

Agriculture MREDI Grant Quarter 5 Sub-project Reports

**Research Center/MAES subproject of the Agriculture MREDI Grant
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Executive Summary

During this past quarter all field plots were harvested and researchers are in the process of collating and analyzing the data. Presented below are state wide data from 11 statewide pea variety trial sites. These data provide information to growers on what are the most productive this their area. In addition, this data when analyzed with weather and fertility data will provide greater insight into what factors-genotype-environment-fertility affect pea protein content. Research has shown that a 250-seed sample is the most reliable sample size for protein analysis. This information will be critical to maximizing profitability of a pea protein fractionation facility. Data from these same sites for lentil and chickpea are undergoing analysis.

While the studies on soil microbiology, and soil fertility factors is not yet complete, data from the Peters lab has elucidated rhizosphere bacterial associations associated and nutrients associated with higher yield and the effects of irrigation (wetter conditions) for a subset of the research locations and similar data will be developed for other locations from samples collected in 2016. The data in this report focus on rhizosphere bacteria. As particular rhizosphere bacteria are associated with higher yields there is the opportunity for MSU to develop bacterial based seed inoculants that will increase yields. Although Dr. Peters is moving to Washington State University, he has indicated that he wants to continue working cooperatively on this research and a recent applied soil microbiology hire at the Central Agricultural Research Center should provide continued progress on developing seed inoculants that will preemptively colonize the rhizosphere.

Data from eight statewide cover crop trials that compared 4 different cover crop seed mixes and 15 individual components have been gathered and analysis for yield is complete and analysis for feed value should be completed soon. Of interest, one of the highest yielding mixes (oats, turnips, canola, pea and safflower) only costs \$22.60/A for seed. This compares to >\$50.00 /A for seed mixes recommended by USDA-NRCS. For the Havre site we also have information on below ground biomass and water infiltration as well as comparison of grazing vs haying returns. In addition, other funds have been identified to examine microbial diversity in soils as affected by the cover crop mixes. This data should be available by next quarter. Finally, although the data is from prior to MREDI, this report shows that producers using a cool season cover crop mix will achieve ~ \$100/A increased cash return compared to using crop fallow for either winter wheat or spring wheat. This data is from 4 years and was done when precipitation was <12 inches/year therefore this is a good proof of concept for integrating cover crops into annual cropping scenarios for most all locations in MT thus providing greater income potential for producers currently practicing crop fallow. Economics on pea and lentil planting as a replacement for fallow should be available soon.

Associated work progress was made in examining direct grazing of annual grass forages and on risk of nitrate poisoning to grazing animals. Work in the Yeoman lab has shown promise for the development of probiotic supplements to address nitrate poisoning.

Considerable progress has been made with the precision Agriculture project. All four field sites have been harvested and data management software using previous yield maps, agronomic data from research centers, soils information, remote sensing and climate information to develop prescriptive fertilizer and herbicide recommendations that will reduce producer risks and maximize profits. Based

on the 4 sites analyzed to date the 2016 work application of the “prescriptions” would have increased revenues statewide by \$5 to 50 million \$ without accounting for herbicide savings.

Work on weed management and avoidance of herbicide injuries to wheat or pulse crops grown in rotation, one of the biggest constraints, was addressed by Dr. Jha at 4 sites. Safe herbicide programs to avoid injury to wheat from pulse herbicides or pulses by wheat herbicides have been developed. In addition, these herbicide program are both highly effective and address the increasing problem of herbicide resistance in our common weeds.

In associated work with the use of hyperspectral optics, progress has been made in identification of dicamba resistant kochia and on detection of weeds in standing crops.

The durum breeding project has identified several lines that are low cadmium and that are well adapted to Montana in term of yield and protein content. Other specific quality characteristics are being determined and should be available in the next quarter.

Work on assessing constrains to adoption of technologies developed by this project through the Participatory Research Networks is well underway as is economic analysis of all components of this project.

Research on the benefits of pulse or cover crop nectar sources to two wheat stem sawfly parasites is ongoing and effects of adjacent cover crops and pulse crops to actual wheat stem sawfly parasitism is underway with stem analysis being done over the winter months. It is interesting to see the important indirect effects of cropping changes on a landscape scale that will impact on of the State’s most serious pests estimated to cause yield losses of \$60-100 million per year.

During the upcoming 6th quarter the majority of data will be analyzed and summarized such that specific conclusions and economic impacts can be made.

Hiring

- Additional temporary help was hired at the EARC and WARC.

Expenditures

- Total Personnel Services: \$130,653.97
- Total Operations: \$7,875.73

Pulse Crop Research subproject of the Agriculture MREDI Grant

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Co-investigators: Yesuf Mohammed, Maninder Walia, Perry Miller, Peggy Lamb, Jessica Torrion, Roger Ondoua, Zachariah Miller, Kent McVay, Patrick Carr

Progress towards milestones

1. Multi-location variety evaluation

This project is coordinated by Eastern Agricultural Research Center. The coordinating center received all the data and subsamples from collaborating researchers. This center arranged and delivered the subsamples for protein content testing at Southern Agricultural Research Center, Huntley. Then, the coordinating center scrutinize and analyze both grain yield and protein data to see the effects of

genotypes and locations on grain yield and grain protein concentration. The results are presented in Tables from 1 to 4.

As shown in Tables 1 and 2 below, variety and location significantly affected both grain yield and grain protein concentration. But their interaction effect was significant only for grain yield. The effects of locations both on grain yield and protein concentration were substantial (as explained by higher sum of squares in the ANOVA table) compared with genotype effects. Since the effect of location was significant when we analyzed the combined data as shown in Tables 1 and 2, it warranted separate statistical analysis for each location. Therefore, the mean grain yield and protein concentration for each genotypes and locations are shown in Tables 3 and 4.

Table 1. Analysis of variance table showing the effects of variety, location and their interaction on grain yield of six dry pea varieties.

| Source | DF | Type I SS | Mean Square | F-Value | Pr > F |
|------------------|----|-----------|-------------|---------|---------|
| Rep | 3 | 7065571 | 2355190 | 9.63 | <0.0001 |
| Variety | 5 | 18875061 | 3775012 | 15.44 | <0.0001 |
| Location | 9 | 535662370 | 59518041 | 243.38 | <0.0001 |
| Variety*Location | 44 | 38787108 | 881525 | 3.6 | <0.0001 |

Table 2. Analysis of variance table showing the effects of variety, location and their interaction on grain protein concentration of six dry pea varieties.

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|------------------|----|-----------|-------------|---------|---------|
| Rep | 3 | 3.59 | 1.19 | 0.74 | 0.5279 |
| Variety | 5 | 112.74 | 22.55 | 13.98 | <0.0001 |
| Location | 9 | 1341.34 | 149.04 | 92.42 | <0.0001 |
| Variety*Location | 45 | 84.85 | 1.88 | 1.17 | 0.2390 |

The mean grain yield was maximum at Richland site (5455 lb/ac) followed by Creston (5338 lb/ac) (Table 3). But from the interaction (variety by location) means, the highest mean grain yield was recorded from the variety Nette 2010 at Creston (6845 lb/ac). This was also the record high grain yield from the 2016 Montana statewide dry pea variety evaluation project result. Similar to grain yield, mean grain protein concentration was higher (25.10%) for the variety Hampton at Richland site (Table 4). The lowest mean grain yield was recorded from Huntley dryland mainly due to hail damage during flowering time. From Statewide dry pea variety evaluation project, for the same location, we have noticed that supplemental irrigation minimized the reduction in grain yield due to stress from hail damage. The lowest mean grain protein concentration was recorded from Conrad (16.21%) followed by Havre (17.60%). This lower protein content needs further research to develop management practices that can enhance dry pea grain quality.

