

**Agriculture MREDI Grant  
Quarter 4 Report  
August 22, 2016**

**Executive Summary**

This project has made progress on all objectives and this executive summary will place this progress in context. At this time all plots have been planted and harvested and data analysis is taking place.

**Objective I. Improve income from lands previously fallowed.**

Planting alternative crops on the 4.6 million acres of fallow land offers the opportunity to gain profit from otherwise fallowed land. Pulse crops (30 pea, 15 lentil and 10 chickpea varieties) were planted and harvested at seven MAES Research Centers. Data from these trials will produce data on yield and protein content under the varying environments encountered during the 2016 cropping season. In addition, seven pea varieties were selected for more intensive work on protein content and water use with trials again planted at all seven MAES Research Centers. These trials will provide critical information on local adaptability of the various pulse crop varieties and their protein content and water use under the varying environments encountered at the Research Centers. This data will help in better defining the relationships between genotype and environment in terms of protein content—a factor important to business plans for a pea protein fractionation plant in MT. The water use information will be critical to re-crop decision making in our traditional crop fallow production systems. One of the limiting factors to integrating pulse crops into wheat rotations is herbicide carryover risk to pulses from wheat herbicides and pulse herbicides to wheat. Dr. Prashant Jha conducted trials at four MAES Research Centers and has identified herbicides that will not have carryover problems and that will control weeds without further exacerbating herbicide resistance problems (see addendum report pages 37-39).

Cover crops harvested for forage either by haying or by grazing animals (cows or sheep) were planted at the seven MAES Research Centers plus Bozeman. Cover crops are an alternative to pulse crops for previously fallowed acres. Cover crop trials were seeded at two different times; a time that would be appropriate for planting cool season species and when appropriate for warm season species at each location. There were four polyculture (cocktails) mixes selected for the trial.

1. Cool – Radish, Turnip, Spring Pea, Safflower, Oat;
2. Warm – Radish, Turnip, Chickpea, FabaBean Sunflower Sorghum;
3. Cool/Warm – Radish, Turnip, Spring Pea, Safflower, Oat, Chickpea, FabaBean Sunflower Sorghum;
4. Alternative – Radish, Turnip, FabaBean, Black Bean, Teff, Indian Corn, Sorghum.

Each of the poly cultures were planted at each seeding time point. The remainder of the study was each individual component that made up the poly cultures was planted alone. Thereby allowing the comparison of how each competes in the mix and how each contributes to the polyculture. Data being developed includes yield. This will be done for above yield (above ground biomass), forage quality (CP, ADF and DM) and nitrate level of the harvested forage.

Related to both pulse and cover crop work is their effect on two common wheat stem sawfly parasitoids. Data gathered to date (see addendum report pages 35-37) indicates that availability of sugar greatly increases parasite longevity and that parasite emergence was higher where either cover crops or peas were planted compared to fallow and that the parasites were larger and had better energy reserves where pulse or cover crops were adjacent to wheat fields. Fall collections from the four research sites will provide data on whether parasitism was affected by having cover crop or pulse nectar sources available to the parasite. Thus, the impact of cover crop or pulses on previously fallowed acres will likely have impacts beyond direct increases of income but may have positive impacts on wheat pest management on an agroecosystem scale. This MREDI grant will also provide data on soil health effects and soil microbiology (pages 11-20 in addendum).

All of this data will be supplied to Anton Bekkerman for economic analysis.

**Objective II. Develop new, improved or quality differentiated products or crops.**

Addendum reports pages 1-10 provide information on progress towards better understanding the progress on understand pea protein variety (genotype) and environment interactions, development of new Montana oriented Durum varieties (addendum reports pages 33-34), new microbial inoculants (addendum reports pages 11-20) and precision weed control using advanced optic and sensor based technologies/weed imaging (addendum reports pages 37-42). This later project is likely to develop some patentable technologies but this is dependent on completion of this research to identify weeds in crop canopies and identification of herbicide resistant weeds. Work in the Peters lab on the nitrogen fixing AZBB163 and other soil microbiology may generate potential patents and products. Work in Yeoman's lab on identifying microbes to be used in feed supplement to combat nitrate poisoning in livestock (broad leafed plants in cover crop mixes have this potential) has identified potential microbes and is now ready for the animal testing phase. Again, new patentable products may be identified in this project.

**Objective III. Develop on farm precision agriculture tools and technologies.**

This objective has several components including field trials to study optimization of wheat yield and protein, weed management, maximizing sustainable use of soil water resources, development of data management technologies and artificial intelligence programs that will develop predictive optimal economic and sustainable recommendations for crop fallow and continuous crop scenarios. Four OFPE (On-Farm Precision Experiments) have been planted and harvested with data on optimal fertility relative to yield, grain protein and weed management having been taken and being used in economic analysis and development of computer software to develop predictive optimal economic and sustainable recommendations for crop fallow and continuous crop scenarios. Progress in these areas are found in addendum pages 24-31. A weather and soil moisture network was installed by the University of MT Climate Center (addendum pages 31-33). The information from this network and other data bases available to the Climate Center will be critical in developing water use models and predicting soil moisture for re-cropping and fertility modeling for predictive models.

A critical component of this research is the development of a participatory research network composed of growers, industry and researchers to identify barriers to the use of these new technologies (addendum pages 44-45). Software developed by the Sheppard and Izurieta groups may lead to patents and marketable products.

**Final comments**

This project has been presented at all seven MAES Research Centers plus Bozeman at Field Days this summer and is on track to deliver data and conclusions on all objectives. Outputs will be refereed journal publications in all areas and extension publications detailing the agronomic and animal research on potentials for cover crops or pulse crops to successfully replace fallow acres. In addition, critical data will have been developed to support the business plan for a pea protein fractionation plant, development of patents and products, and new competitive research grants. A very important output will be development of more refined questions to identify effects of landscape scale cropping changes on soil health, water use, pest control, weed management and optimization of sustainable economic cropping scenarios for MT farmers.

Details of individual sub-project reports can be found in the addendum.

## **ADDENDUM – Agriculture MREDI Grant Quarter 4 Sub-project Reports**

### **Research Center/MAES subproject of the Agriculture MREDI Grant**

**41W225 – Principal Investigator:** Barry Jacobsen; Email: [bjacobsen@montana.edu](mailto:bjacobsen@montana.edu)

#### Progress towards milestones

Report detail of entire project listed in Executive Summary above.

#### Hiring

- Temporary summer help was hired at the EARC, NWARC and WARC.

#### Expenditures

- Total Personnel Services: \$77,699.65
- Total Operations: \$4,333.39

### **Pulse Crop Research subproject of the Agriculture MREDI Grant**

**41W211 – Principal Investigator:** Chengci Chen; Email: [cchen@montana.edu](mailto:cchen@montana.edu)

#### Progress towards milestones

- Pea variety trials from dryland and irrigated sites of Eastern Agricultural Research Center has been harvested. Agronomic data of these trials from the field were collected as planned. Currently, grain samples are being processed in the seed lab. The Richland site will be harvested in two weeks. In addition, most collaborating research centers have harvested the trials at each research center. Seed lab work will be completed in a month from now and then the coordinating center (EARC) will prepare a report.
- To evaluate water use efficiency (WUE) and biological nitrogen fixation (BNF) of selected pea varieties from statewide dry pea variety trial, soil samples were collected before planting and after harvest at EARC. We also asked collaborating researchers to collect these samples. For this purpose, we develop soil sampling protocols for BNF and WUE studies and emailed to cooperating researchers. Once the grain yield data are available from the seed lab, water use efficiency of the selected six dry pea varieties (two early, two medium and two late maturing varieties) will be calculated considering the difference in soil moisture before planting and after harvesting, and precipitation during the growing season. In addition, part of these soil samples collected after harvest will be submitted to Dr. John Peters' lab for biological studies.
- To evaluate different varieties/lines of green and yellow peas for their growth vigor and nitrogen fixation capacity in relation to high protein content and yield, we have conducted the following lab and greenhouse tests:
  - Yellow pea varieties/lines samples collected from different years and locations have been evaluated for protein content on NIR and combustion, the varieties/lines with high protein content and also check varieties/lines have been chosen to be grown for evaluating nitrogen fixation in greenhouse conditions.
  - The equipment i.e. gas chromatograph, for analyzing the nitrogen fixation samples by acetylene-ethylene reduction method is brought to working conditions.
  - The selected yellow peas have been run on both NIR and also N analyzer to calibrate the NIR instrument located at EARC site.

- The pots have been filled with soil during the summer, 2016 work for initializing the green house experiment.
- Research protocol to measure the nitrogen fixation on peas by acetylene-ethylene reduction method have been finalized. F) The gas supplies (standards) to run the procedure have been ordered.
- NIR analysis for the samples from 2012-2014 showed the results in the following. Based on these results a manuscript has been prepared for publication. **Summary and conclusion from this manuscript is:**
  - The effects of environments on nutritional qualities and grain yield is much greater than genetic effects.
  - Grain yield and ash concentration correlate positively indicating the possibility of increasing yield and improving mineral concentration simultaneously. But the correlations between protein and starch, and starch and ash were negative.

Table 1. Analysis of variance showing the effects of genotype, environment and their interaction on protein, starch, ash and grain yield of dry pea across Montana from 2012 to 2014.

Source of variations	DF	Protein		Starch		Ash		Grain yield	
		F-value	Pr > F	F-value	Pr > F	F-value	Pr > F	F-value	Pr > F
Replication	3	0.4	0.7699	2.0	0.1144	0.62	0.6051	6.1	0.0005
Genotype (G)	5	32.5	<0.0001	76.9	<0.0001	88.54	<0.0001	11.9	<0.0001
Environment (E)	21	179.4	<0.0001	47.2	<0.0001	60.53	<0.0001	134.3	<0.0001
G * E	10	4.5	<0.0001	3.6	<0.0001	8.87	<0.0001	1.6	<0.0001
	1								

Table 2. Genotype means for protein, starch, ash and grain yield of dry pea across Montana from 2012 to 2014

Genotype	Genotype means			
	Protein	Starch	Ash	Grain yield
	----- (g kg <sup>-1</sup> ) -----			(kg ha <sup>-1</sup> )
Arcadia (Ar)	209 cd	570 a	25.2 c	2590 a
Bridger (Br)	212 bc	551 b	25.0 c	2547 a
CDC Striker (St)	223 a	529 d	23.0 d	2273 b
Cruiser (Cr)	207 d	538 c	24.7 c	2269 b
Montech 4152 (Mo)	215 b	526 d	27.6 a	2720 a
SW Midas (Sw)	209 cd	537 c	26.3 b	2624 a
Mean	213	542	25.3	2504

Table 3. Environmental means for protein, starch, ash and grain yield of dry pea across Montana from 2012 to 2014

Environment (code)	Environmental means			
	Protein	Starch	Ash	Grain yield
	----- (g kg <sup>-1</sup> ) -----			(kg ha <sup>-1</sup> )
Bozeman 2012 (BO12)	264 a	484 i	27.4 abcde	1115 k
Bozeman 2013 (BO13)	228 cde	512 h	23.4 jk	2273 f
Bozeman 2014 (BO14)	234 cd	526 fgh	26.4 cdefg	2623 ef

Conrad 2012 (CO12)	179	m	556	bcd	25.9	efgh	2826	e
Conrad 2013 (CO13)	204	ij	554	bcde	28.5	a	1956	gh
Conrad 2014 (CO14)	169	n	564	b	25.0	ghij	3223	d
Creston 2012 (CRr12)	232	cde	521	gh	25.8	fghi	3838	c
Creston 2013 (CR13)	238	c	538	efg	27.0	abcdef	4474	b
Creston 2014 (CR14)	223	efg	527	fgh	28.2	ab	5112	a
Havre 2012 (HA12)	201	ijk	559	bc	28.4	abc	2117	g
Havre 2013 (HAa13)	214	gh	544	cdef	26.5	bcdefg	1976	gh
Havre 2014 (HA14)	166	n	581	a	24.7	hij	2165	g
Huntley dry 2012 (HD12)	224	def	513	h	27.7	abcd	1742	hi
Huntley dry 2014 (HD14)	216	fgh	551	bcde	22.4	k	1136	jk
Moccasin 2012 (MO12)	216	fgh	549	bcde	20.7	l	1287	j
Moccasin 2013 (MO13)	184	lm	539	def	26.3	defgh	1884	gh
Moccasin 2014 (MO14)	207	hi	530	fgh	27.4	abcdef	2066	gh
Richland 2012 (RI12)	213	gh	547	bcdef	24.6	hij	3795	c
Richland 2013 (RI13)	195	jk	518	h	25.4	ghi	3918	c
Richland 2014 (RI14)	248	b	553	bcde	22.2	kl	1252	jk
Sidney 2012 (SI12)	227	def	562	bc	23.6	ijk	1501	ij
Sidney 2014 (SI14)	191	kl	590	a	19.0	m	2807	e
Mean	212		542		25		2504	

- As a collaborating center for cover crop research project, EARC has planted both cool and warm season cover crops. Samples have been collected, fresh and dry biomass weight have been recorded, and samples were ground. These samples will be shipped to the coordinating center very soon for quality test. Above ground fresh and dry biomass yield of cool and warm season cover crops is shown in Fig. 1.

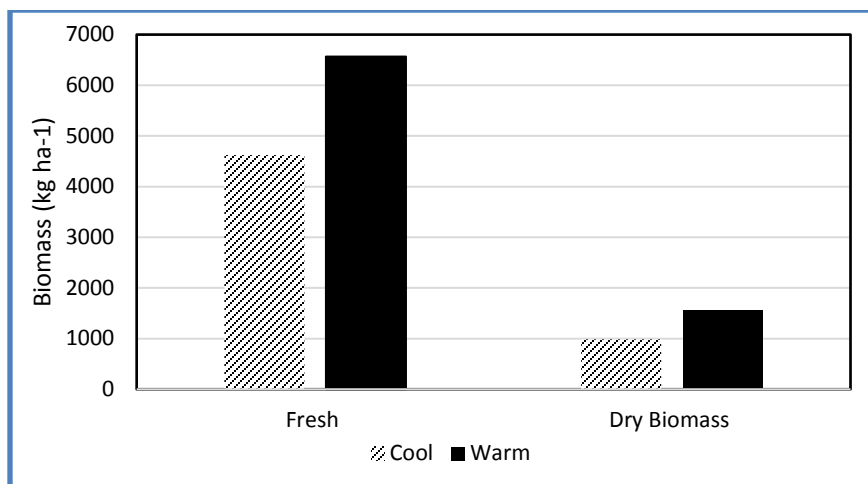


Figure 1. Mean fresh and dry biomass ( $\text{kg ha}^{-1}$ ) of cool and warm season cover crops at dryland in Sidney.

- As a collaborating center for herbicide carryover project, EARC has planted pea, lentil, and chickpea. Pea and lentil have been harvested and seeds are in seed lab for processing. The chickpea will be harvested in a week time from now.

### Future Plan

- Peas (both yellow and green) collected from the experiments located at various research locations will be run on NIR and also near-infrared spectral (reflectance spectra) analysis instrument.
- Soil moisture content and precipitation data will be obtained from the different centers and water use efficiency of the different dry pea genotypes will be calculated.
- The greenhouse studies will be started to evaluate the selected varieties for nitrogen fixation and to find out if various treatment effects on pea protein content and yield. Nitrogen fixation project will help us to better understand the factors affecting plant growth, protein content, and yield. It will also provide us understanding if there is any influence of nitrogen fixation on protein content of peas.
- Dry pea, lentil and chickpea samples for herbicide carryover study will be processed in the seed lab and data will be submitted to coordinating center (SARC).
- Samples from cover crop study will be shipped soon to coordinating center (NARC) for forage quality testing.

### Hiring

- Summer students were hired to help field work.

### Expenditures

- Total Personnel Services: \$31,895.32
- Total Operations: \$11,447.40

### Soil Microbiology and Pea Protein subproject of the Agriculture MREDI Grant

1) 41W212 – Principal Investigator: Perry Miller, Email: [pmiller@montana.edu](mailto:pmiller@montana.edu)

### Progress towards milestones

Additional combustion and NIR analysis was done in step e of the project and are highlighted below:

#### **Combustion and NIR Analysis—step e.**

The aforementioned field samples (n=81) have been analyzed for protein content using combustion analysis (LECO analyzer). The specific method measures percent grain nitrogen (N) on a dry weight basis, and a multiplier of 6.25 is used to convert grain N to protein percentage. Each field sample has been run in duplicate (i.e. two separate subsamples of 50 seeds) to account for within sample variation. The mean protein content for field samples is 23.4, but more importantly, the mean range and standard deviation in subsampled duplicate pairs are -0.12 % and 1.49 % respectively (Fig 1.).

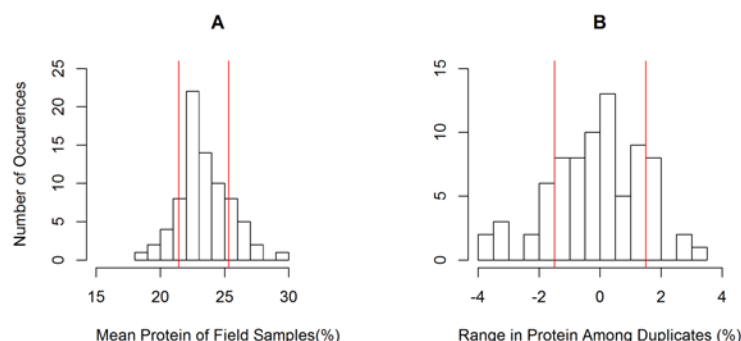


Fig 1. Distributions of mean yellow pea protein content of analyzed field samples (A) and range in subsampled duplicates from field samples (B). Vertical lines are  $\pm 1$  SD on the mean.

The high standard deviation (~1.49 %) in mean range among duplicates suggests that there is potential for high within sample variation. Within sample variation likely arises from both inter and intra-plant variation. All-Khan and Youngs (1972<sup>1</sup>) observed protein could range to ~9% among plants, and Atta et. al. (2004<sup>2</sup>) showed that intra-plant protein could range by ~10% depending on nodal position and variety. Such variability needs to be addressed for two reasons:

1. Mean protein responses produced from combustion analysis are used as points for NIR calibration curves. With potential for high variability among calibration points, NIR calibration will not be precise. If NIR becomes the standard for measuring yellow pea protein and awarding premiums on an industrial scale, it will be critical to ensure precise NIR calibration so that premiums are awarded fairly.
2. Large measurement uncertainty in protein response will reduce statistical power for identifying how M x E interactions affect protein content in pea.

