affected differences in duplicated whole seed and flour measurements, using wheat as a control. Results from this experiment showed that there was a tendency for error in protein measurements to become smaller as sample sized increased, especially using pea flour. Measurement error (i.e. differences between duplicate subsamples from the same pea sample) averaged 0.82 and 0.65% for whole seed and flour, respectively, compared to 0.29 and 0.28% in the wheat control. Protein measurement uncertainty was much greater in yellow pea relative to wheat. At this time we would recommend a minimum of 3 tablespoons of peas be ground prior to standard combustion analysis of pea protein.

Finally 46 yellow pea samples previously measured using the combustion method were compared with protein predictions made on NIR. Results showed that measured and predicted protein content were precise to a mean absolute error (MAE) of 1.02%. It may be difficult to achieve greater precision with NIR considering the high laboratory measurement uncertainty in yellow pea, reported above. The degree of precision in current commercial NIR instruments is not known, but may even be less precise than what we reported here.

This observational data has been useful to light pathways for needed research to improve management and measurement of pea protein. Agronomic research into timing and amounts of nitrogen provision to yellow pea is needed to better understand protein formation in this legume crop. In order to generate more precise NIR calibration, more work should be focused at pinpointing sources of protein measurement uncertainty in yellow pea. A standard set of samples could be developed to test NIR prediction precision, if this is of interest to commercial entities who buy peas for protein fractionation.

Project Scope and Objectives

Consumer demand for 'clean label' plant-based protein is rising. This adds intrinsic value for producers growing yellow pea in Montana through protein fractionation. Specifically, if Montana establishes itself as a source of yellow pea with consistently high protein, it is feasible that markets will target yellow pea grown in Montana via bid price and location of delivery facilities for protein fractionation. This will translate to greater revenues for the Montana agricultural sector.

Currently little scientific information is available relating environment (e.g. soils and climate) and management (e.g. nutrient rates, inoculation, seeding date, etc.) to yellow pea protein across Montana. This project's **primary objective** therefore is to identify *standard management* of yellow pea and identify if *standard management* is affecting yellow pea protein across Montana's water-limited growing environments.

Further, if pea protein becomes an important marketing factor, it will be necessary to verify that traditional laboratory control methods for measuring yellow pea protein are in agreement with streamlined technologies used to measure pea protein on an industrial scale. This will ensure that both researchers and industry provide consistent yellow pea protein measurements to producers and consumers alike. Hence a **secondary project objective** is to assess uncertainty in protein ***measurements*** made by the control laboratory method, or combustion method, (Abatzoglou, 2013)and verify that protein ***predictions*** made by the streamlined near-infrared (NIR) method commonly used on an industrial scale agree with one another.

Primary Objective Methodology—Comparing the Effects of Standard Management and Drought on Yellow Pea Protein Content.

In order to identify standard management of yellow pea and determine if standard management has an impact on yellow pea protein across Montana, two steps were taken. **First** producers were contacted and asked to complete surveys as well as provide yellow pea samples from their farms. **Second** yellow pea samples were tested for protein, and statistical analysis was performed to identify if the combination of management and environment (e.g. soils and climate) affected yellow pea protein. These steps are detailed below.

Establishing Producer Contact, Surveys, and Sample Collection

Yellow pea producers were informed of the study via flyers, radio/web announcements, phone calls, extension agents, industry contacts, and presentations at pulse grower meetings and field days. Once informed of the study, producers were asked to complete a short survey covering field legal location and management of their yellow peas. Producers were then asked to send a representative yellow pea field sample (~ a 1 qt. Ziploc Bag) corresponding to each survey to Bozeman for protein testing.

Yellow Pea Protein Testing and Statistical Analysis

Once in Bozeman, yellow pea samples were tested for protein using the combustion method. Put simply, this means that yellow peas were ground into a fine flour, combusted in a crucible, and the amount of nitrogen gas detected from the combusted pea flour was used to calculate the percent protein in each ground sample.

The combustion method only requires a small volume of seeds to make a protein measurement, so in order to get a better estimate of the protein in the overall sample or *bulk sample*, protein was measured twice per sample or in *duplicate*. Duplicated protein measurements were then averaged to estimate the bulk sample protein.

Bulk pea protein measurements were then linked to respective survey responses to address how growing season conditions and management affected protein. Notably seeding date and field legal location information was used in conjunction with available climate (Abatzoglou, 2013), soils (Soil Survey Staff-NRCS, 2015), and pea growing degree day models (Miller and Holmes, unpublished data 2004) to simulate drought stress patterns over the crop cycle. Cluster analysis was then used to group similar drought patterns into discrete drought environments, and select management variables were tested to assess how protein was affected across drought environment using ANOVA.

Primary Objective Results

General Protein Content and Standard Management

A total of 138 yellow pea samples were collected from 73 Montana farms spanning the 2013-2016 growing seasons. The average protein content of these samples was 24%, but protein ranged from 18-29%. (Fig 1.). The primary management variables that were ***similar*** across farms were as follows.

1. Yellow pea is seeded in April
2. Yellow pea is grown with conventional fertilizer and pesticide inputs
3. Yellow pea is grown on no-till ground
4. Yellow peas are inoculated prior to seeding
5. Yellow peas are seeded following a cereal—generally spring wheat, winter wheat, or durum.

Management variables that were ***not similar*** across farms are as follows:

1. Variety selection varies both within and across farms. The two predominant varieties are CDC Meadow and CDC Treasure. These varieties constitute 29, and 23% of field samples respectively, while the remaining 48% are comprised of Delta (~11%) , Montech 4193 (~7%), AC Agassiz (~6%), Montech 4152 (~5%), Mellow, Korando, Spider, Nette, Salamanca, Trapeze, Bridger, CDC Golden, Capris, Early Star, Jet Set, Admiral, Proseper, and Navarro, and unreported varieties (~19%) (n=138 total samples).
2. Most farms use either granular or peat-powder inoculant. Forty-four percent of farms use granular inoculant and the remaining 54 % use peat-powder inoculant. One farm reported using liquid inoculant, and two farms did not specify if they used inoculant (n=73 separate farms).
3. Nutrient management varies across farms. Thirty-eight percent of farms do not use fertilizer and 62% use various blends and rates of N-P-K-S fertilizer. Of the 62 % of farms that ***<u>do</u>*** apply fertilizer (n=45 separate farms that use fertilizer), the proportion that apply N-P-K and S are as follows:
    a. N: 93 %--Reported rates vary from 2 to 11 lbs ac^{-1}
    b. P_2O_5: 100 %--Reported rates vary from 15 to 52 lbs ac^{-1}
    c. K_2O: 18 %--Reported rates vary from 5-10 lbs ac^{-1}.
    d. S: 64 %--Reported rates vary from 3-8 lbs ac^{-1}.

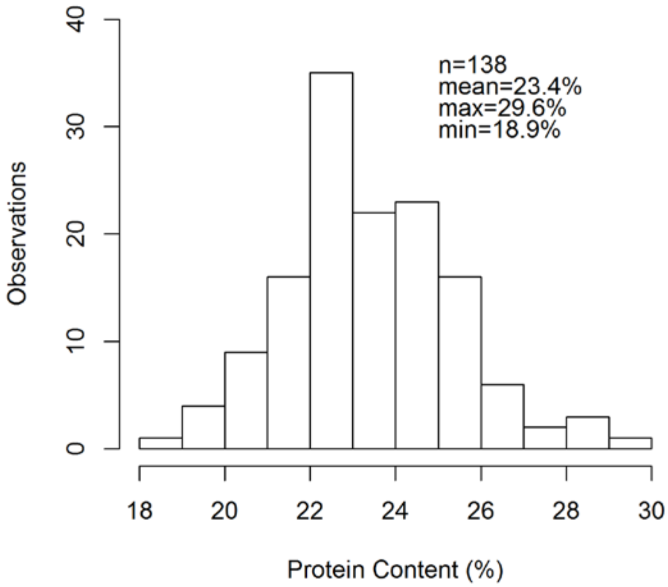


Figure 1. Summary of yellow pea protein content from on-farm samples

The above mentioned management can be considered ***current*** standard management for yellow pea grown in Montana.

Typical Drought Environments for Yellow Pea Grown Across Montana

Individual drought stress patterns were simulated for each yellow pea sample (n=138) based on seeding date and field legal locations from producer survey, soils (Soil Survey Staff, 2015), climate data (Abatzoglou, 2013), as well as growing degree models to estimate crop growth stages (Miller and Holmes, unpublished data 2004). Each line in Fig. 2 A. represents a simulated drought stress pattern. Drought intensity shown on the vertical axis is expressed as a ratio of 0-1 with a value of 0 indicating no drought stress and a value of 1 indicating extreme drought stress. Growing degree days determined from reported seeding dates and daily temperature data are shown on the horizontal axis. A more detailed description of the mechanics of the simulated drought patterns can be found in (Bestwick, 2016).

Clustering techniques were applied to group simulated drought stress patterns into three drought environments (Fig. 2 B.). The three drought environment can be interpreted as favorable (green), moderate (yellow), and severe (red). In the low stress drought environment, pea did not undergo significant drought stress until after flowering. Low drought stress was due to timely precipitation, heavy soils, and mild temperatures or any combination any combination of these environmental factors among others. Conversely, in the high drought stress environment, pea was subjected to drought stress beginning in the V-stages, increasing to R-stages (e.g. flowering), and lasting to maturity. A high drought stress environment is indicative of lacking rainfall, hot temperatures, and soils with low water holding capacity.

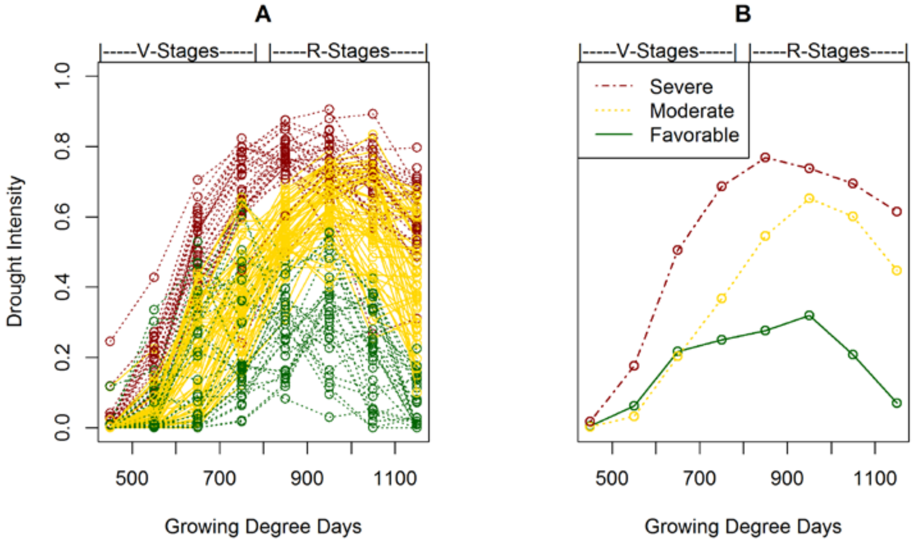


Figure 2. (A) Individual drought simulations, and (B) typical drought patterns derived from clustering techniques. Based on these simulations and characterization techniques, Montana producers can anticipate that their yellow pea crop will typically be subjected to either a favorable, moderate, or severe drought environment. It is further possible that differences in management within each of these drought environments could impact pea protein differently.

Effects of Drought Environment and Management on Pea Protein

Because variety selection, inoculant type, and application of fertilizer represented the most variable standard management factors across farms (*see General Protein and Standard Management*), these were the select management variables tested to address how protein content was affected across typical drought environments.

The specific effects of management by drought environment on pea protein were made as follows:

1. The effect of inoculant type (granular vs. peat powder) and drought environment.
2. The effect of variety and drought environment. Only the varieties CDC Meadow and CDC Treasure were compared since they provided a adequate sample size.
3. The effect of nutrient management and drought environment. The specific nutrient management comparisons that were made were:
    a. Producers that applied N vs. Producers that did not apply N.
    b. Producers that applied P vs. Producers that did not apply P.
    c. Producers that applied K vs. Producers that did not apply K.
    d. Producers that applied S vs. Producers that did not apply S.

In general neither drought environment nor management affected yellow pea protein content (Table 1.). However, we did observe that when granular inoculant was used in a severe drought environment, protein increased by 1.64% compared to when peat powder was applied in a severe drought environment. Granular inoculant has been reported to be superior to peat in dry growing conditions at increasing nitrogen fixation (Clayton et al., 2004). Use of granular inoculant in a severe drought environment may have led to better nitrogen fixation and thereby greater protein compared to peat powder.

We also observed that drought environment influenced protein content produced by CDC Meadow and CDC Treasure. Specifically, the highest average protein content across these varieties was 24.4% and was associated with the severe drought environment. Conversely, the lowest average protein content across these varieties was 22.9% and was associated with a favorable drought environment. However, because neither variety produced greater protein relative to the other across drought environments, it does not appear that choosing to use CDC Treasure or CDC Meadow will have an impact on final protein content.

Nutrient management did not have an effect on yellow pea protein. After a comprehensive review of protein formation in pea, however, these results are expected. Specifically studies throughout the Canadian prairies have found that nutrient management can have a significant effect on yield, but protein is often unaffected (McKenzie et al., 2001a; b). The possibility for yield variation but no change in protein may be due to the indeterminate growth habit of pea. That is, unlike in wheat, pea seed and protein formation occur simultaneously. For instance under a favorable drought environment, both the rate of nitrogen transferred to seeds which builds protein and seed number are high. This means yields may be high due to large seed number, but protein does not change since nitrogen is distributed among more seeds. Alternatively, with a severe drought environment, the rate of nitrogen transferred to the seed and seed number is lower. This means nitrogen is distributed to fewer seeds, so protein is unaffected, yet yield diminishes. For more details on protein formation in pea and a review of how management has affected protein in pea.

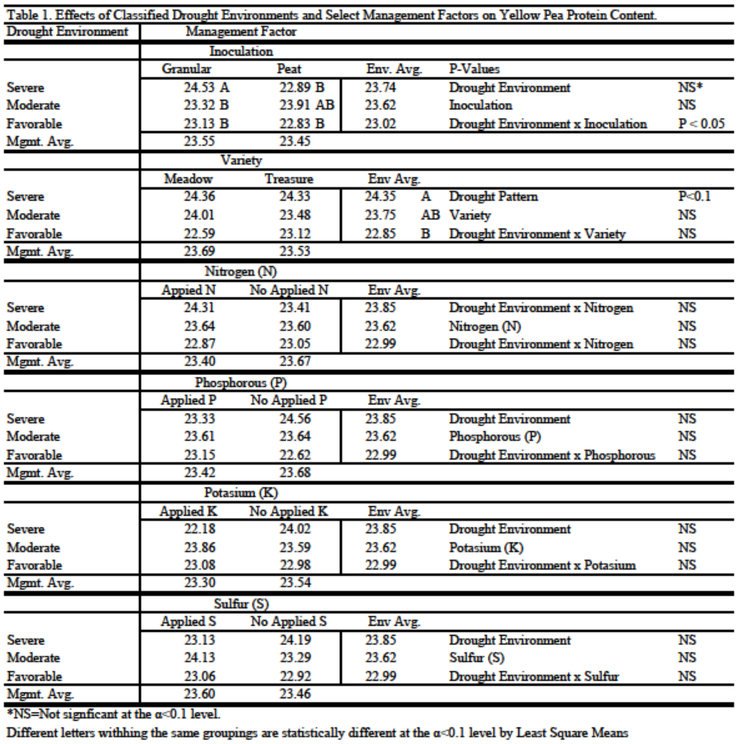


Table 1. Effects of Classified Drought Environments and Select Management Factors on Yellow Pea Protein Content.

Drought Environment	Management Factor					
	Inoculation					
	Granular	Peat	Env. Avg.		P-Values	
Severe	24.53 A	22.89 B	23.74		Drought Environment	NS*
Moderate	23.32 B	23.91 AB	23.62		Inoculation	NS
Favorable	23.13 B	22.83 B	23.02		Drought Environment x Inoculation	P < 0.05
Mgmt. Avg.	23.55	23.45				
	Variety					
	Meadow	Treasure	Env Avg.			
Severe	24.36	24.33	24.35	A	Drought Pattern	P<0.1
Moderate	24.01	23.48	23.75	AB	Variety	NS
Favorable	22.59	23.12	22.85	B	Drought Environment x Variety	NS
Mgmt. Avg.	23.69	23.53				
	Nitrogen (N)					
	Appied N	No Applied N	Env Avg.			
Severe	24.31	23.41	23.85		Drought Environment x Nitrogen	NS
Moderate	23.64	23.60	23.62		Nitrogen (N)	NS
Favorable	22.87	23.05	22.99		Drought Environment x Nitrogen	NS
Mgmt. Avg.	23.40	23.67				
	Phosphorous (P)					
	Applied P	No Applied P	Env Avg.			
Severe	23.33	24.56	23.85		Drought Environment	NS
Moderate	23.61	23.64	23.62		Phosphorous (P)	NS
Favorable	23.15	22.62	22.99		Drought Environment x Phosphorous	NS
Mgmt. Avg.	23.42	23.68				
	Potasium (K)					
	Applied K	No Applied K	Env Avg.			
Severe	22.18	24.02	23.85		Drought Environment	NS
Moderate	23.86	23.59	23.62		Potasium (K)	NS
Favorable	23.08	22.98	22.99		Drought Environment x Potasium	NS
Mgmt. Avg.	23.30	23.54				
	Sulfur (S)					
	Applied S	No Applied S	Env Avg.			
Severe	23.13	24.19	23.85		Drought Environment	NS
Moderate	24.13	23.29	23.62		Sulfur (S)	NS
Favorable	23.06	22.92	22.99		Drought Environment x Sulfur	NS
Mgmt. Avg.	23.60	23.46				

*NS=Not signficant at the $\alpha<0.1$ level.

Different letters withhing the same groupings are statistically different at the $\alpha<0.1$ level by Least Square Means

A final reason why relatively few statistical differences were established about how management and drought environment affect yellow pea protein may be due to uncertainty in yellow pea protein measurements. Of the 138 yellow pea bulk samples measured in duplicate, the average difference in duplicate measurements was 1.1% (Fig. 3). Put simply, this means that there is potential for large measurement uncertainty in yellow pea protein, so establishing statistical differences in management and environment would require a larger sample size.

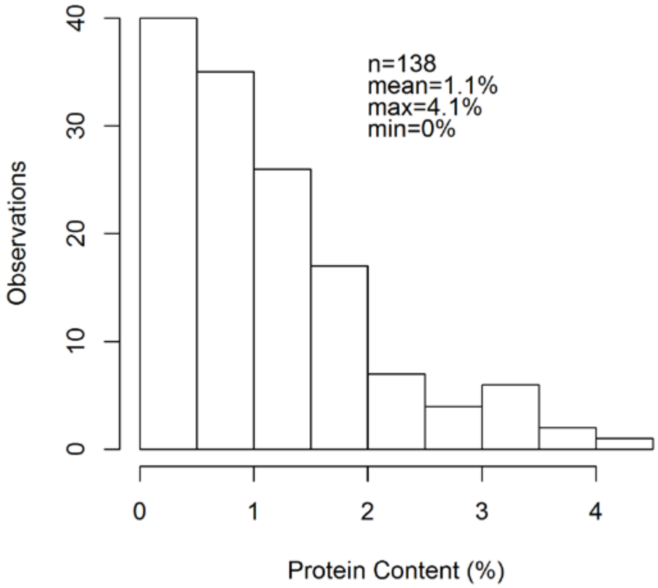


Figure 3. Summary of average difference between duplicated yellow pea protein measurements by the combustion method.

In summary, our results suggest that use of granular inoculant may increase protein, particularly under drought conditions. When comparing only CDC Meadow and CDC Treasure, protein content is determined greatly by growing conditions—higher protein should be expected as drought increases. Use of N, P, K, or S do not appear to influence protein, but the lack of response may be due to the indeterminate growth habit of pea and/or uncertainty in measuring protein content in yellow pea.

Secondary Objective Methodology—Uncertainty in Yellow Pea Protein Measurements by The Standard Laboratory Method and Protein Predictions made by NIR.

There are two primary sources of uncertainty associated with measuring protein using the standard combustion procedure. First there is uncertainty associated with sampling different seeds from the bulk sample (Fig. 4 Left). Second, once seeds have been milled to flour for combustion analysis, there is uncertainty from sampling different portions of the ground sample (Fig 4. Right) One factor that could affect these uncertainties is sample size. For instance, if a duplicate pair of fifty seeds were sampled from the same bulk sample, their respective difference in protein may be larger than a duplicate pair of 100 seeds.

Figure 4. Uncertainty in yellow pea protein measurements may arise from sub sampling different seeds from the bulk sample (left) or sub-sampling different portions of pea flour after whole seeds have been ground (right).

Testing how Subsample volume Affects Uncertainty in Protein Measurements with the Combustion Procedure

To test how sample size affected protein measurement uncertainty, a controlled experiment was established. Four yellow pea and four spring wheat bulk samples were randomly taken from Montana farms. From each bulk sample, four subsample sizes of 1, 2, and 3 tablespoons were ground in a Udy mill with a 1mm screen. Each subsample was then run for total nitrogen using combustion analysis. Protein was then be determined by multiplying total nitrogen by 5.80 and 6.25 for wheat and pea respectively. It should be noted that spring wheat was included in the experiment to serve as a control. In other words, we wanted to determine if there was similar protein measurement uncertainty between yellow pea and spring wheat.

The response of interest was the absolute difference in measured protein for the two subsampled pairs corresponding to each crop type by sample volume combination. Analysis of variance (ANOVA) used to test the effects of crop type and sample volume on absolute differences between protein measurements.

Validating Yellow Pea Protein Predictions with Near Infrared (NIR)

Forty-six of the 138 yellow pea samples that had previously been tested for protein using the combustion method (see part A.) were randomly selected. Selected samples were then run on NIR (FOSS Infratec 1241), and protein predictions made by the NIR were compared against protein measurements made by the combustion method. The distance measure, or metric used to gauge the average difference between NIR predictions and combustion measurements, was the mean absolute error (MAE). A low value for the MAE indicates that predicted and measured protein are precise relative to one another, whereas as a high MAE indicates that predicted and measured protein do not agree with one another.

Secondary Objective Results

Effect of Crop and Sample Size on Protein Measurement Uncertainty

Only crop had a significant effect on differences in protein measurements among duplicates (Table 2.). The average difference in protein measurements for ***duplicated whole seed samples*** was 0.29% and

0.82% for wheat and pea respectively. Similarly the average difference for duplicated ground samples was 0.28 and 0.65% for wheat and pea respectively.

Table 2. Effects of Sample Size and Crop Type on Measured Protein Differences (%) among Seed Duplicates.

Whole Seed Samples

Sample Size	Crop				
	Wheat	Pea	Sample Size Avg.	P-Values	
One Tbsp.	0.22	1.24	0.73	Sample Size	NS*
Two Tbsp.	0.37	0.50	0.44	Crop	$P < 0.05$
Three Tbsp.	0.26	0.71	0.49	Sample Size x Crop	NS
Crop Avg.	0.29 A	0.82 B		Seed Lot (Crop)¶	NS
				Sample Size x Seed Lot (Crop)¶	NS

Ground Samples

Sample Size	Crop				
	Wheat	Pea	Sample Size Avg.	P-Values	
One Tbsp.	0.36	0.99	0.68	Sample Size	NS
Two Tbsp.	0.37	0.59	0.48	Crop	$P < 0.05$
Three Tbsp.	0.11	0.35	0.23	Sample Size x Crop	NS
Crop Avg.	0.28 A	0.65 B		Seed Lot (Crop)¶	NS
				Sample Size x Seed Lot (Crop)¶	$P < 0.05$

NS*-no significant differences at the $\alpha=0.05$ level
¶-Indicates random effect
(Crop)-Nested within crop
Different letters within the same groupings are statistically different at the $\alpha<0.05$ by Fisher LSD

There was a tendency to see smaller differences among duplicated measurements with increasing sample size across crop type for both whole seed and ground samples. That is, differences in protein were smaller as sample sized increased. However our results did not provide strong statistical evidence to verify that increasing sample size would decrease protein measurement uncertainty. Combined these results simply suggest that there is greater protein measurement uncertainty in yellow pea relative to spring wheat.

Uncertainty in yellow pea protein may arise from multiple sources. For instance, All-Khan and Youngs (1972) observed protein could range to ~ 9% among different pea plants. Likewise Atta et. al. (2004) showed that protein could range by ~10% depending on seed nodal position and variety. Considering that the yellow pea samples for this analysis were taken from different Montana farms, they were undoubtedly inconsistent in location and variety, and it’s possible these inconstancies contributed to yellow pea protein uncertainty. Future studies could assess how both variety and location affect uncertainty in yellow pea protein measurements.

Validation of NIR Predictions with Yellow Pea Protein Measurements

Predicted and measured yellow pea protein content determined by NIR and the combustion method agreed with one another with a mean absolute error of 1.02% (Fig. 5). A MAE of 1.02% suggests that yellow pea protein predictions are not highly precise. Lack of precision, importantly, is most likely due to natural variation in yellow pea content as opposed to poor NIR calibration. That is, considering that duplicated protein measurements in pea whole seeds and flour averaged 0.82 and 0.65% *(see above—Effect of Crop and Sample Size on Protein Measurement Uncertainty)*, it would be impossible to calibrate NIR with greater precision.

Other researches have in fact had similar difficulty in producing precise calibration for pea protein with NIR. For instance, Tkachuk et al. (1987) calibrated NIR with a 1.34% standard error of prediction for pea protein. Similarly, Arganosa et al. (2006) achieved a standard error of prediction of 0.94%. Conversely, in wheat, standard error of prediction between measured and predicted protein content has ranged from 0.24% (Williams and Sobering, 1993) to 0.48% (Williams et al., 1985). The better prediction accuracy in wheat relative to pea may simply stem from the lower measurement uncertainty associated with wheat compared to pea.

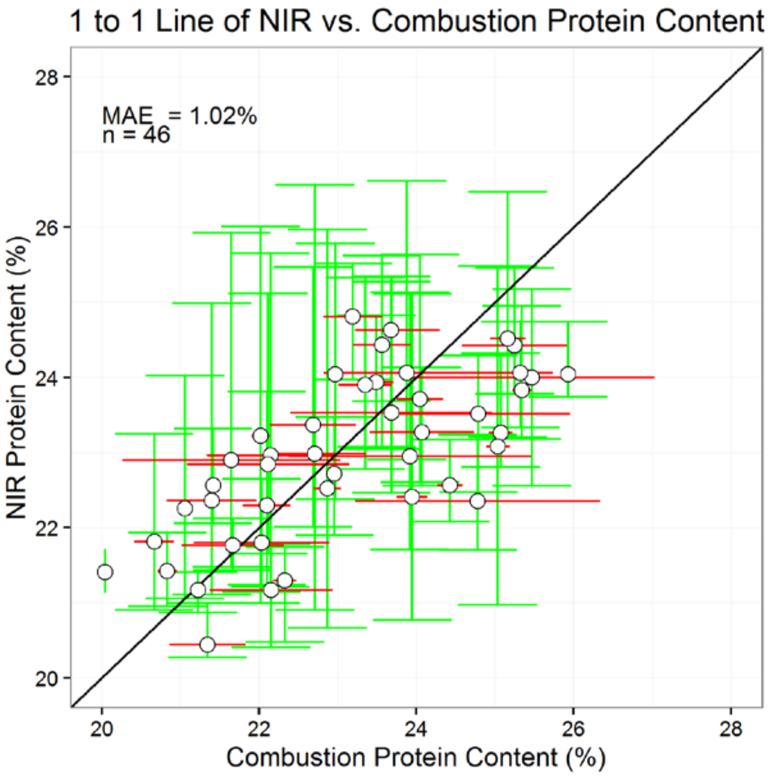


Figure 5. Predicted (NIR) and measured (Combustion) yellow pea protein content. The red horizontal error bars show the range in duplicated measurements from combustion analysis. The green vertical error bars are the range in protein from 5 NIR predictions. The circles represent the averaged protein protein content.

Based on these protein testing findings, more work could be focused on pinpointing the source of protein measurement uncertainty. For instance, if it could be determined that certain varieties demonstrate greater protein variability relative to others, then it may be worthwhile making variety-specific NIR calibration curves. Better understanding the sources of protein variability in yellow pea measurements could ultimately help ensure that both researchers and industry provide precise yellow pea protein measurements to producers and consumers alike

<u>Hiring</u>

- No additional hires in Quarter 7.

<u>Expenditures</u>

- Total Personnel Services: $73,670.17
- Payroll Encumbrance: $5,955.62
- Total Operations: $14,990.77

2) 41W220 – Principal Investigator: John Peters; Email: john.peters@chemistry.montana.edu

Progress towards milestones

Project goal

To monitor and discover optimal soil microbial ecology and macronutrient combinations that support crop productivity. Emphasizing on the effects of pulse growing activity on diazotrophic community structure.

Introduction

Pea and pulse crops are increasingly being used in agriculture due to the economic and environmental benefits. Pea is a legume that has a symbiotic relationship with nitrogen-fixing bacteria and is often rotated with wheat to mitigate nitrogen loss from the soil, reducing the need for fertilizer (Gan 2015). In Montana, pea and pulse crops are an important agriculture staple, with Montana being the country's leader in pea and lentil production since 2011 (Lee 2011). Understanding the soil and rhizosphere microbial ecology, especially the nitrogen fixing community, could potentially lead to higher yield of both pea and grain crops while mitigating the detriments of fertilizer.

The nitrogen-fixing bacteria are called diazotrophs and are a diverse classification that can include symbiotic rhizobia bacteria and free-living soil bacteria. Nitrogen fixation reaction is carried out by the nitrogenase protein which is encoded by the genes nifH, nifD, and nifK. To determine the phylogeny of diazotrophs in soil nifH can be characterized to distinguish the different genus of the bacterium. The diversity of soil bacteria has been correlated to healthier soil and better plant yields (Hartmann 2009). By discovering the changes in the diversity of important diazotrophic communities by pulse crops we can determine a correct strategy for future Montana pulse crop farmers.

Project Design

In order to determine the bacterial communities and the effects on the soil and crop, multiple strategies were developed. First, a statewide pea study was developed with the help of the Montana State University Ag Research Centers. A collection of soil from pulse crop farms was collected over a complete year from seven different geographical locations each with multiple different pea varieties. This soil was tested for bacterial diversity using 16sRNA and nifH target gene techniques. The soil was also tested for chemical composition and agricultural evaluation was done with the collaboration with Chengci Chen. A large amount of data was created that has been used to address multiple hypothesis driven questions about the effects of pulse crop rotation and soil microbial diversity. A second experiment was devolved in a controlled environment at the Post Farm at MSU. In this experiment, relationships between pulse rotation type, fertilizer, microbial diversity, and the diversity of nitrogen-fixing bacteria were tested over a period of 3 weeks. Characterization of the soil microbial community during the rotation from pulse to wheat was performed and the correlation between the bacterial communities to the variables was tested. The progress during the grant term has led to a large amount of data and has proven the viability of these experiments to provide evidence of the effects of soil microbial ecology on pulse crop productivity.