Table 3. Mean grain yield for each variety and locations in 2016 at different locations in Montana

| Varieties | Location | | | | | | | | | | | Variety means |
|-----------|-------------|--------|-----------------|---------|-------------|-------------|---------------|----------|------------|--------------|----------|---------------|
| | Bozeman | Conrad | Corvallis irri. | Creston | Havre | Huntley dry | Huntley irri. | Moccasin | Sidney dry | Sidney irri. | Richland | |
| Delta | 2265 | 3933 | 2519 | 5143 | 2132 | 829 | 1535 | 1406 | 3629 | 4353 | 5459 | 3018 |
| Hampton | 2408 | 3923 | 2270 | 5083 | 2797 | 773 | 1884 | 1445 | 3629 | 4103 | 4023 | 2940 |
| Jet Set | 2560 | 3350 | 3066 | 5570 | 2636 | 851 | 1511 | 1422 | 3812 | 4111 | 6102 | 3181 |
| Majoret | 2067 | 2367 | 1711 | 5024 | 2459 | 693 | 1300 | 1265 | 3819 | 4407 | 4897 | 2728 |
| Navarro | 2167 | 4283 | 2555 | 4364 | 2305 | 467 | 1142 | 1279 | 3765 | 3825 | 5769 | 2902 |

| | | | | | | | | | | | | |
|------------|--------|-------------|-------------|-------------|--------|--------|---------|-------------|-------------|-------------|-------------|-------------|
| Nette 2010 | 2399 | 5329 | 3240 | 6845 | 2508 | 594 | 1814 | 1470 | 4039 | 4459 | 6486 | 3562 |
| Means | 2310 | 3929 | 2530 | 5338 | 2472 | 701 | 1530 | 1380 | 3782 | 4209 | 5455 | |
| P-values | 0.2641 | 0.0183 | 0.0009 | 0.0016 | 0.0008 | 0.3760 | <0.0001 | 0.0010 | 0.8150 | 0.1843 | 0.0003 | |
| LSD (0.05) | Ns | 1292 | 559 | 818 | 217 | Ns | 159 | 89 | Ns | Ns | 721 | |
| CV (%) | 9.32 | 23.27 | 15.61 | 10.84 | 6.22 | 30.22 | 7.36 | 4.58 | 8.98 | 7.36 | 9.35 | |

Bold font indicated the highest yielding variety for a location (within a column). irri. = irrigated (the experiment was conducted with supplementary irrigation). Ns= non-significant.

Table 4. Mean grain protein concentration for each variety and locations in 2016 at different locations in Montana

| Varieties | Location | | | | | | | | | | | Variety means |
|------------|---------------|---------------|---------------------|-------------------|---------------|----------------|------------------|---------------|-------------------|-----------------|---------------|---------------|
| | Bozeman | Conrad | Corvallis* irri. | Creston | Havre | Huntley dry | Huntley irri. | Moccasin | Sidney dry | Sidney irri. | Richland | |
| Delta | 19.83 | 15.68 | | 22.00 | 15.48 | 22.62 | 18.78 | 18.73 | 22.68 | 23.30 | 23.75 | 20.28 |
| Hampton | 22.77 | 16.05 | | 23.18 | 18.90 | 23.88 | 19.75 | 22.23 | 23.35 | 23.40 | 25.10 | 21.86 |
| Jet Set | 20.23 | 16.78 | | 21.20 | 17.68 | 22.57 | 18.09 | 19.05 | 21.98 | 23.15 | 23.13 | 20.38 |
| Majoret | 21.43 | 16.60 | | 22.53 | 18.75 | 24.23 | 20.71 | 20.28 | 23.38 | 23.40 | 23.43 | 21.47 |
| Navarro | 20.68 | 16.45 | | 22.33 | 18.80 | 23.84 | 19.42 | 20.45 | 22.33 | 21.80 | 24.93 | 21.10 |
| Nette 2010 | 19.76 | 15.57 | | 20.93 | 16.00 | 23.41 | 18.87 | 18.48 | 20.73 | 21.48 | 22.28 | 19.75 |
| Means | 20.78 | 16.21 | | 22.02 | 17.60 | 23.42 | 19.27 | 19.87 | 22.40 | 22.75 | 23.77 | |
| P-values | 0.0455 | 0.0175 | | <0.0001 | 0.0188 | 0.3430 | 0.0021 | 0.0003 | <0.0001 | 0.5325 | 0.0025 | |
| LSD (0.05) | 0.7 | 2.1 | | 0.58 | 1.89 | Ns | 0.89 | 1.31 | 0.65 | Ns | 1.07 | |
| CV (%) | 4.69 | 9.49 | | 1.86 | 7.59 | 3.86 | 3.27 | 4.67 | 2.07 | 8.36 | 3.19 | |

Bold font indicated the variety with the highest grain protein concentration for a location (within a column).

*Samples were not submitted for protein testing due to weevil damage, irri. = irrigated (the experiment was conducted with supplementary irrigation). Ns= non-significant.

Summary and conclusion from this progress report:

- The variety Nette 2010 produced the highest grain yield in eight of the twelve locations. Similarly, the variety Hampton resulted in more grain protein concentration in most of the locations.
- The mean grain yields from Richland followed by Creston were substantially high compared with other locations.
- Similar to grain yield, mean grain protein concentration was higher at Richland site. The highest grain protein concentration (25.10%) was recorded from the variety Hampton at Richland site. Mean grain protein was lower at Conrad followed by Havre. This need further investigation to improve protein concertation in the grain.

2. Evaluation of pea varieties for early growth and seedling vigor

Project scope and Objective: To evaluate the different varieties/lines of green/yellow peas with high protein content and check (low protein content) lines for seedling establishment, growth in greenhouse conditions. 2. To evaluate the effects of different seed treatments such as PGPR, humic acid etc. influence the early growth in few varieties of peas.

Progress: A) The yellow/green pea varieties/lines have been evaluated for protein content on combustion, the varieties/lines with high protein content and also check varieties/lines have been chosen to be grown for evaluating them for early growth vigor in greenhouse conditions. B) The greenhouse

experiment was done to evaluate those pea varieties for early growth evaluation (seven leaf stage). E) The pea varieties/lines were also evaluated for seedling vigor indices in incubator.

Results: The pea plants were harvested at seven leaf stage to evaluate the early growth in green house conditions. The plants were evaluated for number of nodules per plant's root system, total nodule fresh weight, root and shoot heights (Table 1). The above- and below-ground biomass was also measured after drying the samples at 65°C for 96 hrs. The measured parameters will be correlated with protein content, yield to measure the relationship between early plant growth on protein content and also yield.