An experiment was conducted to test if different subsample sizes (seed number) or number of subsample replicates (one or two) could reduce within sample variation for measuring protein on LECO. Specifically response of interest was the absolute difference in protein measurements between a 500-seed sample (assumed to be the best representative protein measurement of the bulk sample) and the protein measurements associated with the individual and the duplicate means of 50-, 150-, and 250-seed subsamples (see attachment 2 for detailed methodology). The results of the experiment are summarized below.

1. Absolute differences in protein were smallest between the 500- and 250-seed samples (0.51%) and increased with decreasing seed number (Fig 2. A.).
2. Absolute differences in protein between the 500-seed samples and individual or duplicate subsample averages did not statistically differ (Fig 2. B.).
3. Most variation in bulk samples can be attributed to different seed lots. That is, some lots showed small absolute differences in protein between the 500-seed sample and the seed number x subsample number combinations, whereas other lots showed large differences.

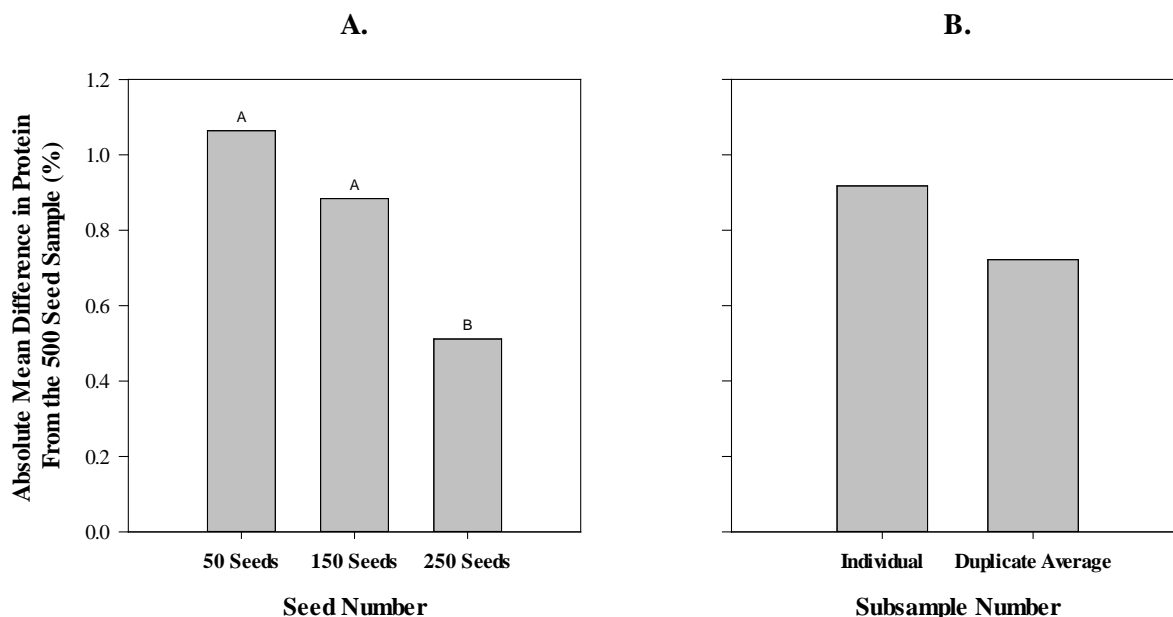


Figure 2. (A) Mean absolute difference in protein content between the 500 seed sample and seed number. (B) Mean absolute difference between the 500 seed sample and subsample number. Different letters indicate statistical differences at the  $\alpha=0.05$  level.

Based on the results of the experiment, we initially recommend that MSU researchers measure yellow pea protein with the combustion method and a subsample size of 250 seeds until formal NIR calibrations have been

established. A 250-seed subsample can efficiently be ground in an Udy Mill, and measuring protein via combustion will eliminate uncertainty that may arise from different NIR instruments currently in used at the Bozeman campus and the MAES centers throughout the state.

Formalizing a reliable NIR calibration will require a) obtaining seed lots with low within sample variation, and b) using the obtained seed lots to generate a NIR calibration from corresponding protein measurements derived from combustion analysis. Steps a. and b. are outlined below:

- a. Obtaining seed lots with low within sample variation—Uncalibrated NIR readings will be used to screen for seed lots with low within-sample variation. Specifically NIR is capable of taking 10 subsample measurements per seed lot. Seed lots that show low standard deviations based on the 10 uncalibrated NIR measurements will be withheld for subsequent NIR calibration and validation.
- b. Calibrating and Validating NIR—When enough seed lots with low within sample variation have been identified (~100 lots), a subsample of 250 seeds will be removed from each lot, ground, and analyzed for protein via LECO. Whole seed lots will then be split into a training (~85 lots) and test set (~15 lots) (Matt Clancy, personal communication, June 2016). The training set and corresponding LECO measurements will be used to formally calibrate the NIR. The NIR calibration will then be validated using the observed and predicted protein values corresponding to the test set. Validation will be evaluated based on the coefficient of determination ( $R^2$ ) and root mean square error (RMSE) between observed and predicted values of the test set.

Near infrared (NIR) calibration will initially be conducted using the FOSS machines in the Cereal Quality Lab in Bozeman. If satisfactory NIR validation is established in Bozeman, we recommend that the training and test sets be distributed to the MAES centers with NIR capability, and we recommend those instruments are calibrated following the procedure outlined herein. This will help guarantee consistent NIR documentation and calibration and for future MSU research purposes pertaining to yellow pea.

### Definitions

*Bulk Sample:* Refers to a yellow pea sample

*Subsample:* Refers to a smaller proportion of the yellow pea bulk sample.

*Duplicate:* Refers to two subsamples taken from the same bulk sample.

### Scope and Objective

Variation in yellow pea protein may arise from inter and intra-plant variation. Early Canadian work showed interplant seed protein ranges of 21.8-30.0 % to 24.2-25.7% depending on cultivar (Ali-Khan and Youngs, 1972<sup>1</sup>). Similarly Atta et. al. (2004<sup>2</sup>) observed that seed protein content could vary by ~10% depending on nodal position and cultivar in Europe.

Preliminary measurements made on 81 yellow pea bulk samples using combustion analysis (LECO Corporation, St. Joseph, MI<sup>3</sup>) suggests that similar variation in seed protein content exists in current Montana varieties. Summary statistics and the distribution of difference between duplicate protein measurements (~50 seeds per subsample) are shown in Table 1.

*Table 1. Summary statistics for difference in duplicated protein content from initial LECO analysis. All units are in percent. (n=81).*

Minimum	1 <sup>st</sup> Quantile	Median	Mean	3 <sup>rd</sup> Quantile	Maximum	STD
-3.83	-1.14	-0.04	-0.12	1.04	3.21	1.49

Assuming differences in protein measurements are normally distributed, it can be expected that 68% of future duplicate measurements will have a range less than or equal to 1.5 % whereas the remaining 32% will exceed 1.5%. This level of variability indicates that duplicate averages may not precisely reflect the true mean protein



content of yellow pea bulk samples. Gaining a more precise estimate of mean protein content is important on two fronts. Namely:

1. Mean protein responses produced from combustion serve as points for NIR calibration curves. With potential for high variability among calibration points, NIR calibration will not be precise. If NIR becomes the standard for measuring yellow pea protein and awarding premiums on an industrial scale, it will be critical to ensure precise NIR calibration so that premiums are awarded fairly.
2. Large measurement uncertainty in protein response could reduce statistical power for identifying how management variables such as crop rotation, inoculant types, and starter nutrients affect protein content in yellow pea.

Potential avenues to increase precision on bulk sample estimates with combustion analysis are a) increase subsample size (i.e. increase the number of seeds per subsample) or b) increase subsample number. The objective of this experiment is to a) determine how subsample number and size affects precision on mean bulk protein estimates and b) determine if subsample number, subsample size, or sample source (e.g. seed lot) results in the greatest variation in protein measurements.

## Methods

### **Sample Population**

The sample population constituted 10 yellow-pea bulk samples sent to the Montana State Seed Lab over the 2015 fiscal year (July 1, 2015-June 31, 2016) from Montana farms. The ten bulk samples were randomly collected from the seed lab for the experiment.

### **Response of Interest, Treatment, and Statistical Model**

Each of the 10 selected bulk samples (ie. replicates) were randomly subsampled twice at 50, 150, and 250 seeds per subsample and a final time at 500 seeds per subsample. Subsamples were ground in an Udy Mill with a 1 mm screen and run on LECO for total nitrogen (N). Nitrogen content was converted to seed protein as  $N \times 6.25$  on a dry weight basis.

The response of interest was the absolute difference in protein measurements between the 500 seed sample (assumed to be the best representative protein measurement of the bulk sample) and the protein measurements associated with individual and the duplicate means of 50, 150, and 250 seed subsamples. This constituted a two-factorial experiment with the following factor level combinations.

1. An individual subsample of 50 seeds
2. An individual subsample of 150 seeds
3. An individual subsample of 250 seeds
4. The duplicate mean of 50 seeds per subsample (*current sampling procedure*)
5. The duplicate mean of 150 seeds per subsample
6. The duplicate mean of 250 seeds per subsample

Random sampling was implemented to select the individual subsample that was placed at factor level combinations 1-3.

The statistical model to test how seed number ( $\tau_i$ ) and subsample number (e.g. individual or duplicated means) ( $\alpha_j$ ) affected protein differences from the 500 seed subsample was:

$$y_{ijkl} = \mu + \beta_i + \tau_j + \alpha_k + \tau\alpha_{jk} + \varepsilon_{ijkl} \quad \text{Eq 1.}$$

Where  $y_{ijk}$  is the absolute difference in protein measurements,  $\beta_i$  is the random block effect corresponding to each of the 10 bulk samples,  $\tau_{\alpha_{jk}}$  is the fixed interaction of seed and subsample number on protein differences, and  $\varepsilon_{ijk}$  is the error term assuming model residuals are  $N(0, \sigma)$ . Treatment effects were considered significant at the  $\alpha=0.05$  level, and post-hoc multiple comparisons were made via a Tukey's test. A Levene's test was conducted to verify homogeneity of variance assumptions at the  $\alpha=0.05$  level. A random effects model using the same mean structure as Eq 1. was lastly run to estimate variance components by REML. This was done to quantify if more model variability was associated with the different sampling procedures ( $\tau_i, \alpha_k, \tau_{\alpha_{jkl}}$ ) or from the different bulk samples ( $\beta_j$ ). All statistical analyses were conducted using the SAS/GLM procedure (SAS Institute Inc., Cary, NC<sup>4</sup>).

## Results and Discussion

### Effect of Sampling Routine on Protein Measurements

Seed number resulted in highly significant absolute protein differences from the 500 seed sample, while there was moderate statistical evidence suggesting subsample number affected protein responses (Table 2.). The seed number x subsample interaction was not significant.

Table 2. ANOVA Table corresponding to Eq. 1.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block ( $\beta_i$ )	9	11.344362	1.260485	5.87	<.0001
Seed Number ( $\tau_j$ )	2	3.17953	1.589765	7.4	0.0017
Subsample Number ( $\alpha_k$ )	1	0.572937	0.572937	2.67	0.1094
Seed Number x Sub-Sample Number	2	0.066266	0.033133	0.15	0.8575
Error	45	9.666628	0.214814		

Mean absolute differences between the 500 seed sample and the individual subsample of 50, 150, and 250 seeds were 1.06%, 0.88% and 0.51% respectively. Only the 250 seed sample resulted in significantly lower protein responses compared to the 50 and 150 samples (Fig 1.A.). Mean absolute differences in protein responses for the individual and duplicate averaged subsamples were 0.91% and 0.72% respectively but did not significantly differ (Figure 2. B.).

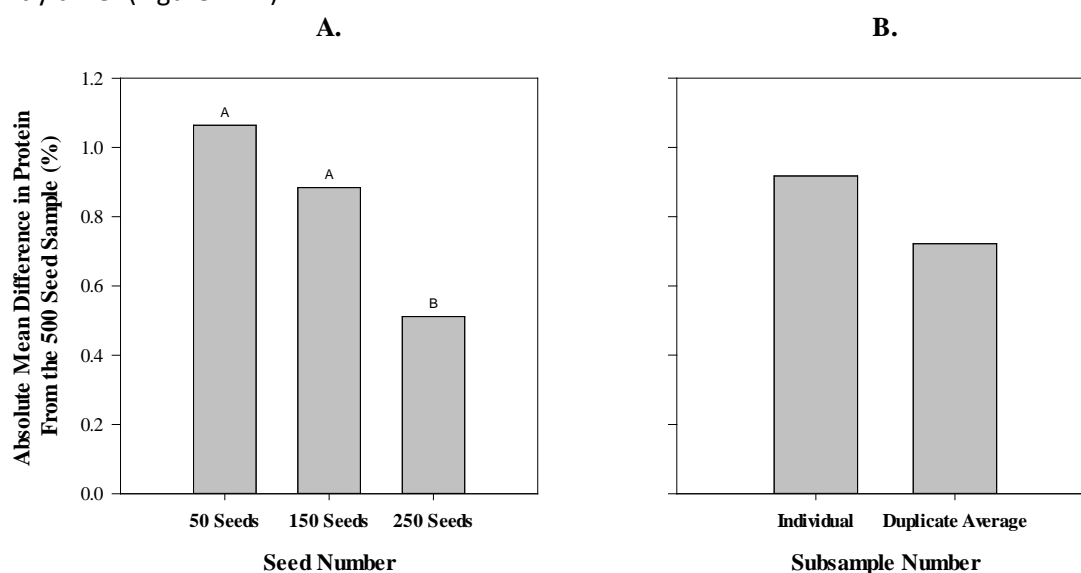


Figure 2. (A) Mean absolute difference in protein content between the 500 seed sample and seed number. (B) Mean absolute difference between the 500 seed sample and subsample number. Different letters indicate statistical differences at the  $\alpha=0.05$  level.

In practical terms, these results indicate that increasing subsample size will better reflect the *true* bulk-sample protein content relative to increasing subsample number. More specifically increasing subsample size to 250 seeds will on average differ by 0.51% from the *true* bulk-sample protein content whereas a subsample size of 50 or 150 seeds will differ by 0.97%.

Even with a subsample size of 250 seeds, importantly, an average difference of 0.51% will make it difficult to validate NIR calibrations and could explain why Tkachuk et al., (1987<sup>5</sup>) was only able to achieved a standard estimate error of (Sy) 1.34 % when validating NIR from duplicated protein measurements using the macro-Kjeldahl method (Figure 2.).

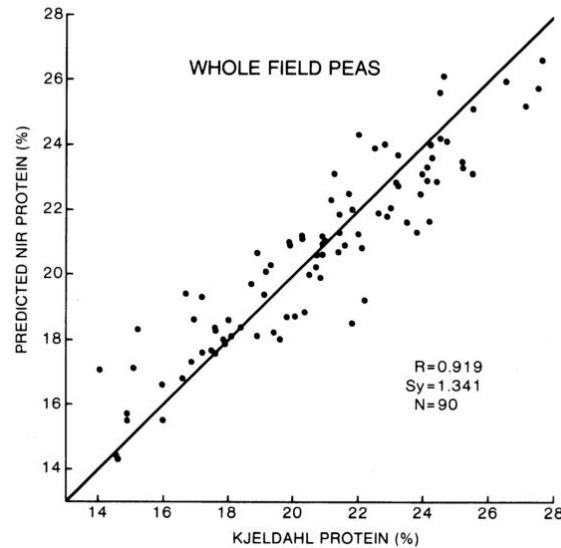


Figure 3. One-to-one line of predicted protein content from NIR analysis and observed protein content based on duplicated measurements using the macro-Kjeldahl measurements taken from Tkachuk et al., (1987). The scatter about the line could be due to the sampling routine or variable protein content from bulk samples.

**Variance Components Estimates for Bulk Sample and Sampling Routine**

The variance component estimate for bulk sample was roughly two and a half times and fifteen times greater than those for seed number and subsample number respectively (Table 3.). The variance component estimate for the seed number x subsample number interaction was essentially zero. This means that the variability in protein responses arises mainly from different bulk samples—not seed or subsample number. In other words, the relatively high variance component estimate for Bulk Sample indicates that seed protein content was not uniform across seed lots.

Table 3. Variance component estimates associated with the random effects model given by Eq 1.

**REML Estimates**

Variance Component	Estimate
Bulk Sample or Block	0.17557
Seed Number	0.06913
Sub-Sample Number	0.01220
Seed Number x Sub Sample Number	0.00000
Error	0.20708

Variation in bulk sample seed protein could result from any combination of the following.

1. Plant Genetics—In certain varieties, individual seed protein has been observed to range by as much as 10% depending on nodal position whereas individual seed protein remains constant in other varieties (Atta et. al. 2004).
2. Interplant Variation—Different pea plants collected from small research plots have produced seed protein ranging from 21.8-30.0 % (Ali-Khan and Youngs, 1972).
3. Bulk Sample Source—It is unknown if the bulk samples initially sent to the seed lab were representative of a single or multiple fields. If the bulk samples were a composite of multiple fields, it is feasible that management (e.g. seeding date, variety selection etc.) may have been inconsistent across fields which could add to protein uncertainty.

Ensuring more uniform protein within each bulk sample will help a) establish if different sampling procedures affect protein measurements and b) provide a stronger NIR calibration sample set.

<sup>1</sup>Ali-Khan, S.T., and C.G. Youngs. 1972. Variation in protein content of field peas. *Can. J. Plant Sci.* 53: 37–41.

<sup>2</sup>Atta, S., S. Maltese, and R. Cousin. 2004. Protein content and dry weight of seeds from various pea genotypes. *Agronomie* 24: 257–266.

<sup>3</sup>LECO Corporation. 2004. St. Joseph, MI.

<sup>4</sup>SAS Institute Inc. 2012. Cary, NC.