Project Results

The dataset collected from fall 2015 of the statewide pea study was used for proof of concept work. The DNA extraction and PCR reactions protocols were tested successfully and applied to the larger group of samples. The 16sRNA PCR fragments (F515-R806) were sent to Michigan State University for sequencing and raw sequencing data was received the spring of 2016. The 16sRNA data was processed using the

MOTHUR SOP into phylogenic diversity outputs such as operational taxonomic units (OTU) and Shannon-Simpson index (alpha diversity). Data created in the MOTHUR pipeline was visualized using R studio packages such as ggplots2, vegan, and phyloseq (Figure 1). This pipeline which was developed over the fall and winter of 2016 has determined microbial diversity in tested soils. This developed pipeline is now ready to process the incoming data from the spring and summer of 2016. Through data visualization and statically analysis in R differences in overall community structure and diversity throughout the statewide experimental stations are observed.

In the statewide pea study, single variable changes are shown due to variation throughout the fields. A difference in yields across the farm locations and a difference in alpha diversity (Figure 2a,2c) reproduces previous studies showing better productivity with more diverse microbial communities (Heijden 2008). Outliers are kept for interpretation of geographical diversity changes in the soil microbiome (Figure 2b, 2d). An overall trend in the statewide farm sites that show a correlation in-between the increased alpha diversity and the yield of the farms (Figure 3). These simple observations give initial statistical markers for effects of variables such as yield, moisture or chemical content on bacterial community structure.

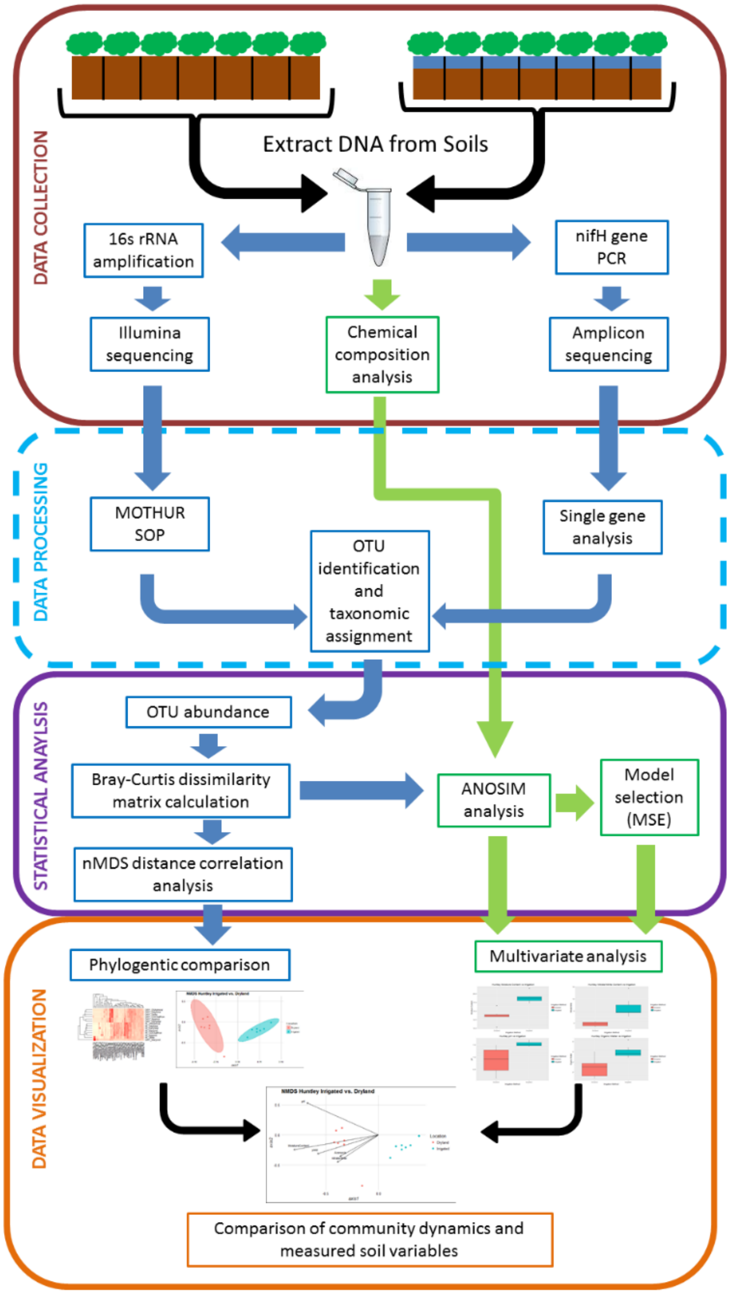


Figure 1: A walkthrough of the sample processing pipeline that is being used on all samples collect.

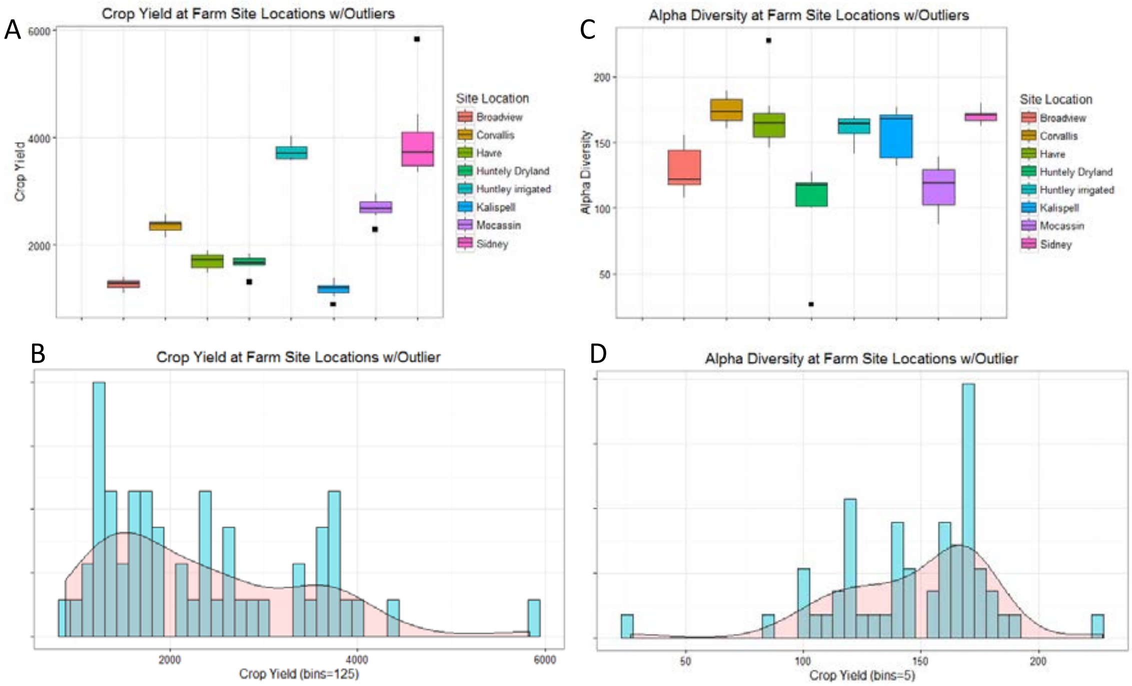


Figure 2: Crop yield varies between geographical locations ANOVA testing shows significance between geographical locations (p-value =$8.37x10^{-23}$). Alpha diversity varies in between some of the locations (p= $2.38x10^{-9}$). Noticeably Corvallis, Harve, Huntley Irrigated, Kallispell, and Sidney show a high alpha diversity. The alpha diversity is representative of the spices richness of the plots.

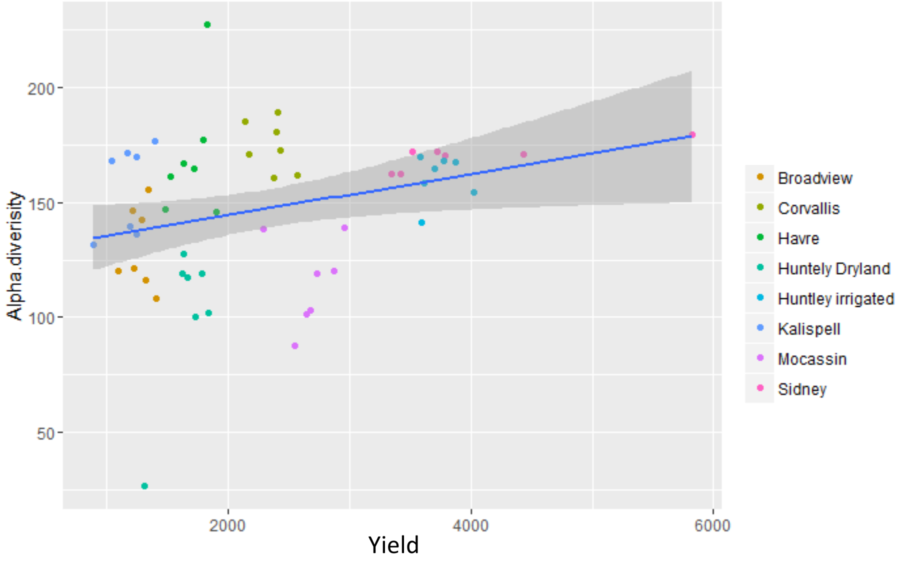


Figure 3: Alpha diversity and yield is correlated (p value=0.02631) with a positive incline. We can also see grouping of alpha diversity and yield by location (colors). Linear regression is shown with (r=0.29) and error in grey is a (95% confidence).

By looking at the community structure of the statewide study we were able to see clustering of the communities to their geographical locations (Figure 4). Yet many variables are involved in defining the microbial community structure of a certain region the clustering represents changes in the soil which may affect the microbiome. With further investigation into the community structure and its correlated soil characteristics, a more prominent observation can be determined for this project.

Huntley irrigation proof of concept analysis

To test the statistical methods used and the ability of the experiment to answer key questions a case study was started by comparing two plots at the Huntley ag station (SARC) that have the most similar soil characteristics but differ in the irrigation methods. Interestingly through the initial investigation, the bacterial community structure is distant between the irrigated and non-irrigated plots (Figure 4). These farm plots and the single variable difference can be used as a proof of concepts experiment to test the strategies for analysis of the large data set. There is a noticeable difference across many soil characteristics between the irrigated and non-irrigated plots (Figure 5).

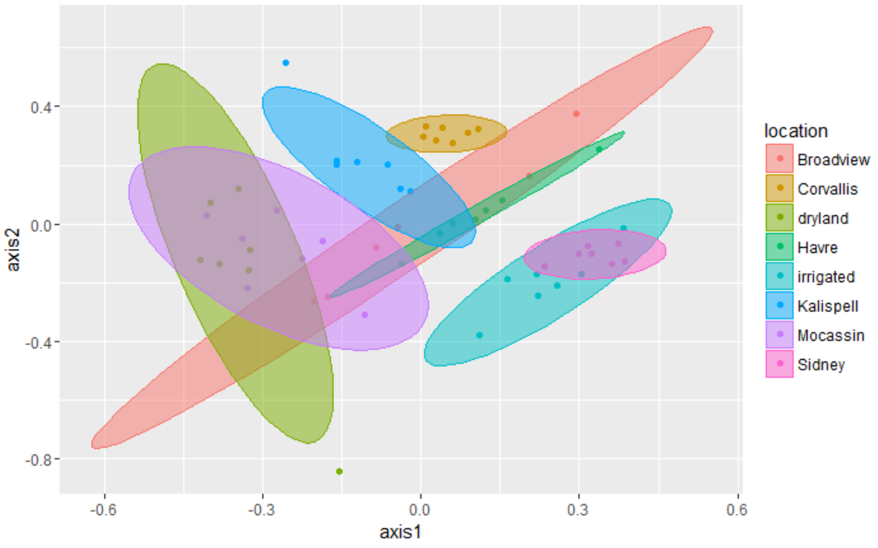


Figure 4: nMDS plot shows the distance characteristics between each plot and variety sample. Each point is a single pea variety at a single sample site. The stat ellipse package was used in r to produce a bivariate density ellipsoid with the confidence level of 0.95. The ellipse statistics shows grouping of geographical variables within in the community structure data. The higher yielding stations (SARC irrigated and EARC) are grouped together with positive influence contributed from axis 1. While other stations show large stratifications across the plot which is due to the large distances in the community trees present in the soil.

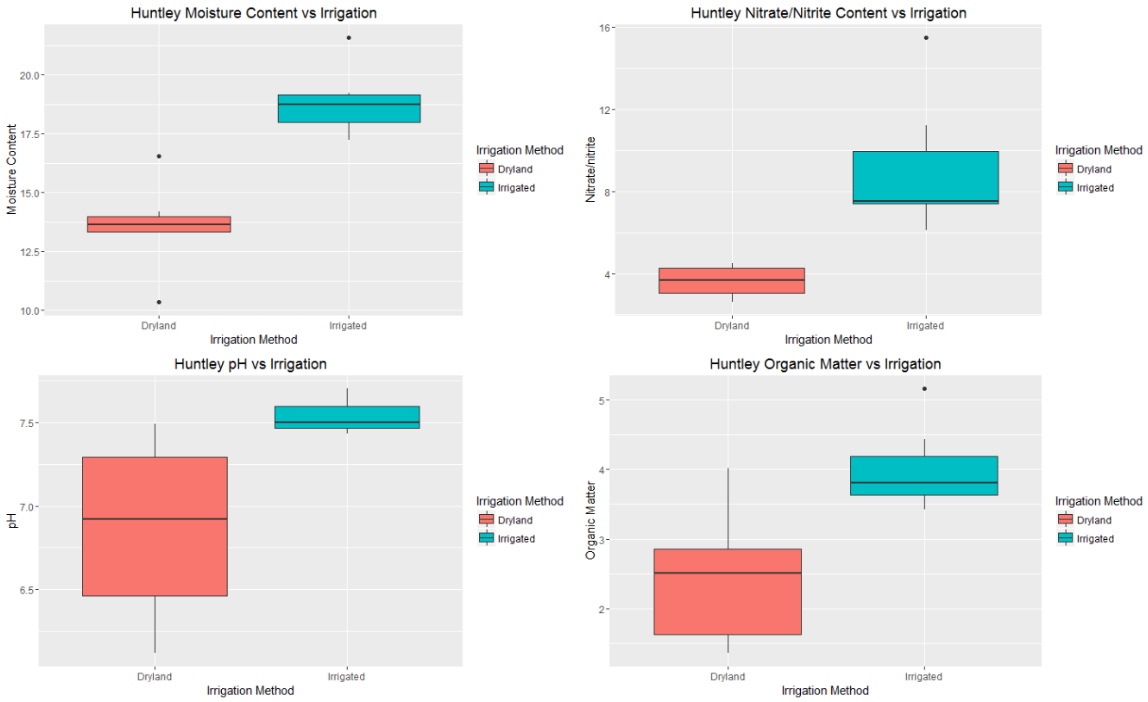


Figure 5: By looking at the single variables of the SARC plots we can compare irrigation methods and outcome variables. A) A difference in measured moisture content and constant moisture (small STD) across the pea variety was measured (p=2.774e-11). B) The nitrate and nitrite content consistency is seen in the dryland plots but see some outlier charateristics are present in the irrigated plots (p = 0.0037). C) There is also a pH diffrence in between the two irrgation methods with dryland having a broad range from 6.5 to 7.25. As irrgated has a constitant pH measurment (p = 0.015). D) Organic matter was meausred in the soil and has a signficance diffrence inbetween two site(p =0.0035).

With these differences between the irrigated and non-irrigated plots, a closer look at the variables effect on the community diversity can be done by assigning specific taxonomic changes to each of the plots. OTU's were taxonomical aligned with the greengenes database and identified down to genus and species level. The composition of the taxonomy through different data visualization methods was compared. First, a bar plot was made using the phylum abundance to compare each of the samples (Figure 6). The comparison of the phylum is a satellite view of the community but show small changes of acidobacteria and proteobacteria which are consistent with cited plant growth promoting bacteria and diazotrophs (Bruto 2014). Actinobacteria also make up a majority of the population of bacteria in the both of the samples. This is in contrast to other reports of farm soil which usually has actinobacteria ranked as third or fourth most abundant phylum, more literature research will be done to determine this difference. For more detailed analysis, a heatmap with clustering trees was made to compare genus level abundance. Clustering shows a clear distinction between differing irrigation methods and similar grouping of variety types (Figure 7). The clustering of certain genus shows distinct interactions in the soil with the change of an irrigation method. An nMDS plot that compares the community structure of the two irrigation methods and the soil fertility measurement shows influence on the community structure (Figure 8). Moisture content, nitrogen, and yield vectors overlay with the dryland nMDS points, vectors have similar response profiles to influence in microbial community structure. This could be explained by an overall effect of irrigation methods on nutrient profiles or microbial communities effect on nutrient profiles. More statistical interpretation is needed to create a correlation.   Future results of with specific diazotrophs community profiles will also lead to more powerful data that could correlate certain bacterial clusters that either directly affects or are affected by the changes in soil characteristics.

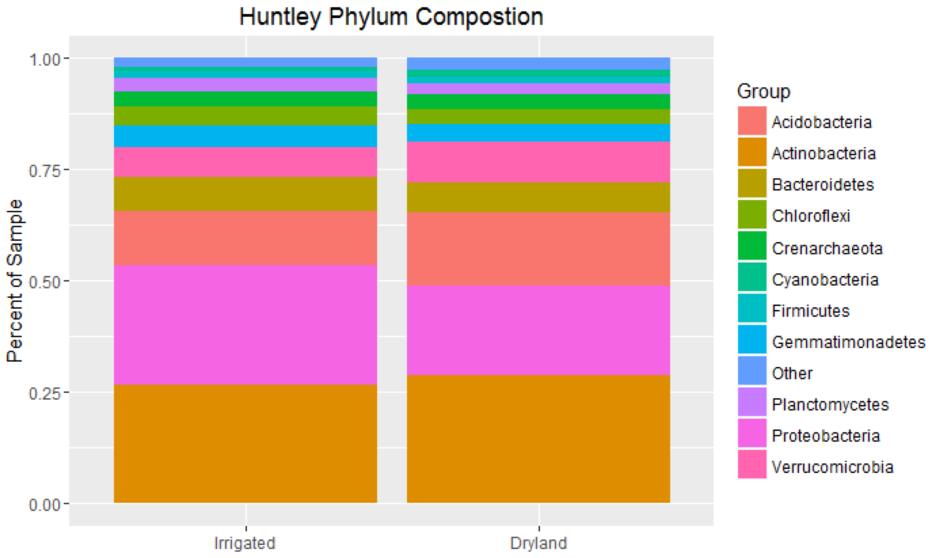


Figure 6: Bar plot showing the top 12 abundant phylum in the samples. Smalk difference on the phyla level between the irrigation methods can be shown. A more detailed look is needed to decipher the true changes in these certain bacterial strains.

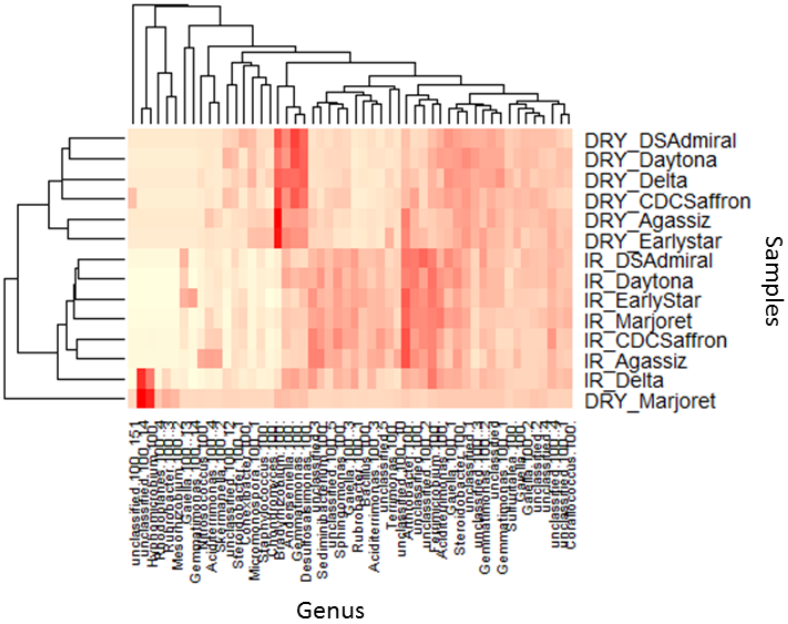


Figure 7: Heatmap shows specific samples clustered into similar variables and genus OTUs are shown clustered on the bottom axis. Abundance data is then applied to show color low abundance white to high abundance red. Bray-curtis clustering program was used Distinct patterns can be seen the shed more insight into the differences between irrigation methods than standard bar plots do. A star labels a branch in the phylogenetic cluster that shows difference between irrigation methods.

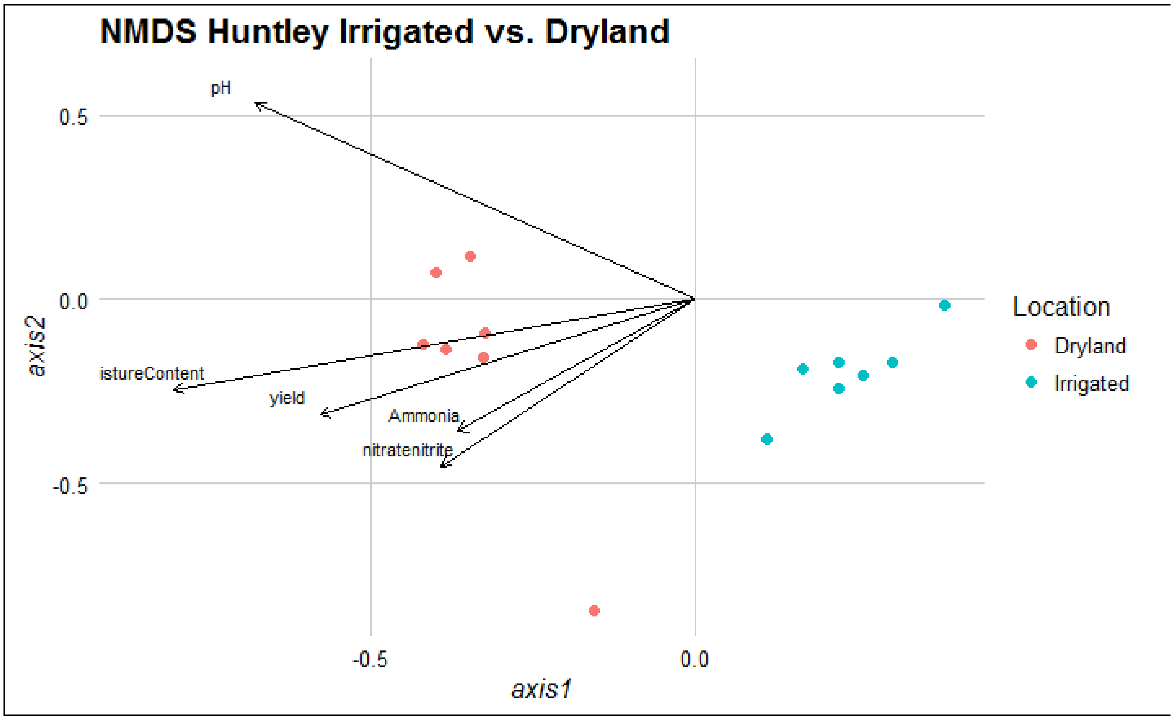


Figure 8: nMDS plot of the distances compassion of the irrigation methods. There is a distinct grouping of irrigation methods with irrigated being more positively affected by axis1 than dryland samples. A bi-plot was transposed on top of the nMDS data to show the effects of chemical data on the community structure. The length of the vector and direction are used to show correlation of the variables yield, ammonia, nitrate/nitrite, and moisture.

Diazotrophs diversity in pulse crop rotations

Another of the main objectives of the pulse crop project was to determine the family structure of diazotrophs and nifH genes present in free-living soil bacteria. The nifH gene codes for the nitrogenase enzyme that reduces atmospheric nitrogen to ammonia and supplies the local communities with reduced nitrogen. To determine the effects of pulse rotation on the bacterial communities nitrogen fixation ability we looked at the abundance and diversity of nifH. A technique has been developed through the utilization the 16s rRNA taxonomical data and predictive genomic function algorithm called PICRUSt which predicts the nitrogen metabolism in specific samples. Though this algorithm gives a good forecast of the nitrogen metabolism of certain groups it does not give enough detail in the structure of these diazotrophs. To be more specific with the determination of diazotrophs community structure another method is to amplify the nifH gene out of the soil using universal primers and PCR to make a gene library. This experiment has been started in the spring of 2017 by sending the genomic DNA to Molecular Research DNA Laboratory to perform next generation sequencing on the nifH gene. These future results will give us detailed community structure of all the diazotrophs present in all the samples.

Post Farm Analysis

In Parallel to the statewide pea study, the control environment at the post farm at MSU was also tested for the chemical composition and 16sRNA gene diversity. This experiment tested the effects of fertilizer, different pulse crops, inoculates, microbial diversity, and the diversity of nitrogen-fixing bacteria over time. OTU tables have been created with the Post Farm data. The interpretation of this data is similar to the methods used for the statewide data. Plots are created showing the nMDS correlation of the differently treated soil and the community structure (Figure 9). There are signs of a grouping of treatment groups in the dissimilarity matrix data. A difference in the pulse variety, time, or chemical composition shows little correlation to the microbial community structure. More research and data analysis are needed to make any conclusions to the post farm data. After confirmation of the nifH gene analysis at the different statewide samples, a similar protocol will be used for the post farm samples.

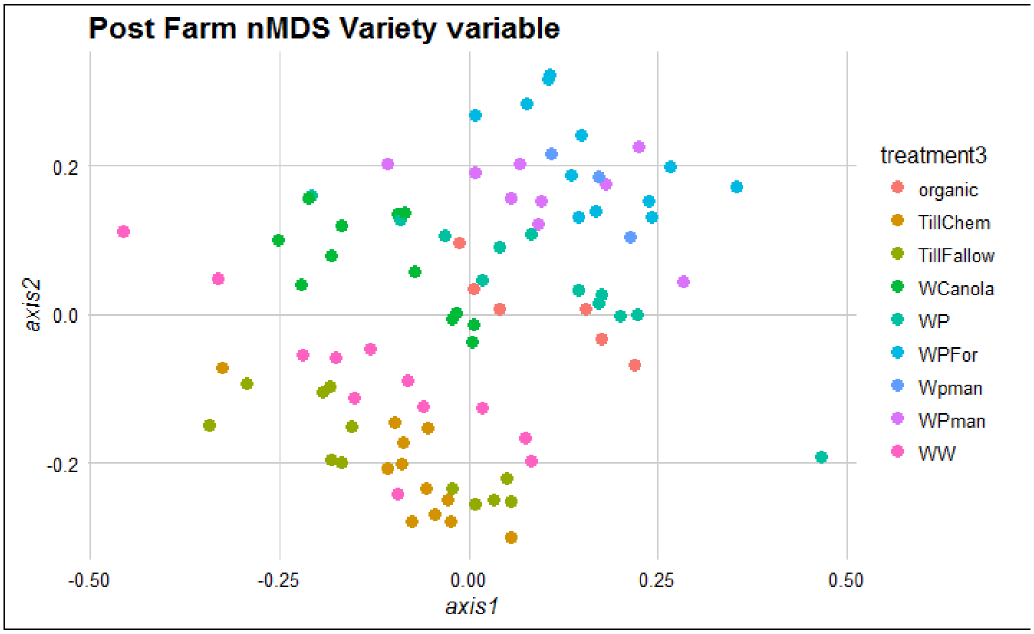


Figure 9: nMDS plot showing treatment variables (colors) grouping with each other. The plot shows that certain groups have similar or dissimilar community structure present. Treatment such as amount of nitrogen added (fertilizer) and previous crops growth (winter wheat) effects the microbial community structure.

Future Data

Chemical data has been received for Spring and Summer 2016 samples and data analysis is currently being performed. DNA extraction and PCR gene testing has also been completed for all of the 2016 samples and has been sent off for next generation sequencing at MR DNA and should be completed by the end of May. After all of this data has been received complete variables including geography, nutrient application, irrigation methods and pea variety will be grouped for a meta-analysis. A pipeline has already been refined in fall of 2016 for multivariate statistics to analyze this large data set. Data processing and hypothesis testing should take place over the summer of 2017 with the finalization of the work by Fall 2017.

Future experimentation

This two-year project has supported an immense amount of work that produced high-quality data that is currently being interpreted to more deeply understand the impacts of pulse crop agriculture on the microbiological ecosystem of Montana soil. These initial studies have set up the groundwork to address the best approach to microbiome sustainability in the soil. Further experimentation can take place to test the initial hypothesis. A longer control farm study may be needed to look at specific changes happening throughout the year in the soils of Montana. This will allow for more understanding of irregularities in the seasonal change of the soil microbiome. This can also apply to the post farm experiment where longer time interval can be taken in order to observe more of a difference in the diversity of the soil.

Conclusion

The project goals to determine factors of pulse crop rotations that effect the diazotrophic bacterial community has been obtained and laid the foundation for future analysis and study of this topic. The immense amount of data collected over the past two years has been invaluable in the study of the pulse crop rotation. Microbial community interactions are on the leading edge of soil and crops science. The progress during this grant will help farmers in the future decide the right pulse rotation, bacterial inoculate, or fertilizer for a better economic output.

Hiring

- No additional hires in Quarter 7.

Expenditures

- Total Personnel Services: $78,227.78
- Total Operations: $21,946.55

3) 41W213 – Principal Investigator: Carl Yeoman; Email: carl.yeoman@montana.edu

Progress towards milestones

Direct-Fed Microbials To Reduce Rumen Nitrate & Nitrite Levels

Significant opportunities exist to more productively use Montana's 4.6 million acres of crop fallow land each year. One opportunity being explored is terminating fall or spring seeded cover crop mixtures by grazing or ensiling, which could result in substantial economic benefits through delaying the turn out of livestock on summer pastures (increasing carrying capacity and livestock production), and allowing both crop and cattle producers to capture significant market revenues within an integrated production system. However, common cover crops such as brassicas and small grains can accumulate high levels of nitrates (up to 20,000 ppm) which can be toxic to livestock species in doses >5,000 ppm. Under normal physiological conditions, lower levels of ingested nitrate are efficiently converted to nitrite, then to ammonia, amino acids, and finally protein by rumen bacteria. Some bacteria are more efficient at this process and may therefore be useful as direct-fed microbials applied during periods of cover crop grazing to bolster the numbers of these bacteria to mitigate the risk of nitrate poisoning during cover crop termination. This project set out to i) identify and isolate microbes that could be stably introduced to the rumen of cattle and sheep and efficiently reduce nitrate and nitrite to microbial protein; and ii) test the efficacy of 1-2 of these strains for use as direct-fed microbials (**DFMs**) in an animal trial.