In incubator, for seedling vigor index, 15 seeds/variety in replication of four were kept moist on germination trays for 8 days at 30°C to measure the seedling vigor characteristics mentioned in Table 2 i.e. length (cm) of the seedling, radical and plumule; oven dried weight after drying at 65°C for 72 hrs. Ten seedlings were randomly taken to measure these characteristics. The germination test was also run in incubator by placing 50 seeds for each variety to record germination percentage.

Seedling vigor was calculated using following formula:

Vigor Index I = Germination% x seedling length

Vigor Index II = Germination% x seedling dry weight

Table 1: Evaluation of different pea varieties/lines for early growth in greenhouse conditions.

| Variety | No. of Nodules /plant | Nodule fresh wt.(g) | Aboveground dry wt./plant (g) | Belowground dry wt./plant (g) | Root length (cm) | Shoot length (cm) |
|----------------|-----------------------|---------------------|-------------------------------|-------------------------------|------------------|-------------------|
| PSO877MT632 | 13.53 | 0.149 | 1.30 | 0.65 | 18.58 | 26.04 |
| PSO826MT290 | 5.77 | 0.062 | 1.57 | 0.74 | 19.09 | 35.37 |
| PSO826MT460 | 8.11 | 0.139 | 1.40 | 0.80 | 20.76 | 19.21 |
| PSO877MT457 | 10.74 | 0.098 | 1.34 | 0.67 | 18.67 | 23.48 |
| Delta | 7.85 | 0.079 | 1.36 | 0.68 | 18.32 | 20.69 |
| DS Admiral | 7.77 | 0.094 | 1.46 | 0.84 | 17.79 | 20.08 |
| CDC Striker | 6.73 | 0.087 | 1.58 | 0.84 | 18.93 | 21.29 |
| Majoret | 6.23 | 0.051 | 1.49 | 0.89 | 17.79 | 21.08 |
| Cruiser | 8.60 | 0.152 | 1.54 | 0.84 | 21.14 | 19.66 |
| PSO826MT190 | 12.88 | 0.138 | 1.48 | 0.69 | 21.34 | 22.51 |
| Mean | 8.82 | 0.105 | 1.45 | 0.76 | 19.24 | 22.94 |
| P-value | 0.0071 | 0.232 | 0.569 | 0.292 | 0.477 | <0.0001 |
| LSD | 4.47 | NS | NS | NS | NS | 6.08 |

Table 2: Evaluation of different pea varieties/lines for vigor indices in incubator.

| Variety | Shoot length (cm) | Root length (cm) | Vigor index I | Vigor index II |
|-------------|-------------------|------------------|---------------|----------------|
| PSO877MT632 | 3.20 | 9.07 | 1202 | 44.03 |
| PSO826MT290 | 8.16 | 11.21 | 1898 | 47.97 |
| PSO826MT460 | 2.31 | 9.20 | 1128 | 47.34 |
| PSO877MT457 | 2.35 | 11.28 | 1335 | 45.29 |
| Delta | 1.19 | 9.33 | 1052 | 44.87 |
| DS Admiral | 2.45 | 7.02 | 946 | 49.37 |
| CDC Striker | 0.71 | 6.99 | 754 | 45.61 |
| Majoret | 2.65 | 7.57 | 981 | 44.40 |
| Cruiser | 0.92 | 9.19 | 971 | 47.04 |

| | | | | |
|----------------|-------------------|-------------------|-------------------|--------------|
| PSO826MT190 | 2.11 | 8.82 | 1093 | 47.13 |
| Mean | 2.60 | 8.97 | 1136 | 46.3 |
| P-value | <0.0001 | <0.0001 | <0.0001 | 0.038 |
| LSD | 0.82 | 1.71 | 236 | 3.25 |

Key points:

- A significant difference was observed among different pea varieties/lines only for number of nodules/plant and shoot length only.
- The highest and least number of nodules/plant was observed in pea line PSO877MT632 and PSO826MT290, respectively.
- The longest shoot length was observed in pea line PSO826MT290, which was significantly different from the PSO877MT632.
- No significant difference was observed in shoot length of other 8 pea lines/varieties.
- For vigor indices, significant difference was observed among pea lines/varieties for shoot length, root length, vigor I and vigor II.
- Similar to greenhouse study, the significantly highest shoot height and vigor I was observed in pea line PSO826MT290.
- A significantly highest root length was observed in two pea lines i.e. PSO826MT290 and PSO877MT457 as compared to other lines/varieties.
- Significant highest vigor index II was observed in PSO826MT290, PSO826MT460, PSO826MT190, DS Admiral, and Cruiser pea lines/varieties.

Hiring

- No additional hires in Quarter 5.

Expenditures

- Total Personnel Services: \$49,954.00
- Total Operations: \$16,975.07

Soil Microbiology and Pea Protein subproject of the Agriculture MREDI Grant

1) **41W212 – Principal Investigator:** Perry Miller, Email: pmiller@montana.edu

Progress towards milestones

Further investigation has been undertaken to a) determine how sample size and number of sample replicates affects measured protein content by combustion analysis b) test the prediction accuracy of the FOSS Infratec 1241 default calibration against measured pea protein from combustion analysis, and c) generate a custom calibration curve for pea flour on the FOSS Infratec 1241.

Results for steps a-c are summarized below.

A. Effect of seed number and replicate number on final protein content measured by combustion

Previous results showed that the standard deviation of range in protein content of 81 yellow pea samples run in duplicate pairs using combustion analysis was 1.49%. The high standard deviation suggests that there is potential for highly variable protein content within a given yellow pea sample. Such variability needs to be addressed for two reasons.

1. Protein responses generated by combustion analysis serve as calibration points for near infrared (NIR). If protein is not precisely measured by combustion analysis, then calibration points for NIR will also be imprecise.

2. Large measurement uncertainty in protein response will reduce statistical power for identifying how genetic, management, and the growing environment affect yellow pea protein content.

An experiment was conducted to test how seed and replicate number affected measured protein content by combustion analysis. The response of interest was the absolute difference in measured protein content of a 500 seed sample control to protein measurements from individual and duplicate means of 50, 150, and 250 seeds. The main results of the experiment are summarized below.

1. The mean absolute difference in protein content between the 250 seed subsample and the 500 seed control seed was significantly less (5.1 mg kg^{-1} ; $p\text{-value} > 0.05$) compared to mean absolute differences between the 150 seed subsample and 500 seed control (8.8 mg kg^{-1}) and the 50 seed subsample and 500 seed control (10.6 mg kg^{-1}) (Figure 1. A.).
2. Moderate statistical evidence ($p\text{-value} = .11$) indicated that the mean absolute differences in individual and duplicated protein measurements between the 500 seed control differed and were 9.1 mg kg^{-1} and 7.2 mg kg^{-1} respectively (Figure 1 B.).
3. Variance component analysis suggested that absolute differences in protein responses from the 500 seed control is more attributable to seed lot relative to seed size or subsample number (Table 1). In other words, some seed lots appear to have highly uniform protein content, whereas others do not.