<sup>5</sup>Tkachuk, R., F.D. Kuzina, and R.D. Reichert. 1987. Analysis of protein in ground and whole field peas by near-infrared reflectance. *Cereal Chem.* 64(6): 418–422.

### **Summary and Moving Forward**

Variation in yellow pea protein makes it challenging to accurately estimate the mean protein content of a bulk sample by combustion analysis. Increasing subsample size to 250 seeds will help better control for within sample variation compared to using duplicate subsamples. However, results from this experiment indicate that the mean protein content of a 250 seed subsample could misrepresent the *true* bulk sample protein content by 0.51 %, which is likely not suitable for NIR calibration.

Most variation between measured and *true* protein (e.g. 500 seed sample) content arises from different bulk samples relative to seed and subsample number. This means it will be critical to identify specific bulk samples with low within sample protein variation for NIR calibration. Once bulk samples with low within sample variation have been identified, a 250 seed subsample from each bulk sample can be run on LECO for subsequent NIR calibration.

The NIR instrument has potential to help identify bulk samples with low within sample variation. Specifically, NIR is capable of making ten protein measurements per bulk sample (~400-500 gram bulk samples). Bulk samples with low variances or standard deviations based on uncalibrated NIR readings are more likely to be those with low within sample protein variation.

Moving forward, we will use uncalibrated NIR readings to identify bulk samples with low within sample protein variation. We will then run 250 seed subsamples from identified bulk samples on LECO and attempt to formally calibrate NIR.

Intermittently we recommend that a subsample size of 250 seeds should be ground in an Udy mill, and yellow pea protein measurements should be made via combustion analysis. A subsample of 250 seeds is a feasible sample size that can efficiently be ground in an Udy mill. Further determining protein using the combustion method could reduce measurement uncertainty arising from different NIR calibrations used throughout the state. This will ensure consistency across experiments in which yellow pea protein is a response variable.

## Hiring

- No additional hires in Quarter 4.

## Expenditures

- Total Personnel Services: \$36,499.09
- Total Operations: \$2,839.60

2) **41W220 – Principal Investigator:** John Peters; Email: [john.peters@chemistry.montana.edu](mailto:john.peters@chemistry.montana.edu)

## Progress towards milestones

### **Background and Objectives**

Pea and pulse crops are increasingly being used in agriculture because of their economic and environmental benefits. Pea is a legume that has a symbiotic relationship with nitrogen fixing bacteria and is often planted to mitigate nitrogen loss from the soil, reducing the need for fertilizer. Other benefits of pulse crops include improvement of soil quality because of increased microbial diversity, breaks in disease cycles, and high return crops such as peas for human consumption. 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. 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.

We have started analysis of soil samples from a pea trial study from Montana Agricultural Experimental Stations and soil and rhizosphere samples from a wheat/pea rotational study at the Post Farm in Bozeman, MT. The pea trial study consists of seven pea varieties and has locations across the state making it possible to study geographical differences. Bulk soil from the Post Farm was sampled in two week intervals after harvest in 2015 and the wheat rhizosphere sampled in spring of 2016 with the objective of capturing changes over time and gain insight into the wheat/pea rotational dynamic.

This project has multiple objectives. With the pea trial study, we are interested in better understanding the soil microbial diversity, nitrogen fixing diversity, nutrient, and pea variety combinations that produce the best yields. With the Post Farm, we are interested relationships between treatment, fertilizer, microbial diversity, and the diversity of nitrogen fixing bacteria over time. We are also interested in characterizing the soil microbial community during the rotation from pea to wheat. Further, we are evaluating the inoculation of wheat wild-type and ammonia excreting strains of a common non-symbiotic nitrogen fixing organism *Azotobacter vinlandii*, in an effort to understand the effects of this strain on wheat health and yield. This was conducted in the green house using soil from the MSU Bozeman Post Farm and that has just very recently come to completion. We are analyzing phenomic data and have conducted molecular analysis of the microbiome dynamics over the course of wheat germination and early stage growth. This is a unique study to evaluate inoculants, in controlled conditions with soil taken from a long-term agricultural use site.

The overall objective of 2016 is to use multivariate statistics to analyze the immense amount of data we have collected from soil and molecular analysis to gain insight into a key microbial and micronutrient factors associated with high crop productivity. The initial analysis of the data is presented in this report.

### **Sampling locations and study sites:**

*MSU Bozeman Post Farm:* The Post Farm is a wheat/pea rotational site. In 2015 plots were WPman = winter pea left on site for manure, WPfor = winter pea planted for forage, WP = winter pea, organic = tilled and organic, WW = winter wheat, chem fallow- left fallow and treated with chemicals, till fallow = fallow and tilled, canola = canola. Each plot, except organic, was treated with high and low amounts of fertilizer. We sampled soil from

these plots every two weeks after harvest for a total of six time points. In 2016 all plots were planted with winter wheat. In spring of 2016 we sampled the rhizosphere in each plot.

*Pea Trial Study:* The pea trial study being conducted at Montana Agricultural Experimental Stations throughout the state including Northern, Western, Southern, Western Triangle, Eastern, Central and Northwestern. For this study seven yellow and green pea varieties were planted including, Early Star, Daytona, Agassiz, CDC Saffron, DS Admiral, Delta, and Marjoret. Summer samples from all stations are arriving and are being stored for analysis.

**Progress: 05/20/16-08/20/16**

**Microbial community and chemistry analysis**

*Statewide study:* We first analyzed our large data set by using a multiple linear regression model where we can compare multiple variables to yield in order to see correlation without bias. As presented in the table below regression statistics that show the variables in relation to crop yields. From our preliminary analysis moisture, potassium and vanadium appear to have the most significant impact. In order to interpret these results further other single variable comparisons were made in order to check the data.

*Table 1. Variables in relation to crop yields.*

Effect	Coefficient	Standard Error	Std. Coefficient	Tolerance	t	p-Value
MOISTURE CONTENT	149.428	50.822	0.840	0.009	2.940	0.006
ALPHA DIVERSITY	8.380	4.376	0.493	0.011	1.915	0.065
OTUABUNDANCE PHYLUM	-0.007	0.132	-0.011	0.019	-0.055	0.957
BARIUM	-1.369	4.231	-0.093	0.009	-0.323	0.749
CALCIUM	0.018	0.020	0.140	0.030	0.890	0.381
CHROMIUM	43.620	69.195	0.350	0.002	0.630	0.533
COBALT	229.334	197.049	0.677	0.002	1.164	0.254
COPPER	64.548	87.029	0.355	0.003	0.742	0.464
IRON	0.032	0.038	0.225	0.010	0.852	0.401
LEAD	-6.033	13.976	-0.047	0.063	-0.432	0.669
MAGNESIUM	-0.285	0.154	-0.750	0.004	-1.856	0.074
MANGANESE	-3.289	2.446	-0.499	0.005	-1.345	0.189
MOLYBDENUM	35.696	94.985	0.020	0.264	0.376	0.710
NICKEL	-58.386	52.175	-0.459	0.004	-1.119	0.272
PHOSPHORUS	4.431	2.548	1.139	0.002	1.739	0.093
POTASSIUM	1.445	0.624	1.675	0.001	2.318	0.028
SULFUR	0.916	1.665	0.174	0.007	0.550	0.586
VANADIUM	-163.420	66.603	-2.102	0.001	-2.454	0.020
ZINC	3.009	18.607	0.078	0.003	0.162	0.873
AVAIL PHOSPHORUS	-50.770	26.628	-0.355	0.021	-1.907	0.067
AVAIL POTASSIUM	1.153	4.586	0.095	0.005	0.252	0.803
BORON	-1,028.058	1,048.959	-0.210	0.016	-0.980	0.335
NITRATE/NITRITE	-18.086	61.986	-0.042	0.035	-0.292	0.773
AMMONIA	151.366	84.355	0.188	0.066	1.794	0.083
ORGANIC MATTER	5.954	26.263	0.014	0.182	0.227	0.822
PH	-326.479	209.206	-0.922	0.002	-1.561	0.129
SULFATE/SULFUR	.	.	.	.		

### Single Variable Analysis

A basic analysis of the single variable data has been performed in order to understand large trends throughout the statewide and post farm samples. Simple single variable approach allows a grasp on the data and sets up questions that will lead to a larger multi variable study. These tests are susceptible to the data dredging bias and need to be confirmed by further larger scale analysis.

The state-wide study contains 16s abundance and chemical data from soil samples. We have compared certain variables that could test hypotheses that have been presented in the past. In order to better understand the differing amount of bacterial classes  $\alpha$ -diversity was used to express the amount of classes present in the soil. We see that there is a statistical significance between the farm stations and the diversity of the soil ( $P < 0.001$ ) (Figure 1). Yield varies significantly in-between sites (Figure 2), in order to see if the  $\alpha$ -diversity has any effect on yield we have compared the two variables. By plotting yield of the peas against the  $\alpha$ -diversity of the soil we see correlation ( $p = 0.035$ ,  $R^2 = 0.0808$ ) (Figure 3). With such a low  $R^2$  value we cannot say that  $\alpha$ -diversity and yield have a linear relationship but there is a correlation between the two. We are currently attempting to analyze the data with a larger sample size and a reduction of outliers in order to better understand the relationships. Variety of different peas in respect to yield and diversity show little change from the site-based analysis. No noticeable grouping of different varieties are seen in the plot at which can be interpreted that location is more responsible for yield than variety. This can also be shown in the standard plot of variety and  $\alpha$ -diversity (Figure 4). These results can give a larger look on the effect of a diverse soil micro-environment to the yield. First we can see that there a significant change in yield due to the  $\alpha$ -diversity of the soil. Also there is no change in diversity of the farm soil due to variety of pea. This leads to a more complex question of what type of diversity affects the yield of peas.

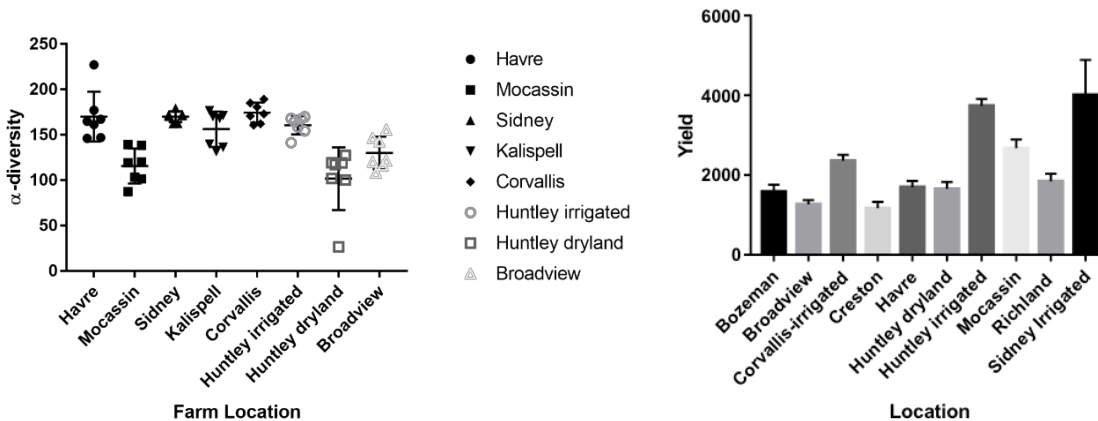


Figure 1. The comparison of alpha diversity in between different locations. There is a slight difference of diversity between different locations.

Figure 2. A difference in yield is seen in between locations around the state. Some yield is different due to irrigation and precipitation differences. There could be multiple variables that lead to these differences in yield.

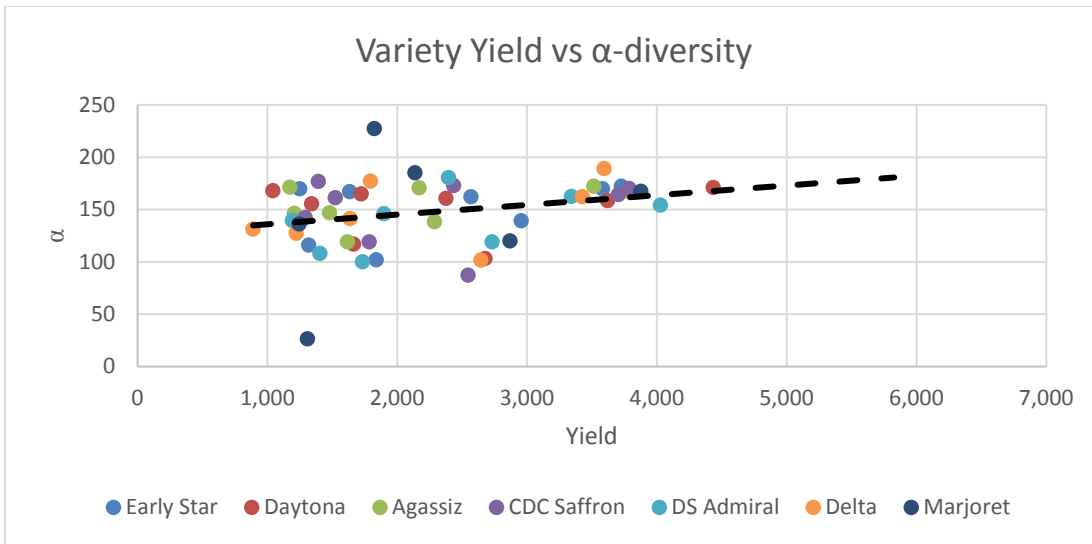


Figure 3. Variety yield and  $\alpha$ -diversity are correlated ( $p=0.035$ ,  $R^2=0.0808$ ) when compare by linear regression. Location grouping cannot be seen therefore variety does not have an effect on diversity or yield.

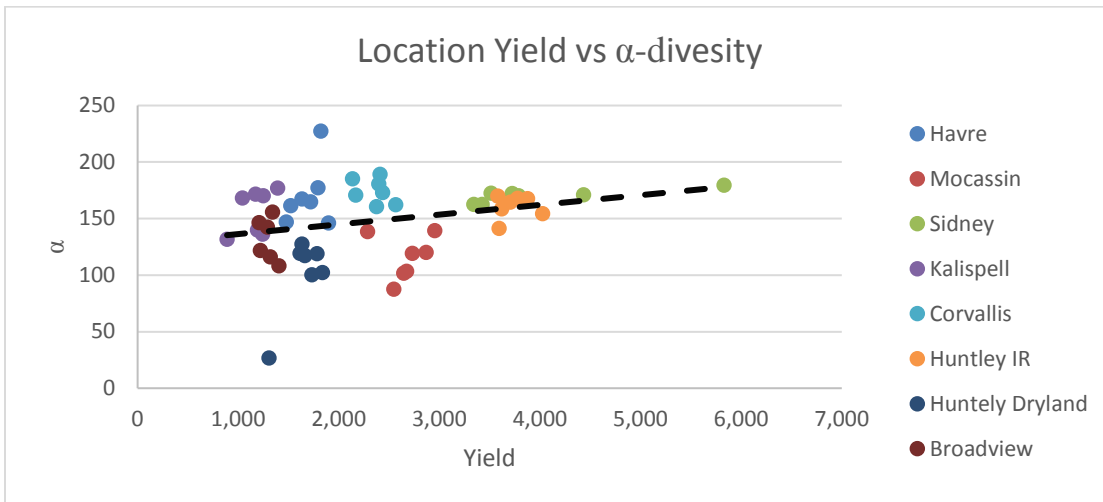


Figure 4. Location yield and  $\alpha$ -diversity are correlated ( $p=0.035$ ,  $R^2=0.0808$ ) when compared by linear regression. Location grouping can be seen, meaning yield and diversity are dependent on location.

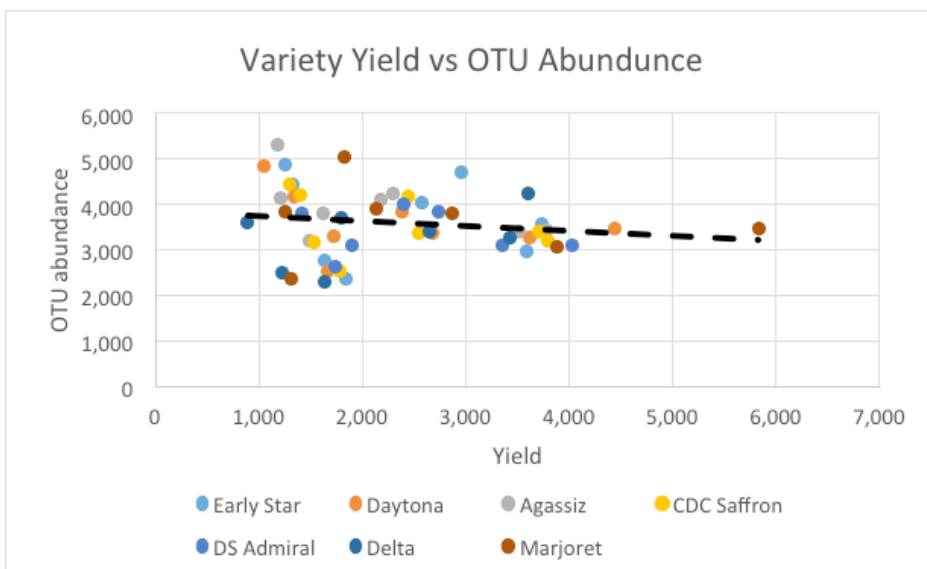


Figure 5. Variety yield and OTU abundance is negatively correlates showing high abundance of bacteria might inhibit yield. No grouping of variety can be seen. Showing that location is more important in abundance and yield.