Results

The initial phase of the trial set out to isolate nitrate and nitrite utilizers and screen each to determine the rates at which they were able to carry out this process. As these strains would ultimately be required to successfully colonize and carry out these metabolic processes in the rumen of domestic livestock species, we only attempted to isolate microbes from rumen samples. These included rumen samples maintained in our collection from domestic cows (n=2), domestic sheep (Rambouilet, n=4), Bison (n=8), Goat (grass-fed Nubian cross n=4), Elk (n=6), Whitetail deer (n=2), Mule deer (n=1), Moose (n=3) and Antelope (n=2). As a positive control, two strains of rumen-isolated bacteria (*Selenomonas ruminantium* and *Wollinella succinogenes*) previously reported to degrade nitrate and nitrite (IWAMOTO, ASANUMA, & HINO, 2002) were purchased from the American Type Culture Collection (ATCC) for comparison. Isolation of bacteria was performed with media adapted from that described by (Kenters, Henderson, Jeyanathan, Kittelmann, & Janssen, 2011). Briefly, (cas)amino acids and peptone were replaced with 5mM sodium nitrate and 3mM sodium nitrite, rumen fluid was allowed to ferment at room temperature for 24h following collection to reduce background levels ammonia, amino acids, and protein, and solutions were cooled under 95% CO_2, 5% H_2 to make anoxic. For plates, media were solidified with the addition of 15g/L of agar for plates. For each sample (cow, sheep, bison, goat, deer, elk, moose, and antelope), 0.5ml was serial diluted $10^3 – 10^7$ in anaerobic broth media (without addition of agar) and spread plated in an anaerobic chamber (95% CO_2, 5% H_2). Plates were incubated for 24h at 39 °C. Fifty microbial colonies were selected from all plates and each colony was transferred to fresh plates and streaked for isolation. Streak plates were again incubated for 24h at 39 °C. Of the fifty plated colonies, 23 grew and were able to be isolated (Table 1). Cell isolates were inoculated in stoppered serum bottles containing 50ml fresh broth containing 5mM nitrate and 3mM nitrite. Growth was monitored by subsampling 200ul hourly for evaluating optical density (absorption at γ=600 nm) using our labs Biotek spectrophotometer. When cultures were found to be in the exponential growth phase, additional 100ul samples were collected and snap frozen at -80 °C. These samples were divided into two 50ul aliquots and diluted 125x and 75x and assayed for nitrate and nitrite levels, respectively, using the Abnova nitrate/nitrite colorimetric assay kit (Abnova, Walnut Creek, CA). Rates of nitrate and nitrite reduction (Table 1) were then determined using linear regression across all points that were collected during the exponential growth phase. Seventeen of our isolates and the two ATCC strains were found to reduce nitrate concentrations in the media at rates ranging from 0.03 – 0.21 mM/h and eight isolates and the ATCC strains were found to reduce nitrite at rates of 0.01 – 0.16 mM/h. The rates determined for the two ATCC strains were similar to those previously reported (IWAMOTO et al., 2002)and were greater than those of any of our isolates and so were selected for further study as DFMs. Alongside, the DNA of all isolates were extracted using PowerFecal DNA isolation kits (MoBio Laboratories, Inc., Solana Beach, CA) and following manufacturer's protocols, amplified for the V3-V4 region of the 16S rRNA gene by 25 cycles of PCR using the KAPA HotStart PCR Kit (Kapa Biosystems, Wilmington, MA), and pooled and sequenced by Illumina MiSeq with a V3 600 cycle sequencing kit. Reads were assembled with PandaSeq (Masella, Bartram, Truszkowski, Brown, & Neufeld, 2012) using default settings curated to remove low quality (<Q30) sequences using the FASTX Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/index.html) and chimeric sequences using mothur's implementation of UCHIME (Edgar, Haas, Clemente, Quince, & Knight, 2011) and then classified using mothur's implementation of the RDP classifier using the SILVA database (Release 123), and further examined by BLASTn alignment of the most representative sequence (by mothur's definition this is the sequence that is closest to all other sequences clustered into the OTU) to Genbank's refseq_RNA database. If best BLASTn alignments were ≥97 % species identity was inferred, otherwise genera were

only reported. The RDP classifier only reports genus level classifications (Cole et al., 2009) and no disagreements were seen between RDP and BLASTn identities at the genus level. Taxonomic identities inferred from this process for each isolate are shown in Table 1.

Table 1. Microbial Isolates Obtained

Source	**Isolate ID**	**Nitrate (mM/h)**	**Nitrite (mM/h)**
ATCC	*Wollinella succinogenes*	0.21	0.16
ATCC	*Selenomonas ruminantium*	0.16	0.09
Sheep 3	*Selenomonas ruminantium* S3.3	0.14	0.05
Sheep 1	*Veillonella sp.* S1.2	0.14	0.01
Sheep 3	*Selenomonas* sp. S3.2	0.13	0.02
Cow 2	*Prevotella ruminicola* C2.3	0.13	n/d
Mule deer 1	*Propionibacterium sp.* MD1.1	0.12	n/d
Sheep 3	*Megasphaera elsdeni* S3.4	0.11	0.03
Cow 2	*Megasphaera sp.* C2.2	0.11	0.03
Cow 1	*Prevotella sp.* C1.3	0.11	0.01
Cow 2	*Prevotella ruminicola* C2.4	0.11	n/d
Goat 4	*Veillonella parvula* G4.2	0.11	n/d
Bison 1	*Selenomonas* sp. B1.2	0.10	0.06
Goat 2	*Coprococcus sp.* G2.1	0.10	n/d
Bison 1	*Propionibacterium sp.* B1.1	0.09	0.01
Sheep 1	*Prevotella sp.* S1.1	0.09	n/d
Mule deer 1	*Propionibacterium sp.* MD1.2	0.09	n/d
Sheep 3	*Veillonella alcalescens* S3.5	0.09	n/d
Cow 1	*Prevotella sp.* C1.1	0.03	n/d
Cow 2	*Anaerovibrio sp.* C2.1	n/d	n/d
Sheep 1	*Butyrivibrio* sp S1.3	n/d	n/d
Goat 4	*Clostridium sp.* G4.1	n/d	n/d
Goat 1	*Prevotella bryantii* G3.1	n/d	n/d
Sheep 3	*Prevotella ruminicola* S3.1	n/d	n/d
Cow 1	*Ruminococcus sp.* C1.2	n/d	n/d

Animal Trial

The second part of the trial was carried out with five graduate students as part of the ARNR521 class Advanced Ruminant Nutrition. Therein 20 Rambouillet smut lambs were transported to the Montana State University (MSU) Bozeman Agricultural Research and Teaching (BART) farm, fitted with electronic transponder ear identity tags, and co-housed in a single pen with access to four GrowSafe nodes to monitor intake (Fig. 1C). The GrowSafe system (GrowSafe Systems Ltd., Airdrie, AB, Canada) measures intake by recording the presence of each ear tag at the feed bunk and every second assesses feed disappearance by reductions in total feed bunk weight. The system has previously been shown to be effective in assessing feed intake in both cattle (DeVries, Keyserlingk, Weary, & Beauchemin, 2003) and sheep (Redden, Surber, Grove, & Kott, 2014). Access to feed bunks was limited to individual animals using head gates and elevated platforms (Fig. 1C).

Figure 1. Animal Trial: *Twenty Rambouillet Lambs (A) were provided one of three DFM treatments or a control (B) and fed a nitrate-enriched hay feed. Feed intake was measured by GrowSafe system (C) and rumen (D) and blood serum samples were collected weekly to measure nitrate and nitrite levels and microbial community composition.*

Lambs were fed a grass hay with 2000 ppm Nitrate added to the hay by spray bottle at 10 ml / Lb of a 100g/L solution of Sodium Nitrate and mixed prior to feeding. Over the first week lambs were stepped up to final nitrate levels as follows (0 ppm day 1, 300 ppm day 2, 600 ppm day 3, 900 ppm day 4, 1200 ppm day 5, 1500 ppm day 6, 1800 ppm day 7, 2000 ppm days 8 – 21). After the first week lambs were sorted into one of four treatment groups: T1) Lambs were given a 20ml dose of 10^6 cells/ml *S. ruminantium* (~2 x 10^7 total cells/day) daily; T2) Lambs were given a 20ml dose of 10^6 cells/ml *W. succinogenes* (~2 x 10^7 total cells/day) daily; T3) Lambs were given 10ml each of 10^6 cells/ml *S. ruminantium* and 10^6 cells/ml *W. succinogenes* (~1 x 10^7 cells of each bacterium/day, ~2 x 10^7 total cells/day) daily; and a control group received sterilized spent-media and no direct-fed microbial daily (i.e. A 50:50 mix of the two culture media that the two bacteria were grown in following autoclave-sterilization to kill those bacteria) (Fig. 2B). Lambs were sorted to ensure groups did not differ in weight ($P > 0.33$), in daily-intake measured over the first week of acclimation ($P > 0.4$), or in intake adjusted to metabolic rate using Kleiber's law ($P > 0.34$) (Kleiber, 1932). These measures were balanced to, as best possible, attempt to ensure groups consumed and endogenously metabolized Nitrate in the feed at a near-identical rate. These measures are shown in Table 2. Treatments were given once daily between 06:00 and 08:00 through week 2 (days 8 – 14). Rumen samples were collected by stomach tube and blood serum samples were collected by jugular venipuncture at day 8 prior to lambs receiving treatment and then at days 14 and 21. No treatment was given the final week to determine if DFMs would persist in the rumen and effectively reduce ruminal and serum Nitrate and Nitrite levels over that period (both considered essential features of any potential product). Rumen and blood serum samples were measured for nitrate and nitrite concentrations using MQuant colorimetric test strips. Initial tests were run using strips with a standard concentration range (2 – 80 mg/L of nitrite and 5 – 225 mg/L of Nitrate) and these assays were repeated with high-sensitivity kits with concentration ranges of 0.5 – 10 mg/L for both Nitrite and Nitrate. All rumen and blood serum samples failed to show any detectible nitrate or nitrite concentration with the exception of a single control animal (M6558) at week 2 (Fig. 2). The week 2 sample of M6558 revealed a concentration of 10 mg/L Nitrate and 5mg/L Nitrite in in rumen samples. However, based on the absence of detection in other animals the trial was determined to be inconclusive.

Table 2 Lamb Treatment Group Metrics

Treatment	**Lamb**	**BW$_i$**	**Intake$_{W1}$**	**Intake/BW^k**
Control	**M6410**	**9.75**	**4.25**	**0.77**
Control	**M6499**	**11.65**	**3.40**	**0.54**
Control	**M6558**	**8.45**	**1.23**	**0.25**
Control	**M6448**	**8.45**	**2.30**	**0.46**
Control	**M6461**	**9.85**	**2.14**	**0.38**
Average		**9.63 ± 1.32**	**2.66 ± 1.17**	**0.48 ± 0.19**
T1	**M6557**	**9.15**	**3.60**	**0.68**
T1	**M6459**	**10.2**	**2.90**	**0.51**
T1	**M6421**	**7.6**	**1.30**	**0.28**
T1	**M6444**	**11.8**	**3.00**	**0.47**
T1	**M6434**	**11.6**	**2.70**	**0.43**
Average		**10.07 ± 1.75**	**2.70 ± 0.85**	**0.48 ± 0.14**
T2	**M6562**	**14.75**	**5.00**	**0.66**
T2	**M6518**	**7.55**	**2.20**	**0.48**
T2	**M6408**	**9.5**	**1.90**	**0.35**
T2	**M6463**	**9.4**	**2.70**	**0.50**
T2	**M6441**	**9.1**	**2.30**	**0.44**
Average		**10.06 ± 2.73**	**2.82 ± 1.25**	**0.49 ± 0.11**
T3	**M6458**	**9.55**	**3.60**	**0.66**
T3	**M6520**	**13.8**	**3.80**	**0.53**
T3	**M6486**	**7.85**	**1.70**	**0.36**
T3	**M6533**	**9.90**	**2.60**	**0.47**
T3	**T6191**	**7.85**	**2.50**	**0.53**
Average		**9.79 ± 2.43**	**2.84 ± 0.86**	**0.51 ± 0.11**

BW$_i$ = Initial Body Weight (Lbs); Intake$_{W1}$ = Intake during Week 1 (Lbs); Intake/BW^k = Metabolism adjusted Intake (Intake / Weight ^ 0.75 as per Kleibers law)

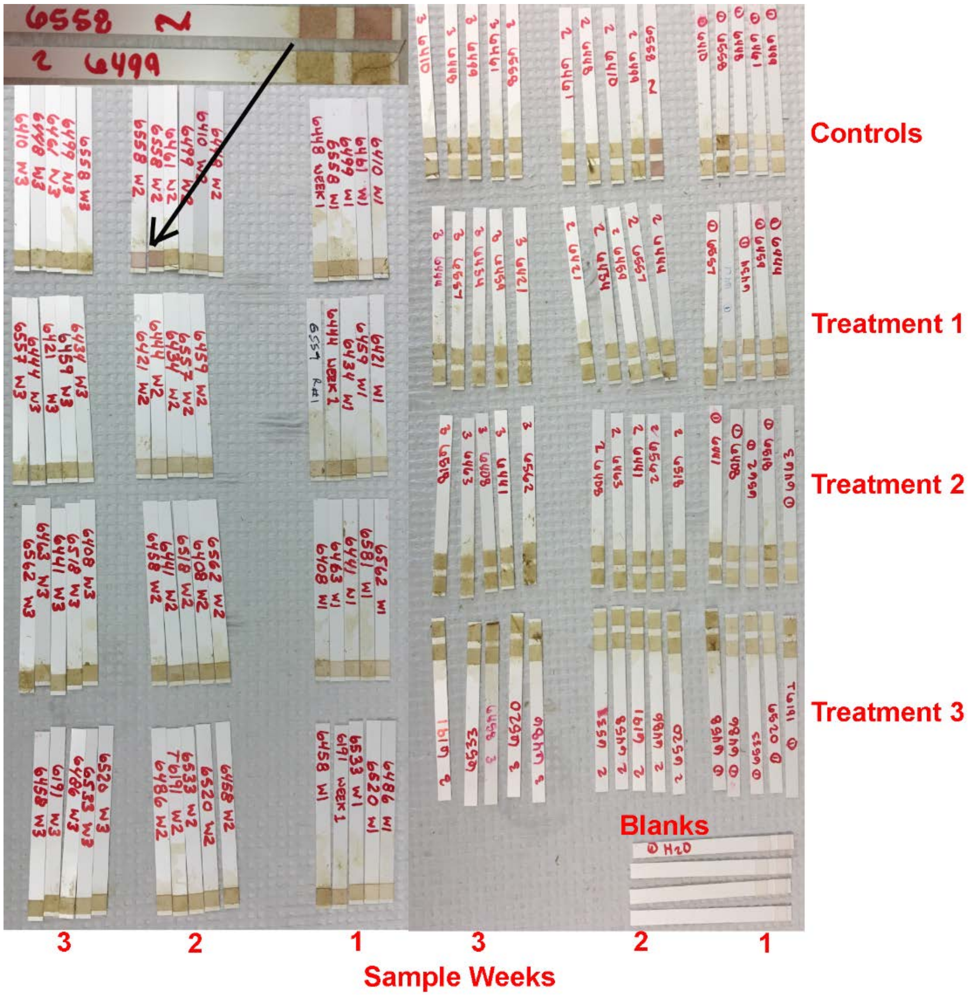


Figure 2. Ruminal Nitrate and Nitrite Colorimetric Tests: *Blood serum and ruminal samples were evaluated for nitrate and nitrite levels. Increasing concentrations of nitrate are determined by a more intense shade of pink to purple as seen in our one positive animal M6558 at week 2.*

Re-evaluation of the trial has led us to conclude the following factors likely limited the success of our trial and should be improved in future trials:

1. Nitrate levels added to feed were too low

In an abundance of caution we selected 2000 ppm nitrate to feed trial animals. This was just 40% of the toxic levels described in the literature. As our objective for this first animal trial was simply to determine the rates of nitrate and nitrite reduction in DFM-treated animals we believed these levels to be sufficient while also avoiding any negative effects on animal health.

2. Stepping up levels of Nitrate in Feed Over the First Week May Have Enabled Animals To Acclimate To The High Nitrate Levels In Feed

Although this was again used in an abundance of caution to avoid poisoning animals, the gradual stepping up of nitrate levels in feed may have favored the enrichment of nitrate/nitrite-reducing ruminal bacteria, allowing more rapid reductions of nitrate levels than would otherwise be expected. We are hoping to get a clearer indication of this through on-going work where we are profiling the ruminal microbes of each animal at each time-point to see how the nitrate diet and treatments affected microbial ecology.

3. Feeding Times Should Have Been Constrained and Sampling Times Should Have Occurred Immediately After Feeding

Allowing animals to spread feed events across the day also spread their exposure to nitrate. Preliminary evaluation of the GrowSafe data further suggests that animals had only ingested small quantities of feed in the hours leading up to sampling times and many had not eaten in the past 6-12 h. For example, on our first sample day (d 8), 3/20 animals were recorded consuming a total of 70g in the 4 hours before sampling, with the most recent consuming 10g approximately an hour prior to sampling. On the second sampling day (d 14), M6558 (the animal with measurable ruminal nitrate and nitrite on this sample day) was recorded at the feed bunk five times in the hours leading up to sampling, including a feeding event where 60g was consumed just minutes before being sampled. The third sample day (d 21) involved 15 different animals consuming a total of 310 g in the 4 hours prior to sampling, with the largest feeding event being 60g approximately an hour before sampling. Because sampling times were between 08:00 and 10:00, these small feeding events likely reflect the feed bunks having little remaining feed after the overnight period. We therefore seek to determine the efficacy of these bacteria supplied as direct-fed microbials to sheep individually and together on rumen nitrate and nitrite levels.

On-going Work

While results from the animal trial were found to be inconclusive, we did obtain rumen fluid from all animals at the three time points (d 8, 14, 21) before, during, and after treatment with DFMs. As an alternative way to examine the efficacy of these DFMs to efficiently reduce nitrate and nitrite levels, we are currently planning in-vitro experiments to longitudinally measure rates of nitrate and nitrite reduction. Undergraduate student, Cassidy Wong has recently joined the lab and is being trained in appropriate techniques to carry out this project.

Laboratory technician, Sarah Olivo, who was partially funded by the MREDI grant is currently preparing ruminal samples for 16S rDNA sequence profiling to determine: i) the relative abundances of the DFMs throughout the trial to assess their background levels, and ability to persist in the rumen after treatment cessation; ii) the longitudinal effect of the nitrate enriched feed on the rumen microbial community (controls); and iii) the ecological effect of the DFMs on the rumen microbial community (Treatments 1 & 2). The first of these outputs will inform us of the viability of these DFMs in a practical setting, as if DFMs are unable to persist beyond the period of inoculation it is likely the daily inoculation regimen would prove too expensive for application in the field. The second and third outputs are of interest to the potential broader impacts of these DFMs as a future product. Specifically, recent research has shown that increasing ruminal Nitrate concentrations can reduce methanogenesis by both replacing CO_2 as a terminal electron acceptor and with Nitrate-reducing bacteria competing with Methanogens for hydrogen (Yang, Rooke, Cabeza, & Wallace, 2016). The broadened benefit of these observations is that by reducing ruminal methane production, we reduce the potential energy lost to this wasteful process (estimated at 2 – 12%) and instead make it available for animal production. As methane is a significant greenhouse gas, its reduction also reduces the environmental impact of animals employed to this system and thereby increases environmental sustainability and stewardship.

Future Directions

We plan to further pursue this project as part of a USDA-NIFA funded SBIR (small business innovation research) grant and are currently looking for potential commercial partners. Our ability to obtain SBIR funding will be greatly increased if our planned in-vitro experiments are successful and demonstrate efficient breakdown of the nitrate and nitrite in rumen samples of DFM-treated animals. Lessons acquired from our animal trial have informed us that we have underestimated the basal potential of the sheep ruminal microbiota to utilize nitrate and nitrite and give us a better foundation to propose higher feed inclusion levels of nitrate for ethics approval. These lessons will also allow us to better design future animal trials that may be funded through a phase I SBIR or other funding opportunity. The grant also provided us with additional technologies, in the form of a bioreactor that will allow us to optimize growth conditions and potentially perform directed evolution experiments to create improved strains – each of these may be proposed in the phase I SBIR.

Hiring

- No additional hires in Quarter 7.

Equipment

- We will not be ordering any additional equipment for this project.

Expenditures

- Total Personnel Services: $38,500.01
- Total Operations: $19,544.35
- Equipment: $8,737.64

Cover Crop/Grazing subproject of the Agriculture MREDI Grant

1) 41W214 – Principal Investigator: Darrin Boss; Email: dboss@montana.edu

Progress towards milestones

Statewide MREDI

In the statewide cover crop trial, targeted mono- and polycultures were evaluated at the seven stations. Species represented cool season species, warm season species and polycultures made up of cool season, warm season, a blend of cool and warm season and an alternative polyculture thought to be very novel in current rotations around the state. The species were determined by input of local agronomists and animal scientists that appeared to have the best opportunity to germinate and produces either above ground biomass or a favorable root structure. There were two planting dates, one appropriate for cool season planting and on appropriate for the warm season plantings. Each of the four polycultures were planted at each planting date. Harvesting occurred when the first species began to head, triticale in most cool season environments and millet in the warm season planting. Thereby preventing additional viable seed production from the cover crop that might contribute to further water use and “weed” problems in future crops.

In seven location the cool season species produced from 615 to 2267 pounds above ground biomass on a dry matter basis with oats being the greatest across all environments. With the polycultures being lower than the monoculture producing the greatest amount of biomass. In all locations when the polycultures were compared across planting dates the warm season outperformed the early planting, however the early harvest was completed around July 8 and the late season harvest date occurred well into August thereby using more soil available water and mimicking a season long cash crop. Nutrient content of the monocultures and polycultures across all locations and both planting dates were very high quality and were across all sites, higher in Crude Protein (CP) and Lower in Acid Detergent Fiber (ADF)than a normal brome hay produced in similar locations. Although not as high in CP or as low in ADF as a first cutting Alfalfa hay but in some cases it was equal to or higher than alfalfa. The forage quality of cover crops followed the well documented forage nutrient quality pattern of as the plant matures CP and other nutrient quality is reduced. Nitrates for the project followed the same maturity patterns. Nitrates for the trial ranged within the guidelines for generally safe for non-pregnant animals (1,000 to 5,000 ppm NO_3), however if fed to pregnant cattle as hay it is recommended to be blended at least 50:50 with hay that does not contain any nitrate. No soil health measurements or the following wheat yields could be determined in the short window of time however, if managed like a cash crop and if the cover crop is allowed to be harvested at the peak of nutrient quality and yields as would an annual forage, it would appear in areas that had via moisture the targeted cover crop species performed well throughout the state.

Large Termination Cover Crop Project

In the large plot termination trial where alternative economics streams of cover crop usage were evaluated, there are some cover crops when used as either a dry forage (hay) or grazing that have shown a $100/acre return over what a transitional winter or spring wheat/fallow rotation. Uses current wheat, hay and grazing prices. There are also cover crops returning less that the traditional wheat/fallow rotations. It should be noted that the harvest date for the cover crops has average July 10 across the entire trial from 2012 to 2016. By harvesting the cover crops as either a dry forage (hay) or grazing at this time point the deep soil moisture is protected for the following cash crops. No cover crop is allowed to produce viable seed if at all possible, and it allows winter wheat to have a chance to be included in the rotation, since the cover crop is terminated after the grazing or haying to allow for fall planting of winter wheat should that be the desire. Over all the years of the trial there has been timely rains during the wheat years and both above and below in crop normal rainfall. There has not been a devastating drought or a below normal rainfall without timely rains during the wheat years. So no assumption can be made about the overall economic two year rotation should a severe drought occur in this rotation.

Soil bulk densities and water infiltration rates were generally unaffected by long term cover crop inclusion in comparison to traditional wheat fallow rotation. However it should be noted changes in soil parameters and how a soil equilibrates to long term rotations takes substantial time, as an example it took several years to alter organic matter as producers adopted chemical fallow or other conservation tillage practices.

Associated Deliverables

The project team along with several Specialists and County agents are targeting an extension report and webpage for integrating managed grazing of cover crops in Montana diversified livestock and farming operations. The guideline will discuss recommendations for species selection, managing elevated nitrate levels or other potential hazards. Educational opportunities from this project are still on-going and will for an extended period to have locally adapted, tested individual species and polycultures at each Research Center to allow producers to meet their goals for utilizing a cover crop. Whether that be to graze the available forage, or to provide cover rather than fallow. If the goal of the producers is to graze late fall, their recommended cover crop selection would be much different than a July grazing period. The biomass from the cool season cover crops would definitely out yield the warm season species in early to mid-July and produce an outstanding amount of biomass and excellent nutrient profile for the cattle. However letting that cool season crop go to seed and graze after a frost or late fall would only add to the seedbank load although it would be tame seed rather than wild the opportunity for volunteer is large. When in fact a choice of warm season species would increase the amount of available biomass and retain higher amount of forage nutrients than the mature cool season species. To date through location field days and industry tours the MREDI cover crop project personnel have presented data and project details to upwards of 700 industry professionals, NGOs and producers around the state about the termination cover crop trial conducted statewide. The data from the statewide project has been accepted as a presentation for the American Society Agronomy Nation l meeting in Tampa Bay, Florida, October 2017. Montana County Agricultural Agents are participating in an in-service learning opportunity at NARC for selecting, implementing and grazing cover crops in Montana. Their county outreach education programs will impact producer selection and implementation. Seed dealers are implementing and using data to provide appropriate seed polycultures to producers, specifically a simple Montana locally adapted blend of oat, or triticale as the cereal, radish, canola safflower, vetch and a modest amount of millet. This blend being less than $20/acre sold by local triangle area seed dealer. Which allows producers to have a positive experience with locally adapted cover crop blends. The project team from the long term termination trial has been invited to present an invited talk at the national Symposium--Cover Crops and Forage Utilization in Integrated Crop-Livestock Systems at the National American Society of Agronomist in Tampa Florida. Currently new faculty scientists have been able to leverage the current MREDI projects by integrating and expanding mini-rhizotron work to elucidate why of how multispecies polycultures alter root and soil interactions. A diversified group of researchers from MSU are leveraging this long term unique to the western states project to prepare a $2.5 million 5 year proposal to the USDA current Agriculture and Food Research Initiative Competitive Grants Program for Foundational knowledge of agricultural production systems A1102 thereby enhancing the understanding of biotic an abiotic stressors of wheat and their effects on different production systems including cover crops.

Figure 1. Pictures representing the termination techniques but also the alternative economic endpoints when cover crops are used in a winter or spring wheat rotation.

Hiring

- No additional hires in Quarter 7.

Expenditures

- Total Personnel Services: $16,524.24
- Total Operations: $28,821.90

2) 41W227 – Principal Investigator: Emily Glunk; Email: emily.glunk@montana.edu

Progress towards milestones

This research shows promise in utilizing cover crops as a forage source throughout Montana. Several items can be taken away from the data:

Annual forages have the potential to be a strong competitor to perennial forages as a grazing source. As evidenced by the data published in the manuscript "The preference and forage quality of 13 cultivars of forage barley and 2 cultivars of oats when grazed by sheep" (published in the International Journal of Experimental Agriculture 15 (2): 1-7, 2017), many annual forages in use and being developed for livestock grazing have exceptional forage quality and palatability. All forage cultivars sampled in this particular project have the ability to meet or exceed nutrient requirements of livestock at most reproductive stages. This is encouraging for producers, and their ability to include annual forages into their livestock management program.

When evaluating the utility of annual forages compared to commonly-used perennial forages, we see the competitiveness, and similarities between the two. The nutrient quality of most cultivars tested are comparable to good (crude protein between 18-20%, acid detergent fiber between 29-32%, and total digestible nutrients between 52.5-54.5%) or premium (crude protein between 20-22%, acid detergent fiber between 27-29%, and total digestible nutrients between 54.5-55.9%) quality alfalfa (Table 2; USDA, 2016). In fact, if just evaluating the total digestible nutrients and fiber values, as we know alfalfa is a legume and a better source of protein, the cultivars tested would be similar to fiber and energy values of a supreme quality alfalfa, the highest quality of alfalfa available, meaning they are extremely high quality livestock forage sources.

The other benefit of annual forages, making it potentially more competitive to perennial forages, is that we expect higher yields than compared to their perennial counterparts. A study conducted over an 11 year period at Central Ag Research Center (CARC) in Moccasin, MT found that winter cereals produced about 10 T/ ac more than alfalfa grown at the same station compared to alfalfa or intermediate wheatgrass, a commonly-used cool-season perennial grass. At CARC, they were averaging approximately 2.45 T/ ac. The cultivars evaluated in Bozeman were ranging from 3.5- 5.36 T/ ac. Comparing that to average alfalfa production for the state, which is approximately 1.5 T/ ac on dryland and 3.2 T/ ac on irrigated, we see a huge yield boost with the annual forages. When combining the yield advantage with the high nutrient quality, it is evident that these perennial forages show a strong ability to be competitive in Montana as a grazing and preserved forage source.

Table 2. Nutrient quality analysis of the fifteen cultivars of cereal forage cultivars tested

Cultivar	Species	CP	ADF	CF	TDN	NEm	Nitrate
				%			% NO_3
Haybet	Barley	18.7	30.2^{a}	2.6	63.4^{acd}	0.64^{abd}	1.02^{ab}
Hays	Barley	21.4	26.7^{b}	3.5	63.3^{a}	0.64^{a}	1.19^{a}

Haymaker	Barley	20.1	28.0^{ab}	2.5	62.9^{a}	0.64^{ab}	0.98^{ab}
Lavina	Barley	18.7	29.3^{ab}	3.4	64.1^{abc}	0.65^{abde}	0.82^{b}
MT103083	Barley	21.5	29.3^{ab}	2.9	60.5^{cd}	0.61^{cb}	1.11^{ab}
Haxby	Barley	17.8	26.9^{ab}	4.3	64.3^{a}	0.66^{d}	0.89^{b}
Horsford	Barley	16.2	30.4^{a}	2.8	62.1^{abcd}	0.63^{abcde}	0.77^{b}
Pronghorn	Barley	18.5	33.4^{ac}	3.9	63.0^{ac}	0.64^{ab}	0.46^{b}
MT10397-1	Barley	20.0	29.6^{ab}	2.9	62.7^{acd}	0.64^{abcd}	1.19^{ab}
MT103038-6	Barley	20.8	25.9^{b}	2.7	62.8^{ac}	0.64^{abd}	1.23^{ab}
MT103038-4	Barley	19.1	26.5^{ab}	3.2	62.6^{abc}	0.63^{abd}	1.09^{ab}
MT103089-3	Barley	18.6	31.3^{a}	3.1	61.4^{acd}	0.62^{abc}	0.97^{b}
MT103101-5	Barley	17.3	27.8^{abc}	3.0	65.0^{b}	0.66^{e}	0.76^{b}
Otana	Oats	18.5	35.0^{c}	2.7	62.3^{c}	0.63^{b}	2.53^{c}
Stampede	Oats	20.3	27.0^{ab}	3.7	60.3^{d}	0.60^{c}	2.10^{c}

^{a,b} Means without a common superscript within a column differ ($P \leq 0.05$)

CP: crude protein, ADF: acid detergent fiber, CF: crude fat, TDN: total digestible nutrients,
NEm: net energy for maintenance

Annual forages have potential to be an economically viable alternative, particularly in dry years

Based on the information above, we can see the economic implications of producing more tonnage per acre, with that tonnage being high-quality forages. Seeding costs do need to be considered, as seeding will need to occur every year versus every couple of years. However, there are other advantages of using annual forages. One is that they can be very resilient in dry environments compared to perennial cool-season forage species, and often use less water per ton of dry matter produced.

We often observe a "summer slump" in cool-season perennial forages immediately after the summer equinox due to root shedding, as well as high rates of evapotranspiration, and low rates of precipitation. Annual crops may use more available soil moisture during establishment due to their rapid growth, but are typically considered very water efficient later in maturity. Because of this, they are often called "emergency forages" for drought years, because they can grow with relatively low moisture requirement.