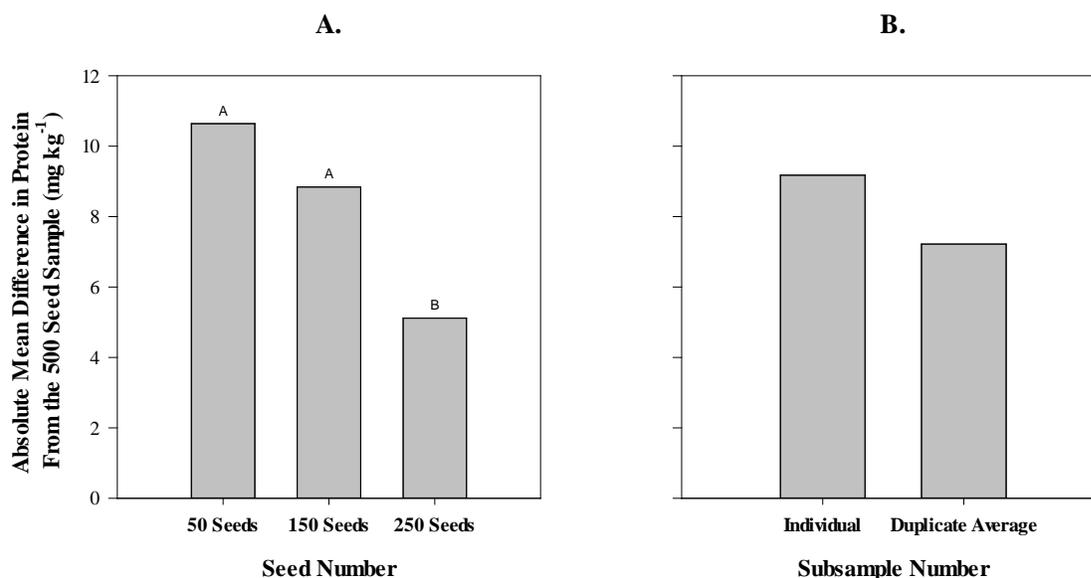


Figure 1.(A) Mean absolute difference in protein content between the 500 seed sample and seed number. (B) Mean absolute difference between the 500 seed sample and subsample number. Different letters indicate statistical differences at the $\alpha=0.05$ level.

Table 1. Normalized restricted expected maximum likelihood (REML) variance component estimates

| Variance Component | Proportion of Variance |
|--------------------------------|------------------------|
| Seed Lot | 0.38 |
| Seed Number | 0.15 |
| Subsample Number | 0.02 |
| Seed Number x Subsample Number | 0.00 |
| Error | 0.45 |

Based on these preliminary results, we recommend processing a 250 seed subsample for measuring protein by combustion analysis. A 250 seed subsample appears to be the most representative of a 500 seed control and is still a reasonable sample volume that can fit easily fit into standard benchtop mills

required for combustion prep work. Additional work is being conducted to test the how running subsamples in triplicate affects absolute differences between the 500 seed control.

B. Validation of the FOSS Infratec™ 1241 Whole-Seed Default Calibration Curve for Predicting Yellow Pea Protein

The FOSS Infratec™ grain analyzer is equipped with a factory calibration equation to predict protein content in whole pea seed samples using NIR. Five scans are made per sample, and final protein predictions are taken as the average protein prediction of the five scans. The prediction accuracy of the calibration equation was validated against 46 whole seed samples measured for protein using combustion analysis in with ~50 seeds in duplicate. The main results regarding the predictive accuracy of the default calibration to are as follows.

1. The mean absolute error (MAE) between the average predicted and measured protein was 10.2 mg kg⁻¹ (Figure 2). In other words NIR predicted the lab-measured protein to within 1% on average.
2. The average range in measured protein of laboratory duplicate samples was 11.3 mg kg⁻¹ or 1.1% protein
3. The average predicted range in protein of five NIR scans per sample was 22.4 mg kg⁻¹ or 2.2%.

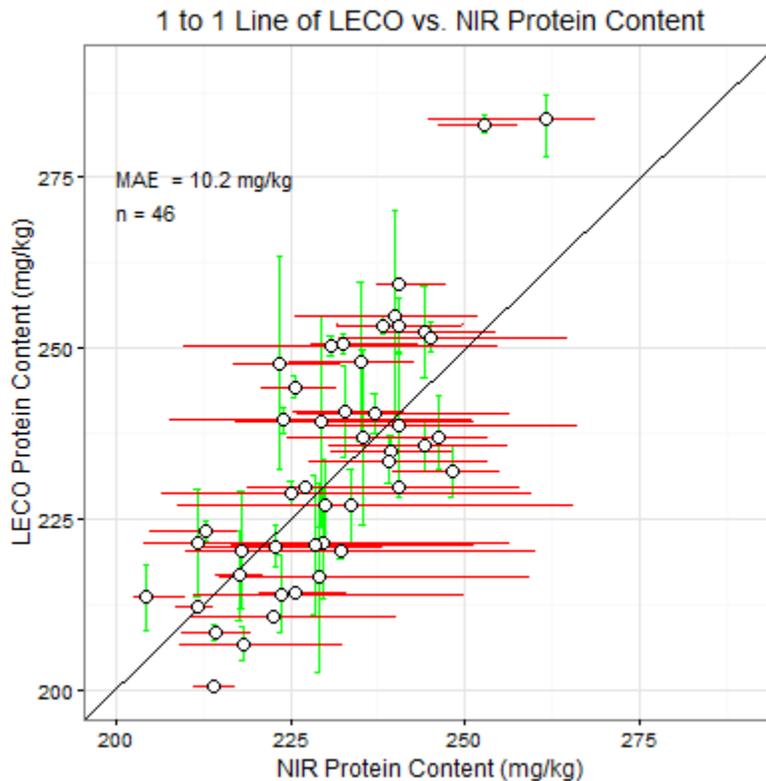


Figure 2. 1-to-1 line of LECO-Combustion (Measured) vs NIR (Predicted) protein content in yellow pea protein based on the FOSS Infratec 1241 factory calibration. Green lines represent ranges in measured protein content from LECO combustion analysis based on duplicates of 50 seeds per subsample. Red lines represent ranges in predicted protein content based on five scanned subsamples.

The high Mean Absolute Error (10.2 mg kg⁻¹) potentially arises from variable protein content within each bulk sample (see step A; i.e. 50-seed subsamples). Utilizing revised sampling procedures (e.g. using 250 seed subsamples or identifying seed lots with low protein variation) prior to measuring protein with combustion analysis may provide better NIR validation. The 46 samples used to validate the NIR

calibration will therefore be reanalyzed by combustion analysis with 250 seed subsamples to test this hypothesis.

C. Custom calibration curve for pea flour on the FOSS Infratec 1241.

Predicted and observed protein content resulted in a mean absolute error of 10.2 mg kg⁻¹ using the FOSS default calibration on whole seed samples (see step B). This seemingly high discrepancy in measured and predicted protein content may arise from heterogeneous protein in yellow pea bulk samples (see step A). Milling pea samples and performing NIR analysis on yellow pea flour—as opposed to whole pea seeds—could help homogenize protein content and reduce prediction uncertainty across scans.