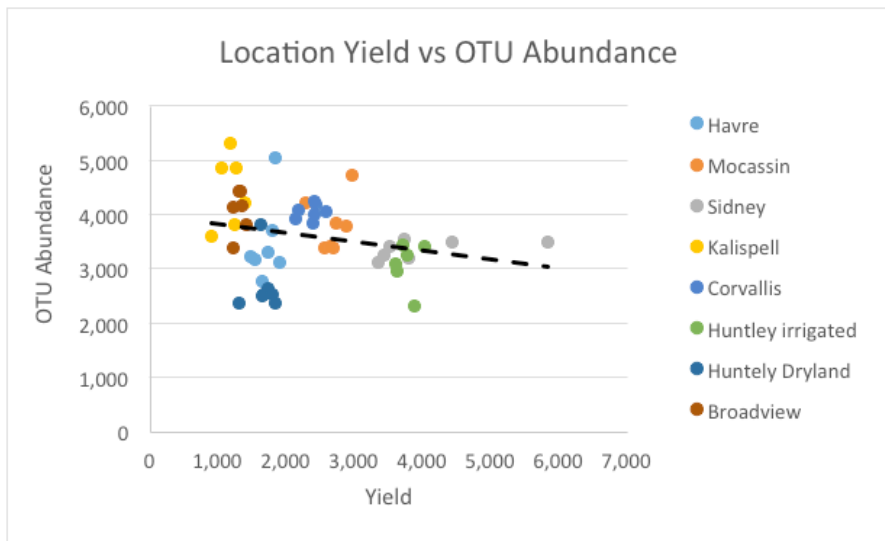


Figure 6. Location yield and OTU abundance are negatively correlated ( $P < 0.001$ ) meaning the large amount of OTU's will inhibit the yield of the peas. There is grouping of the locations. Showing the Location is determining yield more than variety.

OTU abundance can also be used as an indicator of the amount of bacteria in the samples. There is a change in the abundance of OTU across the different sites. OTU is not the best indicator of diversity because there could be multiple OTUs from a single genus or species of bacteria. This indicator can give us an estimate of the abundance of bacteria in the soil relative to other sites. Since there is a negative correlation in between OTU abundance and yield we can suspect that there is some over population in the soil that is out competing (more OTU's) and disturbing the diversity of the soil (Figure 5 and 6). This could lead to an interesting hypothesis that there is a relationship between microbiome composition, soil chemistry, and crop yield.

As anticipated, soil nitrate in the soil and yield are positively correlated. This should be seen in the statewide farms and can lead to further proof that certain bacterial classes might be correlated with nitrite/nitrate levels which lead to better yields. The first step of nitrification is the production of nitrite which is performed by ammonia oxidizing bacteria. The relative abundance of nitrite in the soil will have an effect on the plants ability for nitrogen uptake and yield. Analyzing the nitrate+nitrite abundance vs ammonia abundance as it pertains to yield can lead to conclusion on what types of soil bacteria are having an effect on the nitrogen availability and balance in these different environments (Figure 7). These chemical analyses will be reinforced by looking at specific genus level diversity of diazotrophs in each soil. The analysis will also benefit by the specific molecular analysis to probe for diazotrophs.

With the understanding of the simple variables involved with crop yield we can now analyze large scale correlations. In order to determine what variables affect  $\alpha$ -diversity and therefore effecting yield we must look a groupings of the bacteria phylum that are involved.

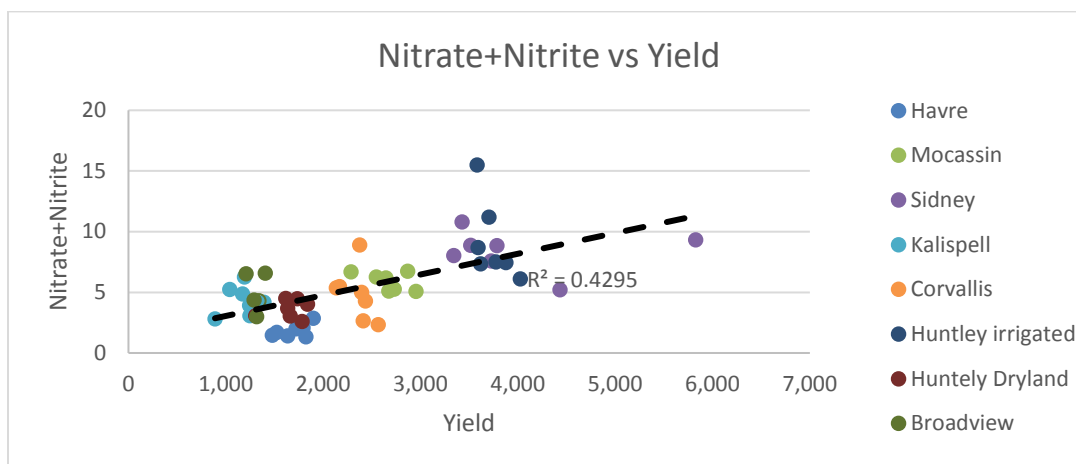


Figure 7. Nitrate+Nitrite values are correlated to yield ( $p < 0.001$ ,  $R^2 = 0.4295$ ) Showing that the higher concentration of nitrates will lead to better crop yield. This should be correlated and known previously. This measurement is of interest in future analysis on the changing of nitrate+nitrite compared to the difference of microbial diversity. Also a study on the difference of nitrate+nitrite over time through cycles of pulse crop rotation.

### Microbiome composition across the state

By applying non-metric multidimensional scaling (NMDS) we can articulate the dissimilarity of bacterial groups in different location. This allows us to see the difference in the  $\alpha$ -diversity and can lead to ideas on what groups of bacteria are affecting yield the most.

From the data presented we can see that there is a clustering of bacterial classes that is particular to each of the farm stations. A good example of this is Sidney station (Blue circle Figure 8) which shows a close cluster of OTU similarity for the site. Certain branches of the tree (Figure 9) have a potential to being a diverse community that will produce a better yield. A preliminary analysis of these data sets provides evidence that the composition of the microbiome varies in soils from different cropping scenarios and different soil chemistries across the state (Figure 10). We are in the process of further analyzing the data to provide a detailed understanding of these differences and to understanding the specific relationships between microbiome structure and crop yield.

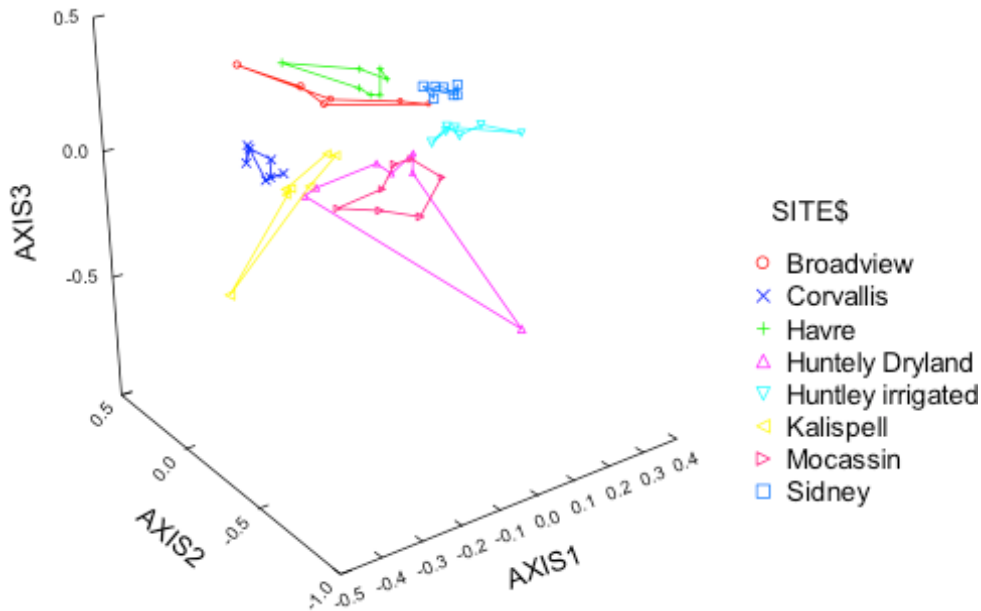


Figure 8. NMDS graph showing clustering of bacterial groups at different locations. This is most obvious in the Sidney farm (blue squares) where there is close clustering meaning bacterial communities have highly coordinated OTUs within its own community.

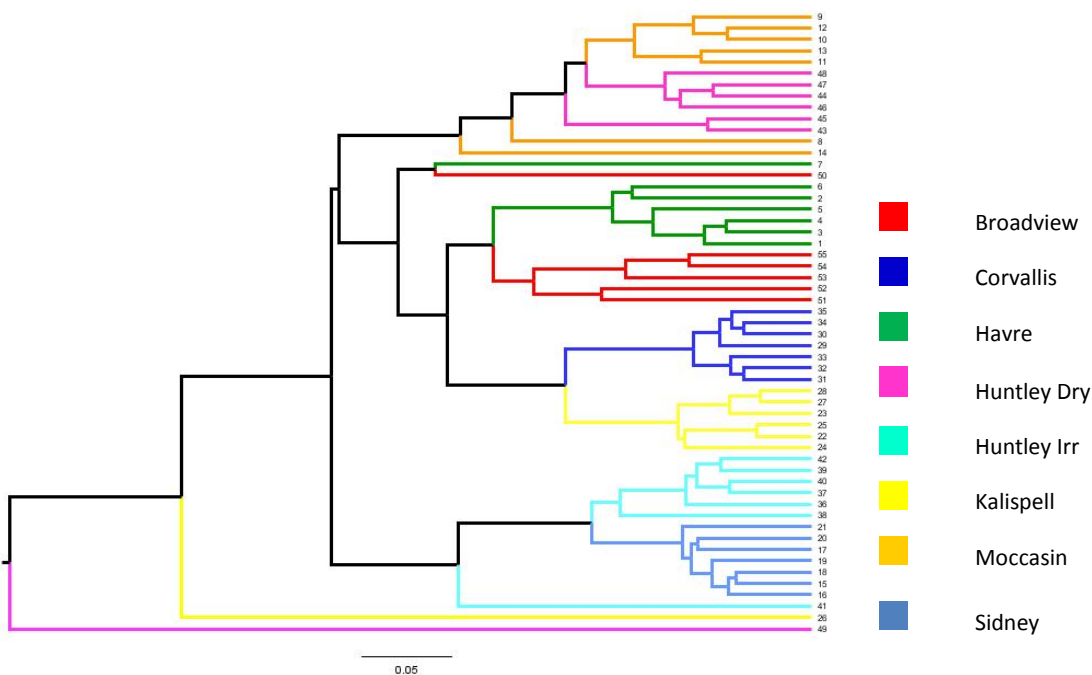


Figure 9. A clustering tree is shown; colors correspond to different location. In different locations there is a similarity between bacterial groups.

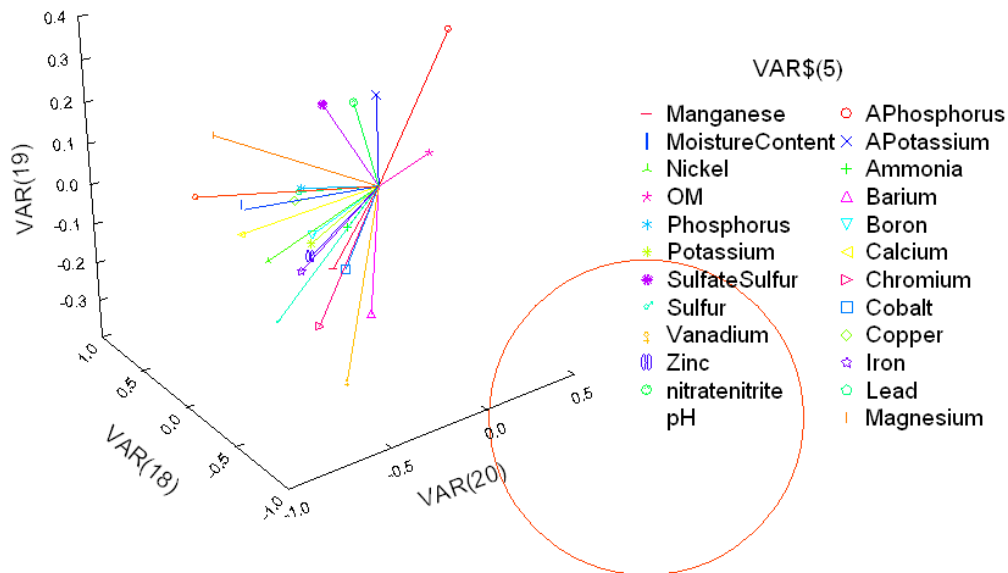


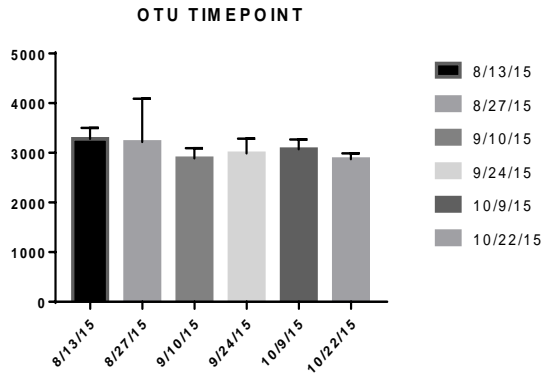
Figure 10. Principle coordinate analysis shows the correlation of different chemistry with the given variables.

### Post Farm

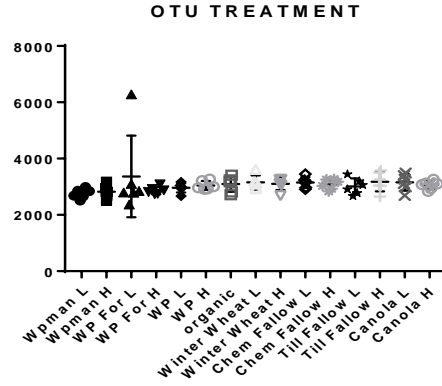
The Post Farm study allows us to conduct experiments under a more controlled environment. It also allows for more time points to be taken and compared. Here we analyze the results of the post farm study by looking at the OTU abundance compared to the treatment and time points. We saw little change in the abundance of bacteria in the soil in the different cropping scenarios (Figure 11A, 11B). The inverse Simpson diversity normalizes the OTU abundance giving a more accurate representation of species diversification in the soil (Figure 11C, 11D). With these numbers we still do not see any change in the amount of diversity due to time or treatment. However, further examination that there are changes in microbiome composition and structure in the different cropping scenarios over time. There is specific clustering of different samples that are due to treatment and time (Figure 12). This is in contrast of the clustering we see in the statewide study which might be due to geographical location. These clustering of soil communities at the Post Farm give evidence that temporal or chemical variables are responsible for shaping of the community structure. A correlation that can be picked out of this preliminary multivariable data is the dramatic difference of community clustering in-between winter pea rotations and tilled rotations (Figure 13). In the tree present the fallow community clusters on the top half of the tree (blue and red) while the winter pea rotation cluster on the bottoms (light blue and pink). This is might lead to evidence of differing the pulse crop leads to different bacterial communities.

The need for a more detailed look into the community structure can be provided with the addition of NifH data. The effects of fertilizer treatments on the NifH operon lead to conclusion not only how fertilizers affect the community structure but also gene regulation. More multivariate analysis needs to be performed to determine which variables are responsible for the changes in community. A more detailed graph will be developed to show the change in community clustering over time.

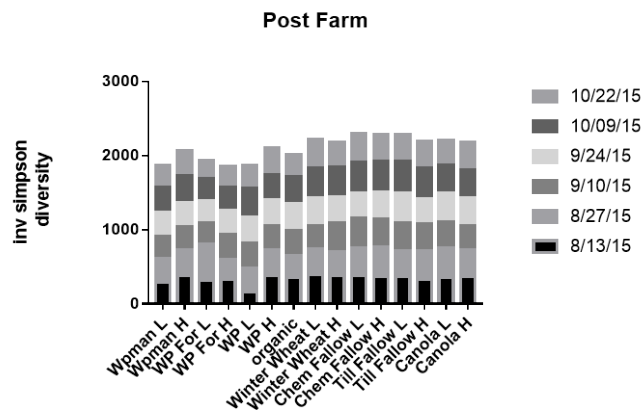
A)



B)



C)



D)

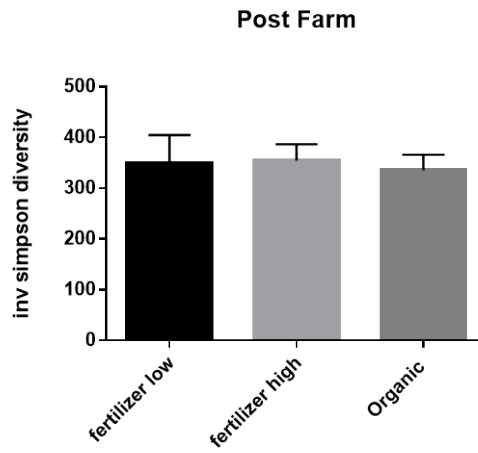


Figure 11. A) The OTU abundance does not change temporally. B) There is no change in OTU abundance between treatments of winter wheat at the post farm. Meaning the size of the bacterial community does not change due to the treatment. C) Inverse Simpson diversity does not change over treatment time or temporally. D) The amount of fertilizer does not have an effect on the inverse Simpson diversity.