Forage quality in species evaluated meets requirements for most livestock in Montana

As outlined above, the forage quality of the cultivars tested meets or exceeds that which is required for most classes of livestock. A cow at her peak requirement, typically early lactation, will usually require a maximum of 13-14% crude protein. All cultivars tested meet and exceed that requirement. Total digestible nutrients, an estimate of energy availability, is also said to be "good" if above 60%, which all cultivars tested in this trial were.

An important part of the quality of harvested or grazed annual forages is the maturity at which those forages are at harvest. The more mature the forage, the less digestible and nutrient dense that forage will be. Based on this research, harvesting within 5-10 days after heading is appropriate for producing a high-yielding, high-quality forage source.

Evaluation of anti-quality components (nitrate levels) is important information for producers, particularly concerning not just acute, but chronic nitrate toxicity.

Anti-quality factors are an important part of forage evaluation. The data is in agreement with previous research, finding that oats typically have the highest risk of nitrate accumulation (Bolan and Kemp, 2003; Crawford, et al., 1961; Gul and Kolp, 1960). All cultivars evaluated were at elevated levels, which may need to be dilute fed to animals that are being used for reproduction, as it may cause abortions and other complications. However, the oat entries were at levels almost double that of the forage barley varieties tested. These should not be fed at all to reproductive livestock, and should be limit-fed to other livestock in order to avoid acute toxicities.

Hiring

- No additional hires in Quarter 7.

Expenditures

- Total Personnel Services: $10,467.40
- Total Operations: $9,094.49

On-Farm Precision Experiment subproject of the Agriculture MREDI Grant

1) 41W215 – Principal Investigator: Bruce Maxwell; Email: bmax@montana.edu

The OFPE team of PIs and key collaborators (farmers and industry representatives) meet every 2 weeks to discuss progress, data management and research approaches. See our website: (https://sites.google.com/site/ofpeframework/) for detailed information about the project.

Progress towards milestones

PI Maxwell and Technician Davis (MSU LRES)

We have made major progress in applying our on-farm experiments for top-dress nitrogen management on 9 new fields bring our total to 17 fields where OFPE is being implemented (Table 1). Analysis of 2016 growing season data continues and is nearly complete. Results suggest that the optimized (profit maximizing) site-specific (variable rate) nitrogen application strategy (VRA) consistently increases net returns over optimized uniform application rates even when a technology cost for VRA is charged. However, the optimized VRA approach does not always decrease the total amount of nitrogen fertilizer applied to the field when compared to the profit maximizing uniform nitrogen rate strategy.

Table 1. New fields brought into the OFPE system in 2017

2017 Fields		
Farmer	**Field Name**	**Area (ac)**
Broyles	Section 35 West	111
	Section 1 East	194
Wood	Carlin West	113
Vandyke	Portnell South 1	59
	Davidson Midwest 2	66
Merja	SRE 9-10	155
	Grams/Moms	318
Bailey	Bailey-1	105
	Bailey-2	98

Hiring

- No additional hires in Quarter 7.

Expenditures

- Total Personnel Services: $180,596.13
- Payroll Encumbrance: $23,763.28
- Total Operations: $178,395.59
- Total Equipment: $90,730.00

2) 41W226 – Principal Investigator: John Sheppard; Email: john.sheppard@coe.montana.edu

Progress towards milestones

Optimization and Precision Experimentation

Prescription Maps

General description before alterations

The prescription software is intended to generate nitrogen fertilization rates for a given field that maximize the information able to be gleaned from a field, while also minimizing stress on fertilization application equipment. Specifically, this software ensures that there is sufficient replication of nitrogen rates in a particular range of yield values. The input data consists of data concerning the specified field and the yield points for that field with their values and location. The field information includes the shape of the field by GPS coordinates, the name of the farmer, and the name of the field. The information used from the yield file is limited to the yield value and its UTM location values; this information is saved into a yield-point data structure.

After reading the CSV files and saving the required information, the field is created from the obtained coordinates, and plots are created within the field based on the desired plot size, which is determined by input from the farmer (based on the size of the equipment). The plots are created based on the largest width and length of the obtained polygon, resulting in a rectangular shape, of the field. This means that some plots are created that fall outside of the field; therefore, the array of plots is iterated over to check if it falls within the field boundaries and plots outside of the field are deleted. Once the final plots have been determined and saved into an array containing the plots as "rectangle" data structures, yield points are added to the plots. The entire array of yield points is iterated over, and for each yield point, it is determined in which plot it is situated. In other words, for each plot the yield points are saved into the "rectangle" data structure, within the array of the field.

For each block within a field, the average yield is calculated based on the yield points within that block. These averages are used to divide the blocks evenly into three categories: high, medium and low yield (where each category has the same number of blocks). However, sometimes there is no yield-data available for certain blocks, rendering them uncategorized, or "uncat." This categorization is implemented to make sure there are enough plots within each category so there is a wide range of different circumstances that produce each yield category.

The actual Nitrogen application rates are chosen by the farmers. The first application rate is chosen randomly from these values and applied to the first block within the field array. The next blocl in the field is assigned the next application rate from the farmer-chosen application values. This continues on until there are no rates left, after which it assigns a zero application rate and starts going back up again. Figure 1 shows an example of a prescription for a particular field.

Figure 1 *Prescription for OFPE N-fertilizer experiment on Broyle's field. Different colors represent different N-fertilizer rates.*

Alterations to the model

Strip

The code described above has been altered to include the possibility to add a strip in the field with a uniform application rate. This addition required alterations to the internal data structures to enable easy assignment of a vertical strip that has more than two blocks. Once the strip is chosen, a uniform rate is applied, which is also chosen by the farmer. An example of a prescription with the added strip can be seen in figure 2. In this example, the uniform strips is the second vertical strip from the right (green for color renderings).

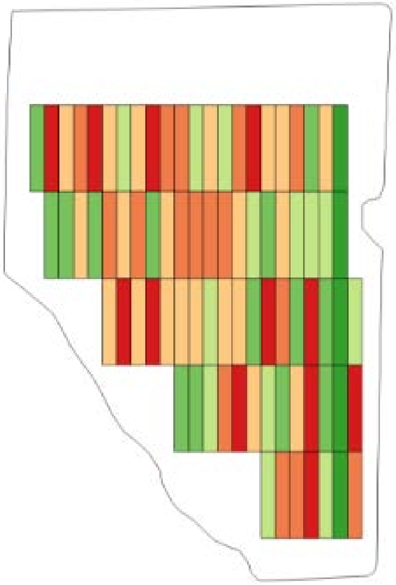


Figure 2 *Prescription for OFPE N-fertilizer experiment on a Broyles' field with strip*

Protein

Aside from yield, protein values can be included as an extra parameter. The calculation of average protein follows the same logic as described above for yield values.

Zero assignment

Some farmers do not want as many zero assignments as the normal prescription software defines, so the option to reduce the number of zero assignments has been added in.

Command line interface

To enable easier usage of the prescription software, a command line interface has been implemented. Furthermore, code commentary has been added to make the software easier to understand, and guidelines were created to explain how to use the command line interface.

Future work

In the future it would be useful to add a Graphical User Interface so the application becomes more user friendly for farmers. In addition, other ways to create fertilization application prescriptions could be added so farmers can choose the way they wish to apply their nitrogen, based on the equipment they have available. For example, instead of jumping back to zero after the highest value has been reached, the prescription rate would lower back down towards zero and then be raised again. Additional future work would also involve incorporating the yield and protein prediction and optimization as to ensure that farmers would have the option to set yield and protein targets for the field, and have the software optimize nitrogen inputs in an attempt to reach those targets.

Optimization

Multi-Layer Perceptrons

A neural network is defined as a parallel, distributed information processing structure. This structure contains processing elements connected to each other with unidirectional signal channels (connections). Each of these processing elements possess local memory and can therefore process local information, all the processing within an element depends solely on the current value of the input signal and on values stored within the local memory. The elements each have a single output (with an output signal of any mathematical type) branching into as many collateral connections as necessary. (Hecht-Nielsen, 1989)

In a feed-forward neural network (also called a multi-layer perceptron) all nodes of the network pass information forward through a sequence of layers until reaching the output layer. The updates to the weights in the interior of the network are defined by the backpropagation algorithm. An example of a feed-forward neural network is shown in Figure 3. Back propagation is one of the most popular techniques for training neural networks. It was introduced by Rumelhart, Hinton, and Williams. A multi-layer perceptron uses the back propagation algorithm where the updates are propagated from the output layer back toward the input layer. The gradient of them mean squared error with respect to the vector of weights in the network is computed, and this gradient is used to determine how to update the weights to minimize error. (Sheppard, 2016)

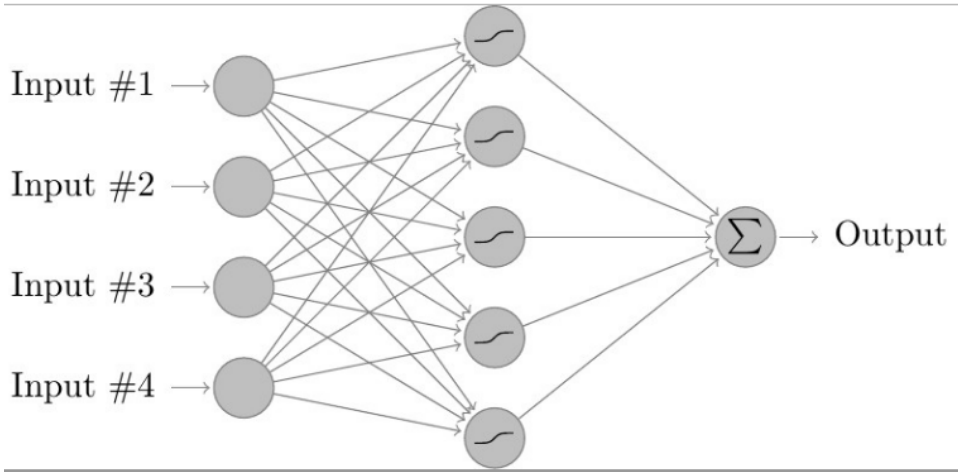


Figure 3 *Feed Forward Neural Network*

Stacked auto encoder

Deep Learning refers to a set of machine learning algorithms that attempt to model high level abstractions in a set of data. Deep Learning is typically implemented in the form of a multi-layer neural network, but it tends to be more involved, adding more layers to derive better abstractions. A traditional neural network can only have about two hidden layers before the training methods start to fail, and as such is limited in the abstractions it can model. A Deep Learning network (usually called a deep network) can have as many layers as we need.

The Deep Learning method we are using is referred to as a stacked auto-encoder. An example of this network is seen in Figure 4. Auto-encoders take in a set of inputs and transform that input, usually reducing dimensions. In the training process, and then attempt to convert the input back to its original form, using the difference between the original and decoded outputs to modify the transformation. After training, when we use the auto-encoder, only the input-to-transformed input part is used to generate a new set of training data. This new data is used to train a new auto-encoder.

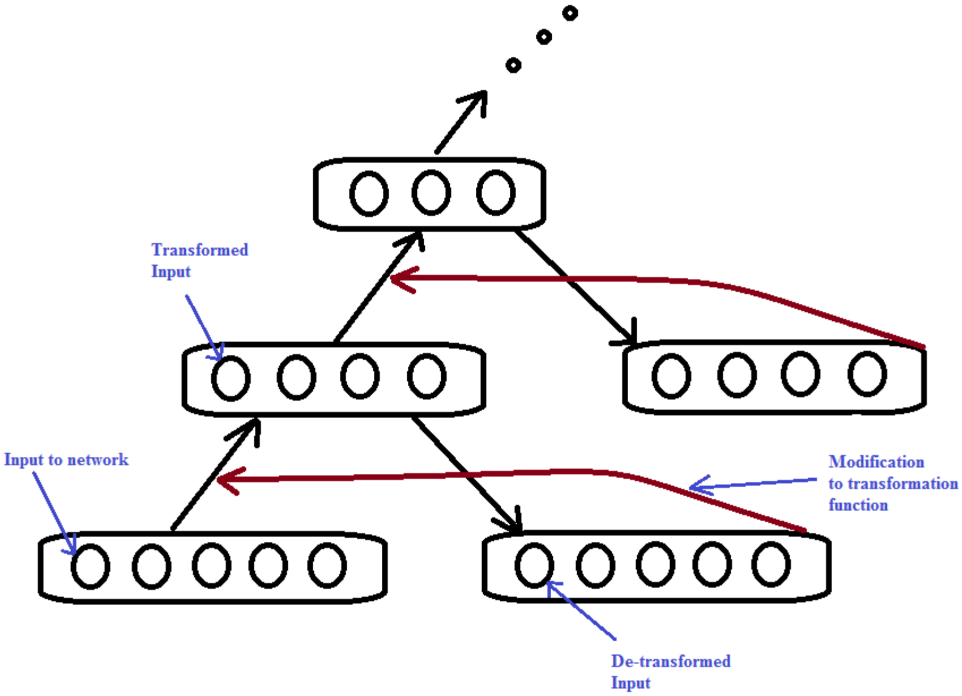


__Figure 4__ Example of a stacked auto-encoder. Inputs feed into a layer, and get transformed as they go into the next layer (also reducing the dimensions). Then, to make sure the transformation works the way we want, we try to reconstruct the original input. The difference between the original and reconstructed input is used to modify the transformation and make it better.

When we stack the auto-encoders, higher auto-encoders in the stack tend to recognize more abstract features. For example, if we have an image that is being fed through the stacked auto-encoders, the first auto-encoder might identify lines in the image. The next auto-encoder might identify shapes and the auto-encoder after that might identify relationships between shapes. This makes stacked auto-encoders a powerful tool for accurate prediction in very complex systems. As we will discuss later, traditional neural networks can predict protein with an accuracy of about 40% in the best case. We hypothesized and found that stacked auto-encoders would provide much higher accuracy.

The Numerical Intelligent Systems Laboratory at MSU developed a stacked auto-encoder implementation for predicting wind-speeds using TensorFlow and Keras. (McAllister, 2017) The TensorFlow section of the implementation is mostly used for parallelizing the network with Graphical Processing Units. The Keras section is used as scaffolding around network creation and training. The remainder of the framework was created by Richard McAllister in the programming language Python. This framework allows for communication between network primitives (e.g. nodes, layers). The framework also uses json files (JavaScript Object Notation) to store and run different network configurations. For this project, the initial implementation was modified for use in a Precision Agriculture setting.

To evaluate the ability for different neural network methods to predict yield and protein, we performed experiments comparing feed-forward networks, stacked auto encoders, and linear regression models. To compare these models, we applied cross-validation and used both means squared error and the Coefficient of Determination (R^2). R^2 represents the amount of variation in the data that can be explained by a particular model. Higher R^2 values correspond to more accurate models. We also used Root Mean Squared Error (RMSE) divided by the range of values for each variable. Higher RMSE values indicate less accurate models.

Through a tuning process, we selected the number of layers and the number of nodes in each layer in both the stacked auto-encoder network and the feed-forward network. The highest performing stacked auto-encoder had 3 layers and 1000 nodes in each layer. The highest performing feed forward network had 2 layers and 10 nodes in each layer. We also evaluated the effect of adding spatial context for each point on accuracy. We did this by finding the eight nearest points to each point. The R^2-values for linear regression, the feed-forward network, the feed-forward network with spatial context, the stacked auto-encoder, and the stacked auto-encoder with spatial context are shown below. The stacked auto-encoder with spatial context had by far the best R^2 for both protein and yield.

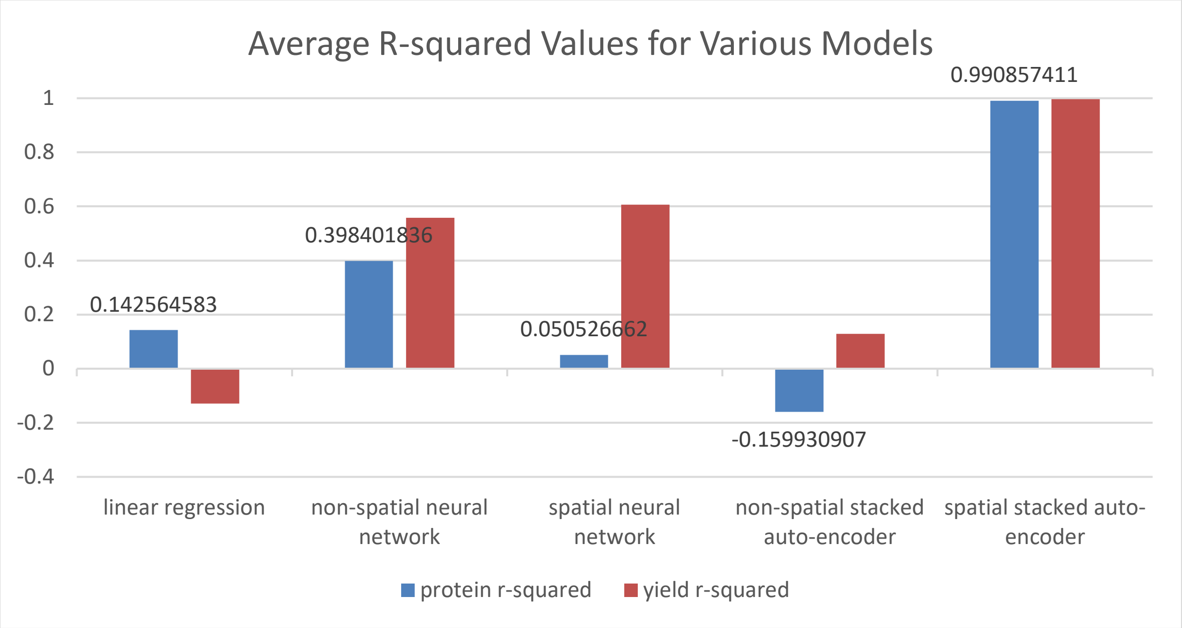


***Figure 4** Average R-squared value for various model*

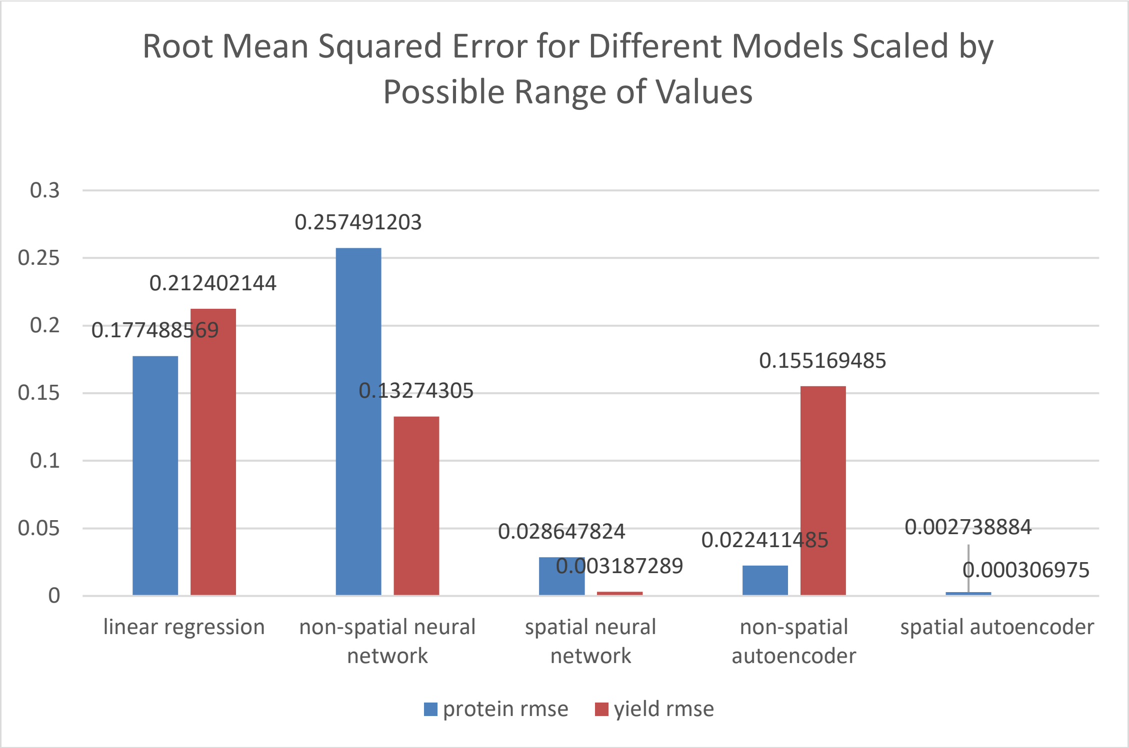


Figure 5: *Root mean squared error for different models scaled by possible values*

Discussion

As shown in figure 4 and figure 5 above, there is a significant improvement using the spatial stacked auto-encoder. The root mean squared error shows the opposite of the R-squared value, instead of assessing how well the model performs, it shows its error rate. To discover what exactly makes this model perform almost 50% better than all other models, especially where protein is concerned, more research would be necessary. A possible explanation is the fact that the code references to less complex iterations of itself multiple times in different ways due to the larger number of hidden layers, thus improving learning and leading to more accurate results. It would be interesting to investigate whether other types of spatial sampling lead to similar results.

The most interesting and confounding part of the results, are the negative R-squared values. Especially in the linear model, the negative value for yield indicates that the yield values are going in a downward trend and that the line drawn throughout these results is worse than the average line. This may indicate problems with the values or with the way the linear regression is performed. There is also a negative R-squared value for protein when applying the non-spatial stacked autoencoder. This result is especially interesting due to the large contrast to the results from the spatial stacked autoencoder. This makes sense considering all the used data is related to specific locations. However, when looking at the difference between spatial and non-spatial shallow neural networks, the logic behind the results concerning the autoencoders does not hold for protein values. More research into these issues is required to discover the underlying cause for these results.

Future Work

There are several methods of including spatial context not described in this work. We would like to evaluate the relative performance of including spatial context. Currently the only type of spatial sampling that has been applied is the K Nearest Neighbour method, in future research we would also explore Equal

Distance and Random Selection as spatial sampling methods. Additionally, multiple linear regression is another method for predicting protein and yield we would like to investigate.

The current stacked autoencoder that is being implemented was designed for a different project, so it may be useful to see whether the autoencoder can be modified to suit this specific project. Furthermore, there are still parts of the code that require more in depth research to fully grasp how it is calculating the results, which could lead to a better understanding and hopefully to improving the model.

Hiring

- No additional hires in Quarter 7.

Expenditures

- Total Personnel Services: $45,922.67
- Payroll Encumbrance: $10,293.34
- Total Operations: $14,792.95

3) 41W228 – Principal Investigator: Clem Izurieta; Email: clem.izurieta@gmail.com
Co-investigator: Rob Payn

Progress towards milestones

Payn and Izurieta are managing the team focused on design and implementation of the data management and workflow technology. The underlying software for data management has been named the Object Oriented Environmental Data System (OOEDS). The system is based on state-of-the-art "NoSQL" database technologies, and will handle transfer and storage of digital information for the data import, model calibration, experimental design, yield optimization, and application prescription phases of OFPE process. There have been no new hires to the team managed by Payn and Izurieta during the past quarter (February-May). The larger team, including Thomas Heetderks, Seth Kurt-Mason, and Michael Trenk continue to meet approximately every other week to track project progress and address the shifting priorities inherent in a research and development project. MSU's "Box" cloud service is being used as a central document repository for the project, and a "Github" service is being employed to provide centralized management of code organization and revision during software development.

The last quarter saw progress on the following activities. In each section, we also include a brief description of what we expect to accomplish by the end of June 2017 (end of funding).

1. OOEDS Data Model

The abstract schema of the data model is complete. We have made minor modifications that allow for a future feature-set that will track data quality, though implementation of this feature set is not planned for this round of funding. This design will be communicated in the manuscript being prepared, as described in part 4 of this report. Resources allocated to data model development are currently more strongly allocated to the manuscript development.

Specific objects in the schema will continue to be added and evolve through the life of the project. The schema is designed to be extensible to allow new data types to be added, as required by new datasets or data relationships used in the agronomic models. We expect continual new requirements will force modifications; however, the modifiability by design will make this work simple.

Activity and completed tasks

- Designed the specific “model” and “activity” objects that are necessary for storing collated data that are spatially rectified with wheat yield or protein models.
- Adapted the schema to include a data quality assurance / quality control measurement context. Data may need to be sorted by various quality types that are tied to the measurement of analysis techniques. More than one context for quality assurance may be necessary, so a given evaluation may be associated with objects in multiple QAQC measurement contexts.

Plans through end of funding

- Design evaluation and model classes necessary for storing fertilizer prescriptions.

2. OOEDS Web Interface

We continue to develop a basic data management interface based an open-standard authentication mechanism (OAuth), using a web development framework (Flask). The interface will provide secure access to the MongoDB database for basic workflow management (Figure 3). The authentication system will be installed on the production server and will be used with MongoDB’s user database system to manage data security. Efforts have been focused on implementing the basic features that will be necessary for future construction more detailed user interface tools to a precision agriculture workflow (Figure 5).

Activity and completed tasks

- OOEDS programming interface and the associated back-end software is complete.
- User interface prototype is complete (Figure 4).

Plans through end of funding

- Workflow tools (described below) will be integrated with user interface as they are completed.

3. Workflow software products:

Over the course of the project, an end-to-end OFPE data acquisition and optimization process has been clearly defined. There are now clear abstractions of individual workflows that must occur in order, regardless of the details of what property of a crop is being optimized or the type of optimization model being used. We summarize the input, activity, and output of each of these processes below, along with reporting progress on each one. Note that the output of a given workflow (with the exception of the prescription workflow) is identical to the input of the next workflow in the overall process (with the exception of the yield editor data input workflow).

a. Yield Editor data input workflow

Input (from farm): Raw data from harvest machinery.
Activity: Processing of raw data using external “Yield Editor” software. This is a complex decision process that primarily verifies that data from the harvester is associated with the appropriate location in the field, based on harvester speed, lag time of grain movement through the harvester, etc.
Output (to OFPE database): Tables of yield data appropriately aligned with field locations for a given harvest of analysis. Note that there is a similar process with different software for protein data.

Activity and completed tasks

- Programmers interface features necessary for the yield editor workflow are complete.
- Tools for loading yield editor workflow output to the database have been updated to use the completed OOEDS programmers interface.

Plans through end of funding

- Integrate yield editor workflow tools with the user interface.
- Load existing yield editor outputs to the database.

b. Data rectification workflow

Input (from database): Tables of yield data appropriately aligned with field locations for a given harvest of analysis.

Input (from other data sources): Data (multiple forms) from the associated field that is expected to be a useful explanatory variable for spatial variation in yield or protein within the field.

Activity: Data for explanatory variables are spatially rectified to the yield or protein data points. This rectification is mainly carried out with GIS software, and is necessary to ensure that later application of optimization models are associating the yield or protein at a given location with the appropriate value of the explanatory variable that is driving that yield or protein level.

Output (to OFPE database): Tables of yield or protein data that are appropriately aligned with the spatially associated value for the explanatory variable. There is one additional column in this table for each explanatory variable.

Activity and completed tasks

- Programmers interface features necessary the data rectification workflow are complete
- Tools for loading output of data rectification workflow to the database are complete

Plans through end of funding

- Implement data query tool for rectified data tables
- Integrate data query tool with the user interface
- Load existing rectified data tables to the database.

c. Optimization workflow

Input (from database): Tables of yield or protein data that are appropriately aligned with the spatially associated value for the explanatory variable. There is one additional column in this table for each explanatory variable.

Activity: A calibrated model is created by optimizing parameters for the accurate prediction of yield/protein for the given year of analysis based on data from the previous year. Then, the calibrated model is used to generate a fertilizer prescription that is optimized for both information from experimental plots and yield/protein from production plots.

Output (to OFPE database): A shape file of varied fertilizer application prescription plots across the field.

Activity and completed tasks

- Programmers interface features necessary for the optimization workflow are complete.

Plans through end of funding

- Implement data query tool for extracting rectified data tables from the database.
- Integrate data query tool with the user interface

d. Prescription workflow

Input (from database): A shape file of varied fertilizer application prescription plots across the field.

Activity: The shape file is input into third-party field equipment manufacturer software, which translates it to the file formats necessary to control the application rates in field machinery.

Output (to application equipment): Files controlling application rates.

Activity and completed tasks

- Database schema design for classes necessary to store shape files is complete

Plans through end of funding

- Design tools for downloading prescription in a format suitable for import to third-party nitrogen application software packages.

4. Manuscript

We are actively working on developing a manuscript for an environmental informatics journal (e.g. Environmental Modeling and Software). The topic of the manuscript will be to introduce an extensive objective oriented data model suitable for storing environmental data in NoSQL (or object-oriented) databases. Rob Payn, Seth Mason and Clem Izurieta are meeting on a bi-weekly basis to develop this manuscript.

Activity and completed tasks

- Outline and initial figures have been developed. Figure 1 is the example of the core abstractions of the data model from the manuscript, and Figure 2 is an example of the OFPE case study data model that is being used to demonstrate the utility of the schema.
- Initial draft is approximately one third complete

Plans through end of funding

- We expect that the first complete draft of the manuscript will be complete by the end of the funding period, with submission to the journal by the end of the summer.

5. Documentation

Plans through end of funding

- The design team will be creating technical documentation for the design and implementation of the programmers interface and user interface. These documents are geared toward providing continuity in training new developers for future system development work.