In order to address this, protein in yellow pea flour for 306 samples was measured by the combustion method and subsequently run on NIR to generate calibration curves from spectral NIR output. A total of 60 calibration curves were generated based on a combination of different data preprocessing methods and principal components (1-10) using principal components regression (PCR) and partial least squares (PLS). The specific data preprocessing methods were 1) no baseline removal, 2) baseline correction using the first derivative Gap-Filter, and 3) baseline correction using the second derivative Gap-Filter. Leave-one out cross validation (LOOCV) was lastly performed and evaluated based on the mean absolute error of cross validation (MAECV).

Results of this analysis showed that the MAECV ranged from 18.86-8.95 mg kg⁻¹ depending on model (Table 2.). The lowest MAECV validation was obtained using the first derivative Gap Filter with five principal components, but all regressions with 4 or more principal components were roughly similar.

Table 2. Mean absolute error of cross validation (MAECV) corresponding to each calibration equation.

| Model and Preprocessing Method | Number of Principal Components | | | | | | | | | |
|--|--------------------------------|-------|-------|------|------|------|------|------|------|------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | RMSECV (mg kg ⁻¹) | | | | | | | | | |
| PCR-No Pre | 18.86 | 10.75 | 10.31 | 9.33 | 9.02 | 9.03 | 9.02 | 9.06 | 9.07 | 9.09 |
| PCR- 1 st Der | 15.11 | 9.22 | 9.19 | 8.99 | 8.96 | 8.99 | 8.98 | 8.99 | 8.99 | 9.00 |
| PCR- 2 nd Der | 17.23 | 10.13 | 9.20 | 9.16 | 9.17 | 9.00 | 9.03 | 8.97 | 8.99 | 8.99 |
| PLS-No Pre | 16.69 | 10.46 | 9.81 | 9.13 | 8.99 | 9.04 | 9.06 | 9.13 | 9.15 | 9.33 |
| PLS-1 st Der | 12.15 | 9.16 | 9.13 | 8.96 | 8.95 | 9.03 | 8.98 | 8.96 | 8.99 | 8.97 |
| PLS-2 nd Der | 12.26 | 9.60 | 9.17 | 9.10 | 9.00 | 9.01 | 8.98 | 9.02 | 9.06 | 9.06 |
| MAECV—Mean absolute error of cross validation | | | | | | | | | | |
| PCR—Principal Components Regression | | | | | | | | | | |
| PLS—Partial Least Squares | | | | | | | | | | |
| No Pre—No data preprocessing | | | | | | | | | | |
| 1 st Der—Data preprocessed using 1 st derivative of Gap Filter | | | | | | | | | | |
| 2 nd Der—Data preprocessed using 2 nd derivative of Gap Filter | | | | | | | | | | |

These results do not indicate that milling pea flour will greatly improve NIR prediction accuracy compared with the factory NIR calibration run on whole seed samples (see step B). However, these results are preliminary, and as more calibration points are added, calibrations could improve. Additional yellow pea samples collected from the 2016 growing season will therefore be utilized for NIR calibration points, and formal test and training sets will be split to validate NIR protein prediction accuracy based on the calibration curve with the lowest MAECV.

Report Summary and Moving Forward

Measuring yellow pea protein is difficult due to potential for high protein variation within a bulk sample (see step A.). Such variation makes it challenging to verify that the factory NIR calibration on the FOSS Infratec™ 1241 is predicting yellow pea protein within an acceptable level of precision for research or industrial purposes (see step B.). For instance, in wheat, protein predictions by NIR are considered acceptable if they fall within 1.0 mg kg⁻¹ of measurements by combustion analysis (Matt Clancy—*Next Instruments*, Personal Communication, August 2016). Revised sampling procedures such as increasing subsample size to 250 seeds prior to measuring protein by combustion or identifying seed lots with low protein variation may provide a better standard sample data set which may lead to better precision between NIR predictions and combustion measurements. Preliminary NIR custom calibration curves with pea flour, as opposed to whole seed samples, do not greatly improve prediction accuracy compared to NIR run on whole seed samples (see step C.).

Moving forward the following steps will be taken to further address protein variation and NIR calibration uncertainty.

1. Subsamples of 50, 150, and 250 seeds will be run in triplicate to test how the mean protein content differs between the protein content of a 500-seed control. This will be done because there was statistical evidence suggesting that the average protein content from duplicated combustion measurements provided a better representation of the 500-seed control (see step A). If the average of triplicate protein measurements better resembles the 500-seed control, it may be advisable to use three subsamples—not just two—for estimating the protein content of a yellow pea bulk sample.
2. The forty-six samples already used to validate the factory NIR calibration equation on the FOSS Infratec™ 1241 (see step B.) will be re-analyzed for protein content by combustion analysis using a subsample size of 250 seeds instead of 50 seeds. The NIR calibration will then be validated against the revised protein measurements.
3. Custom calibrations for pea flour (see step C.) will be updated as more samples are acquired from the 2016 growing season. This will be done since more samples could potentially improve pea flour calibration equations.

Hiring

- No additional hires in Quarter 5.

Expenditures

- Total Personnel Services: \$51,572.83
- Total Operations: \$2,849.99

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

Progress towards milestones

In the fifth quarter, we have focused on the analysis and interpretation of the 16s rRNA data and optimization of the experimental protocol for extracting and sequencing nifH. We have separated the data into three main goals; analysis of statewide agriculture research centers, comparison of SARC irrigation methods and post farm analysis. In each one of these data sets, we are refining a pipeline of analysis to enable an efficient way to process the data with respect to the changes in bacterial communities and nitrogen fixation in soil.

- Statewide Data Analysis

- The dataset collected last fall of the pea rotation soil from the agriculture experimental stations is the largest dataset we have produced. Over the previous quarter, the 16s rRNA data was processed using the MOTHUR SOP into phylogenetic diversity outputs such as operational taxonomic units (OTU) and Shannon-Simpson index (alpha diversity). Data created in the mother pipeline was visualized using r studio packages such as ggplots2, vegan, and phyloseq (Figure 1).

- Plots created during this quarter show change in overall community structure and diversity throughout the statewide experimental stations. There is a difference in yields across the farm locations and a difference in alpha diversity (Figure 2a,2c). Outliers are kept for interpretation of geographical diversity changes in the soil microbiome (Figure 2b, 2d). There is an overall trend in the statewide farm sites that show a correlation in-between the increased alpha diversity and the yield of the farms (Figure 3). Diversity index number (alpha) comparisons should be taken into consideration only if there is similar sampling depth taken throughout the farm locations (Figure 4). We then took a dissimilarity matrix of the classified OTUs and applied non-metric multidimensional scaling (nMDS). By plotting of the nMDS points grouping of geographical farms site can be seen (Figure 5).

- The results from the statewide data lead to broad conclusions but lead the project in the right direction. Upon analysis of the large data set, we decided to focus in on the Huntley (SARC) center in order to elucidate more details from our 16s rRNA data.