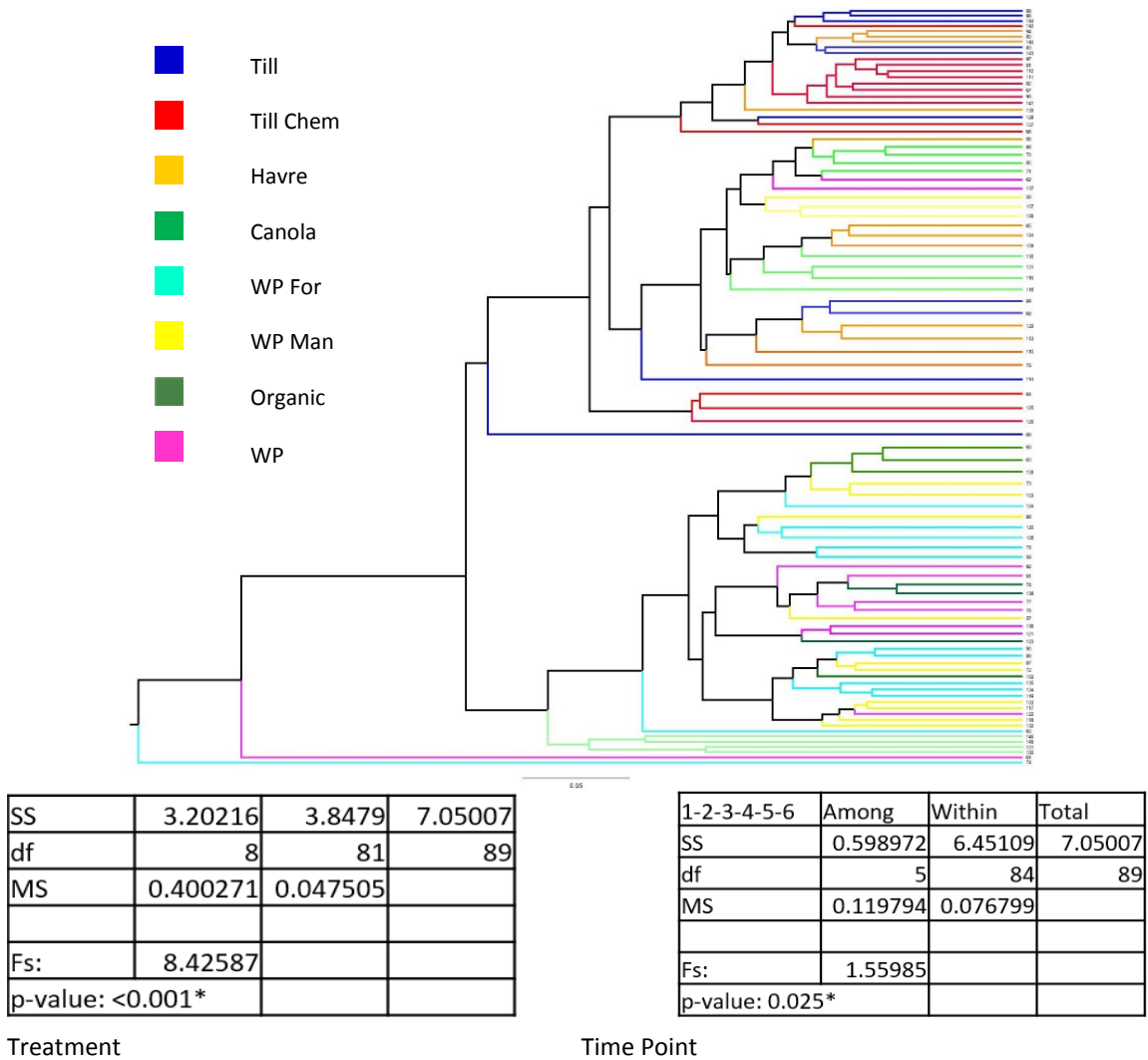


Figure 12. A change of community structure can be shown as significant due treatment and temporal variables ( $P < 0.001$ ,  $P = 0.025$ ). In the tree produced we can see clustering of classes due to different treatments.

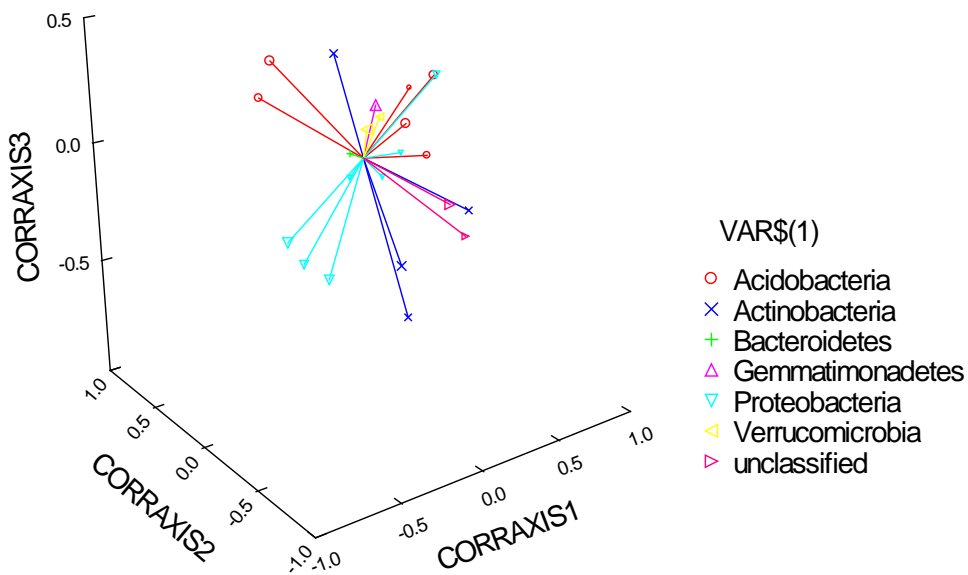


Figure 13. The NMDS analysis shows clustering of the phylum of bacteria in the post farm soil samples. The longer the vector higher the correlation, Corraxis represent a correlation factor to other variables. As we can see proeobacteria, acidobacteria, and actinobacteria all are highly correlated together.

### **Evaluation of AZBB163**

Since an overarching aim of this project is to understand and evaluate pea crops, a plant used specifically for its nitrogen fixing capabilities, we are also in the process of sequencing NifH. NifH is a gene encoding a sub-unit of the nitrogenase enzyme, some bacteria possess this gene and are able to fix nitrogen. Examples are Rhizobia, the symbiotic bacteria with pea, and *Azotobacter vinlandii*, a free living nitrogen fixing bacteria. Understanding the nitrogen fixing community and diversity will be a key step in interpreting factors affecting pea yield, for the statewide samples, and differences in treatments and fertilizer usage at the Post Farm.

We designed and implemented a greenhouse experiment using the soil sampled from the Post Farm and spring wheat inoculated with an ammonia producing strain of *Azotobacter vinlandii* (AZBB163). For this experiment we have measured germination rate, sampled for rhizosphere microbiome analysis, have sampled leaves to look at fixed nitrogen incorporation, and measured photosynthetic efficiency. This experiment will provide valuable information about the effectiveness of AZBB163 as an inoculant for wheat growth and yield. The data analysis of this project is in progress.

### **Future Work**

For the Post Farm and statewide structure, we will continue to analysis the data for correlation of variables and yield of crops. Goals are to complete analysis of the fall samples by the end of this quarter. We are still in the process of sequencing NifH from all sites and NifH for the Post Farm rhizosphere. The goal is to organize a researcher and complete sequencing in order to begin analysis. Understanding the NifH community and diversity will provide insight into the variability of soil chemistry at all study sites.

We will continue the AZBB163 greenhouse experiment and analyze the results which can lead to key insights into using an ammonia excreting strain as a wheat seed inoculant. This project will ultimately give a big picture idea of the relationship between nitrogen from pea, nitrogen from fertilizer, crop productivity, soil chemistry and the microbial community. Understanding and interpreting this complex list of variables has the potential to be applicable to pea and wheat health and yield and contribute to more sustainable agriculture in Montana. New samples from the statewide study have been stored for future testing. Spring 2016 and summer 2016 are ready for sequencing analysis. These samples can give insight into the change of soil microbiology throughout the year and throughout different crop rotations.

### Hiring

- Graduate student Alex Alleman has been hired to help conduct data analysis.

### Expenditures

- Total Personnel Services: \$48,606.38
- Total Operations: \$14,745.39

### **3) 41W213 – Principal Investigator:** Carl Yeoman; Email: [carl.yeoman@montana.edu](mailto:carl.yeoman@montana.edu)

### Progress towards milestones

We are in the process of organizing a delivery date for the bioreactor and have obtained AACUC and animal use approval to run our animal trial starting the middle of September.

### Hiring

- No additional hires in Quarter 4.

### Equipment

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

Expenditures

- Total Personnel Services: \$42,685.53
- Total Operations: \$15,472.22

**Cover Crop/Grazing subproject of the Agriculture MREDI Grant**

**1) 41W214 – Principal Investigator:** Darrin Boss; Email: [dboss@montana.edu](mailto:dboss@montana.edu)

Progress towards milestones

The statewide Cover crop trial has been harvested for above ground forage biomass yield and forage nutrient analysis. All seven sites were successful in establishing a stand. Varying weather patterns at each location will be the driving force for the year as far as forage above ground biomass at each location. For instance, the Havre site, NARC is over 140% of normal rainfall during the growing season and forage yields unanalyzed out of the field show that increase in rainfall. Other locations were under normal to below normal rainfall patterns. All sites have completed their initial forage harvest for the cool season plantings with most sites have completed the warm season harvest. The samples at each location are being ground to be shipped of for forage quality (NDF, CP and nitrates).

The large plot cover crop termination trial location at NARC, where cover crops are being terminated by either cattle grazing (Figure 1), swathing and haying operation or herbicide treatment has completed the winter and spring wheat harvest on field B3, completing the fourth year of the Cover Crop-Wheat-Cover Crop-Wheat cycle. Along with field B2 which finished that four year rotation the data set is ready for the economic analysis. The economic analysis will be investigating Fallow-wheat rotations profitability compared with continuous cropping represented by Barley annual forage and with 15 different cover crops then wheat, but after four years or two full rotations.

<u>Year 1</u>	<u>Year 2</u>
Fallow	Wheat Yield both winter and spring
Barley Annual forage	Wheat Yield both winter and spring
Cool season	
CC Mix1	Wheat Yield both winter and spring
CC Mix2	Wheat Yield both winter and spring
CC Mix3	Wheat Yield both winter and spring
CC Mix4	Wheat Yield both winter and spring
CC Mix5	Wheat Yield both winter and spring
Warm Season	
CC Mix6	Wheat Yield both winter and spring
CC Mix7	Wheat Yield both winter and spring
CC Mix8	Wheat Yield both winter and spring
CC Mix9	Wheat Yield both winter and spring
CC Mix10	Wheat Yield both winter and spring
Cool/Warm season	
CC Mix11	Wheat Yield both winter and spring
CC Mix12	Wheat Yield both winter and spring
CC Mix13	Wheat Yield both winter and spring
CC Mix14	Wheat Yield both winter and spring
CC Mix15	Wheat Yield both winter and spring

Along with the economic analysis soil samples were taken before and after the current cover crop on field B2 (over 612 cores 48 inches deep). To quantify any differences after several cover crops rotations with wheat on



water use, chemical and physical characteristics of the soil. These would be bulk density, N, P & K, CO<sub>2</sub> respiration rate and Haney tests. The team collected data with infiltrometers (Figure 2) on how fast water uptake was taking place after the cover crop was terminated by haying, grazing or chemical means. Early after germination plastic probes were inserted into the ground 48 inches deep to record below ground root structure and mass between various cover crop treatments.

To date through location field days and industry tours the MREDI cover crop project personnel have presented data and project details to upwards of 500 industry professionals, NGOs and producers around the state, Figure 3.



*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.*







*Figure 2. Infiltrometer measurements investigating how fast the soil can take water after cover crop termination. Right hand picture is of a root scan collected with a minirhizotron (below ground camera) to monitor underground root profile differences just prior to termination of the cover crops.*



*Figure 3. One of the 6 field day tours of the statewide MREDI Cover Crop trial, NARC June 22, 2016.*

#### Hiring

- No additional hires in Quarter 4.

#### Expenditures

- Total Personnel Services: \$16,025.07
- Total Operations: \$4,219.98

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

#### Progress towards milestones

The cover crop grazing trial was completed on 7/21/16. Sheep were grazed for a total of 3 days, with 1 replication per 24-h period. Initial herbage mass samples were taken prior to grazing, as well as immediately after each grazing event. Plots were sampled for nitrate levels using a QuikTest prior to grazing, with all testing at safe levels. Samples are in the process of being ground for nutrient analysis, as well as quantitative nitrate analysis. Data is in the process of being analyzed.

We are also beginning to sample a field that has been in a several year study, under the direction of Drs. Perry Miller, Clain Jones, and Cathy Zerbinski, evaluating rotation of winter wheat with cover crops. Sampling will begin this August of previously harvested cover crops for forage quality, as well as soil samples to determine the correlation between soil nutrients and plant nutrient uptake. Dr. Tony Hartshorn and his students are helping on the soil analysis.

#### Hiring

- We will soon be hiring undergraduate students to help with analysis.

#### Expenditures

- Total Personnel Services: \$1,111.20
- Total Operations: \$58.57

### **On-Farm Precision Experiment subproject of the Agriculture MREDI Grant**

**1) 41W215 – Principal Investigator:** Bruce Maxwell; Email: [bmax@montana.edu](mailto:bmax@montana.edu)

#### Progress towards milestones

The OFPE team of PIs and key collaborators (farmers and industry representatives) meet every 2 weeks throughout the summer to discuss progress, data management and research approaches. We have created a website (<https://sites.google.com/site/ofpeframework/>) to have a central place to gain information about the project. No new hires were made during this quarter.

Pre-harvest calibration data and harvest data has been collected and some has been cleaned and placed in the temporary database (MSU Box account).

Pre-harvest sampling of winter wheat heads for protein was conducted on one field per farm where soil samples and weed density counts were taken. Protein will be measured with a benchtop analyzer and thus can be used to help calibrate the on-combine CropScan analyzer. All samples have been collected and threshed, but still have a couple of fields to get protein measurements.

Weed density survey data was collected on one field per farm at all farms. Soil sample results have been returned from Agvise and aggregated.

We have on-combine CropScan Protein Analyzer data from 3 of the 6 harvested fields and we have combine yield monitor data for 2 of the harvested fields. Two fields have yet to be harvested.

All data is being assembled and made ready for yield and protein analysis and optimization for next winter wheat year's nitrogen fertilizer prescription.

#### Expenditures

- Total Personnel Services: \$89,594.94
- Total Operations: \$65,480.86
- Total Equipment: \$90,730

**2) 41W226 – Principal Investigator:** John Sheppard; Email: [john.sheppard@coe.montana.edu](mailto:john.sheppard@coe.montana.edu)

#### Progress towards milestones

Dr. John Sheppard is managing the team focused on designing and implementing the model calibration, yield optimization and application prescription phases of the On-Farm Precision Experimentation (OFPE) process.

Over the past quarter, Janette Rounds (graduate student) has prototyped a feed-forward neural network for yield optimization.

A neural network is a collection of simple computing units, sometimes called nodes or neurons, connected by directed links, which approximates a function. Each node, or neuron, approximates the function by first calculating the weighted sum of its inputs, then applying an activation function to derive its output. In the case of a feedforward neural network, these links go in only one direction, with no loops. The ability of the network to approximate the function is dependent on the number of layers in the network. The network developed for the prototype exploits spatial information in the field in order to more accurately predict yield. As data from the 2016 harvest is currently being obtained, the network was tested on data obtained by Lawrence, Rew, and Maxwell (2015)\*.

A process called cross-validation is often used in machine learning. Cross-validation separates data into two independent subsets where one subset is used for training and the other for testing. The purpose is to provide independent data with which to evaluate how well the trained model can generalize to new situations. When multiple “folds” of cross validation are performed, the data set is then changed so as to produce a different training and testing set. This allows the experiment to be repeated. In this experiment, Ms. Rounds looked at three different cross-validation designs. In the first, she withheld one year of data to see if the remaining years of data could predict the withheld year. In the second, as a particular year would only be dependent on subsequent years and not following years, Ms. Rounds also used one year of data to train the network and the immediately following year to test the network. Finally, Ms. Rounds also used all of the data except for the last year for training and then tested on that last year.

Over fitting is a phenomenon where the network learns the noise in the data set rather than the signal (actual function). When this occurs, there is usually very low error on the training set but very high error on the testing set. In order to prevent this situation, the network was trained for a set number of iterations rather than training to a particular error threshold. In the future, alternative “early stopping” rules will be investigated such as using a third, validation test set to detect over fitting. High-level tuning was performed to set parameters of the network. In this experiment, a learning rate of 0.6 was used, as well as a momentum rate of 0.4. The maximum number of iterations the network was trained for was 1000 iterations. Initial results showed prediction error was quite low, although some fine-tuning of the process is still necessary.

The three fields used in this preliminary test were from current OFPE farmer Chuck Merja's farm near Power, MT. Two fields, named *grams* and *mom* had four years of data (2006, 2008, 2010, and 2012) while the final field, named *rosie*, had only three years of data (2006, 2008, and 2010). As such, only the fields *grams* and *mom* were used in the experimental designs that required 3 years of training data. The *rosie* data is reported here but not included in the experimental design comparisons. There were six variables used as inputs to the network. The first variable was whether the field was maintained fallow with herbicides or had a pea crop grown on it the previous year. The second variable was the growing season precipitation, and the third was the amount of urea applied. The fourth, fifth and sixth variables were the topographic wetness index, and electrical conductivity at 15 and 30 cm below the surface. Urea, topographic wetness index and electrical conductivity are site-specific variables, meaning each site (measuring 60 feet by 60 feet) had a distinct value for these variables, as well as for yield. In order to predict the yield for each site, the values for neighboring sites' site-specific variables were also used as inputs to the network.

The performance for the network was reported both in terms of mean-squared error (MSE) and as the coefficient of determination ( $R^2$ ). Mean squared error measures the average squared difference between the actual yield and the predicted yield. This measure is dependent on the size of the output, i.e. it is not an overall error percentage, but can be used to compare the results of two networks. On the other hand, the coefficient of determination is the variation that can be accounted for by the model divided by the total variation. This means that it is a measure of the overall effectiveness of the network. A network with a higher coefficient of

determination is better than a network with a lower coefficient of determination, while a network with a higher MSE is worse than a network with a lower coefficient of determination.

In this experiment, Ms. Rounds compared three different architectures of networks. The simplest of these is the simple-perceptron. This network is equivalent to linear regression and it contains only the output layer. The second network architecture contains two layers, a single hidden layer and an output layer, with seven nodes in the hidden layer. The final architecture contains three layers, two hidden layers and the output layer. Each hidden layer has 25 nodes. Note that additional tuning of the network architecture is likely to be needed.

Initial experiments found that the poorest performing network architecture for the experimental design that trained on three years and tested on the fourth (not necessarily subsequent) year was the simple-perceptron. This architecture had a mean coefficient of determination of 0.6732 and a mean squared error of 0.3376. There was no statistically significant difference between the other two architectures. The two-layer network had a mean coefficient of determination of 0.9083 and MSE of 0.0942 while the three-layer network had a mean coefficient of determination of 0.9082 and MSE of 0.0945.