Figures

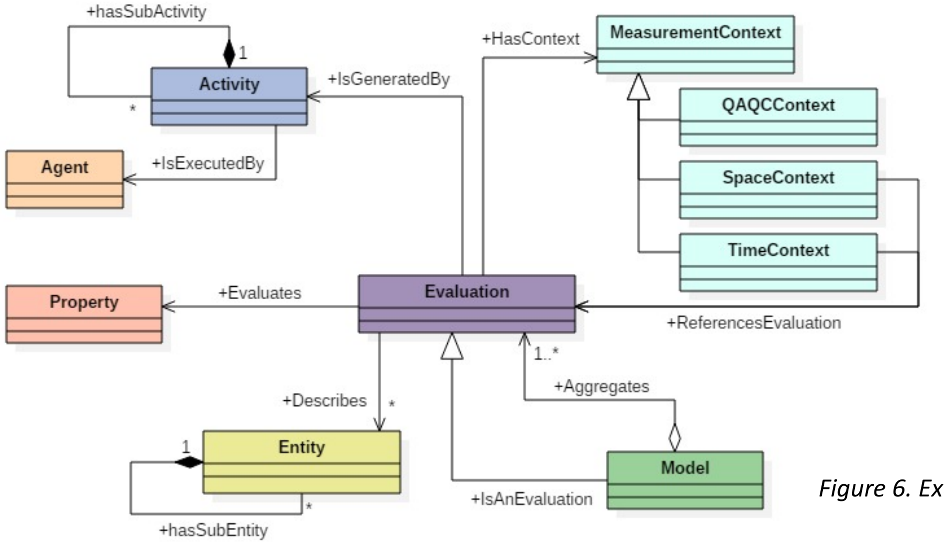


Figure 6. Executive Level Data Model

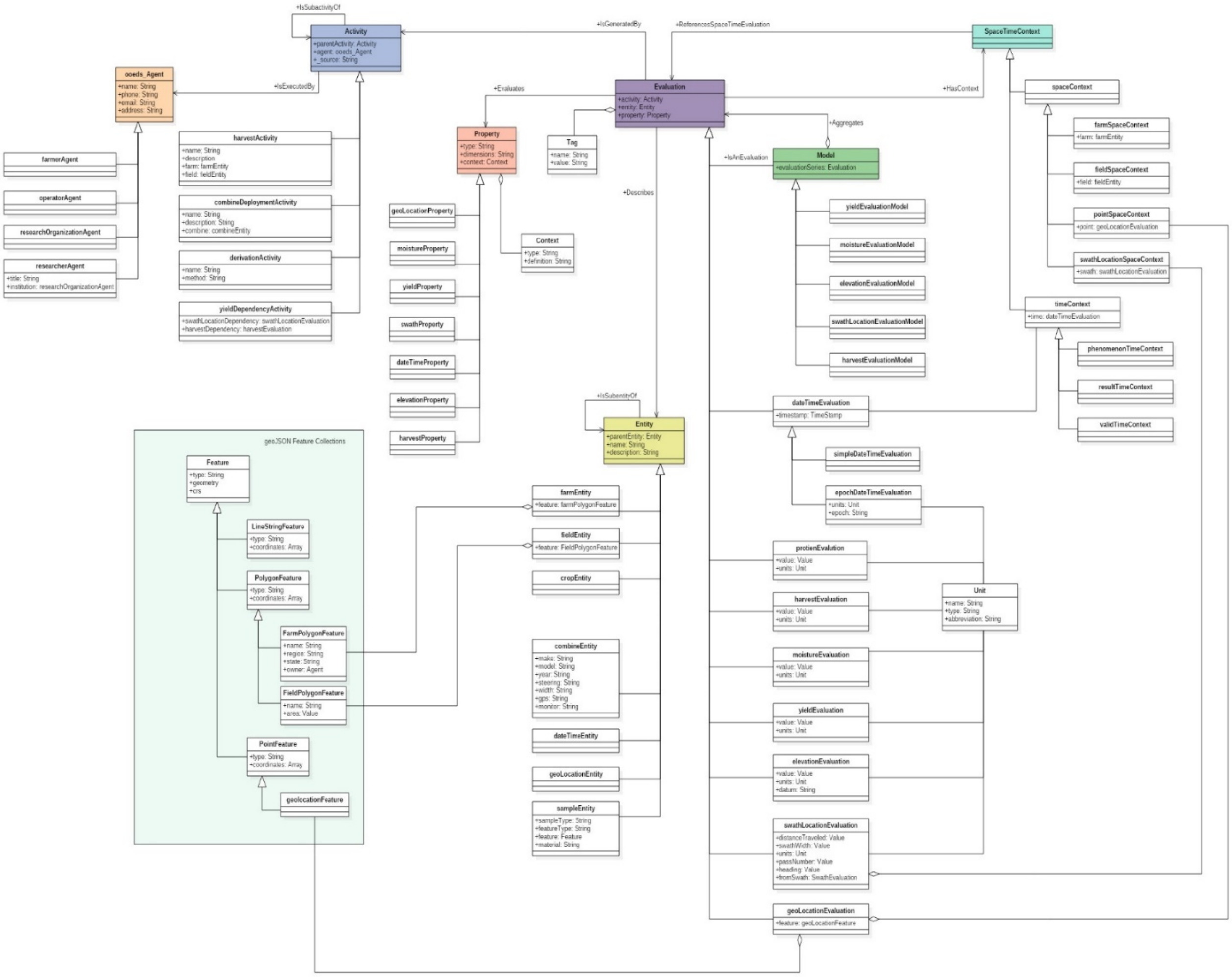


Figure 7. Each component describes an entire subsystem

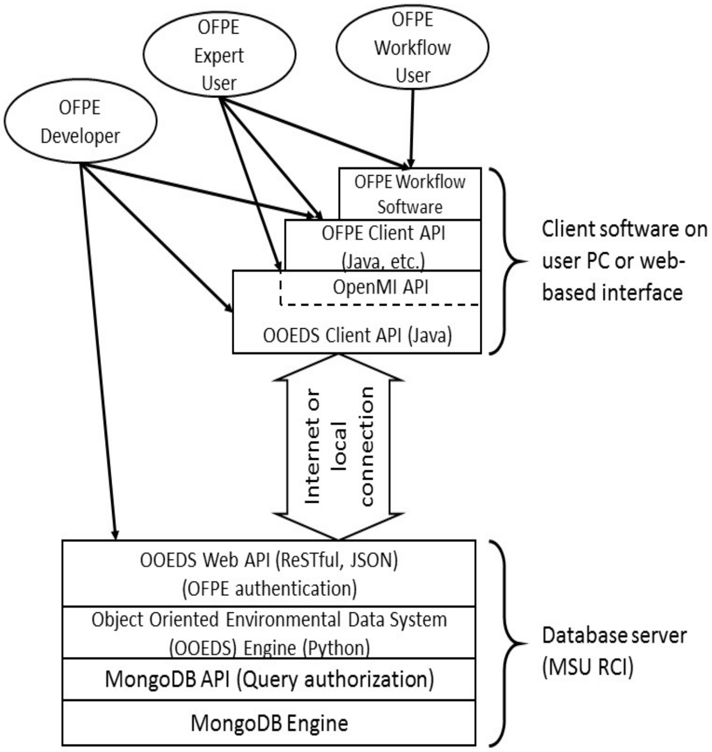
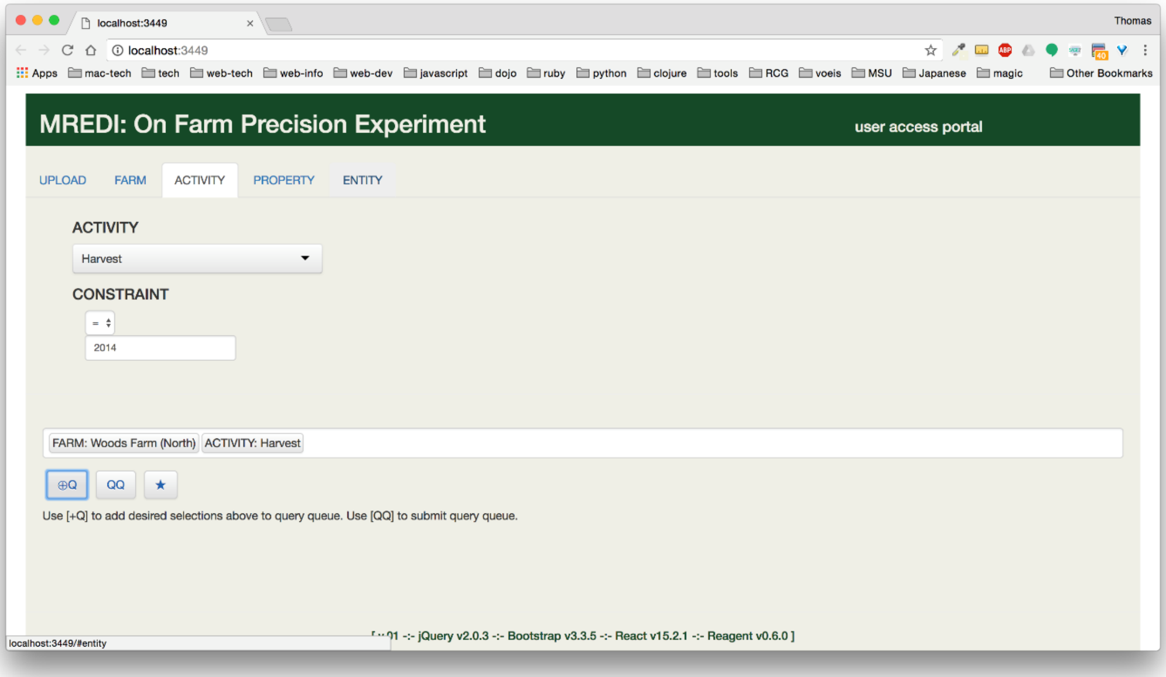


Figure 8. Software Architecture



ACTIVITY

Harvest

CONSTRAINT

=

2014

FARM: Woods Farm (North) ACTIVITY: Harvest

Use [+Q] to add desired selections above to query queue. Use [QQ] to submit query queue.

[·· 01 -:- jQuery v2.0.3 -:- Bootstrap v3.3.5 -:- React v15.2.1 -:- Reagent v0.6.0]

Figure 9. Screenshot from prototype for a basic web interface to the database.

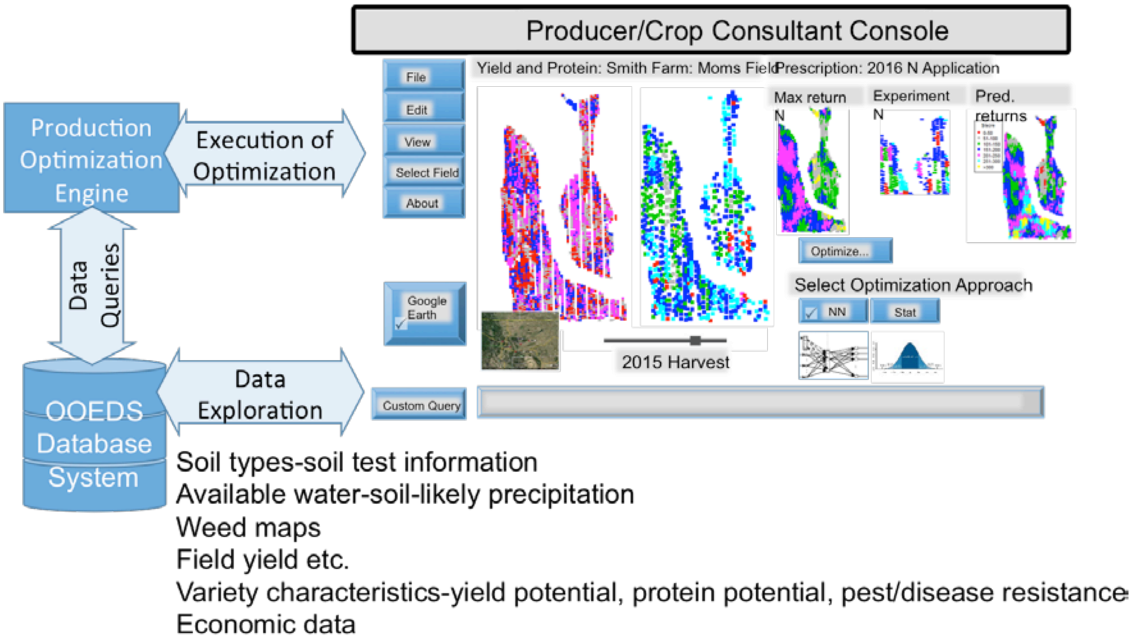


Figure 10. Rough 'mock-up' of the potential user interface for performing optimizations and data explorations for a given field, and the programming interfaces to the lower-level functionality begin developed in this project.

<u>Hiring</u>

- No additional hires in Quarter 7.

<u>Expenditures</u>

- Total Personnel: $123,036.41
- Payroll Encumbrance: $15,412.28
- Total Operations: $23,497.51

4) Industry Match - Dr. Nick Silverman (Adaptive Hydrology) in collaboration with Dr. Kelsey Jencso (UM)

<u>Progress towards milestones</u>

- Adaptive Hydrology has continued to maintain all weather stations at the four participant farms. We are working closely with the Climate Office to install and maintain an additional 5 stations that will support the OFPE project. These stations have been ordered and will be installed by the end of June.
- We have aggregated precipitation and growing degree day data using three separate datasets for each field and have used a quantile mapping algorithm to fill gaps in these datasets. We have used these weather data to help inform an "all farms" model where we have traded time for space to acknowledge water limitations during the growing season. Both non-linear least squares and random forest statistical techniques were used to predict yield.
- We continue to provide technical support to all the participant farmers.
- We have been working with Bruce Maxwell to fine-tune the development of a statistical model for yield and protein in winter wheat; we've developed a random forest model to complement the standard non-linear model.
- We are helping to calculate net economic returns using the non-linear least squares models for each field.
- We are supporting the development of the USDA AFRI-NIFA proposal due in July.
- We have been present and active in all meetings over the last three months.

Hiring

We have continued the employment of Dr. Kevin Hyde (Mesonet technician/coordinator). Dr. Hyde was hired using MCO OFPE funds (December 2016) and will continue to be supported by the BLM and NOAA cooperative agreements.

Durum Quality subproject of the Agriculture MREDI project
41W221 – Principal Investigator: Mike Giroux; Email: mgiroux@montana.edu

Progress towards milestones

Our focus in this quarter was on advancing and creating new breeding populations in both the Bozeman greenhouse and in a winter location in Yuma, AZ and setting up the field trials including the 2017 Montana Statewide Durum Trials. The Montana Statewide Durum Trials relies upon MSU research station cooperators along with Northern Seed (Table 1). We increased the number of entries this year. Whereas there were 15 total entries with 9 named varieties and 6 MT experimental lines in 2015 and 2016, the 2017 trials have 13 named varieties and 12 MT experimental lines (Table 2). The 2017 Statewide Durum Nursery was set up and seed stocks were treated with CruiserMaxx Vibrance (Syngenta). There are three replications of the 15 entries (Table 2) within nine environments (Table 3) along with several additional off station field trials planted by MSU-NARC and MSU-EARC. As in previous years, agronomic data will be collected including but not limited to yield, heading date, plant height, test weight, and grain protein. Grain sub-samples made from a combination of the three replications will be submitted to Linda Dykes (USDA-ARS) for seed and semolina quality analysis. Similar analysis will be performed at the Cereal Quality Lab (MSU-Bozeman).

Table 1. 2017 Statewide Durum Nursery Cooperators

Research Center	Location	Contact	email
MSU-CARC	Moccasin, MT	Patrick Carr	patrick.carr@montana.edu
		Jed Eberly	Jed.eberly@montana.edu
MSU-Bozeman	Bozeman, MT	Mike Giroux	mgiroux@montana.edu
		Andy Hogg	ahogg@montana.edu
MSU-NARC	Havre, MT	Darrin Boss	dboss@montana.edu
		Peggy Lamb	plamb@montana.edu
Northern Seed	Bozeman, MT	Craig Cook	ccook@northernseedmontana.com
		Dale Clark	dclark@northernseedmontana.com
USDA-ARS	Fargo, ND	Linda Dykes	linda.dykes@ars.usda.gov
MSU-WTARC	Conrad, MT	Gadi Reddy	reddy@montana.edu
		John Miller	jhmiller@montana.edu

Table 2. 2017 Statewide Durum Nursery Entries

Line/variety	Source	entry
Mountrail	**NDSU**	**1**
Divide	**NDSU**	**2**
Alkabo	**NDSU**	**3**
Grenora	**NDSU**	**4**
Tioga	**NDSU**	**5**

Carpio	**NDSU**	**6**
Joppa	**NDSU**	**7**
Silver	**MSU**	**8**
Alzada	**WestBred**	**9**
Dynamic	**CDC**	**10**
Fortitude	**CDC**	**11**
Precision	**CDC**	**12**
Vivid	**CDC**	**13**
MT112219	**MSU**	**14**
MTD2016001	**MSU**	**15**
MTD2016002	**MSU**	**16**
MTD2016003	**MSU**	**17**
MTD2016004	**MSU**	**18**
MTD2016005	**MSU**	**19**
MTD2016006	**MSU**	**20**
MTD2016007	**MSU**	**21**
MTD2016008	**MSU**	**22**
MTD2016009	**MSU**	**23**
MTD2016010	**MSU**	**24**
MTD2016011	**MSU**	**25**

Table 3. 2017 Statewide Durum Testing Locations and Environments

Location	Environment	Row Length	Exp #	Rows per Plot	Replicates
Bozeman	Irr	10	D1701	4	3
Bozeman	Dry	10	D1702	4	3
Churchill	Irr	20	D1703	7	3
Havre	Dry	22	D1704	3	3
Sidney	Dry	10	D1705	7	3
Sidney	Irr	10	D1706	7	3
Conrad	Dry	12	D1707	5	3
Conrad	Irr	20	D1708	7	3
Moccasin	Dry	14	D1709	5	3

Durum Breeding Populations

In the most recent quarter, we advanced durum breeding populations in both the greenhouse and in Yuma, AZ in preparation for this summer's field trials. For each of the unique durum crosses seeds were space planted in the field or in the greenhouse and we harvested 250-500 heads for each population. F_3

and F_5 individual plants from each population will be grown in the field in 2017. So far, we have planted more than 600 space planted rows. For both F_3 and F_5 populations we will select plants based on traits such as plant height, head size, agronomic adaptability and maturity date. Lines will then be further screened by seed morphology and seed color.

To continue to create new populations we obtained seed of recent releases from Canada that contain several unique traits. The traits of interest we would like to incorporate into new breeding populations are fusarium resistance, solid stem, low cadmium, and increased straw strength. We have planted the new releases containing these novel traits along with some of our previous best lines for intercrossing in the greenhouse. This will create ~20 new segregating populations that will be in the field in 2018 as F_2 or F_3 plants.

Northern Seed Durum Research Update (Dale Clark and Craig Cook)

The 2016 Durum yield trials focused on diverse germplasm sources mentioned above were harvested and analyzed based on yield, test weight, seed protein %, semolina sedimentation values and semolina color. Based on these evaluations 65 lines were selected for advancement and have been planted in Montana at multiple sites spring of 2017. Heads from these same selections were harvested from the 2016 Bozeman site and were grown in Yuma, AZ to begin the purification process. These purifications were harvested in April and planted in Montana spring of 2017.

Northern Seed has planted yield trials with this material at 5 locations in Montana and one location in Western North Dakota. The sixty-five Montana State Eckhoff lines planted were selected for quality parameters – primarily protein content, bright yellow color and gluten strength and are being compared to Alzada, Joppa and Transcend in this year's yield trials. Purifications that were planted in Yuma were harvested and twelve of them selected for a larger increase at Bozeman. The sixty-five lines will be scored for agronomic characters, disease resistance and yield at the 6 locations and samples will be submitted to quality analysis. Providing one or more of the lines being increased is a candidate for release, Northern Seed will have enough pure seed to plant one to two acres in Yuma this fall. This would give Northern Seed sufficient planting seed in the spring of 2018 to plant 60-120 acres producing Breeder/Foundation seed. Continued yield/quality testing will occur in 2018 and a possible release of Certified seed to the Montana grower would be the spring of 2020.

Hiring

- No additional hires in Quarter 7.

Equipment

- We do not anticipate ordering any additional equipment for this project.

Expenditures

- Total Personnel: $103,333.26
- Payroll Encumbrance: $11,551.56
- Total Operations: $58,463.20
- Total Equipment: $70,994.00

Wheat Stem Sawfly subproject of the Agriculture MREDI project
41W222 – Principal Investigator: David Weaver; Email: weaver@montana.edu

Progress towards milestones
Research Update. Laboratory study of sugar and nectar in the longevity and egg dynamics of the wheat stem sawfly parasitoids.

The two species, *Bracon cephi* (Gahan) and *B. lissogaster* Muesebeck have very different responses to sugar resources that might be provided by existing sugar resources. An important ecological point is that studying the role of flowering crop sugars in these two species has discovered that the biology of the two species is quite different. Before this project the two species were known to be spatially and temporally sympatric using the same host, wheat stem sawfly larvae. This doesn't happen often. Sibling species usually don't compete directly for the same resource using the same biology. The more abundant species, *B. cephi*, uses sugar to be more successful in a different way than the less frequently encountered *B. lissogaster*.

Research Update. Field study of the role of flowering pulse and cover crops in the abundance of wheat stem sawfly parasitoids.

In these field surveys, there were 5 sites with both cover crops next to wheat and adjacent fallow next to wheat. There were 6 sites with both pulse crops (3 pea, 2 lentil, 1 chickpea) next to wheat and adjacent fallow next to wheat. Using field trapping, emergence from residue and postharvest dissection, both parasitoids and wheat stem sawflies were detected in all fields. There were 4 sites with abundant sawflies and 4 with abundant parasitoids. Of these, there was 1 site with abundant parasitoids and few sawflies and another with abundant sawflies and few parasitoids. There were 4 sites that had both abundant parasitoids and abundant sawflies. When trapping sawflies in these fields, there was more sawflies and parasitoids caught at the wheat - flowering crop interface or there were no differences between the wheat- flower and wheat – fallow interface. Dissection of stems for a more accurate measure indicated that significantly more parasitoids were present in fallow next to flower source at 3 sites, with no others being different. Allowing insects to emerge from a bulk of overwinter residue indicated greater numbers of sawflies or parasitoids in wheat next to flowers than in wheat next to fallow. One of these fields had more parasitoids next to fallow and more sawflies next to flowers, which was not expected.

1. LABORATORY SUGAR

Longevity

Material and Methods. Here, we tested the effect of adult sugar feeding on the longevity of *Bracon cephi* (Gahan) and *B. lissogaster* Muesebeck (Hymenoptera: Braconidae), both native, idiobiont and host specific larval parasitoids of the wheat stem sawfly (WSS), *Cephus cinctus* Norton (Hymenoptera: Cephidae). Both generations of parasitoid females were reared on diets of sucrose or water only, and daily evaluations were made to check for mortality. Additionally, a preliminary experiment testing the effect of honey feeding on the longevity of the WSS was made to guarantee that carbohydrate-rich diets do not affect survival of females.

Results. Our results generally showed a significant increase in longevity when sucrose was offered to both parasitoid species and generations (Figure 1). Specifically, first generation females of *B. cephi* lived for a mean ± SE of 11.4 ± 0.7 days with water, and 30 ± 2.5 days when fed with sucrose. Second generation females of *B. cephi* lived for 8.2 ± 2.3 days with water only and 29.2 ± 3.3 days with sucrose. For *B. lissogaster*, first-generation females lived for 8 ± 0.7 days with water and 54.9 ± 8.6 days with sucrose. While second-generation females of *B. lissogaster* lived for 3 ± 0.7 days with water and 46.7 ± 7.3 days with sucrose. Our results also showed significant difference in longevity between generations for both parasitoid species, when females were fed with water. These results suggest that first-generation females could be using more of their metabolic reserves to sustain longevity, compared to second-generation females when they were fed with water only. However, after feeding on sugar, these differences are not significant anymore. Finally, results showed no significant difference in longevity for WSS females treated with water or honey.

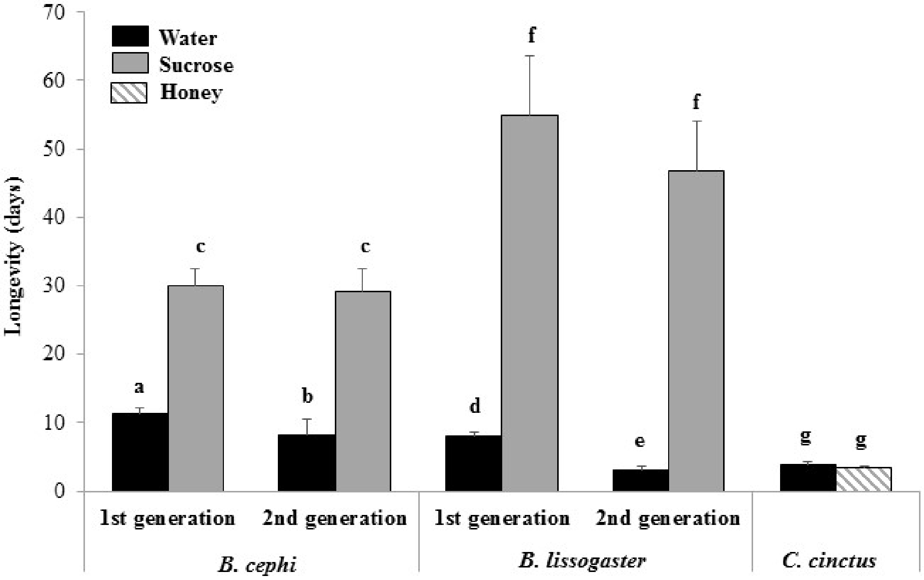


Figure 1. *Average longevity of both generations of B. cephi and B. lissogaster females in response to water and sucrose treatments. Average longevity of C. cinctus females in water and honey diets.*

Egg Load and Egg Volume

Material and Methods. In this experiment we tested the effect of sugar feeding on the egg load and egg volume of both generations of *B. cephi* and *B. lissogaster*. In this case, "egg load" means the number of matured eggs carried by a female parasitoid at a given moment in her lifetime. We determined the egg load and egg volume of parasitoid females at 3 different ages: 2, 6 and 10 days-old. Females were reared on diets of sucrose and water only. To count the number of matured eggs females were dissected under ethanol using a stereomicroscope. Length and diameter of each matured egg was measured, and the egg volume was estimated as $V = (\pi r^2)(L-2r) + (4/3)(\pi r^3)$, where L = length, D= diameter and $r=D/2$ (O'Neill et al. 2014).

Results. Our results show a great contrast in egg load and egg volume between the two native parasitoids of wheat stem sawfly. Females of the more abundant species, *B. cephi*, had less mature eggs in the ovaries at ages 2, 6 and 10 days-old, compared to *B. lissogaster* species (Figure 2). In contrast, egg volume of *B. cephi* females is greater than the egg volume of *B. lissogaster* females (Figure 3). Results also showed that water-fed females of first-generation of *B. cephi* and *B. lissogaster*, as well as second-generation of *B. lissogaster* resorb eggs as they age. Female parasitoids usually do that to reallocate energy resources from egg production to longevity. Sugar feeding affected species and generations differently. First-generation females of *B. cephi* significantly increased egg load and egg volume when fed with sucrose, compared to females that were fed with water only. Second-generation females of *B. cephi* also significantly increased egg load when fed with sucrose, but egg volume did not change. Likewise, first-generation females of *B. lissogaster* increased egg load, but egg volume was the same after feeding on sugar. Finally, for second-generation females of *B. lissogaster* egg load and egg volume did not change when sucrose was provided. These results may provide some insight about the difference in reproductive strategies of *B. cephi* and *B. lissogaster*. Egg size is often correlated with offspring success, thus abundance of *B. cephi* in the field could be a result of a greater investment of females in egg volume instead of egg load. In contrast, *B. lissogaster*, which is also known as a gregarious species, produced smaller, but more numerous eggs. In this case, "gregarious" means that females can lay more than one egg per host. With this strategy, the smaller the eggs, the more change they have to successfully develop in a single host. Therefore, sucrose feeding could potentially enhance reproductive success of *B. cephi* females by increasing egg volume, and for *B. lissogaster* females, by increasing egg load.

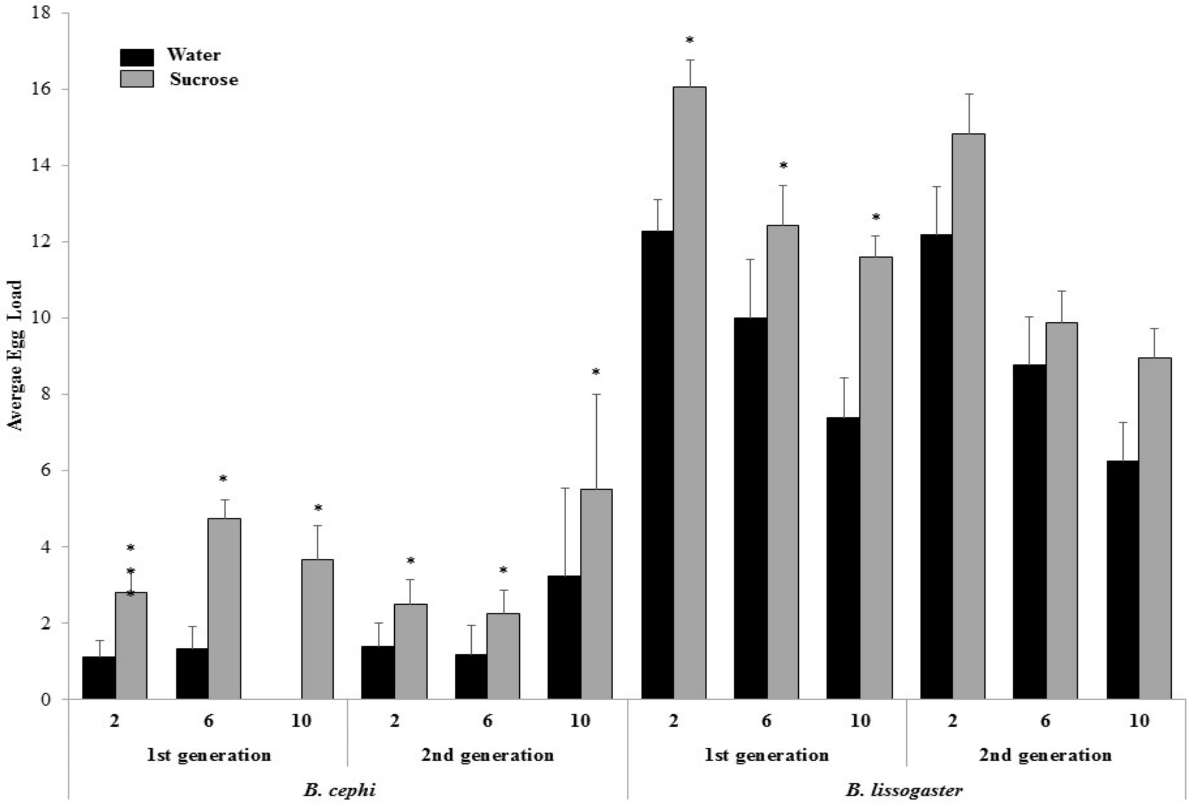


Figure 2. *Average egg load of both generations of B. cephi and B. lissogaster in response to water and sucrose treatments.*

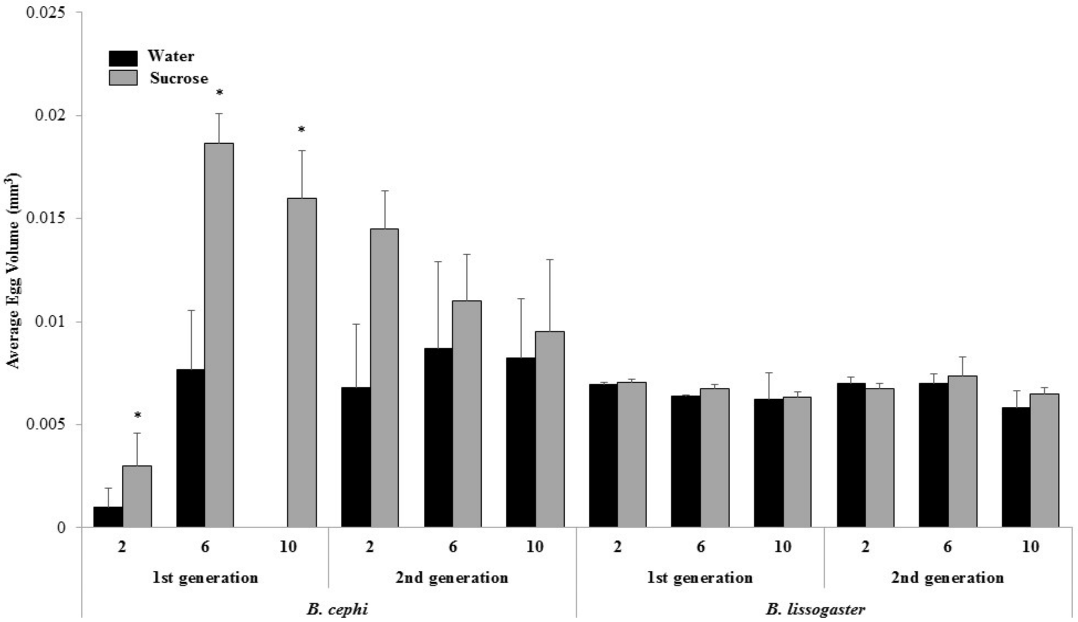


Figure 3. *Average egg volume of both generations of B. cephi and B. lissogaster in response to water and sucrose treatments.*

Greenhouse Experiment

Material and Methods. At the greenhouse we tested the effect of flower resources in the longevity, egg load and egg volume of first-generation females of *B. cephi* and *B. lissogaster*. The flowering plants tested in this experiment were: wheat, alfalfa, flax, buckwheat and peas. Water was offered as a control. Newly emerged females were individually released in cages made of plastic cups, with a recently bloomed flower (Figure 4). Parasitoids were checked daily for mortality. At death, parasitoid females were dissected to count the egg load and estimate the egg volume.