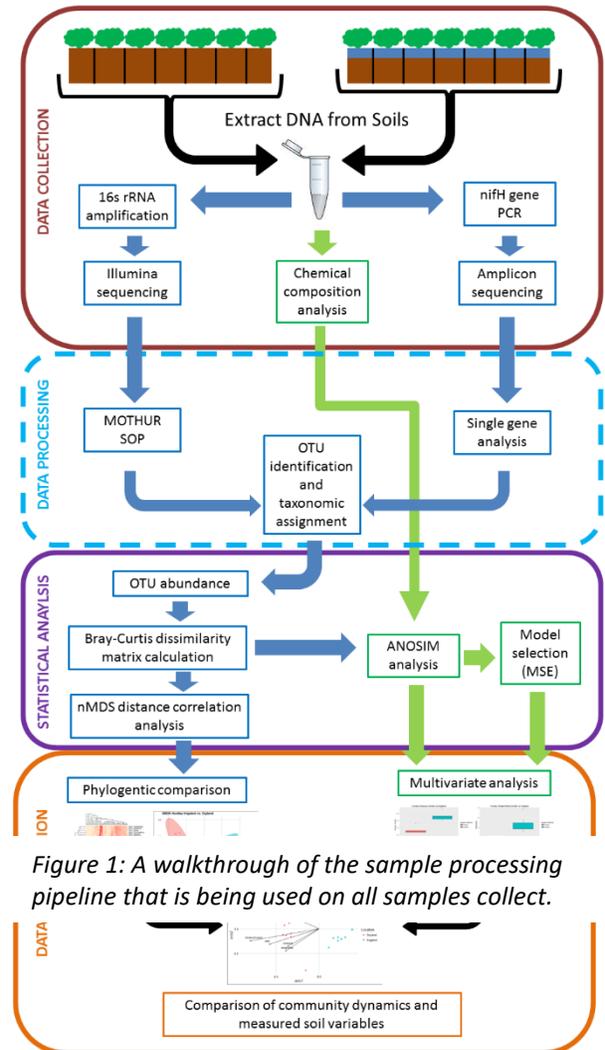


Figure 1: A walkthrough of the sample processing pipeline that is being used on all samples collect.

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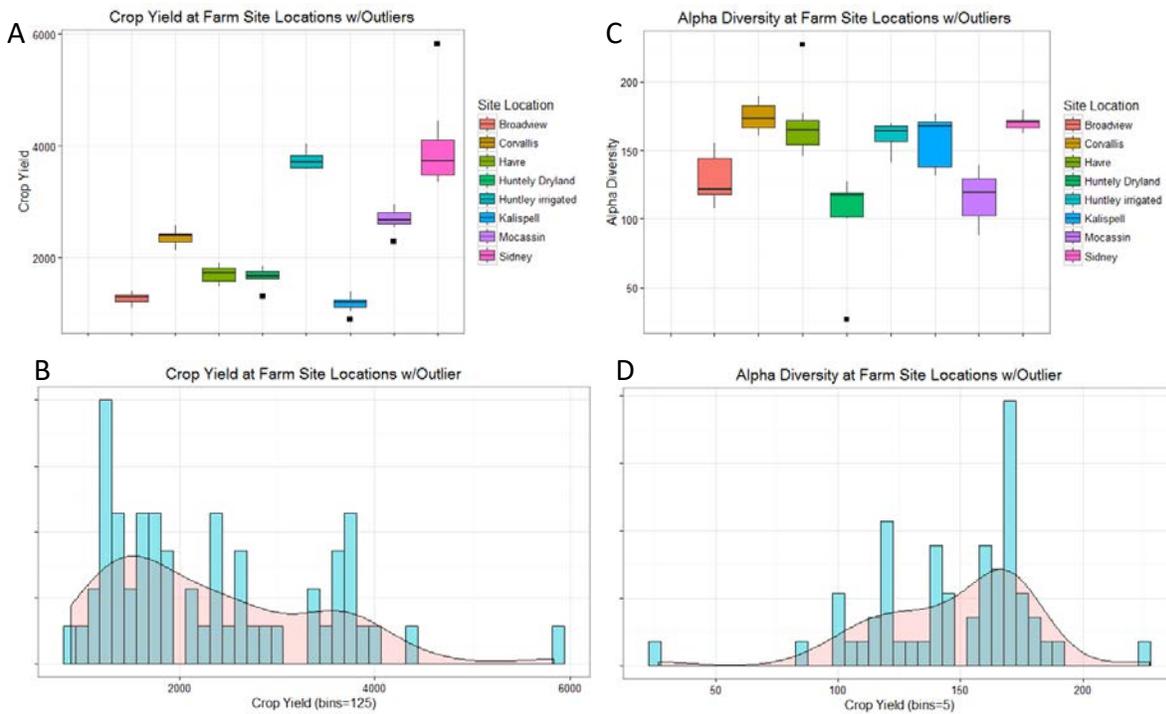


Figure 2: Crop yield varies between geographical locations ANOVA testing shows significance between geographical locations ($p\text{-value} = 8.37 \times 10^{-23}$). Alpha diversity varies in between some of the locations ($p = 2.38 \times 10^{-9}$). Noticeably Corvallis, Harve, Huntley Irrigated, Kallispell, and Sidney show a high alpha diversity. The alpha diversity is representative of the spices richness of the plots.

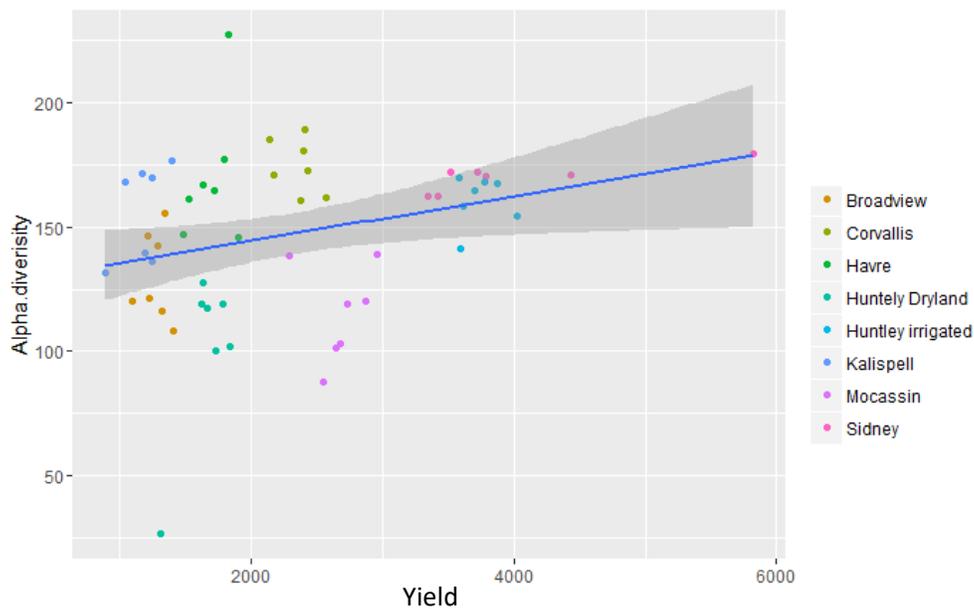


Figure 3: Alpha diversity and yield is correlated ($p\text{ value} = 0.02631$) with a positive incline. We can also see grouping of alpha diversity and yield by location (colors). Linear regression is shown with ($r = 0.29$) and error in grey is a (95% confidence).