**Table 1.** Three network architectures compared by training strategy. Results averaged across fields grams and mom.

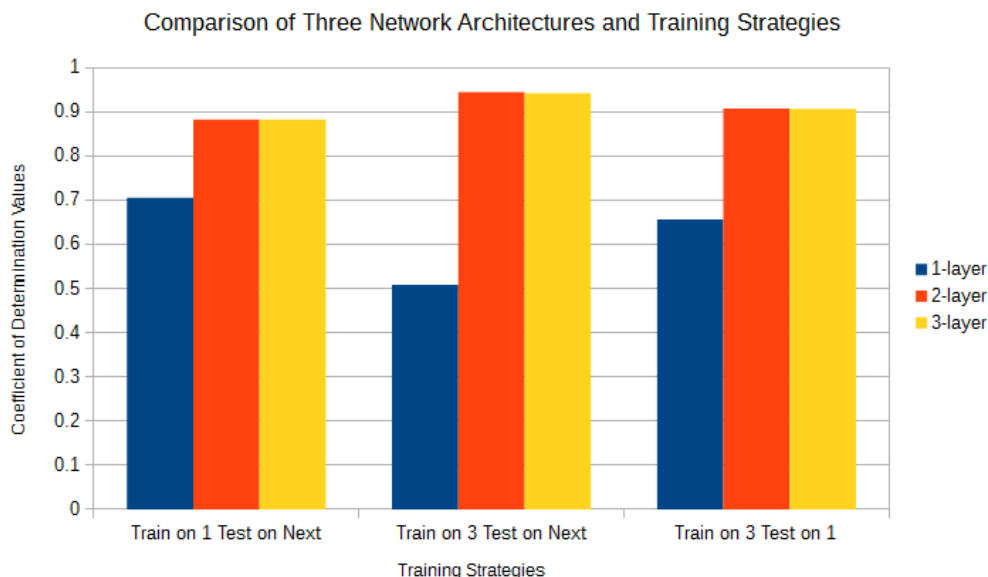
Training Strategy	1-layer		2-layer		3-layer	
	MSE	R <sup>2</sup>	MSE	R <sup>2</sup>	MSE	R <sup>2</sup>
Train on 1 Test on Next	0.3042	0.7057	0.1212	0.8826	0.1212	0.8825
Train on 3 Test on Next	0.5017	0.5081	0.0568	0.9443	0.0587	0.9424
Train on 3 Test on 1	0.3536	0.6563	0.0952	0.9077	0.0959	0.9070

Training on three years and testing on a fourth subsequent year (e.g. training on years 2006, 2008 and 2010 and testing on 2012) raised the coefficient of determination to 0.9443 for the two layer network and 0.9424 for the three layer network. On the other hand training on a single year and testing on the following year decreased the coefficient of determination to 0.8826 for the two layer network and to 0.8825 for the three layer network. However, the coefficient of determination for this training strategy increased for the simple-perceptron to 0.7057 and the MSE decreased to 0.3042. There was no statistical difference between fields using the coefficient of determination.

**Table 2.** MSE and R<sup>2</sup> for three fields by Training Strategy and Network Architecture combination.

Training Strategy / Network Architecture Combination	grams		mom		Rosie	
	MSE	R <sup>2</sup>	MSE	R <sup>2</sup>	MSE	R <sup>2</sup>
Train 1 Test Next / 1-layer	0.2712	0.7378	0.3372	0.6735	0.2765	0.6987
Train 1 Test Next / 2-layer	0.0735	0.9287	0.1688	0.8365	0.0818	0.9506
Train 1 Test Next / 3-layer	0.0736	0.9286	0.1689	0.8364	0.0831	0.9493
Train 3 Test Next* / 1-layer	0.4066	0.6022	0.5968	0.4139	0.2500	0.7572
Train 3 Test Next* / 2-layer	0.0225	0.9780	0.0910	0.9106	0.0289	0.9719
Train 3 Test Next* / 3-layer	0.0221	0.9783	0.0953	0.9065	0.0289	0.9719
Train 3 Test 1* / 1-layer	0.3051	0.7039	0.4021	0.6086	0.2905	0.7182
Train 3 Test 1* / 2-layer	0.1034	0.8998	0.0870	0.9156	0.0924	0.9101
Train 3 Test 1* / 3-layer	0.1043	0.8990	0.0875	0.9150	0.0912	0.9114

\*Train on 2 for rosie field; rosie field not included in averages.



**Figure 1.** Comparison of three training strategies across network architectures.

These results are highly preliminary and further experimentation, parameter tuning and validation is required. In the future, Dr. Sheppard and Ms. Rounds intend to expand these experimental results in several ways. They will fine-tune the yield optimization process, attempt to predict protein, use a prior year's protein to predict the next year's yield and vice-versa, as well as transition the prototype into production-quality code. Furthermore, the most recent data differs from the Lawrence et. al. data in that it contains more variables. The current network will be expanded to take advantage of these new variables. Results of the prototype will be compared against results produced by other implementations of a neural network. Additionally, the experimentation will be expanded to Ms. Rounds and Dr. Sheppard will continue to collaborate with the team managed by Dr. Izurieta and Dr. Payn to design the interface between the database and the experimental design, calibration, and optimization software.

\*Lawrence, Patrick G., Lisa J. Rew, and Bruce D. Maxwell. "A probabilistic Bayesian framework for progressively updating site-specific recommendations." *Precision Agriculture* 16.3 (2015): 275-296.

#### Expenditures

- Total Personnel Services: \$30,779.20
- Total Operations: \$6897.15

**3) 41W228 – Principal Investigator:** Clem Izurieta; Email: [clem.izurieta@gmail.com](mailto:clem.izurieta@gmail.com)

#### Progress towards milestones

Drs. 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 (May – August), however undergraduate student Mike Trenk will change status to graduate student (M.S.) under the direction of Dr. Izurieta.

The larger team, including Pol Llovet, Thomas Heetderks, Seth Kurt-Mason, and Michael Trenk (and occasionally Nick Silverman and Phillip Davis), meet every other week to track project progress and address the shifting priorities inherent in a research and development project. A flexible “Kanban” style project management system

is being used to track project milestones and the tasks necessary to accomplish each milestone. 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:

### **1. OOEDS Data Model**

- We continue to revise and extend the schema of the data model to provide new features necessary for data input, optimization, and prescription workflows (see below). **[In Progress]**
- Our design products in development of the data model provide a valuable contribution to the environmental data management literature; we are revising key figures and outlines for a manuscript targeting one of the environmental modeling journals. **[In Progress]**

#### **Data Schema [Completed]**

- Revised and updated the schema to include classes for agents (individuals or organization performing/undertaking certain activities). geoJSON features (for describing geographic representations of points, lines, and polygons)
- Improved handling of space/time context information
- Updated data model terms and class type names to align better with other data models/ontologies used in environmental and agricultural domains

### **2. OOEDS Web Interface**

- Prototypes have been developed for an open-standard authentication mechanism (OAuth) using a web development framework (Flask) to provide security for access to the MongoDB database. This authentication system will be installed on the production server and will be used with MongoDB's user database system will to manage data security. **[In Progress]**
  - Finished an API authorization flow diagram to validate the design.
  - Implementation is currently on-going.
- As driven by features needed for workflow development (see below), steady progress continues on the OOEDS implementation of the web interface (RESTful API). **[In Progress]**
- Began the development of the database agnostic OOEDS Java Library. This library is an important component for organizing our code base, and will allow for much more rapid development of software using the OOEDS data model in the future. **[New]**
- Development of the MongoDB extension for the OOEDS Java Library. **[New]**

### **3. Workflow software products (in order of current priority):**

#### **3.1 Yield Editor Data Input**

- Based on the data input files from the Yield Editor software, we have defined the structure of the configuration file necessary to input data to the database, and implementation and testing of the code is well under way. **[In Progress]**
- A Python prototype was completed and will be used moving forward to test functionality quicker. It can serve as a staging language before full design in Java. It will help us understand the types of features in the OOEDS library.
- We expect to complete the initial version of the OOEDS-library-based software for this workflow next quarter. The re-definition of the OOEDS Library using Java is forcing additional testing/development. **[In Progress]**

#### **[Completed]**

- Developed Python script for reading YieldEditor data CSV output files and writing data into MongoDB
- Identified query parameters necessary for retrieving harvest data from MongoDB to enable optimization modeling/workflows



- Identified table structures and data formats required for prescription data export files
- Began work to develop Gherkin/Cucumber scripts to support documentation and automated tests.

### 3.2 Optimization [Planning]

- The fundamental activities and sequences to support the workflow have been defined in design documentation. Queries for optimization workflows are a primary source of case studies for development of the OOEDS library (see above); thus progress on these workflow will parallel progress on the library implementation.
- Activity in the next quarter will be to implement the design for querying data for optimizations from the database, and returning the results of optimization with provenance metadata back into the database.

### 3.3 Prescription [Planning]

- No progress this quarter, but we will be starting the design process for this workflow soon, once the design of the optimization workflow is complete and in the process of being implemented.

## 4. Manuscript

Continued working on literature review and development of basic figures for the manuscript. [In Progress]

### Figures

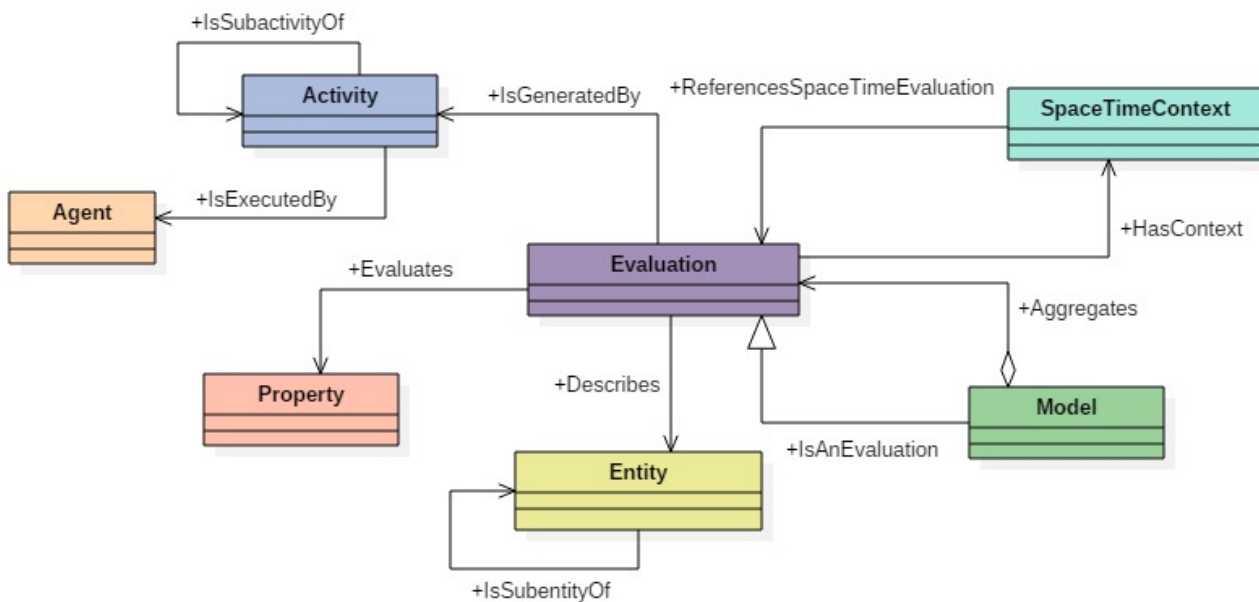


Figure 4. Executive Level Data Model

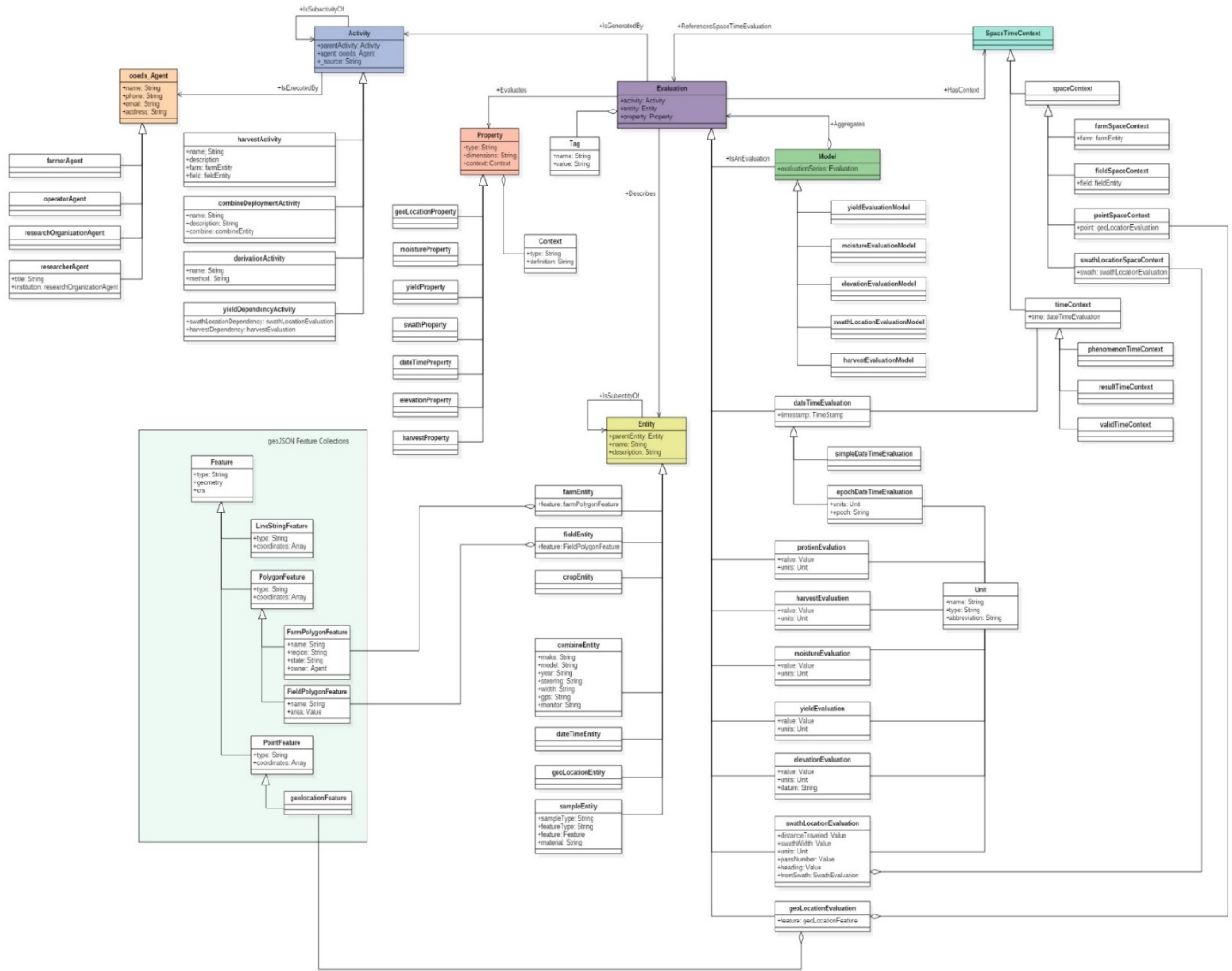


Figure 5. Each component describes an entire subsystem



## Software architecture

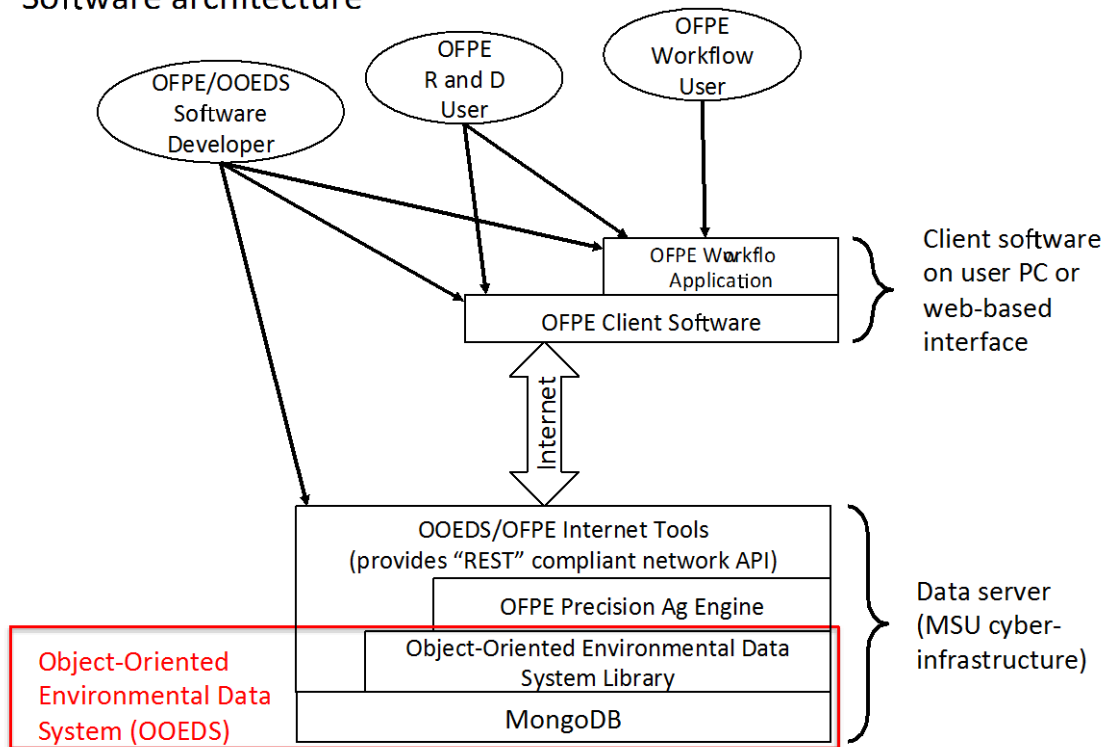


Figure 6. Software Architecture

### Expenditures

- Total Personnel: \$52,802.53
- Total Operations: \$33.56

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

#### Progress towards milestones

The objectives of the Montana Climate Office section of the MREDI OFPE are to develop and maintain a prototype agriculture sensor network at the seven MSU Agricultural Research Centers and additional offsite farms. The MCO will also ensure networking capability and convey the data online through the MCO website in near real time.