Results. Here, our results showed that only buckwheat increased longevity of *B. cephi* (Figure 5-A,B) and *B. lissogaster* (Figure 6-A,B). These results suggest that both species of parasitoids were able to access and consume sugar sources from buckwheat flowers. In contrast, no difference was found between longevity of females of both species treated with control, alfalfa, flax, peas and wheat. No differences in egg load were observed for *B. cephi* females among treatments (Figure 5-C). Because any eggs were found in the ovaries of *B. cephi* females treated with wheat and alfalfa, the egg volume for these two treatments was also equal to zero. Disregarding these two treatments, no difference in egg volume was found in *B. cephi* (Figure 5-D). Egg load of B*. lissogaster* females decreased with buckwheat (Figure 6-C). However, it was previously shown that this species resorb eggs as they age. This could explain why egg load was significantly lower for females that significantly increased longevity in the buckwheat treatment. No difference in egg volume was found in this species (Figure 6-D).

Figure 4. Pictures illustrating the cages, made of plastic cups used to determine longevity of WSS parasitoids in response to flower resources. Females of B. cephi and B. lissogaster were individually placed inside the cages with a recently bloomed flower.

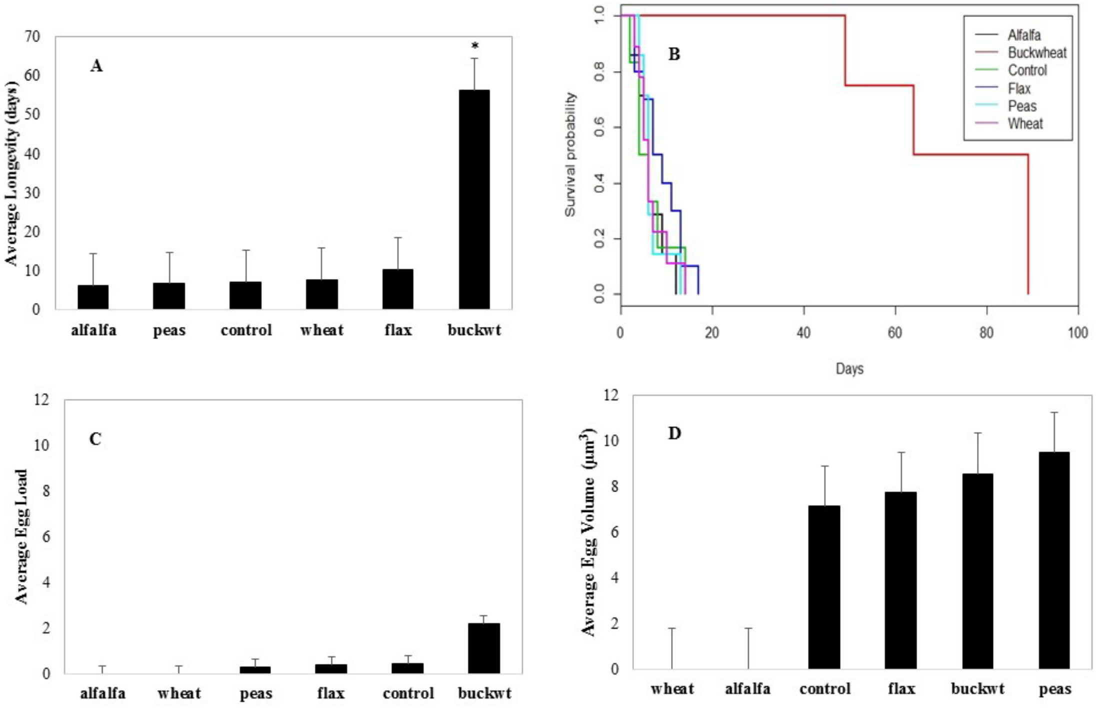


Figure 5. *Comparisons of average values of (A) (B) longevity, (C) egg load and (D) egg volume of B. cephi females in response to the treatments (control, alfalfa, buckwheat, flax, peas and wheat).*

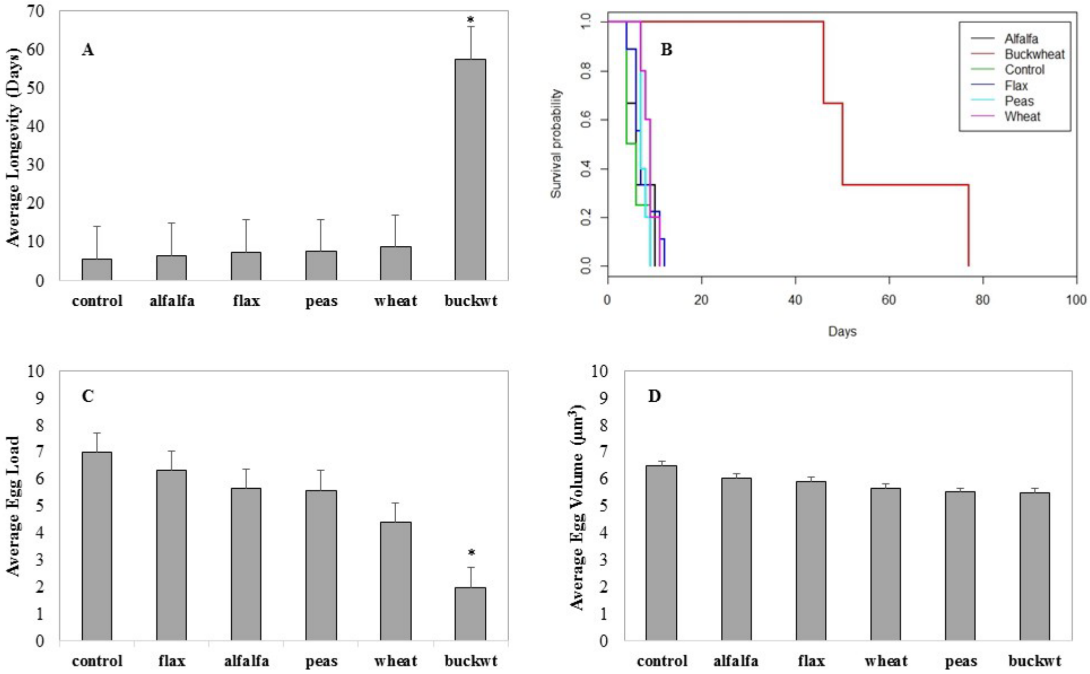


Figure 6. *Comparisons of average values of (A) (B) longevity, (C) egg load and (D) egg volume of B. lissogaster females in response to the treatments (control, alfalfa, buckwheat, flax, peas and wheat).*

2. FIELD SURVEYS

Materials and Methods. Twelve research sites with wheat growing next to pulse crops or cover crops, as well as wheat growing next to fallow fields were selected for this study (Figure 1). Emergence barrels, stem dissection and trapping were all utilized to compare the health and size of the parasitoid populations in the fallow-adjacent wheat compared to flower-adjacent wheat. Emergence barrels and trapping were used to monitor adult populations of sawfly and parasitoids.

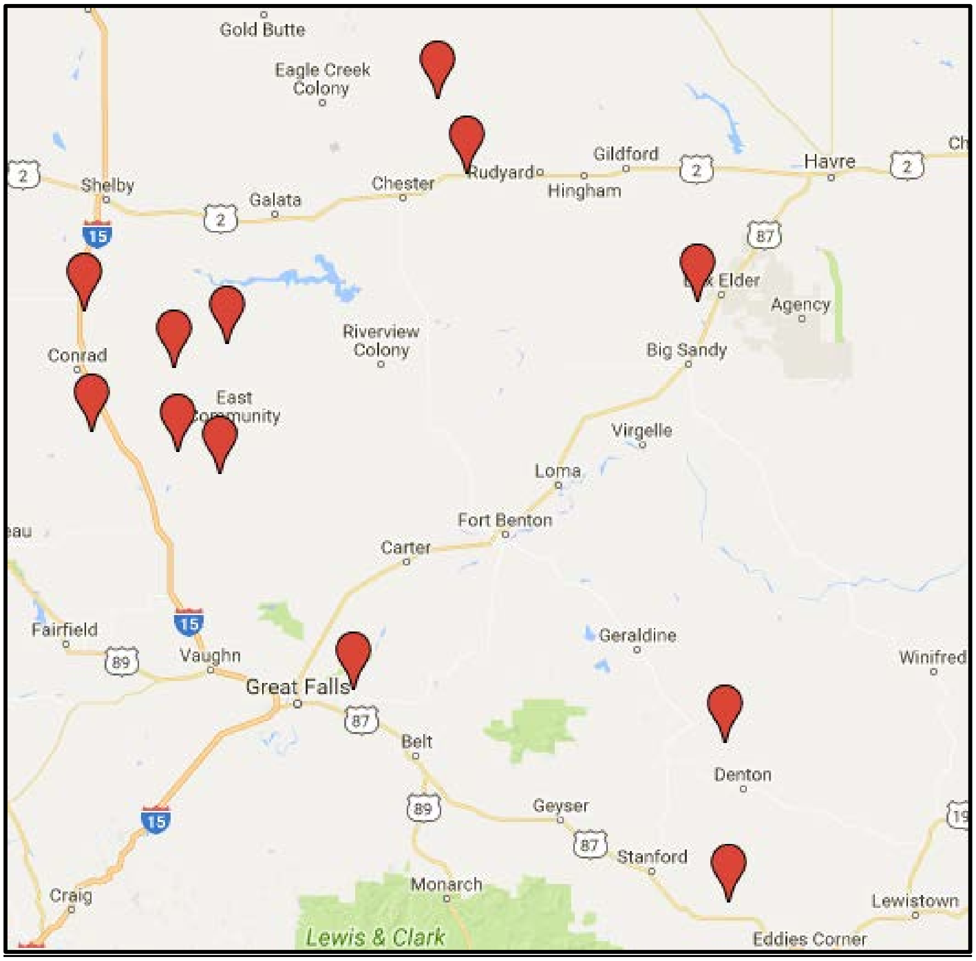


***Figure 1**. Locations of 12 sites where adjacent field comparisons of overwintering wheat stem sawfly and braconid parasitoid numbers were collected. Each site had a flowering field (pulse or cover crop) adjacent to a field of growing wheat and a fallow wheat field adjacent to a field of growing wheat.*

Emergence Barrels

Materials and Methods. Crop residue was placed inside of barrel liners which had a black contractor bag around it to block light. Lids were made by gluing plastic jars to poster board. Barrels were left in the light from the 29th of May to the 9th of July, and insects were collected daily to be weighed and measured. Pre-emergence stubble samples were collected as the plant material used in emergence barrels. Postharvest stubble samples were collected to process stems. There were 7.5 m of stubble were collected for each comparison. Comparisons between numbers emerged were made using a Chi-square or Fisher's exact test. Trap captures were compared using *t* - tests.

Results. There were 12 fields from 2015 that were collected for initial counts of parasitoids (Table 1) and wheat stem sawflies (Table 2) in emergence barrels. At Site 1 there were a significantly greater number of parasitoids in wheat adjacent to fallow, but a significantly greater number of wheat stem sawflies next to the cover crop. At Site 3 there were a small number of parasitoids next to the cover crop only and there were no wheat stem sawflies in either field. At Site 7 there were only 3 parasitoids and these were adjacent to the cover crop, but there were a significantly greater number of wheat stem sawflies next to the cover crop and none next to fallow. At Site 8 there were a significantly greater number of wheat stem sawflies and parasitoids next to the cover crop. At Site 9 the numbers of parasitoids adjacent to the two fields were not different but there were a greater number of wheat stem sawflies adjacent to fallow. Finally, at Site 10 there were a significantly greater number of parasitoids adjacent to chickpea and the number of wheat stem sawflies was not different between the two fields.

Table 1. *The number of parasitoids that emerged from 2015 wheat residue collected adjacent to cover crop or pulse field and the number that emerged from wheat residue collected adjacent to a matched 2015 fallow field.*

	SAWFLIES						
Site	Comparison	Fallow	Flowers	Expected Fa	Expected FL	Value	*P*
1	Cover Crop	15	**35**	25	25	8	**0.0047**
2	Peas	0	0	n/a	n/a	n/a	n/a
3	Cover Crop	0	0	n/a	n/a	n/a	n/a
4	Peas	1	0	0.5	0.5	1	0.3173
5	Cover Crop	1	0	0.5	0.5	1	0.3173
6	Peas	0	0	n/a	n/a	n/a	n/a
7	Cover Crop	0	**40**	20	20	40	**<0.0001**
8	Cover Crop	30	**88**	59	59	28.508	**<0.0001**
9	Cover Crop	**27**	2	14.5	14.5	21.552	**<0.0001**
10	Chickpea	12	13	12.5	12.5	0.04	0.8145
11	Peas	1	0	0.5	0.5	1	0.3173
12	Peas	0	5	2.5	2.5	5	0.21> X >0.091

Table 2. *The number of wheat stem sawflies that emerged from 2015 wheat residue collected adjacent to cover crop or pulse field and the number that emerged from wheat residue collected adjacent to a matched 2015 fallow field.*

	PARASITOIDS						
Site	Comparison	Fallow	Flowers	Expected Fa	Expected FL	Value	*P*
1	Cover Crop	**32**	9	20.5	20.5	12.902	**0.0003**
2	Peas	0	6	3	3	6	0.091
3	Cover Crop	0	**7**	3.5	3.5	7	**0.091> X >0.038**
4	Peas	0	1	0.5	0.5	1	0.3173
5	Cover Crop	2	1	1.5	1.5	0.333	0.5637
6	Peas	2	1	1.5	1.5	0.333	0.5637
7	Cover Crop	0	3	1.5	1.5	3	0.0833
8	Cover Crop	5	**75**	40	40	61.25	**<0.0001**
9	Cover Crop	20	12	16	16	2	0.225
10	Chickpea	2	**22**	12	12	16.667	**<0.0001**
11	Peas	6	2	4	4	2	0.304
12	Peas	5	2	3.5	3.5	1.286	0.500> X >0.304

Trapping at Field Interface

Materials and Methods. Traps were deployed at the beginning of wheat stem sawfly flight period and removed just prior to harvest. Rebel Yellow sticky traps from Great Lakes IPM were used for trapping. Counts of trapped wheat stem sawfly, *Bracon cephi*, and *B. lissogaster* were made weekly. Observations began on June 20th, 2016, and continued until the wheat was harvested. The last observation was made August 4th, 2016.

Results. Monitoring in the growing 2016 crop indicated that there were 2 fields with a greater number of wheat stem sawflies at the wheat - cover crop interface and a field with a greater number of wheat stem sawflies at the wheat - chickpea interface. There was one field that had greater capture of parasitoids at the cover crop - wheat interface, while the number of wheat stem sawflies was not different between the cover crop - wheat interface and the fallow interface (Table 3).

Table 3. *The number of wheat stem sawflies and parasitoids trapped at cover crop or chickpea – wheat interface during the 2016 growing season.*

	SAWFLIES						
Site	Comparison	Fallow	S.E.	Flower	S.E.	*t* value	*P*
A	Cover Crop	7.0	± 0.9	**17.2**	± 2.4	4.1	**0.002**
B	Cover Crop	3.3	± 1.0	35.3	± 15.2	2.1	0.065
C	Cover Crop	7.2	± 1.4	**32.9**	± 7.3	3.5	**0.006**
D	Chickpea	33.3	± 4.4	**48.3**	± 3.6	2.6	**0.023**

	PARASITOIDS						
Site	Comparison	Fallow	S.E.	Flower	S.E.	*t* value	*P*
B	Cover Crop	0.7	± 0.3	**12.3**	± 5.0	2.3	**0.002**

Hiring

- No additional hires in Quarter 7.

Expenditures

- Total Personnel: $32,149.57
- Payroll Encumbrance: $7,563.17
- Total Operations: None to date

Weed Imaging/Pulse Crop Herbicide subproject of the Agriculture MREDI project

1) 41W217 – Principal Investigator: Prashant Jha; Email: pjha@montana.edu
PULSE CROP HERBICIDE EVALUATION

Progress towards milestones

1. ***Weed Control in Pulse Crops***
During this grant period, we focused on dissemination of results obtained from this research to Montana clientele. Findings and recommendations were presented during MSU Research Center Field Days, MABA, and winter grower meetings across Montana. The findings of this research will be available to the growers through extension publications as "*Montana Guide*" and "*MSU Research Bulletin*". Based on the results obtained from this project, we were able to initiate collaborative work with chemical industry and Montana Pulse Commodity group for prioritizing pulse weed control research and registration of new herbicides and optimizing application timing for weed control in pulse crops (pea, lentil, and chickpea). This research will significantly contribute to increased adoption and sustainable production of pulse crops in Montana.

2. ***Light Activated Sensor Controlled (LASC) WeedSeeker Sprayer for Precision Weed Control***
During this grant period, we built a tractor-mounted 30-feet spot sprayer fitted with 30 WeedSeeker units. This technology will be tested during summer of 2017 in grower fields across Montana. The precision sensor units are fitted with TeeJet 6502 flat-fan nozzles spaced 12" apart, calibrated to deliver 20 gal/acre of herbicide spray solution. A pull-type sprayer will be used with a 300 gallon tank. The LASC spot sprayer will be compared for weed control with a conventional broadcast sprayer calibrated to deliver the same volume of herbicide spray mixture.

Figure 1: WeedSeeker sprayer with 30 LASC units for precision (site-specific spot spray) weed control in chemical fallow (2016).

Based on our field evaluations (2015-2017) of LASC technology in no-till fallow and post-harvest wheat stubble, weed control efficacy with LASC sprayer was consistent with the conventional broadcast sprayer. LASC sprayer reduced the herbicide (plus adjuvant) usage by 55 to 70% of the amounts (depending on weed density) used with a conventional broadcast sprayer. The herbicide savings were mainly due to savings in the spray volume using LASC sprayer vs. broadcast application. Based on results from the field research in 2015-2016, use of LASC sprayer reduced herbicide costs per acre by up to 70% compared with the conventional broadcast application for the herbicide programs tested in chemical-fallow/wheat stubble. This technology has proven accuracy in weed detection/sensitivity and spot spray (weed heights from 1 to 8 inches) at operating speeds of 10-12 mph.

I continue to collaborate with Dr. Joe Shaw (MSU Optics, Department of Electrical Engineering) on the hyperspectral imaging (MREDI subproject) to detect herbicide-resistant weeds in-crop (report presented by Dr. Shaw). The initial milestones achieved from that project will be used to leverage more grant funding to further expand this technology for use in UAVs designed with hyperspectral camera to detect and develop herbicide-resistant weed maps in crop fields for site-specific weed management and herbicide resistance mitigation.

Educational Activities

- Presentation on hyperspectral imaging to detect herbicide-resistant weeds in-crop. Weed Science Society of America Annual Meeting, February 6-9, 2017.
- Presentation on advanced optical sensor-based hyperspectral imaging and spot spray technologies for precision weed control. Malt Barley and Sugar beet Symposium, Billings, MT, January 10-11, 2017.

- Presentation on mitigating herbicide carryover and introducing new weed control options in pulse crops in Montana. CHS Grower Meeting, Malta, MT, January 9, 2017.
- Presentation on precision weed control technologies in Montana agriculture. MSU-Extension Crop and Pest Management Convention, Bozeman, MT, January 3, 2017.
- Presentation and demonstration on precision weed control technologies, MSU-SARC, Field Day, Huntley, MT, June 28, 2016.
- Presentation on weed control options for herbicide resistance management in pulse crops in eastern MT, MSU Eastern Agricultural Research Center Field Day, Sidney MT, June 24, 2016.
- Presentation on fall-applied soil residual herbicides in wheat stubble and rotational crop safety and weed control in pulse crops, Northern Agricultural Research Center Field Day DRC-NARC, Havre, MT, June 22, 2016.
- Presentation on management of glyphosate-resistant weeds in wheat-pulse rotation, Divide County Crop Improvement Meeting, Crosby, ND, December, 2016.

Media Contribution

Precision agriculture and site-specific weed management using optical sensors and hyperspectral imaging. Montana Ag Live– Broadcasted by Montana PBS Live TV Show (1 hour). October 16, 2016.

Hiring

The following people continue to work on this project:

- Dr. Vipan Kumar, Postdoctoral Research Associate
- Mr. Shane Leland, Research Technician at SARC, Huntley
- Mr. Charlemange A. Lim, PhD student

Equipment

- A growth chamber ($15,000) has been purchased for performing plant and soil bioassays for rapid detection and mitigation of potential herbicide carry-over concerns in Montana wheat-pulse rotation.

Expenditures

- Total Personnel: $36,950.78
- Total Operations: $4,859.60
- Total Equipment: $15,000.00

2) 41W216 – Principal Investigator: Joseph Shaw; Email: jshaw@montana.edu
PRECISION WEED CONTROL USING ADVANCED OPTICS AND SENSOR-BASED TECHNOLOGIES

Progress towards milestones

During this quarter we made significant progress toward milestones #4 and #5, having to do with completing the research demonstration and commercializing optical imaging methods for the discrimination of herbicide-resistant weeds. A proposal for follow-on research and development was submitted to the USDA in collaboration with Dr. Prashant Jha at the Southern Agricultural Research Center. This proposal is to explore the variability of spectral signatures for herbicide-resistant and herbicide-susceptible weeds, as this variability will help define how broadly and robustly our newly developed methods will work. The commercialization milestone was worked on by our subcontracted company partner, NWB Sensors, Inc. Their report is incorporated below.

Hyperspectral weed imaging

During this quarter we continued to explore broader use of hyperspectral imaging methods for identifying herbicide-resistant weeds. Our previous work has resulted in methods that worked well in controlled lighting conditions, but now we are pushing deeper into the complex reality of partly shaded scenes that result with inter-mixed weeds and crops in direct sunlight. These conditions continue to be a challenge, but we are understanding more about these complexities so that we can better design future experiments in the quest for more robust sensing capabilities. Our focus in this phase of the study is on different ways of inducing more separability in the training data. As noted in previous reports, a large obstacle in generating a general algorithm to discriminate between susceptible and herbicide-resistant Kochia strains in hyperspectral data is that the standard deviations of the spectra from the different weed strains and from the crops themselves overlap over large parts of the spectral range (see Figure 1). The result is that significant numbers of spectra are still being misclassified.

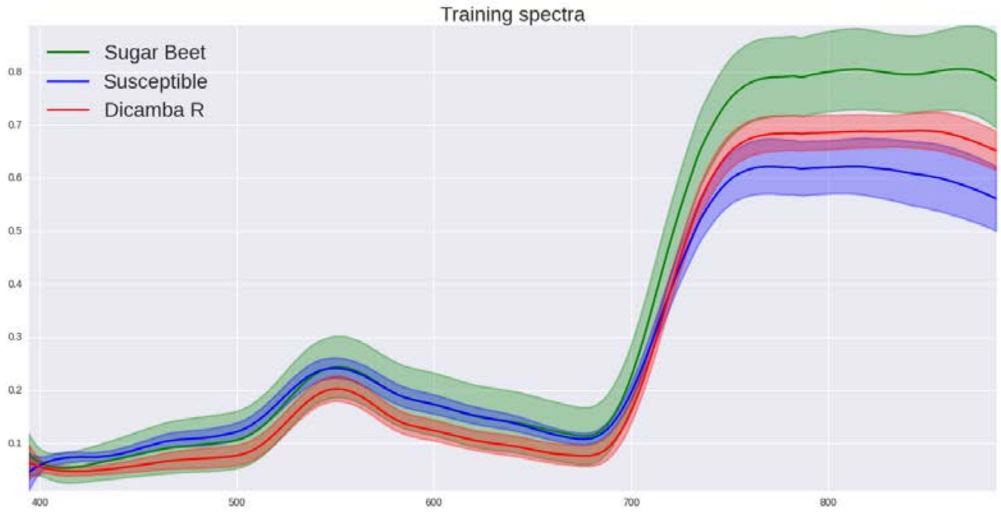


Figure 1. Means and standard deviations of the sugar beet, susceptible Kochia and dicamba-resistant Kochia. In this form, the data are only partially separable.

We attempted to increase the separability of the data by substracting the median vegetation spectrum of the entire data set from each training spectrum; however, this actually decreased the data separability (see Figure 2). This approach was inspired by our previous use of a median vegetation spectrum, but was different in that the data currently being used have been calibrated from digital number to reflectance using a reflectance panel. In the previous work, the spectra were uncalibrated.

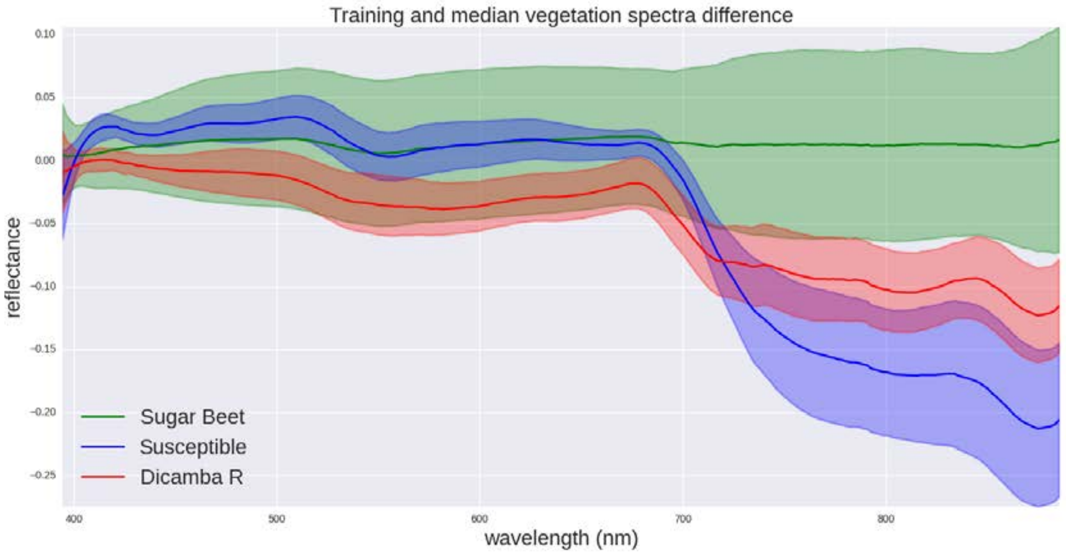


Figure 2. The training spectra subtracted by the median vegetation spectrum.

We next explored the use of ratios of the training data and the median vegetation spectrum. Figure 3 shows that this approach increased the data separability. Further attempts are being made to find specific wavelengths or spectral features that may be useful in further developments of the algorithm.

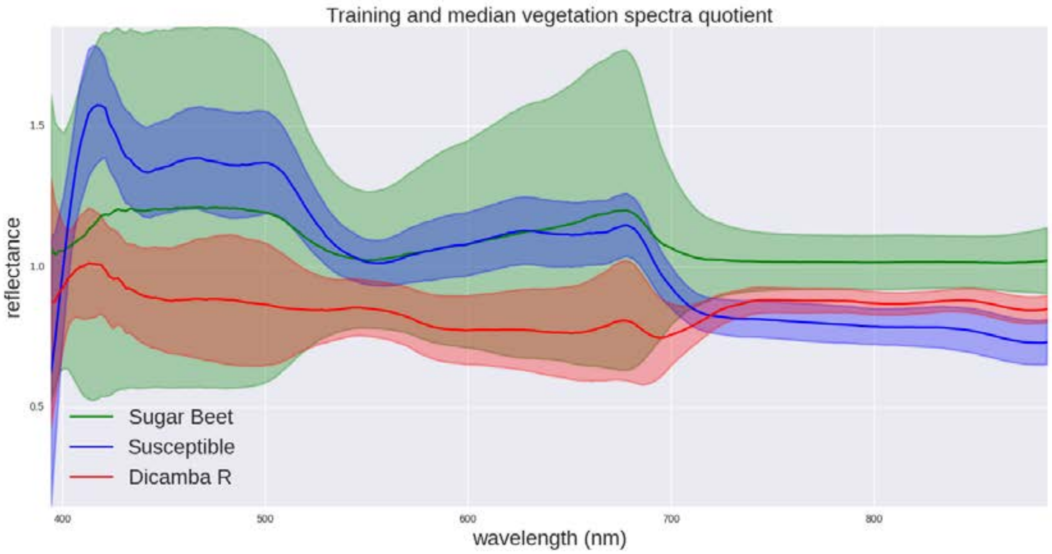


Figure 3. Ratio of the training spectra and the median vegetation spectrum.

Finally, the training spectra were normalized, dividing each training spectra by its integrated sum. Initially, as seen in Figure 4, this had promising results. The data seemed, under these circumstances, fully separable. However, in full testing of the algorithm, the majority of pixels were still being misclassified. The explanation is found partly by plotting the calibrated and normalized spectra of the soil and pots in the images (Figure 5). As one can see, the sugar beet is nearly identical to the pot and dirt. It is suspected that as a result, this is interfering with the workings of the entire algorithm. It is currently unclear why, as well as why this normalization has made the spectra of sugar beet, dirt and pots all look so similar.

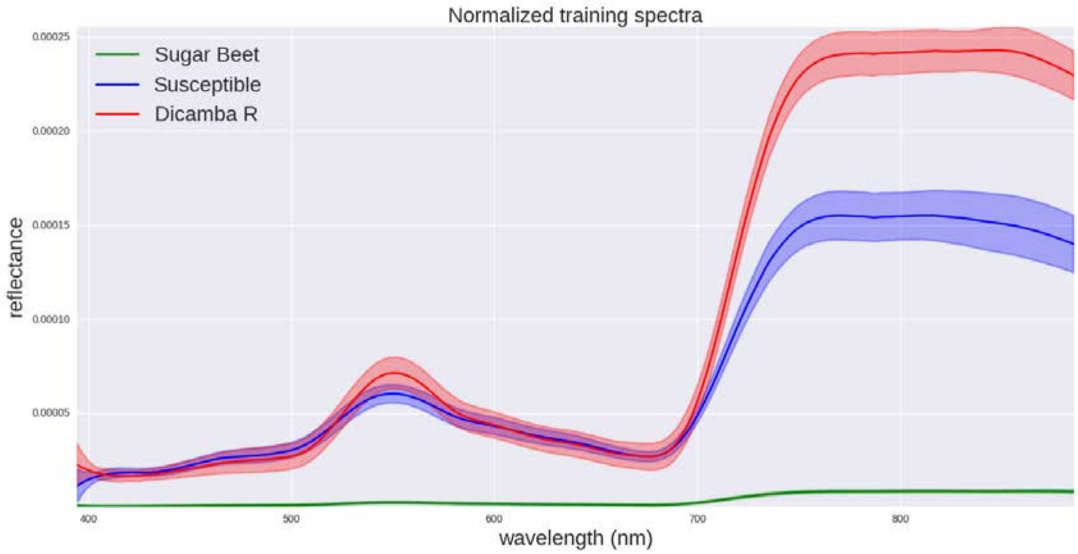


Figure 4: Training spectra for sugar beet, susceptible and herbicide-resistant Kochia that have been calibrated from DN to reflectance than normalized.