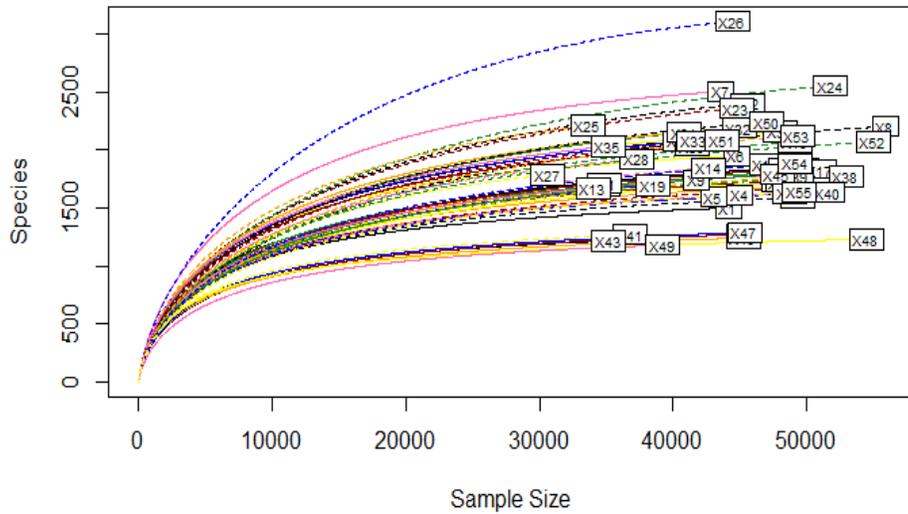


Figure 4: Rarefaction curve of the statewide 16s rRNA sampling data. Rarefaction curve estimates the amount of samples observations need to accurately describe the amount of species in the samples.

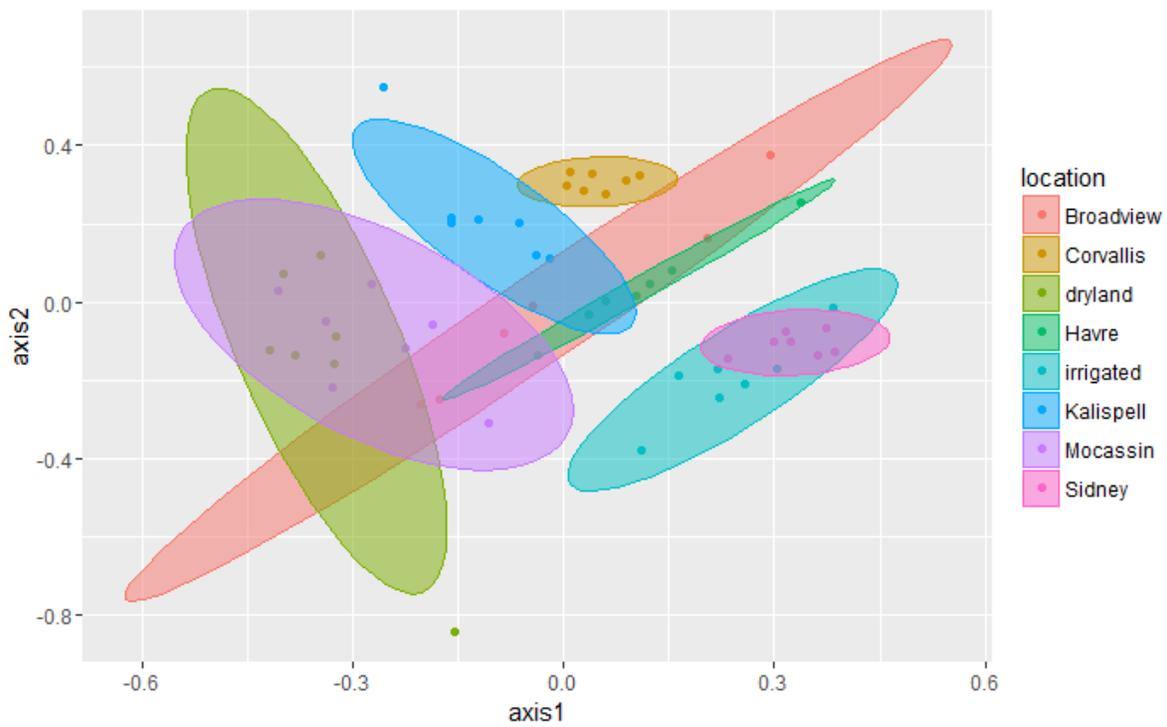


Figure 5: nMDS plot shows the distance characteristics between each plot and variety sample. Each point is a single pea variety at a single sample site. The `stat_ellipse` package was used in `r` to produce a bivariate density ellipsoid with the confidence level of 0.95. The ellipse statistics shows grouping of geographical variables within in the community structure data. The higher yielding stations (SARC irrigated and EARC) are grouped together with positive influence contributed from axis 1. While other stations show large stratifications across the plot which is due to the large distances in the community trees present in the soil.

- Huntley irrigation
 - There is a noticeable difference across many variables between the irrigated and non-irrigated plots (Figure 6). The rarefaction curves also plateau representing good sampling depth at our sequence rates (Figure 7).
 - With these differences between the irrigated and non-irrigated plots, a closer look at the variables effect on the community can be done by assigning specific taxonomic changes to each of the plots. OTU's were taxonomical aligned with the greengenes database and identified down to genus and species level. The composition of the taxonomy through different data visualization methods was compared. First, a bar plot was made using the phylum abundance to compare each of the samples (Figure 8). For more detailed analysis, a heatmap with clustering trees was made to compare genus level abundance. Clustering show clear distinction between differing irrigation methods and similar grouping of variety types (Figure 9). An nMDS plot that compares the community structure of the two irrigation methods and the soil fertility measurement shows influence on the community structure (Figure 10). Moisture content, nitrogen, and yield vectors overlay with the dryland nMDS points meaning the given vectors have similar response profiles to change in microbial community structure. This could be explained by an overall effect of irrigation methods on nutrient profiles or microbial communities effect on nutrient profiles. More statistical interpretation is needed to create a correlation.