Since the last quarter Dr. Jencso completed a one-month trip (July through August) and has installed 10 Decagon Meteorological stations (Figure 1) that also include measurements of soil moisture, soil water electrical conductivity, and soil temperature at four depths. Six of these sites included the Agricultural Research stations. For these initial installations, only the data logger, enclosure and soil moisture probes were installed (Figure 2). Decagon Devices is shipping the remaining sensors that measure wind speed, wind direction, relative humidity, temperature, rainfall, and solar radiation, in late August. Currently the data from these stations are available at 30-minute intervals through a cellular network to each Research Center (Figure 3). The MCO is working to develop a web based GIS interface to host the data online and to convey it in a more accessible format.

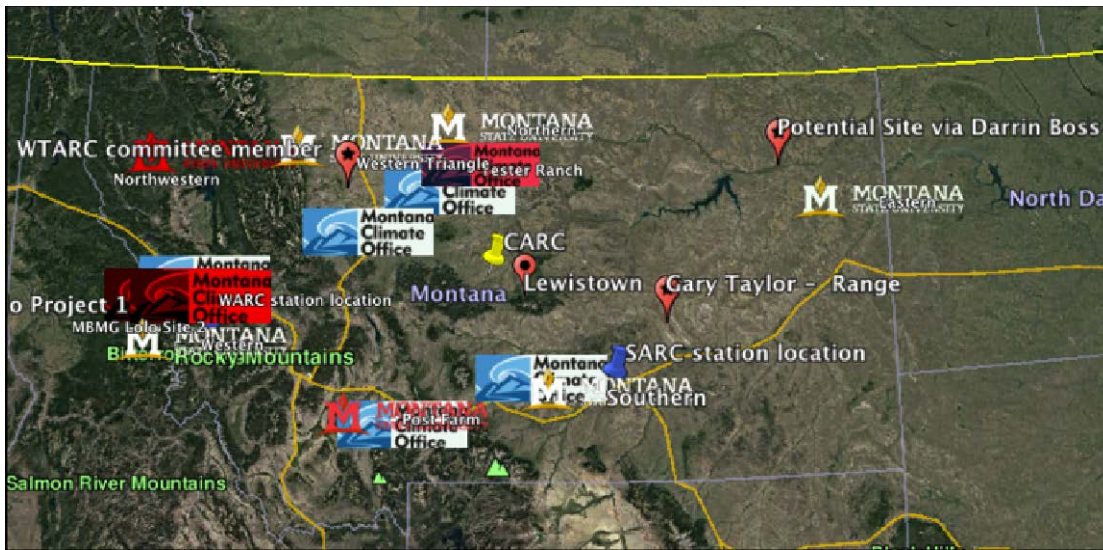


Figure 1: Current locations of stations installed by the Montana Climate Office. Red bubbles indicate potential future sites to be completed in the fall of 2016.



Figure 2: An example station installation with soil moisture, soil water electrical conductivity, and soil temperature measurements at 3", 8", 20", and 36" depths.

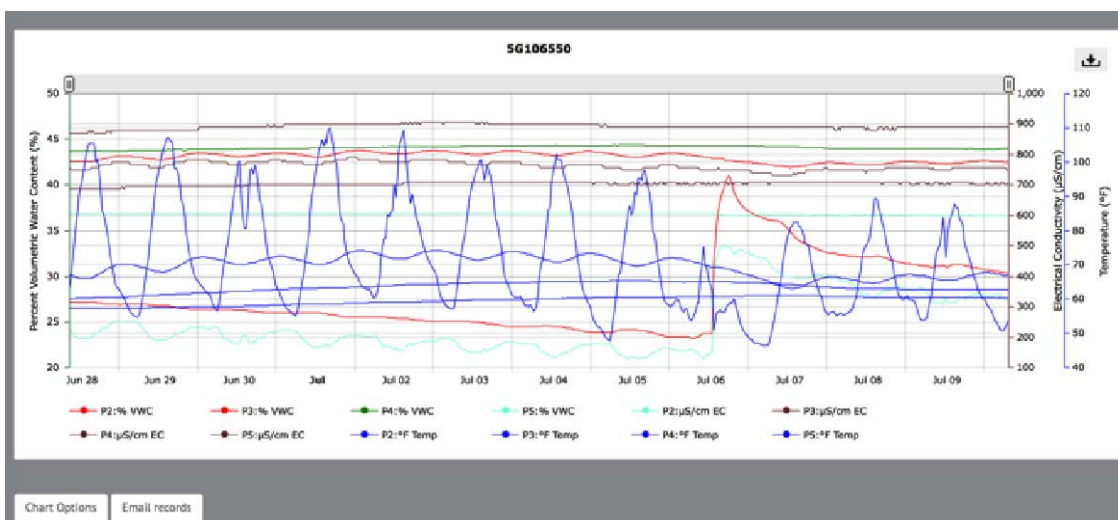


Figure 3: Example online graphical display of soil moisture, soil water electrical conductivity and soil temperature measurements from the Western Triangle Agricultural Research Center.

### Highlights:

- Successful submission of a \$1,500,000 stage 1 grant entitled “A topographically resolved water resources system for assessing water availability across agricultural, forest, and rangelands of the Intermountain West” to the NASA ROSES program. The full proposal is due on September 1 and involves PIs from the Montana Climate Office, MSU, MT DNRC, and the United States Forest Service.
- Establishment of a 5 year cooperative agreement with the Bureau of Land Management to install weather and soil moisture stations across range lands in Montana and to develop web based decision support tools for producers. See grant number L16AS00242 in grants.gov.

### Hiring (Industry Match)

A recently hired technician will complete the above ground sensor installations at each Research Station in September as well as installations at other locations across Montana.

### Equipment Purchased (Industry Match)

- 20 weather station enclosures
- Miscellaneous hand tools for installations (shovel, pick axe, etc.)
- Miscellaneous supplies for installations (concrete, zip ties, signs, etc.)
- 9 Decagon Devices EM50 cellular data loggers with a two-year cellular data plan.
- 9 Decagon Devices meteorological sensor packages (i.e. windspeed/direction, relative humidity, temperature, rainfall, radiation)

### Expenditures (Industry Match)

- Contract Faculty: \$3,394
- Employee Benefits: \$823
- Weather Stations: \$36,444
- Misc. Equipment: \$4,395
- Travel: \$1,506

### **Durum Quality subproject of the Agriculture MREDI project**

**41W221 – Principal Investigator:** Mike Giroux; Email: [mgiroux@montana.edu](mailto:mgiroux@montana.edu)

### Progress towards milestones

Our focus in this quarter was moving forward with durum trials by managing and advancing our new breeding populations. The interstate durum yield trial was planted at nine locations to allow genotype by environment comparisons of 15 entries consisting of nine named varieties and 6 MT experimental lines. We participated in field day presentations to growers at three MT growers to communicate information on current and in development barley varieties. Agronomic data for each location has been collected consisting of yield, heading date, plant height. Harvest season is commencing at many locations and we will soon be able to compile yield, test weight, seed size and grain protein data. 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).

### Durum Breeding Populations

Our durum breeding populations are advancing in the field and space planted F<sub>2</sub> plants are approaching maturity. We plan on harvesting heads from F<sub>2</sub> plants that have desirable plant height, head size, agronomic adaptability and maturity date. Lines will then be further screened by seed morphology and seed color. Selected lines and populations will be advanced in the greenhouse beginning in September.

Our F<sub>1</sub>s from our top cross populations designed to increase the frequency of the low cadmium trait are maturing in the greenhouse. F<sub>2</sub> seed of these populations will be harvested and planted in the greenhouse in October.

#### **2016 Experimental durum breeding material evaluation**

Durum populations created at MSU were evaluated in Yuma, AZ and the results indicated that a small subset of lines were yield competitive with current cultivars with several potentially higher in yield. Protein content and strength was assessed on all lines and those having high protein content and strength were identified. These lines are being grown in replicated trials in Bozeman and Conrad with selections being made based upon plant growth characteristics, yield, and seed quality. Final selections for lines to include in 2017 will be made after harvest and will include preliminary end product quality evaluations. Additional traits of interest in these populations include low cadmium and a unique increased pasta firmness trait. Both of these traits are potentially valuable for increased marketing opportunities for Montana durum.

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

In this quarter, Northern Seed has been continuing to identify improved durum varieties that may be useful to Montana growers by testing a diverse series of germplasm. As described previously, we are evaluating Montana State's Joyce Eckhoff germplasm (JE), Montana State's Mike Giroux germplasm (MG), and that of 2<sup>nd</sup> Nature Research's (NR).

We selected 156 JE germplasm lines to grow in replicated trials in Bozeman this summer. Plant characteristic and agronomic data is being compiled from these plots and after harvest all lines will be assessed for agronomic quality. The goal here is to identify the best lines to grow in multi-location replicated trials in 2017. We will work in collaboration with MSU-Bozeman to complete quality testing and then heads of the best lines will be planted in Yuma, AZ this fall and harvested next April to bring back to Bozeman to begin the production of Breeder Seed.

We planted and are managing replicated trials of MG germplasm being grown at two MT locations. These plots will be harvested in August of 2016 and yield data and seed samples will be supplied to MSU. As with the JE germplasm, the best lines will be selected and grown in replicated trials in 2017.

We planted and are managing over 4000 replicated yield trial plots of NR germplasm at three MT locations. As with the other germplasm groups, we will identify the best lines to advance to multi-location yield trials in 2017. The goal for our work with each germplasm group is the release of high yielding and high end product quality durum for MT growers.

#### **Hiring**

- No additional hires in Quarter 4.

#### **Equipment**

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

#### **Expenditures**

- Total Personnel: \$37,528.90
- Total Operations: \$10,928.36
- Total Equipment: \$70,994.00

Progress towards milestones

We have now fully characterized the effect of sugar availability on the longevity of both species of native parasitoids of the wheat stem sawfly. We had earlier reported preliminary information of sucrose on the survival of these species. The red line indicates survival when parasitoids have access to water, while the black line shows this for sucrose (Figure 1). The longevity of newly-emerged *Bracon cephi* females at 50% survival probability was 8 days for water and 37 days for sucrose. For male *B. cephi* the survival probability was 8 days for water and 30 days for sucrose.

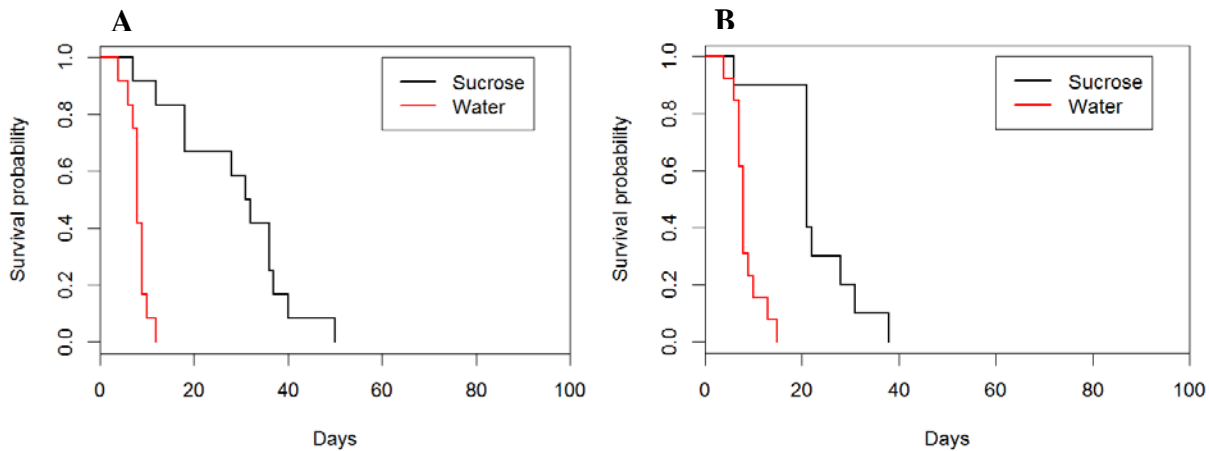


Figure 1. Kaplan-Meier survival probability of *B. cephi* first generation parasitoids when water and sucrose were provided. A) Females; B) Males.

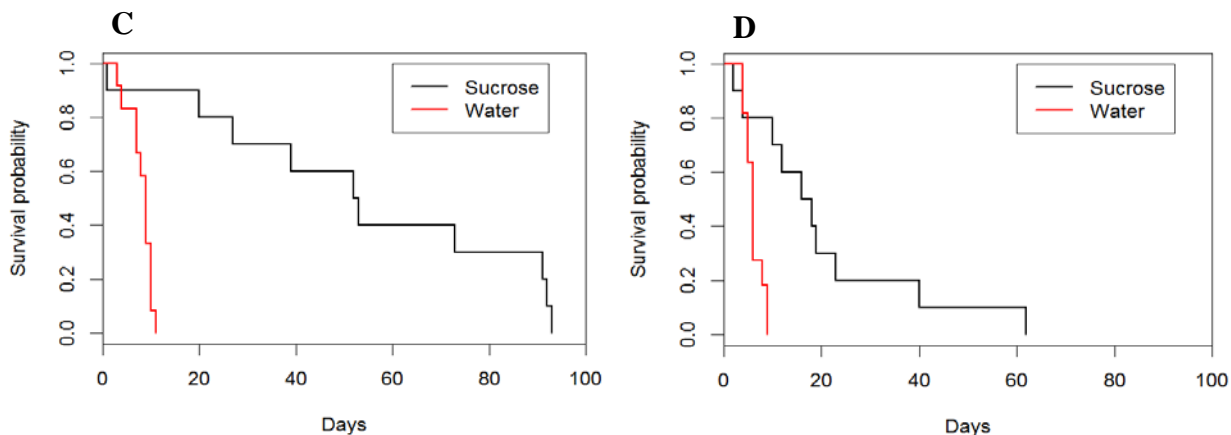


Figure 2. Kaplan-Meier survival probability of *B. lissogaster* first generation parasitoids when water and sucrose were provided. C) Females; D) Males.

For the more fecund and gregarious species, *B. lissogaster*, the differences were even more profound (Figure 2). In this case, “gregarious” means that more than one parasitoid can be produced on a single pest larva, while for *B. cephi* only one parasitoid can be produced for each wheat stem sawfly larva.

The longevity of female *B. lissogaster* at 50% survival probability was 8 days when provided water only and 53 days when provided sucrose. For males of this species, the numbers were 8 days and 22 days.



In the field we looked at the numbering of surviving overwintering parasitoids emerging from 30 cm of a wheat row (2015 crop) for comparisons taken from cover crop fields adjacent to fallow fields and for pulse crop fields adjacent to fallow fields. There were six sites with fallow matched to cover crop and six sites with pulses matched to cover crops. Overall parasitoid emergence was higher in the paired cover crop fallow sites when compared to the paired pulse crop and fallow sites (Figure 3). There is also a trend for greater numbers of parasitoids from wheat fields adjacent to both types of flowering crops than for wheat adjacent to fallow. Figure 5 is for illustrative purposes only. The analytical comparison for each site is influenced by the overall infestation at each site.

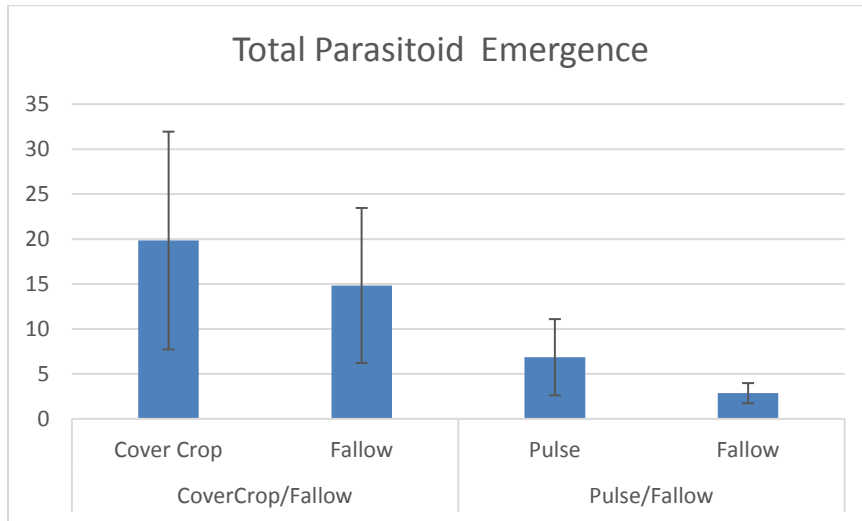


Figure 3. Mean parasitoid emergence from cover crop paired with fallow fields and from pulse crop paired with fallow fields.

To simplify size comparisons we took the mean body mass (Figure 4) and mean tibial length (Figure 5) of parasitoids from each set of field pairs. Mass in grams would account for differences in body lipid content and tibial length is a fixed measure of size. Both measurements are important. Insects can expand in size due to flexible connections between segments and be plumper, while the insect exoskeleton is rigid and cannot expand. The tibia (lower leg) is a standard measure of fixed body size of adult insects.

The cover crop or pulse measure “treated” was divided by the measurement for wheat “control”. In both cases there were no differences between insects emerging from wheat grown next to cover crop or fallow and the paired wheat field, with slightly smaller males from pairs where wheat was grown next to cover crop pairs and the adjacent fallow field than for wheat grown next to pulse crop pairs and the corresponding adjacent wheat grown next to fallow fields.

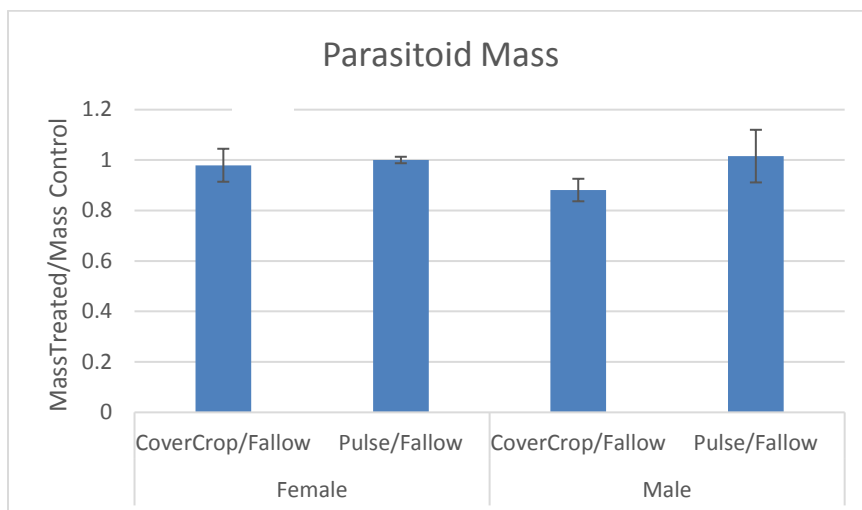


Figure 4. Mean proportion of the mass (grams) of parasitoids emerging from wheat adjacent to cover crops relative to wheat adjacent to the corresponding fallow fields and parasitoids emerging from wheat adjacent to pulse crops relative to wheat adjacent to the corresponding fallow fields.