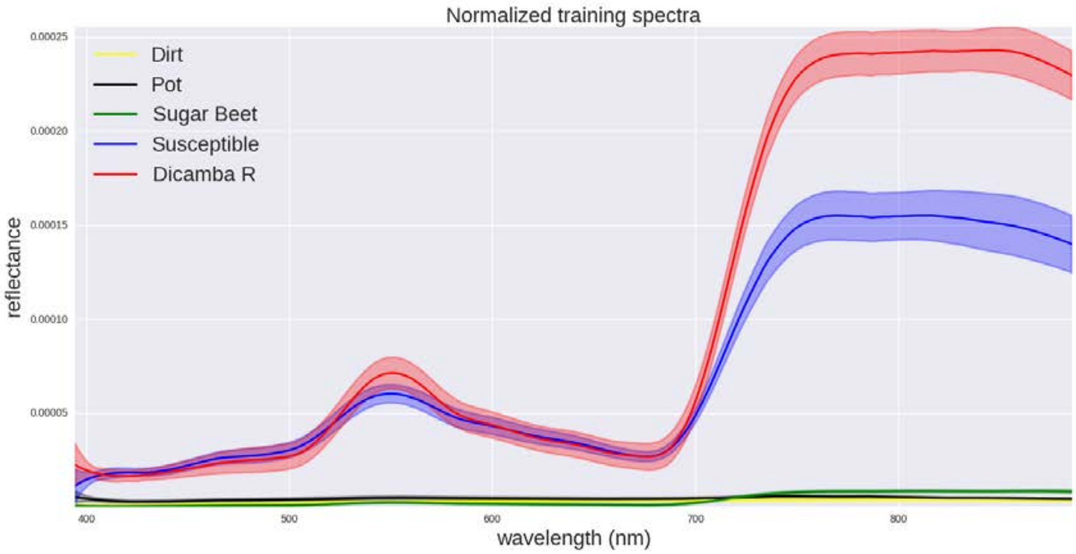


Figure 5: Training spectra for sugar beet, soil, pots, susceptible and herbicide-resistant Kochia that have been calibrated from DN to reflectance, then normalized. Note that sugar beet, soil and pot spectra are all very similar, making the automated classification more difficult.

Commercialization and Dissemination (report from NWB Sensors, Inc.)

Project Overview

This effort is tasked with the development of a commercialization plan for the technology developed by Joseph Shaw and Prashant Jha in the MREDI Weed Imaging/Pulse Crop Herbicide. Our effort has focused on the weed species and herbicide resistance detection technology developed in part 2 of the subproject “PRECISION WEED CONTROL USING ADVANCED OPTICS AND SENSOR-BASED TECHNOLOGIES.” During this quarter, we focused our work on understanding the competing / similar technologies that exist in the market, and working toward identifying competitive advantages of the technology under development at MSU.

Strengths of the Technology

Uniqueness

Utilizing hyper-spectral sensing (either imaging or non-imaging) for herbicide resistance detection is a technology that in addition to the work at MSU is being pursued by multiple institutions worldwide [1]–[3]. However much of this success has been in controlled settings and none of these technologies have seen commercialization. Therefore, if commercialized, this technology will be one of the first solutions on the market.

Fit with Herbicide Resistance Management Best Practices

Current herbicide-resistant management best practices call for improved methods of monitoring the weeds present in the field. The MSU technology addresses three of these practices. Developing historical information about the weeds and resistances present in a field. Enabling site-specifics weed management, including the ability to target resistant weeds. Improving field scouting, through the ability to detect herbicide resistance in the field.

Embodiments of the Technology

We foresee two embodiments of the species / herbicide resistance optical detection at MSU.

i. Field Scouting Device

The first embodiment is a hand spectral analyzer coupled with the herbicide resistance detection algorithms. This detector would be aimed at farm service providers rather than the growers. This would include but would not be limited to agronomists, agricultural cooperatives, and research institutions. It is envisioned the device primarily be used for in-field scouting in response to suspected herbicide resistance post herbicide, or for random sampling tests pre-herbicide. Either tests would enable rapid confirmation of herbicide resistance. Current confirmation with known resistant species requires costly and timely genetic testing. Spectra could be analyzed on the device, with spectral samples sent to university or other labs if expert confirmation was needed. This would take place digitally and could be instant over cellular data networks. If needed this embodiment would include in-field calibration equipment. This embodiment of the technology, feasibility, and potential market will continue to be studied throughout this project.

ii. Spray controller for Site-Specific Management

The second embodiment is a compact, lower-cost spectral sensor designed to enable site-specific herbicide applications. The target of this sensor would be both growers and farm service providers. This sensor could be integrated into the herbicide application system (spray coup or other technology). It would detect the presence of the susceptible / resistant weed in the field and adjust the herbicide mixture accordingly. NWB Sensors, Inc. has begun discussions with the MSU Spectrum lab and Advanced Micro-cavity sensors to determine if the technology they have been developing on a separate MREDI project could meet this need. This embodiment of the technology, feasibility, and potential market will continue to be studied throughout this project.

Difficulties of these technologies

Reactive

One of the difficulties this and similar technologies face in the market is that these technologies are fundamentally reactive to the problem of herbicide-resistant weeds. What is meant by this is that the technology is developed in response to the emergence of an herbicide resistant weed. This creates two problems.

- The technology is always lagging the problem it is trying to address. This can lead to a situation where the user of the technology always has some level of dissatisfaction with the technology since it cannot detect weeds with newly developed herbicide resistance. A product that faces a similar problem is anti-virus software for computers.
- If pro-active herbicide resistance prevention best practices (crop rotation, herbicide rotation, and herbicide use reduction) currently being developed become widely adopted then the need for this technology would be reduced.

However, the existence of herbicide-resistant crops creates an immediate need for these products, at least for the short term. This guides the approach to the market we intend to take.

Slow Adoption

Many precision agriculture products have been slow to be adopted by the market, and it is not expected this technology will be different. Spot spraying technology although it is available and the potential savings outweigh the cost of the equipment this technology has been slow to be adopted by the grower. We feel there is potentially a non-economic aspect to this slow adoption rate and we will be working with marketing experts to explore the causes of this slow adoption as part of our commercialization study in this project. This guides the approach to the market we intend to take.

Lack of Plant Spectral Data

It is currently the position of NWB Sensors that there seems to be a lack of published plant spectral data that includes critical information required to broaden the technology under development at MSU. Most available spectra contain only average spectra with no information about the natural in-species spectral variations, or the lifecycle spectral variations for a plant. It also makes it difficult to determine if the spectral difference between a resistant and susceptible biotype can be attributed to the development of resistance or to natural variation between different plants of the same species. This makes it difficult to develop broadly applicable weed detection routines in unobserved crops. Although this may pose a difficulty to the commercialization of this technology, the development of such a database may present both a commercial and / or research opportunity.

Hiring

The following people continue to work on this project (and continue to collaborate with Dr. Prashant Jha of the MSU Southern Agricultural Research Center):

- Dr. Joseph Shaw: subproject director (receiving partial summer salary)
- Mr. Bryan Scherrer: Ph.D. student
- Mr. Andrew Donelick: Ph.D. student (transitioned to a new research group but is working still with us on plans for a publication reporting the preliminary results he helped us achieve)

At NWB Sensors, Inc:

- Dr. Paul Nugent
- Mr. Austin Beard
- Mr. Seth Berardinelli

Equipment Procurement

- We are working towards purchasing a Resonon Pika L airborne system.

Expenditures

- Total Personnel: $28,951.37
- Payroll Encumbrance: $21,577.09
- Total Operations: $13,614.93
- Total Equipment: $16,716.00

Film Production for the Agriculture MREDI Grant

41W218 – Organizer: Eric Hyyppa; Email: eric_hyppa@montanapbs.org

Progress towards milestones

None to report Q7.

Equipment Procurement

- We do not anticipate ordering any additional equipment for this project.

Expenditures

- Total Personnel: $6,691.17
- Total Operations: $7,283.95
- Total Equipment: $7,999.00

Economic analysis subproject of the Agriculture MREDI project

41W219 – Principal Investigator: Anton Bekkerman; Email: anton.bekkerman@montana.edu

Progress towards milestones

Rapidly, Montana has become the largest producer of pulse crops in the United States and an important participant in global pulse crop markets. Continuing growth of this agricultural sector in the state is important for at least two reasons. First, it represents an opportunity for Montana agricultural producers to diversify their production portfolio and lower their exposure to market risk. For example, many Montana producers have been able to leverage their pulse crop production to lessen the adverse economic impacts of recent weak global wheat markets. Second, the development of Montana pulse markets has provided an economically-effective incentive for producers to convert summer fallow land—which has consistently been shown to not be a sustainable long-term production system—to more efficient use.

However, while the transition to more intensive cropping systems has been significant in some parts of the state, large portions of productive agricultural land remains in the traditional wheat–fallow rotation. For example, Figure 1 shows changes in planted pulse crop acres between 2007 and 2016. The data indicate that the majority of the growth has occurred in northeast Montana, while conversion in large portions of central and northern Montana still remains fairly slow. To a large extent, this is due to the fact that there has been little research investment and a very short time period—relative to Montana's wheat sector—to develop improved pulse crop varieties, management systems, and knowledge transfer mechanisms that can help Montana's farmers to transition in an economically effective manner.

(a) Pulse Acres, 2007

(b) Pulse Acres, 2016

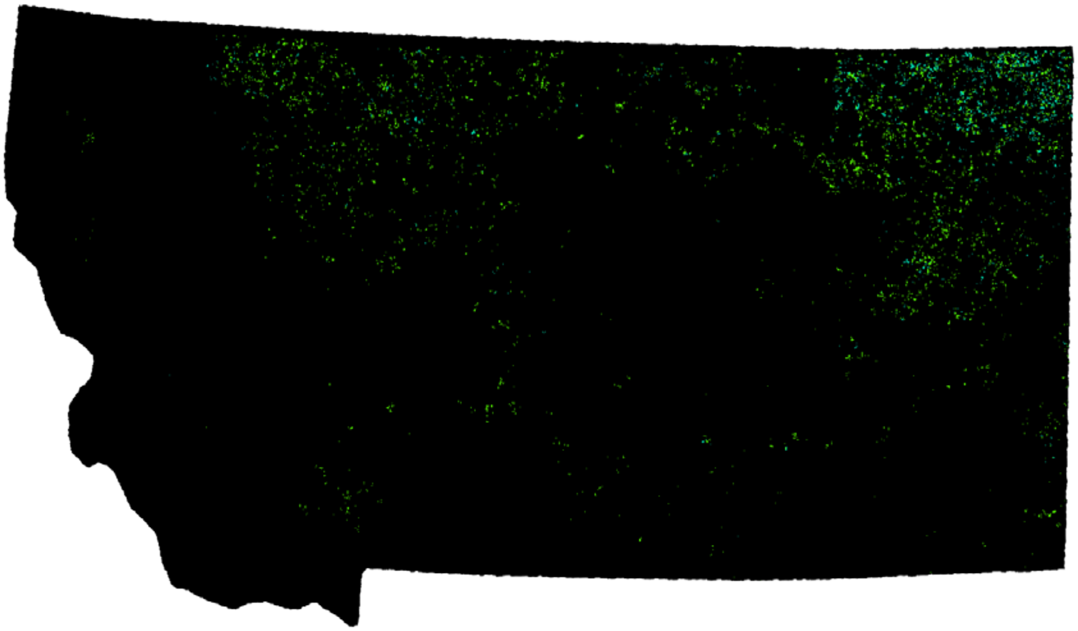


Figure 1. Planted Pulse Acres in Montana, 2007 and 2016
Source: USDA National Agricultural Statistical Service, CropScape (Cropland Data Layer)

While one of the primary goals of this MREDI project is to overcome these challenges and expedite Montana's producers' ability to transition to more intensive cropping systems, it is important to measure the extent to which the state's agricultural sector could benefit from these efforts. Moreover, in developing these measures, it is critical to acknowledge and account for issues such as capacity constraints in the state's grain handling and transportation infrastructure, productivity of newly converted land, and lack of rail infrastructure to ship pulse crops to non-traditional markets.

This report provides a summary of the approaches and methods used to estimate state-level economic impacts resulting from increasing conversion of fallow land into pulse crop production. Specifically, I focus on measuring the additional value that farmers could gain by moving away from wheat–fallow systems, and aggregating that value to the state level to provide a big-picture perspective of Montana's production agriculture sector. Intentionally, the analysis only focuses on profits realized by those directly involved in the production of those crops. While these estimates do not capture all of the potential additional economic activity through various multiplier effects and related sector growth, they are more accurate because they are not distorted by potentially poorly estimated economic multipliers (a common concern when conducting macroeconomic impact studies). Thus, the estimates in this study represent a lower bound of economic effects that are likely as a result of increased pulse crop production in lieu of summer fallow.

Under conditions that most realistically represent the current and short-term future state of Montana's production and marketing landscapes, I estimate that if land conversion continues at its most recently observed pace (approximately 4–5% per year), five-year direct additional value to Montana's production agriculture sector are estimated to be just under $3 million. However, developing improved pulse crop varieties, improving methods for better management techniques for more intensified cropping systems, and transferring new knowledge to producers—all objectives of this MREDI project—are likely to increase farmers' willingness to more quickly convert fallow acres into more productive uses. If a 10% annual conversion rate is assumed for a five-year period—not inconceivable given recent trends and likely outcomes of more intensive research efforts—cumulative state-level additional returns to farmers are estimated to be nearly $5.2 million.

Methods and Data

The general modeling approach is to consider the additional net value that an acre of land would provide to a typical Montana farmer if that acre was to be used for pea or lentil production rather than fallow. Then, determine the aggregate state-level added value by modeling the transition of fallow acres to intensified pulse producing acres. It is important to note that this type of analysis has certain advantages and disadvantages.

The primary advantage is that the methodology provides information about actual farm-level profits, rather than simply looking at sales revenues. Analyses that consider only revenues are likely to overestimate the community and state-level impacts because only the *net* revenues are salient. That is, producers will make decisions based on whether the benefits of intensifying agricultural land use exceed the costs. Moreover, indirect benefits at the community and state levels will only occur if farmers increase their *disposable* income, which can then be used to increase economic activity and trigger multiplier effects.

The main disadvantage of using an aggregated farm-level measure is that it likely represents a lower-bound of potential community and state-level impacts. Other types of impact studies consider macroeconomic, market-level effects and implement numerous estimates of economic multipliers across various economic sectors to provide a total impact value. However, these analyses can be highly sensitive to economic multiplier assumptions and interactions among economic sectors. For Montana, a literature review resulted in very few, often dated multiplier and sector dynamics estimates, implying that results from a macroeconomic impact analysis may be at high risk of highly over- or under-valuing economics effects. As such, the decision was made to provide a more conservative but likely much more accurate analysis using farm-level information.

Farm Budget Analysis

The foundation of the analysis is based on assessing farm-level differences in net returns between a traditional wheat–fallow cropping system and more intensified systems that rotate wheat with pea or lentil crops. Cost of production and net returns budgets for a representative farmer across different cropping systems can be used to make these comparisons. The data required for creating these budgets require estimates of a representative producer's variable costs (those that depend on the operations that occur within a production period), fixed costs (those that occur regardless of a producer's specific production decisions, including ownership and depreciation), and market revenues.

For non-irrigated cropping systems—which characterize the majority of wheat and pulse production in Montana—budgets require information about input costs for operating equipment; labor requirements; seed, fertilizer, and other chemical costs; ownership and repair costs; crop insurance expenses; crop yields; and market prices. Because, as shown in Figure 2, the majority of fallow acres in Montana are primarily located in regions where winter wheat production is predominant, this analysis compares production budgets for a typical winter wheat–fallow operation and an operation that rotates winter wheat with a pulse crop. Furthermore, all operations are assumed to use conventional non-irrigated production and farm management methods, with chemical fallow. Table 1 presents a summary of the inputs used to characterize a typical operation and sources for estimating the most recent cost values. For winter wheat and fallow operations, costs were verified using information from focus group meetings with Montana wheat producers (Bekkerman and Fuller, 2016).

Figure 2. Fallow Acres in Montana, 2016

Source: USDA National Agricultural Statistical Service, CropScape (Cropland Data Layer)

The intent of this budget is to characterize a "typical" Montana producer. While actual producers may be more or less efficient, the "typical" producer is intended to represent an average baseline case. All of the farm-level analysis is completed on a per acre basis, which provides more generalizable insights and comparisons. While there could certainly be some economies of scale for larger operations, the manner in which these potential savings will affect production costs are likely to be idiosyncratic across operations and are difficult to capture and generalize.

In developing the budget analysis, several assumptions were made to more accurately characterize production and marketing decisions of modern Montana farming operations. For crops for which federally-subsidized crop insurance is available—either the Revenue Protection or average production history (APH) policy—producers were assumed to purchase the insurance product at a 70% coverage. These insurance products and coverage level represents the modal policies purchased by Montana producers.

A full budget is available on request. The total per acre costs for each of the crops of interest are as follows:

Winter wheat: $175.47 / acre
Peas: $220.39 / acre
Lentils: $183.46 / acre
Fallow: $62.72 / acre

Revenue Calculation and Price Constraint Considerations

The cost of production budget provides only half of the profit equation. The revenue side is determined by a combination of market prices and yields observed for each crop produced in a particular system (wheat–fallow or wheat–pulse).

To determine wheat and pulse crop yields that are likely to be observed in the next five years—the period of analysis—I collect historical yield information (40 years for winter wheat and 12 years for peas and lentils) from the USDA National Agricultural Statistical Survey data. To improve the accuracy of the analysis, the data are collected to represent three USDA-defined agricultural statistical districts in Montana: northeast, central, and north-central.

Next, I follow the detrending approach of Goodwin and Ker (1998) to ensure that the historical yields are adjusted for differences in technological advancements across time (e.g., to be able to compare 1976 yields to 2016 yields after accounting for the fact that machinery and wheat breeding techniques has advanced substantially since 1976). Specifically, I first regress each of the yields and indices on a time trend variable and then use the regression residuals to estimate the detrended yields. This method allows for all yield and index values to be normalized to the 2016 level.

Table 1: Variables in Cost of Production Analysis

Operation	Variables	Budget category	Units	Data Source(s)	Notes
Tractors	180 HP	VC, FC		NDSU; U. Idaho	For sprayer only
	340 HP	VC, FC		NDSU; U. Idaho	All other pulled equipment
Seeding	Air seeder	VC, FC		NDSU; U. Idaho	
	Roller	VC, FC		NDSU; U. Idaho	
	Seed, pea	VC	lb	NDSU	
	Seed, lentil	VC	lb	NDSU	
	Seed, winter wheat	VC	lb	NDSU; U. Idaho	
Fertilizer	Nitrogen	VC	lb	NDSU; U. Idaho; S. Dakota	
	Phosphorous	VC	lb	NDSU; U. Idaho; S. Dakota	
	Potassium	VC	lb	NDSU; U. Idaho; S. Dakota	
	Sulphur	VC	lb	NDSU; U. Idaho; S. Dakota	
Spraying	Sprayer	VC, FC		NDSU; U. Idaho	
Chemicals	Glyphosate	VC	oz	NDSU; U. Idaho; S. Dakota	
	2-4,D	VC	oz	NDSU; U. Idaho; S. Dakota	
	AMS	VC	oz	NDSU; U. Idaho; S. Dakota	
Custom labor	Urea	VC	lb	NDSU; U. Idaho	
	Application	VC	acre	NDSU	
Harvest	Combine	VC, FC		NDSU; U. Idaho	
	Truck, tandem axle	VC, FC		NDSU; U. Idaho	
	Truck, single axle	VC, FC		NDSU; U. Idaho	
	Truck, pickup	VC, FC		NDSU; U. Idaho	
Fuel	Diesel	VC	gal	NDSU; U. Idaho	Includes lubrication costs
Crop insurance	APH or RP	VC		USDA RMA	
Other	Operating interest	VC		NDSU; U. Idaho	5.50% of VC
	Miscellaneous	VC		NDSU; U. Idaho	
	Land opportunity cost	FC	acre	USDA NASS	Rental rate

Abbreviations: VC refers to variable costs; FC is fixed costs (ownership, repairs, depreciation); NDSU is the North Dakota State University's Enterprise Budget and Custom Rate Survey data for the western North Dakota production region; U. Idaho is the University of Idaho's Enterprise Budget, Estimates for the northern Idaho region; USDA RMA is the USDA Risk Management Agency; USDA NASS is the USDA National Agricultural Statistics Service. Full citations are in the References section.

These normalized wheat, pea, and lentil yields are then used to determine the average yield for all three crops, and a "potential" yield for peas and lentils. The potential yield is calculated as the average yield plus one standard deviation, representing advances that are likely to be influenced by the research conducted as part of this MREDI project. Table 2 summarizes these yield values.

Table 2: Average and Potential Yields, in Pounds Per Acre

	Wheat	Peas		Lentils	
	Average	*Average*	*High*	*Average*	*High*
Yields (lbs)					
Northeast region	2705	1790	1902	1337	1437
North central region	2885	1557	1732	1082	1225
Central region	2780	1348	1498	807	865

Lastly, in calculating revenues from wheat following a pulse crop, yields are assumed to be 7% lower than winter wheat yields in wheat–fallow systems (see Miller et al., 2015). The yield reduction is primarily due to decreased water availability in more intensive cropping systems.

The pricing component of the revenue calculation requires several additional considerations. In a typical market analysis of production returns, it would be reasonable to obtain projected prices from sources such as the Long-term Projections Report published by USDA Office of Chief Economist and U.S. Baseline Projections published by the Food and Agricultural Policy Research Institute (FAPRI). These national prices can then be adjusted using a historical basis for Montana production regions to obtain a local projection. Specifically, the five-year average projected price (using an average of the most recent 2017 USDA and FAPRI estimates) is $4.82 per bushel, with a –$0.60 per bushel basis adjustment for Montana (Bekkerman, Brester, and Taylor, 2016). For peas and lentils, the projected prices are $0.13 per pound and $0.27 per pound, respectively. Montana is the largest pulse crop producer in the United States, and, therefore, no basis adjustment is made because the projected prices likely account for price formation in major production regions. It is important to note that despite wheat and pulse crops representing two of Montana's most important agricultural products, Montana and the United States account for a very small proportion of total global production of these crops. Therefore, using the USDA and FAPRI price projections is appropriate, because those prices largely reflect global market conditions and are unlikely to alter substantially in response to production and grain handling changes in Montana.

In Montana's rapidly changing grain marketing landscape, however, these typical price projections are not sufficient. This is due to the "growing pains" that are likely to occur in a marketing environment in which the grain handling infrastructure was historically designed to handle mostly wheat–fallow cropping systems, but must now handle more intensive grain production. That is, for over 80 years, Montana's grain handling and grain transportation system has operated in production landscapes in which farmers typically grow wheat every other year and have sufficient on-farm storage to have the option to keep much of their produced wheat from delivery. In the last decade, however, a rapidly increasing amount of fallow land has now been used to produce crops in addition to the existing wheat production. This has resulted in placing pressure on grain handling facilities and the transportation infrastructure to:

- Handle significantly higher volumes of products.
- Respond to higher labor demand to provide more handling services (in areas where the labor supply may be relatively low).
- Provide more off-farm storage (because farmers may not have sufficient on-farm storage or may choose not store pulse crops).
- Face increased demand to quickly move pulse crops, which can oxidize and lose favorable aesthetic characteristics that are demanded in retail consumer markets.
- Increase logistical capacity to ship pulse crops to destinations that have not been traditional terminals for wheat (such as shipping pulses south to processing facilities in the Midwest and to export facilities to South America).

For example, Figure 3 shows the current active grain handling facilities in Montana (data are from the Montana Wheat and Barley Committee, 2017). The size of each elevator location (indicated by a circle) represents the relative storage capacity at that location and green circles represent those that accept peas, lentils, and/or other pulse crops. The figure makes evident that despite the growth in pulse production in Montana, there are still many more elevators that do not accept pulse crops, even in the northeast part of the state. In fact, only 34% of elevators in Montana handle pulse crops. Moreover, an average elevator that accepts pulse crops has an average capacity of 563,909 bushels, while an average elevator that specializes in wheat handling has a capacity of 773,307. On aggregate, out of the total available capacity at active Montana elevators, 29% of that capacity represents elevators that accept pulse crops while 71% of capacity specializes in wheat.

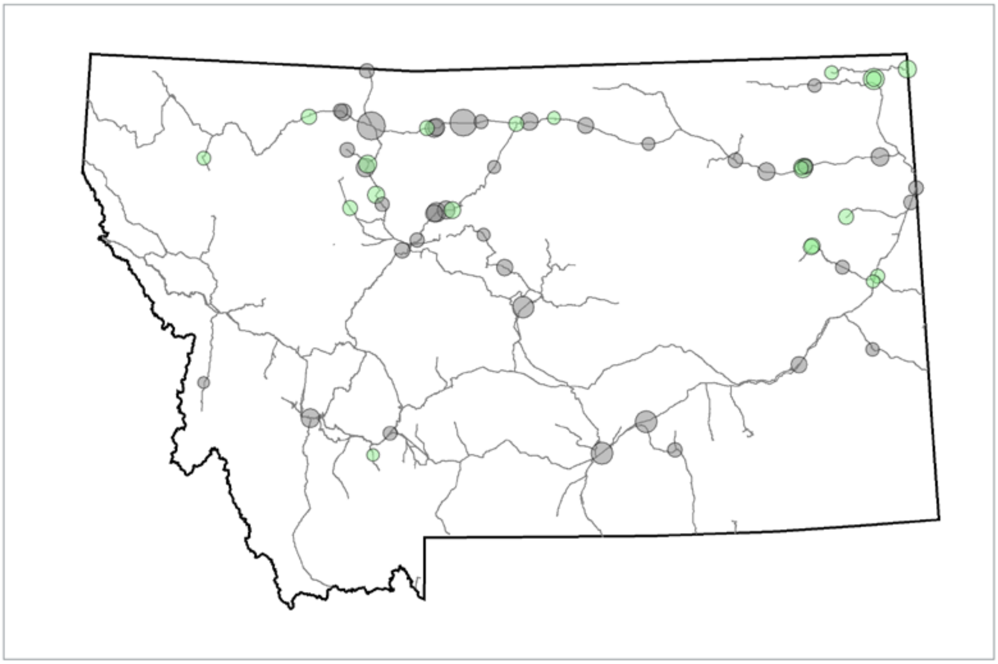


Figure 3. Montana Grain Handling Facilities

Notes: Circles indicate facility locations and the circle size indicates relative storage capacity Green circles are facilities that accept pulse crops.

Figure 3 provides suggestive evidence that despite the growth in pulse crop production, Montana's grain handling industry is still adapting and may still be capacity-constrained in response to the growth in supply. Another issue facing Montana's pulse handling and transportation industry is the fact that, historically, the demand for Montana's grain has been from either Pacific Northwest export facilities or processing and distribution facilities in the Great Lakes region. While a large portion of Montana peas and lentils also heads west to be exported to Asia and India, there has been increasing demand for peas in the U.S. Midwest region, where many pet food processing facilities are located, and for lentils in Central and South America (personal communication, pulse merchandiser at Viterra USA).

Figure 4 shows a map of the U.S. rail infrastructure. The rail lines through much of Montana and western North Dakota—the primary pulse production regions—have no north–south routes, and the only major north–south throughway does not occur until Minnesota. This requires significant additional shipping costs on the part of the grain handler, which are likely to be passed down by grain elevators to farmers in the form of lower price bids.

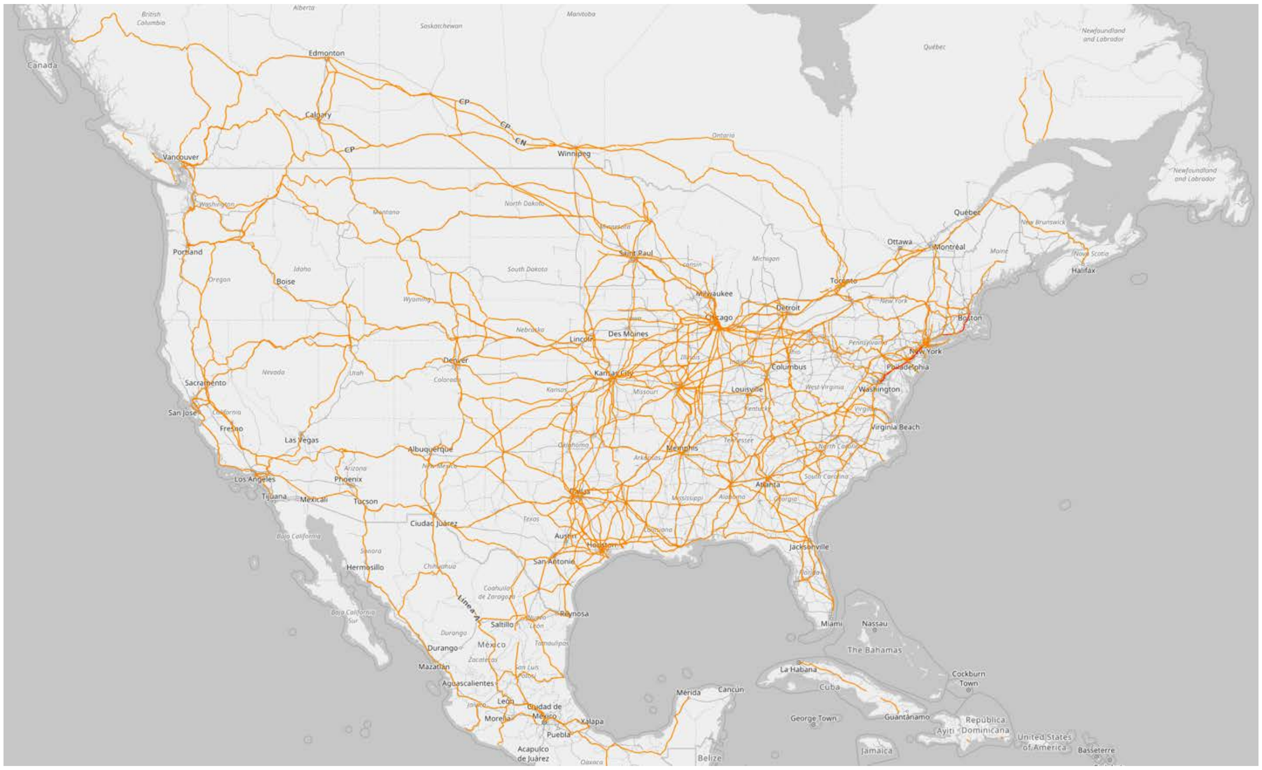


Figure 4. U.S. Rail Infrastructure Map

Source: OpenRailWayMap.org

These issues are expected to result in responses by those in the grain handling sector, and manifest through price dynamics. Specifically, grain elevators can alter the prices they offer to producers in order to increase or decrease the quantities of both wheat and pulse crops delivered to the location and/or to pass through additional costs or cost-savings. For example, if at harvest, the deliveries to an elevator begin to exceed the facility's capacity, they can lower their bids to reduce the demand for their grain handling services. Moreover, it is likely that the reduction will occur for *both* wheat and pulse prices because elevators typically handle both crops.

To empirically measure the extent to which Montana's grain handling may be constrained and whether prices have been impacted, I use historical daily basis wheat data for four Montana regions—northeast, north-central, Golden Triangle, and southeast—between 1998 and 2016. Using an event-study regression analysis, I estimate monthly basis values before and after 2009, which is when the major increase in pulse production began occur in Montana. The model controls for differences in location, seasonality, and differences across years.

Figure 5 shows a visual representation of the regression results for the four regions. The figure shows that there was a clear reduction in wheat basis (and, thus, prices paid to farmers) after the major expansion in pulse production. That is, relative to basis *prior* to the major pulse expansion, elevators offered lower basis bids after the expansion. On average, wheat basis decreased by an estimated 26%, with much of the decrease occurring at harvest time when there is the greatest influx of pulse crops. As the supply of pulse crops wanes throughout the marketing year, wheat basis returns to its historical normal levels.

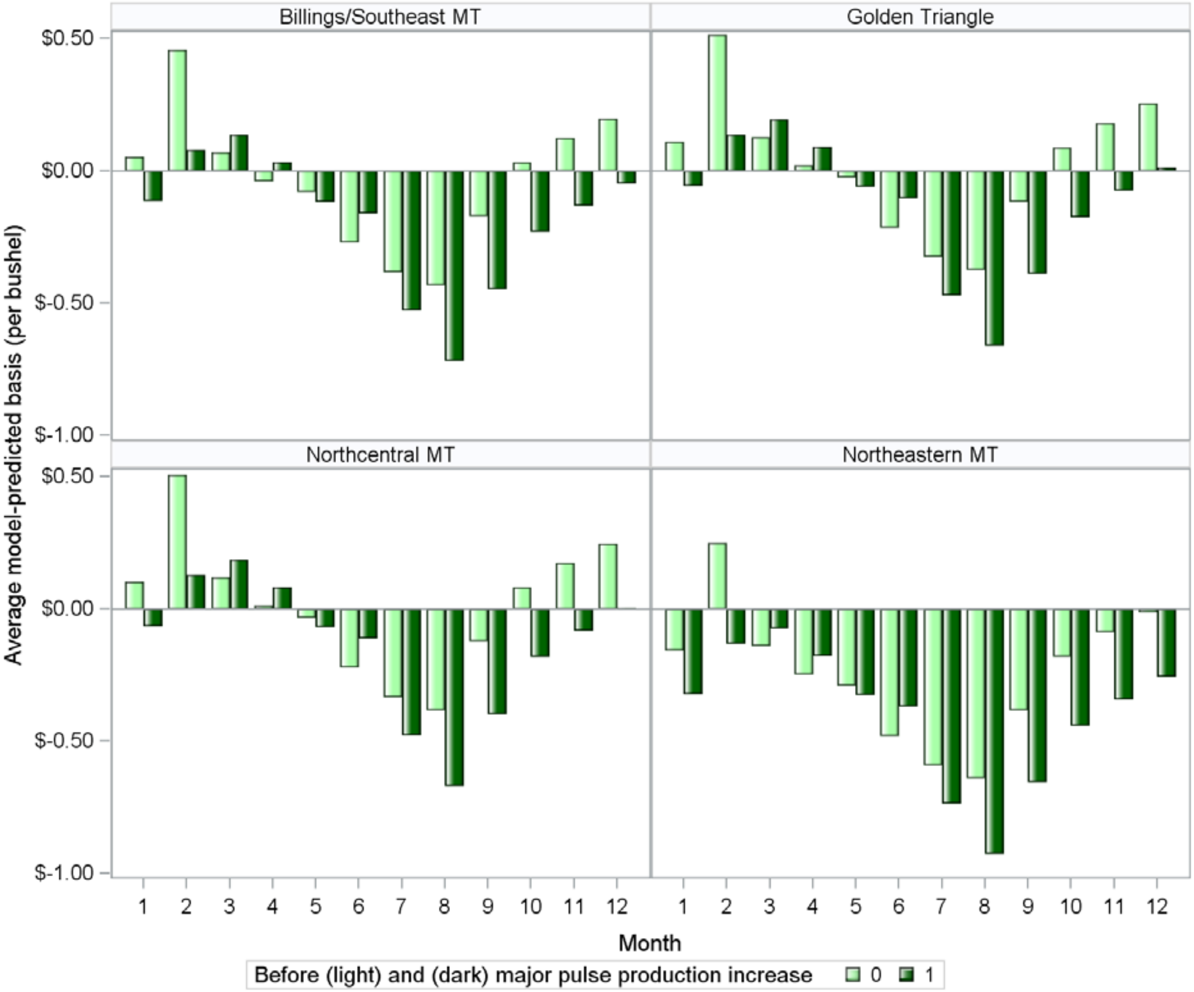


Figure 5. Estimation Results of Basis Regression Model

The results of the empirical analysis indicate that when evaluating farm-level returns from pulse production and marketing and aggregating to the state level, it is necessary to account for price reductions when calculating revenues. However, it is also important to note that as the industry continues to develop and the grain handling and marketing infrastructure expands, prices for both wheat and pulse crops are likely to strengthen. While it is difficult to know how quickly this expansion will occur, it is also useful to examine the potential returns in an environment that is not constrained by the capacity and transportation limitations.

Finally, the net returns are projected for the period 2017–2021. In order to ensure that the time-value of money is appropriately accounted for before comparing returns from alternative cropping systems, I calculate five-year discounted net present value (NPV) for each system's net return. The five-year NPV helps capture potential forgone longer-run opportunities that a producer would have if they were not able to invest revenues across the five-year period. For example, if a farmer chooses one production approach an earns an additional $1 relative to an alternative production system, then the farmer can invest that extra dollar and earn interest. The producer who was not able to earn the additional $1 would not have the choice to invest it. The discount rate used to calculate the NPV is 1.84%, which is the 2016 five-year U.S. treasury bill yield rate. This characterizes a risk-free investment that can be made by any producer.

State-level Land Conversion and Aggregated Net Returns Estimation

After calculating farm-level net returns for a representative acre of land under alternative cropping systems, state-level net returns can be calculated by aggregating returns on converted acres. In performing the aggregation, there are three issues that needed to be considered and addressed:

- How many fallow acres should be assumed to enter production annually?
- When fallow acres enter, what proportion are likely to be allocated to pea production and to lentil production?
- Should the overall productivity of a new converted acre be assumed to be the same as the overall productivity of a previously converted acre?

First, to determine the likely entry of new fallow acres into agricultural production, I look to historical conversion data. Records from the 1997 and 2012 USDA Censuses of Agriculture indicate that between those years, there was a 37.9% decrease in summer fallow acres in Montana. Annualizing this fifteen-year change suggests that an average annual reduction in fallow acres is approximately 2.5%. Because the 2017 Agricultural Census has not yet been completed, more recent information can be gleaned by using satellite imagery published by the USDA National Agricultural Statistical Service, CropScape (Cropland Data Layer). These data indicate that in Montana between 2012 and 2016, fallow acres decreased by 9.1%, or a 2.3% annualized rate of decrease.

It important to also note that in recent years, there is suggestive evidence that fallow acre conversion may be increasing. For example, from 2015 to 2016, 9.7% of fallow acres entered production. Moreover, the number of acres that can be enrolled into the Conservation Reserve Program has been continually lowered over the past decade and is now at a maximum ceiling of 24 million acres nationwide. This implies that farmers may be unable to enroll idle acres into the program. Lastly, the combination of rapid entry of acres into pulse production in Montana (see Figure 1 above), relatively high pulse price with respect to wheat prices, and continued research and improvements in pulse varieties and production techniques, may suggest that future fallow land conversion may be more rapid than observed in the past. As such, I use a 5% annual land conversion rate as a benchmark for the next five years, 3% as a lower bound (representing historical trends), and 10% as a potential upper bound.

The second consideration is a farmer's decision to allocate converted fallow acres into pea or lentil production. Using historical regional planted acreage data from the USDA National Agricultural Statistical Service, the main pulse production regions tend to allocate approximately 70% of land to pea production and 30% to lentils. Table 3 provides a summary of regional land use in Montana.

Table 3. Summary of Land Allocation, by Agricultural Statistical District

Land	Northeast	Central	North-central
Fallow, 2016 acres	527,658	337,316	2,180,339
Winter wheat, 2016 planted acres	200,453	443,807	1,401,038
All wheat, 2016 planted acres	2,230,498	576,886	2,429,507
Fallow as proportion of all land in wheat, 2016	19.13%	36.90%	47.30%
Historical allocation lentils relative to peas	31.20%	12.90%	31.20%

Notes: Agricultural statistical districts are defined by the USDA.

The last modeling consideration is the treatment of the productivity of additional converted acres. Economic theory suggests that farmers will first convert the most productive acre of land. They will then convert the next most productive acre of land, and so on. This theoretical intuition is reflected in farmers' decisions to convert pulse acres in Montana. For example, Figure 1 shows that between 2007 and 2016, much of the new pulse production has occurred in the northeast portion of the state, where pulse production conditions are most favorable. In the Golden Triangle, where less moisture has made growing pulse crops less productive, conversion has been slower.

This implies that as additional fallow acres are added into pulse crop production, it is reasonable to expect decreasing marginal returns to those acres. As such, each annual influx of fallow acres into production is expected to have lower potential aggregate state-level returns (i.e., state-level returns are likely to still be positive, but will be less than the returns observed in the preceding year). To model this behavior, I use an inverse distance weighting function based on the percentage of fallow acres remaining in an agricultural region. That is, if 100% of Montana's acres were fallowed, then the first acre to be converted would have the highest productivity after conversion and would receive the highest "productivity" weight. The further fallow acres move from 100%, the lower "productivity" weight they receive. Figure 6 helps visualize the weighting scheme used for aggregating farm-level productivity to the state-level.

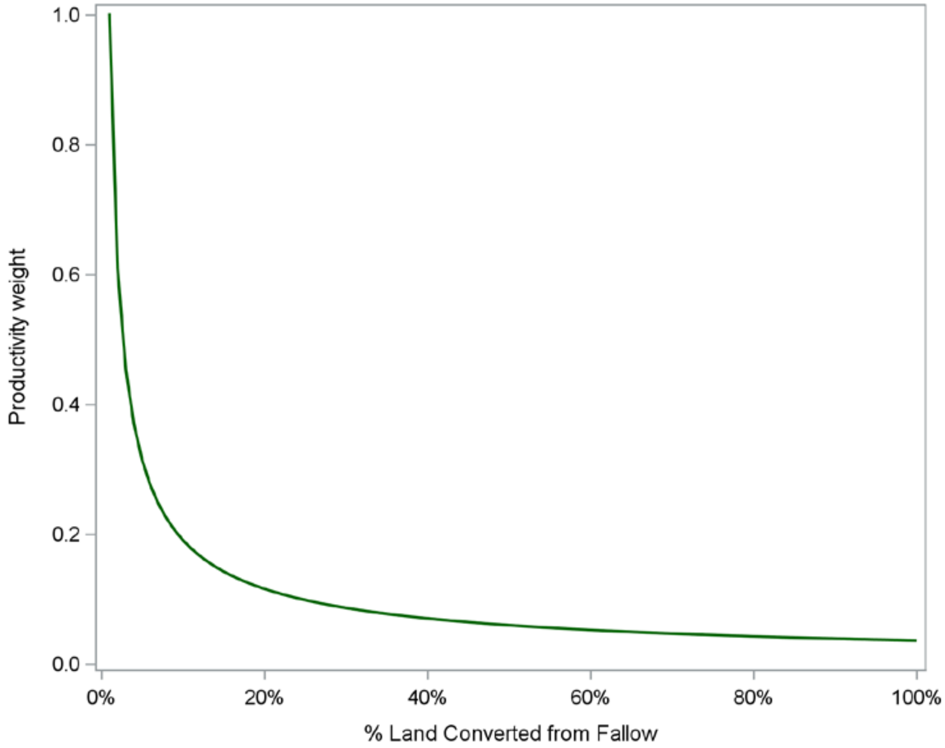


Figure 6. Land Productivity Weighting Function for State-Level Aggregation

Analysis Results

Figures 7–9 provide visual summaries of the economic analysis for projecting aggregate state-level returns to farmers. Again, it is important to note that the figures represent *additional* value that is provided by converting a wheat–fallow cropping system to a wheat–pulse system (not the total return from a wheat–pulse system). Each of the three figures represents impacts associated with the three grain handling and transportation infrastructure constraint conditions. Figure 7 represents the assumption that Montana's grain handling infrastructure is so constrained that both wheat and pulse crop prices are not at their maximum potential. Figure 8 represents the scenario that only wheat prices are lower due to constraints, but that pulse crops prices are at their efficient market levels. And Figure 9 characterizes a scenario that assumes both wheat and pulse crop prices are fully unconstrained by the evolving grain handling infrastructure.

In each figure, the solid green line represents the annual additional state-level value of fallow acres converted to pulse production. These baseline estimates assume a 5% annual land conversion rate. The light green band around the solid line represents value added if fallow land conversion is assumed to be between 3% and 10%

annually. The dark green bars represent cumulative net present added value under the 5% fallow land conversion assumption. That is, each bar represents the sum of value added from the current year and all previous years. The whiskers represent cumulative returns if fallow land conversion is assumed to be between 3% and 10% annually. Lastly, the top panel of each figure represents aggregate returns if pulse crop yields are assumed to remain at historical averages. The bottom panel represents state-level returns to farmers if pulse crop yields are higher as a result, for example, from research associated with this MREDI project.

The fully constrained assumption (Figure 7) provides the most conservative market characterization. Under the average pulse crop yield assumption, state-level added value is between $256,000 in 2017 and $160,000 in 2021. If higher land conversion rates are assumed, these returns increase to approximately $829,000 in 2017 and $408,311 in 2021. Cumulative five-year returns range between $629,000 (3% conversion rate) and $1.84 million (10% conversion rate), with the baseline cumulative returns of $1.01 million. However, significantly higher returns are expected if pulse crop yields increase as a result of research and increase knowledge transfer. Specifically, even under the most price-constrained market scenario, the baseline five-year cumulative state-level aggregate net returns are estimated to be $1.64 million, and up to $2.99 million if fallow conversion is assumed to occur at a 10% annual rate.

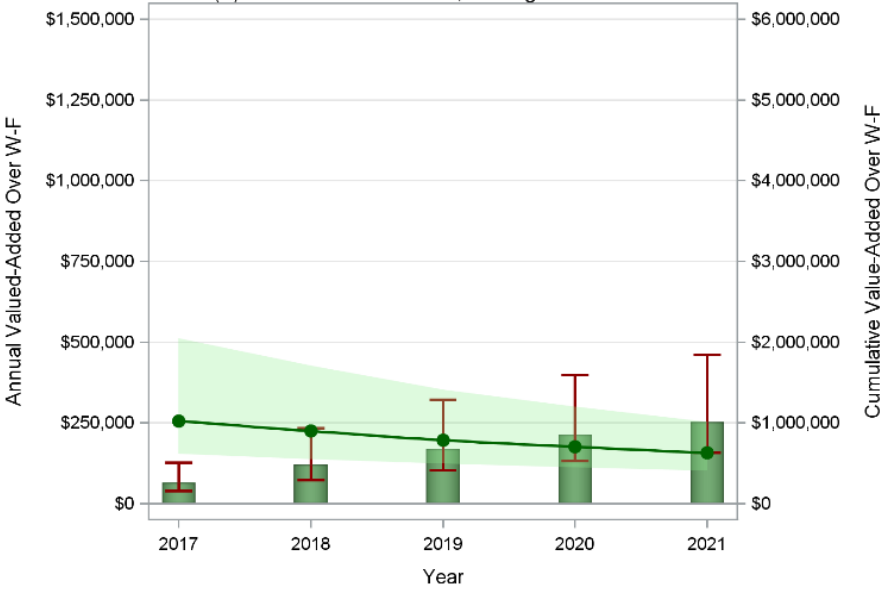
Figure 8 shows perhaps the most representative model for Montana's current markets, in which wheat prices are likely constrained but pulse crop prices are becoming less constrained as global and domestic demand has expanded and stabilized significantly. Under the average pulse yield and baseline land conversion assumptions, annual returns range between $524,000 in 2017 and $320,000 in 2021. Under these assumptions, cumulative five-year state-level returns are estimated to be $2.07 million. That is, if land that would have been fallowed was converted to pea and lentil production, aggregate farm-level returns to Montana producers would increase by over $2 million dollars in a five year period. Under alternative land conversion assumptions, these returns can range between $1.29 million and $3.77 million.

Figure 8 also shows that significantly higher additional farm-level returns can be realized if pulse crop yield potential can be improved. Under a baseline land conversion assumption, cumulative five-year returns are estimated to be $2.82 million. However, developing improved pulse crop varieties, improving methods for better management techniques for more intensified cropping systems, and transferring new knowledge to producers is likely to increase their willingness to more quickly convert fallow acres into more productive uses. If a 10% annual conversion rate is assumed for a five-year period—not inconceivable given recent trends and likely outcomes of more intensive research efforts—cumulative state-level returns to farmers are estimated to be nearly $5.2 million.

It is again important to note that this cumulative additional value likely represents a lower bound of overall state-wide economic impacts. That is, the value characterizes returns that directly go to agricultural producers. It is likely that as Montana's grain handling and transportation industry continues to adapt to the changing production environment, there will be increased demand for labor and services. Moreover, while some of the additional returns to farmers will be re-invested in their operations, some portion is expected to enter and enhance other economic sectors in the state. As such, associated economic multiplier effects will likely increase the overall economic impacts.

Lastly, Figure 9 shows the results under the assumption that grain handling and transportation constraints are sufficiently low that they do not impact wheat or pulse crop prices. Interestingly, these results do not differ substantially from those in Figure 8. This suggests that even when wheat prices recover, it is unlikely that producers who maintain a wheat–fallow cropping system would outperform those with a pulse crop rotation (conditional, of course, on wheat yields within a pulse system maintaining only a moderate reduction relative to a fallow system and on pulse crop prices remaining relatively stable).

(a) All Prices Constrained, Average Pulse Yields



(b) All Prices Constrained, Potential Pulse Yields

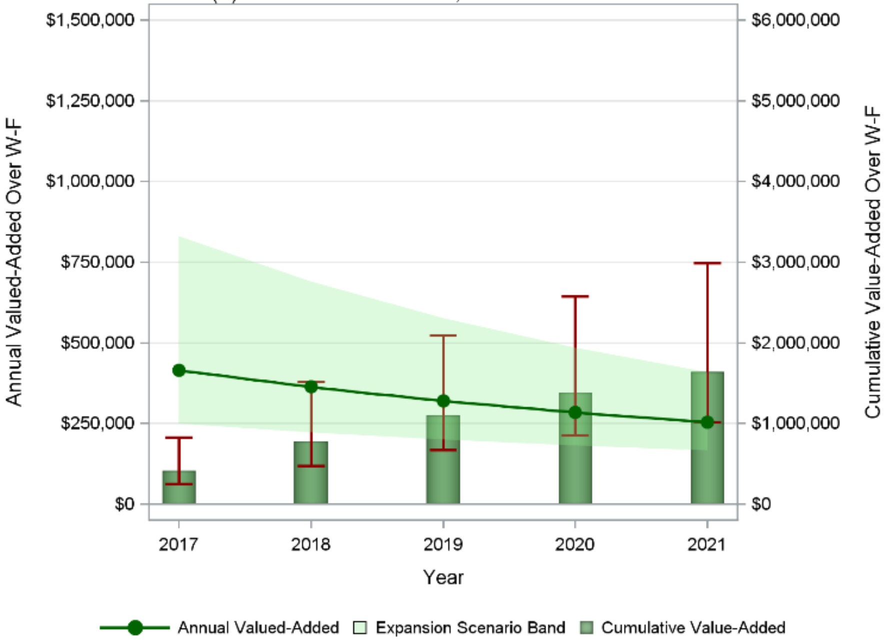


Figure 7. State-level Returns, Constrained Wheat and Pulse Price Assumption

Notes: Green line indicates returns to wheat-pulse cropping systems relative to wheat-fallow under the assumption that there is an annual 5% of fallow land converted to production. The light-green band indicates additional returns if fallow land conversion is assumed to be between 3% and 10% annually. Bars represent cumulative net present value of added value under the 5% fallow land conversion assumption, and whiskers represent cumulative returns if fallow land conversion is assumed between 3% and 10% annually.

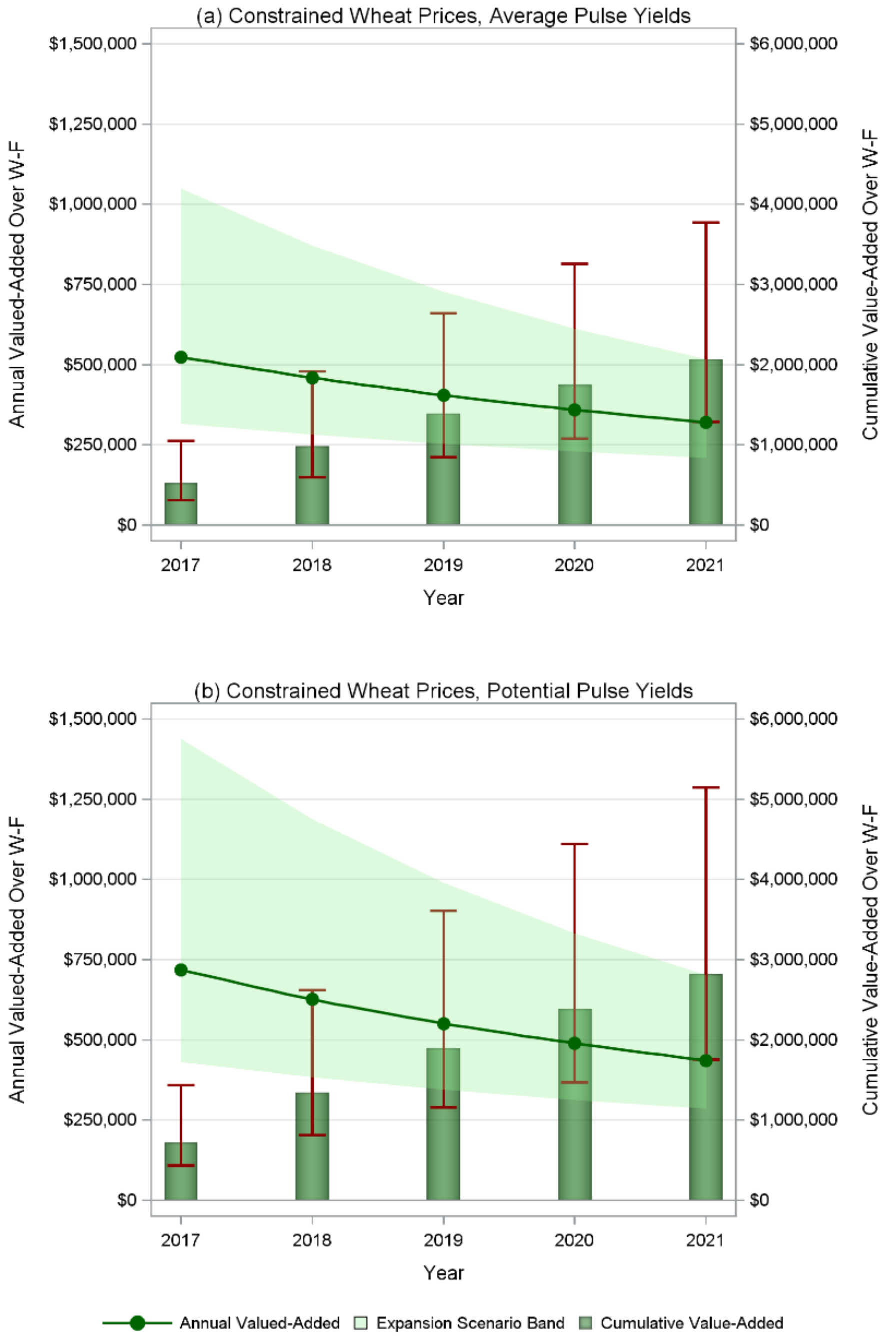


Figure 8. State-level Returns, Constrained Wheat Price Assumption

Notes: Green line indicates returns to wheat-pulse cropping systems relative to wheat-fallow under the assumption that there is an annual 5% of fallow land converted to production. The light-green band indicates additional returns if fallow land conversion is assumed to be between 3% and 10% annually. Bars represent cumulative net present value of added value under the 5% fallow land conversion assumption, and whiskers represent cumulative returns if fallow land conversion is assumed to be between 3% and 10% annually.

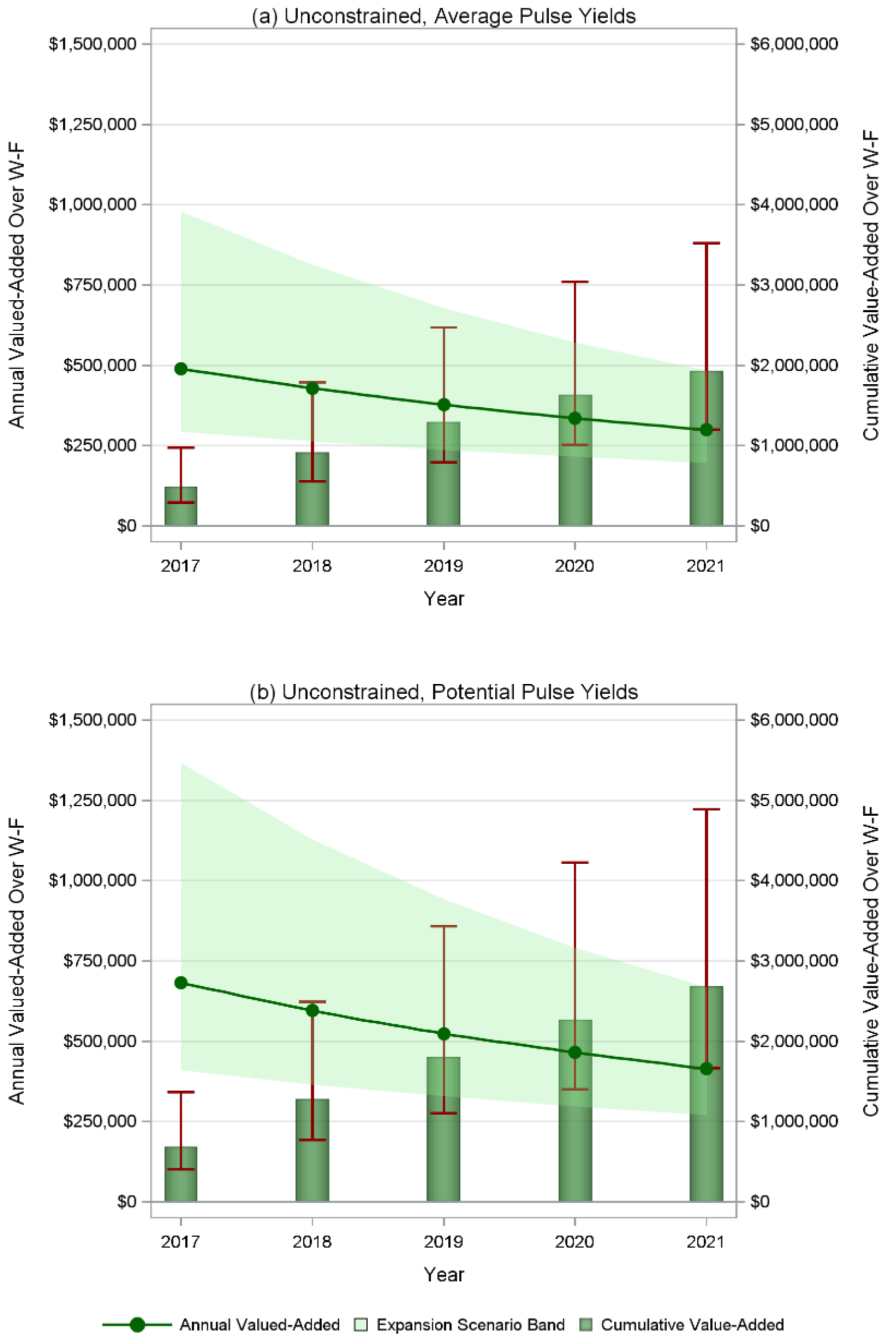


Figure 9. State-level Returns, Unconstrained Price Assumption

Notes: Green line indicates returns to wheat-pulse cropping systems relative to wheat-fallow under the assumption that there is an annual 5% of fallow land converted to production. The light-green band indicates additional returns if fallow land conversion is assumed to be between 3% and 10% annually. Bars represent cumulative net present value of added value under the 5% fallow land conversion assumption, and whiskers represent cumulative returns if fallow land conversion is assumed to be between 3% and 10% annually.

Hiring

- No additional hires in Quarter 7.

Expenditures

- Total Personnel: $42,527.06
- Total Operations: $11,613.00

Participatory research network subproject of the Agriculture MREDI project

1) 41W224 – Principal Investigator: George Haynes; Email: haynes@montana.edu

Progress towards milestones

The cost:benefit and participatory research surveys have been completed with producers. Three of the four producers collaborating with MSU on the precision agricultural (variable rate technologies) intervention were interviewed in April and May about fixed cost considerations (primarily, equipment purchases), barriers to technology adoption, and the success of the participatory research effort with MSU. All primary data collection needed for the final report has been completed.

Hiring

- No additional hires in Quarter 7.

Expenditures

- Total Personnel: $14,887.67
- Total Operations: $2,604.28

2) 41W223 – Principal Investigator: Colter Ellis; Email: colter.ellis@montana.edu

Progress towards milestones

In addition to Dr. Haynes' cost:benefit analysis, Colter has been working to develop a short report that provides an overview of the primary themes of from the data collected. This information will be printed and sent to study participants. Please find a preliminary demographics sheet representing the 68 farmers interviewed in Table 1.

Table 1. Preliminary demographics

Demographic Category	**Results**
Average Age	49 years
Male	88.89%
Female	11.11%
Percent did not complete high school	
Percent completed high school	6.120%
Percent completed some college	18.370%
Percent completed Assoc. or 2-year degree	12.240%
Percent completed Bachelor's degree	53.060%
Percent completed Grad. or Prof. degree	10.200%
American Indian	0%
Alaskan Native	0%
Asian	0%

Black or African American	0%
Hispanic or Latino	0%
Native Hawaiian, Other Pacific Islander	0%
White	100%
Other	0%
Married	88%
Living w/ partner (not married)	0%
Divorced/separated	2%
Single	8%
Widowed	2%
Other	0%
Under $9,999	0%
$10,000 - $19,999	2%
$20,000 - $29,999	2%
$30,000 - $39,999	4%
$40,000 - $49,999	9%
$50,000 - $59,999	4%
$60,000 - $69,999	15%
$70,000 - $79,999	13%
$80,000 - $89,999	9%
$90,000 - $99,999	0%
$100,000 - $109,999	7%
$110,000 - $119,999	4%
$120,000 - $129,999	2%
$130,000 or more	28%

Hiring

- No additional hires in Quarter 7.

Expenditures

- Total Personnel: $14,405.19
- Payroll Encumbrance: $4,361.91
- Total Operations: $7,429.53