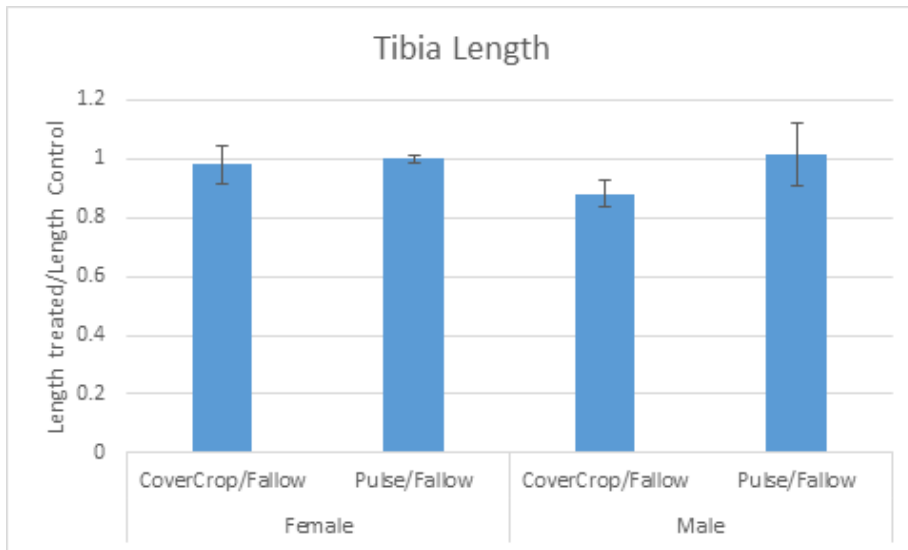


Figure 5. Mean proportion of the length of the tibia (mm) of parasitoids emerging from wheat adjacent to cover crops relative to wheat adjacent to the corresponding fallow fields and parasitoids emerging from wheat adjacent to pulse crops relative to wheat adjacent to the corresponding fallow fields.

Thus, the parasitoids emerging from wheat grown next to flowering fields are not smaller than those emerging from the adjacent fallow field. This is encouraging, given that greater numbers of parasitoids emerge when next to flowering crops.

We also compared trap capture of parasitoids during the growing season in 2016. We used four fields with cover crops paired to adjacent fallow fields and eight fields with cover crops adjacent to pulse crops. Quantifying the actual number of parasitoids in the crop is difficult. Using sweep nets is greatly challenged by weather conditions and time of day. We controlled for this by using a trap fixed in place to quantify parasitoids. This has never been attempted before for these braconid species, so it is encouraging that it works. However, these data are not definitive. The parasitoids have two generations and all of the wheat fields were harvested before the second generation of parasitoids had emerged. The traps have to be removed before harvest and the second generation is difficult to trap while flying over a newly-harvested field. The trapping data will be supplemented by an autumn collection of standing residue for stem dissection, which will allow us to accurately determine the benefit of the flowering species environment. These samples will be collected at the end of September.

#### Hiring

- No additional hires in Quarter 4.

#### Expenditures

- Total Personnel: \$1,993.10
- 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](mailto:pjha@montana.edu)  
**HERBICIDE CARRY-OVER STUDIES FOR SUCCESSFUL INTEGRATION OF PULSE CROPS IN CEREAL-BASED CROPPING SYSTEMS OF MONTANA**

#### Progress towards milestones

Field studies were established in the fall of 2015 across multiple locations: Huntley, Moccasin, Havre, and Sidney, MT. There are three major objectives of these field studies:

Objective 1. Effect of fall-applied soil residual herbicide programs on pea, lentil, and chickpea tolerance and weed control (*progress report as below*)

Objective 2. Effect of Group 2 Sulfonylurea herbicides applied in the fall PRE and spring POST in winter wheat (including Clearfield wheat varieties) and carry-over to pea, lentil, and chickpea (*Progress: plots established in fall 2015, winter wheat planted in fall 2015, herbicides applied in fall 2015 and spring 2016, winter wheat harvested this summer, pulse crops will be planted-back in spring of 2017*).

### **Objective 1.**

**Effect of Fall-Applied Herbicides on Lentil.** In general, the lentils were found to be the most susceptible among the three pulse crops to the soil-residual herbicides across all four locations. The hierarchy of crop susceptibility was lentil > pea > chickpea. The carryover effects of few herbicide treatments were dose-dependent and varied among locations (Fig. 1 to 3).

At the Huntley site, fall-applied Sencor at 4 to 8 oz/a, Valor 3 to 6 oz/a, Anthem Flex 3.24 to 7.28 oz/a, Prowl 16 to 32 oz/a plus Outlook 18 to 36 oz/a were safe to lentils. An unacceptable injury (>40%) was observed with Corvus applied alone at 4 to 8 oz/A or tank-mixed with Sencor (4 or 8 oz/a) at the first rating (early spring 2016). The observed injury symptoms included chlorosis, stunting, plant deformation, reduction in plant height, and stand loss across all locations. Spartan charge (12 oz/A) and Authority MTZ (16 oz/A) also caused significant injury to lentils at the first rating. The injury symptoms from Spartan charge and Authority MTZ included chlorosis, stunting, leaf burn, and necrosis. However, injury from these herbicides declined at the subsequent evaluation dates (<25%).

At Sidney location, the first injury rating and symptomology on lentils with most of the treatments was consistent with the Huntley location. Sencor, Anthem Flex, Prowl plus outlook treatments were safe on lentils. Valor at 3 to 6 oz/A caused 25% injury to lentils at this site.

At Havre and Moccasin locations, the injury response on lentils with majority of the treatments was comparable. There was a greater recovery of lentil plants from the injury caused by Spartan charge, Authority MTZ, or Corvus at these two sites.

**Effect of Fall-applied Herbicides on Pea.** Irrespective of rates, fall-applied sencor, valor, Anthem Flex, Prowl plus outlook, Spartan charge 6 to 12 oz/a, Authority MTZ 8 to 16 oz/a were safe to lentils, and did not cause any significant injury. Corvus consistently showed visual injury on peas at all ratings across all four locations. However, the magnitude of injury was significantly different among locations. For example, the pea injury from carryover of Corvus ranged from 30 to 35% at Huntley, 40 to 45% at Sidney, 15 to 20% at Moccasin, and less than 15% at Havre throughout the growing season.

**Effect of Fall-applied Herbicides on Chickpea.** Chickpeas were the most tolerant among three crops. Irrespective of rates, fall-applied Sencor, Valor, Anthem Flex, Prowl plus Outlook, Spartan charge 6 to 12 oz/a, Authority MTZ 8 to 16 oz/a were safe on chickpea. At Huntley, the maximum injury (25-30%) on chickpea was observed with Corvus-based herbicide program. However, injury was reduced by the second rating (5 to 15%), which may not be economically significant. Injury on chickpeas from these Corvus-based herbicide programs did not exceed 15% throughout the growing season at other three locations (Sidney, Havre, and Moccasin).

**Effects of Fall-applied Herbicides on Residual Weed Control.** Herbicide treatments including Sencor (8 oz/a), Spartan charge (6 to 12 oz/a), Valor (3 to 6 oz/a), and Authority MTZ (8 to 16 oz/A) applied in the fall of 2015 provided excellent season-long kochia control (>90%) throughout the growing season in 2016, across all four locations. Kochia control with Anthem flex (3.6 to 7.2 oz/a) and prowl + Outlook (32 + 36 oz/a) was also good (70 to 80%) through the growing season across all four locations.



***Crop Yield and Soil sampling.*** We are still in the process of harvesting the pulse crops across all four locations. Therefore, the yield data will be analyzed and presented in the next quarter report. Soil sampling will be conducted this fall across all sites for herbicide residue analysis. The soil properties (pH, OM) at each site will be correlated with the herbicide injury to further optimize rates for reduced crop injury and extended residual weed control from these fall-applied soil residual herbicides in pulse crops in MT.

#### Hiring

The following people continue to work on this project:

- Dr. Vipin Kumar, Postdoctoral Research Associate
- Mr. Shane Leland, Research Technician at SARC, Huntley

#### Equipment

- The purchase of a growth chamber is under process and will be completed soon (August).

#### Expenditures

- Total Personnel: \$7,132.13
- Total Operations: None to date

**2) 41W216 – Principal Investigator:** Joseph Shaw; Email: [jshaw@montana.edu](mailto:jshaw@montana.edu)

#### **PRECISION WEED CONTROL USING ADVANCED OPTICS AND SENSOR-BASED TECHNOLOGIES**

#### Progress towards milestones

Our next milestone is to complete collection of field data by 30 September 2016. Accordingly, our primary activity this quarter was field deployment of a hyperspectral imager for outdoor imaging of weeds and crops and processing of the data.

During 5-7 July 2016 we deployed the hyperspectral imager at the Southern Agricultural Research Center in Huntley, Montana. We recorded spectral images of herbicide-resistant and herbicide-susceptible weed, primary of the Kochia species, but also of common crops such as wheat, barley, and beans, as well as combinations of Kochia with those crops. We collected measurements under direct sun and indirect sun. We also imaged the plants in configurations ranging from clusters of plants down to individual leaves. Whereas previous measurements were made inside a greenhouse or in isolated outdoor conditions, these measurements were made exclusively outdoors with potted weed plants arranged among actual crops.

#### **Measurement summary:**

Our hyperspectral imager was used to record hyperspectral datacubes with 240 spectral channels per spectrum over the wavelength range of 396 – 885 nm. The measurements were sorted by the crop type, with labels for the Kochia strains in each image. Calibration and dark correction measurements were taken at the same time to convert instrument output from digital numbers to reflectance for each pixel at each wavelength. Illumination was primarily direct sunlight, with measurements recorded primarily between 10:00 am - 2:30 pm Mountain Daylight Time, with the sun elevation angle above 44°. Every image (unless noted) included all four types of weeds. Data on framerate, integration time, gain, scan speed, solar elevation were also collected, along with all-sky visible images to record cloud cover and sky conditions. During the measurements we noted that slight breezes could cause a blurring in the hyperspectral images, so we attempted to record images with minimal winds.

#### Observations on 5 July:

- Spring Wheat (visual browning): diffuse solar illumination
- Barley: diffuse solar illumination
- Spring Wheat: oblique solar illumination

Observations on 6 July:

- Sugar Beet: direct solar illumination
- Soybean: direct solar illumination
- Short Corn: direct solar illumination
- Spring Wheat: direct solar illumination
- Wild Oat (no control plants): direct solar illumination
- Fallow: direct solar illumination
- Lentils: oblique solar illumination

Observations on 7 July:

- Chick Peas: direct solar illumination
- Lentils: direct solar illumination
- Safflower: direct solar illumination

Figure 1 is a photograph showing the initial stage in which the hyperspectral imager was pointed directly at a high-reflectance calibration plate to map differences in the pixel-to-pixel response. Figure 2 shows a similar scene, but with the calibration plate lying horizontal within the field of view of the scanning, tripod-mounted hyperspectral imager. Figure 3 shows an example of measurements made with the potted weeds surrounded by the crops. Figure 4 is fisheye view of the measurement area and the overhead sky during a portion of the experiment. Figure 5 shows an example infrared image at 840-nm wavelength, one of many images currently being processed to develop algorithms for identifying weed species in outdoor measurements.



*Figure 1: Tripod-mounted hyperspectral imager recording pixel-to-pixel variations by viewing a high-reflectance calibration plate prior to recording spectral images of the potted weeds and crops.*





*Figure 2. Tripod-mounted hyperspectral imager recording spectral images of potted weeds and crops.*



*Figure 3. Tripod-mounted hyperspectral imager recording spectral images of potted weeds and crops, with the potted weeds surrounded by crops.*





*Figure 4. Fisheye photograph of the hyperspectral imager scanning across the potted weeds in a field of soybeans under a clear sky with direct sunlight.*



*Figure 5. Infrared image (840 nm wavelength) of potted weeds among safflower and wild Kochia (susceptible variety).*

### Hiring

The following people continue to work on this project:

- Dr. Joseph Shaw: subproject director (receiving partial summer salary)
- Dr. Paul Nugent: Research Engineer who successfully defended his Ph.D. during this quarter (receiving partial calendar year 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)

### Equipment Procurement

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

### Expenditures

- Total Personnel: \$25,335.53
- Total Operations: \$8,466.08
- Total Equipment: \$16,716.00

## **Film Production for the Agriculture MREDI Grant**

**41W218 – Organizer:** Eric Hyypa; Email: [eric\\_hyypa@montanapbs.org](mailto:eric_hyypa@montanapbs.org)

### Progress towards milestones

MontanaPBS has filmed several MREDI related activities at multiple locations including: pulse crop seeding at the Northern Agricultural Research Center in Havre; the producer's Field Day at the same location; an interview with Darrin Boss; and winter wheat crop termination at the Post Farm site in Bozeman. MontanaPBS will soon be conducting interviews with several key figures in the grant.

Images from these shoots can be found

at: [https://www.dropbox.com/sh/1xr2m9xwqaax7xr/AAAUh50FTIXLT6N4I86\\_pzkHa?dl=0](https://www.dropbox.com/sh/1xr2m9xwqaax7xr/AAAUh50FTIXLT6N4I86_pzkHa?dl=0).

### Equipment Procurement

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

### Expenditures

- Total Personnel: \$0.00
- Total Operations: \$6,813.44
- Total Equipment: \$7999.00

## **Economic analysis subproject of the Agriculture MREDI project**

**41W219 – Principal Investigator:** Anton Bekkerman; Email: [anton.bekkerman@montana.edu](mailto:anton.bekkerman@montana.edu)

### Progress towards milestones

The economic analysis of the emerging Montana pulse market is intended to demonstrate farm-level and state-wide impacts of technological improvements for traditional wheat–fallow systems, which have dominated the state's landscape for a century. During the fourth quarter of the MREDI project, Bekkerman was involved in three components of this objective:

1. Bekkerman worked with pulse crop rotation data provided by Dr. Chengci Chen to develop production budgets to evaluate the profitability of alternative pulse and oilseed cropping systems in central and north-central Montana. Yield and protein data were generated from plots at the Central and Northern Agricultural Research Centers. The budget results indicate that while the wheat–fallow system remains the most profitable the majority of the time, the system that included lentils outperformed all other alternative systems in the plot experiments.
2. Bekkerman began to take a more active role in the modeling and assessment component of the precision agriculture portion of the MREDI project. The group is nearing completion of data collection and model development, and Bekkerman is helping advise and work with the group to provide input about economic modeling for the results.
3. Bekkerman is nearing completing of the model that will be used to evaluate how technological improvements in the production of wheat and pulse crops is expected to affect Montana's agricultural economy. The empirical model follows the seminal methodology developed by Alston, Norton, and Pardey (1995). The approach is known as the "Dynamic Research Evaluation for Management" (DREAM), and has been used to estimate the magnitude and distribution of the economic benefits of agricultural research and development. That is, the assessment of the dynamic economic impacts across related sectors (in this case, wheat and pulse crops) as technological changes are distributed throughout the sector. The model captures the trade-offs associated with the economic opportunities and constraints within the system.

The model incorporates spillover effects across Montana regions as well as nearby states and global markets. This is particularly important to the evaluation because both Montana wheat and pulse crops are primarily sold to international consumers. As such, Montana is modeled as a small open economy, where prices are determined exogenously on the world market but small variations are allowed to account for local heterogeneity.

The primary challenge (and current on-going work) is to accurately represent the underlying economic parameters associated with market dynamics and the assumptions associated with technological disbursement (i.e., the rate with which farmers will adopt alternative cropping systems, management strategies, and machinery equipment). Data were collected to inform many of these parameters, but scenario analyses will be necessary to provide a range of possible outcomes. This is especially necessary due to the high degree of market uncertainty that is pervasive in agricultural markets and, in particular, in Montana's dryland production environment.

#### Hiring

- No additional hires in Quarter 4.

#### Expenditures

- Total Personnel: \$20,873.59
- Total Operations: \$11,613.00

### **Participatory research network subproject of the Agriculture MREDI project**

#### Progress towards milestones

This quarter, Colter Ellis made two weeklong data collection trips. Visits were made to areas in the Conrad, Sunburst, Chester, Joplin, Havre, Malta, and Whitewater areas. During these trips, 19 individual interviews were collected with producers. These interviews total over 23 hours of recorded time. Interviews are now being transcribed and qualitative analysis of this data will begin early Fall 2016. These interviews are in addition to the five focus groups previously conducted. In total, 58 individuals have been included in this study.

On August 10, 2016, preliminary data from these data were presented at a paper session on "On-Farm Data and Agricultural Technology" at the Rural Sociological Society's annual meeting in Toronto, Ontario. The presentation was well received.

Moving forward, we hope to collect additional interviews with producers who have been specifically involved with the MREDI project. George Haynes has developed a survey instrument and requested contact information from all project directors. He will be interviewing collaborating producers in the next few months.

**1) 41W224 – Principal Investigator:** George Haynes; Email: [haynes@montana.edu](mailto:haynes@montana.edu)

#### Hiring

- No additional hires beyond graduate student, Tom Woods.

#### Expenditures

- Total Personnel: \$13,211.65
- Total Operations: None to date

**2) 41W223 – Principal Investigator:** Colter Ellis; Email: [colter.ellis@montana.edu](mailto:colter.ellis@montana.edu)

### Hiring

- None to date

### Expenditures

- Total Personnel: None to date
- Total Operations: \$4,631.55