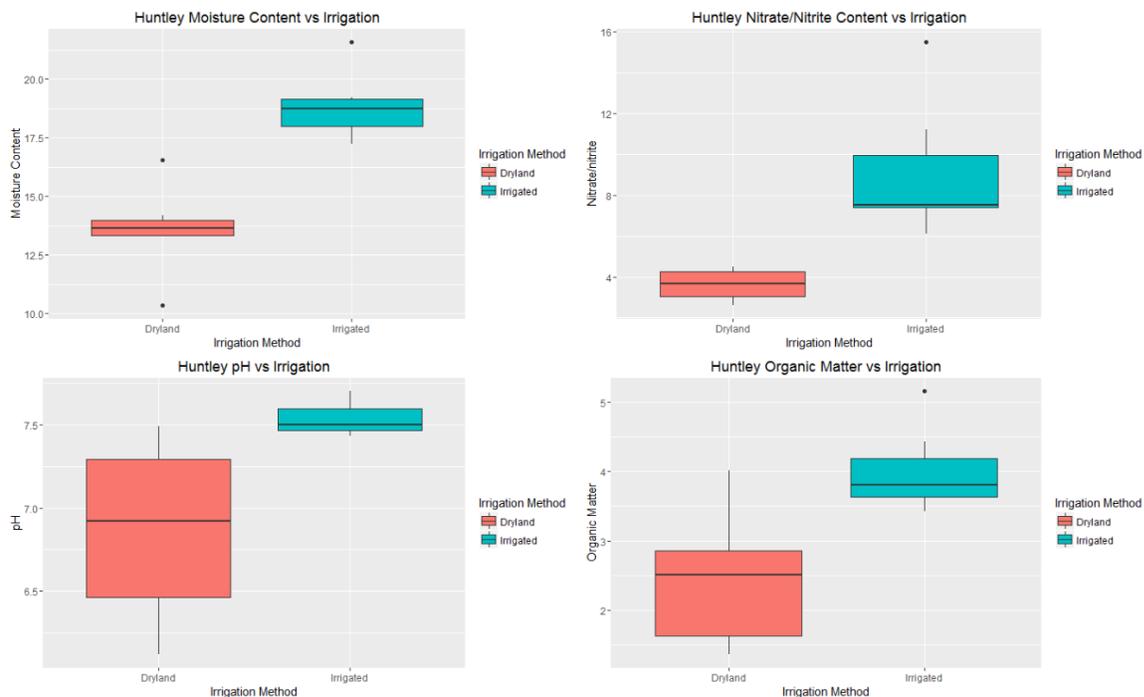


Figure 6: By looking at the single variables of the SARC plots we can compare irrigation methods and outcome variables. A) A difference in measured moisture content and consistent moisture (small STD) across the pea variety was measured ($p=2.774e-11$). B) The nitrate and nitrite content consistency is seen in the dryland plots but see some outlier characteristics are present in the irrigated plots ($p = 0.0037$). C) There is also a pH difference in between the two irrigation methods with dryland having a broad range from 6.5 to 7.25. As irrigated has a consistent pH measurement ($p = 0.015$). D) Organic matter was measured in the soil and has a significant difference in between two sites ($p = 0.0035$).

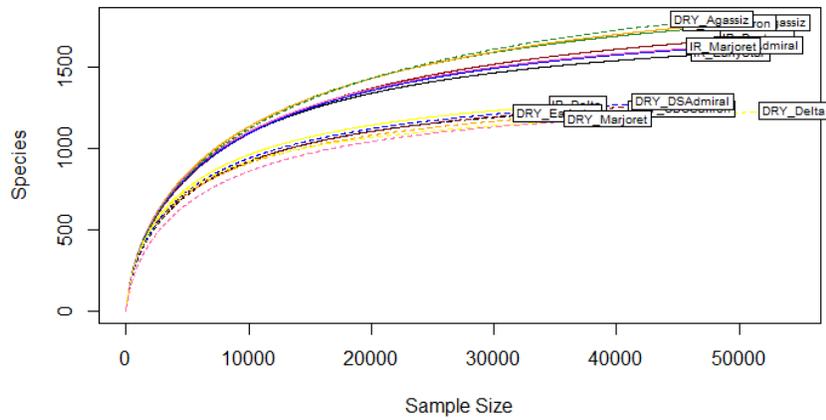


Figure 7. Rarefaction curve shows specie richness in the SARC. Rarefaction curves are used to predict if the number of observed species (OTUs) is representative of the actual species in the sample. We observed on average ~26,000 OTU across the samples giving us plateauing affect which can be used to assume an accurate representation of the samples. There is also a distinct splitting of the rarefaction curve in the above plot, the spilt does not correlate to irrigation strategy or plant variety.

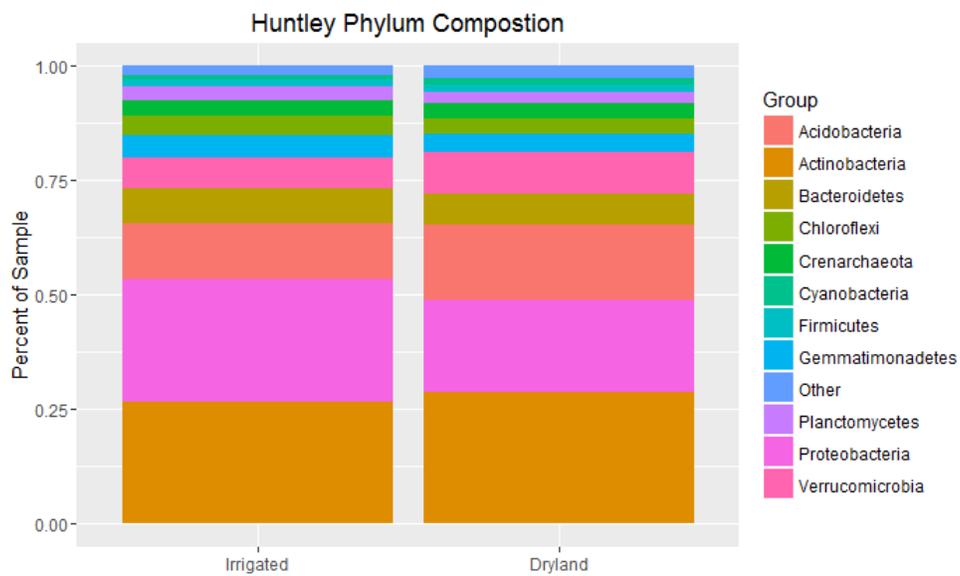


Figure 8: Bar plot showing the top 12 abundant phylum in the samples. There is no obvious difference on the phyla level between the irrigation methods. We can see a small difference in acidobacteria and proteobacteria which consist of many cited plant growth prompting bacteria and diazotrphs. A more detailed look is needed to decipher the true changes in these certain bacterial strains. Actinobacteria also make up a majority of the population of bacteria in the both of the samples. This is in contrast to other reports of farm soil which usually has actinobacteria ranked as third or fourth most abundant phylum, more literature research will be done to determine this difference.

- nifH progress
 - Another of the main objectives of the pulse crop project was to determine the family structure of diazotrophs and nifH genes present in free-living soil bacteria. The nifH gene codes for the nitrogenase enzyme that reduces atmospheric nitrogen to ammonia and supplies the local communities with reduced nitrogen.
 - We are approaching this hypothesis from two different experimental methods. First, we can utilize our already taken 16s rRNA taxonomical data and pull out the know diazotrophs. This can also be done in parallel with predictive genomic function algorithm called PICRUST that can then predict the nitrogen metabolism of specific samples. The second method is to amplify the nifH gene out of the soil using universal primers and PCR to make a gene library. This library will then be sequenced and we could then cluster the nifH gene and compare differences between samples.
 - We have found a universal nifH that works on our diverse soil DNA samples and we are now in the process of optimizing the process in order to create our nifH gene library. We have started with the SARC samples in order to simplify the process.

- Post Farm Analysis
 - OTU tables have been created with the Post Farm data. The interpretation of this data is similar to the methods used for the statewide data. Plot are created showing the nMDS correlation of the differently treated soil and the community structure (Figure 11). There are signs of a grouping of treatment groups in the dissimilarity matrix data. More research and data analysis is needed to make any conclusions to the post farm data.

Future work will continue the analysis of the data present in order to create a publication on our findings. Further soil process is also occurring with the newly received samples from spring and summer of 2016. Chemical analysis and DNA extraction experiments will be done by the end of the year.

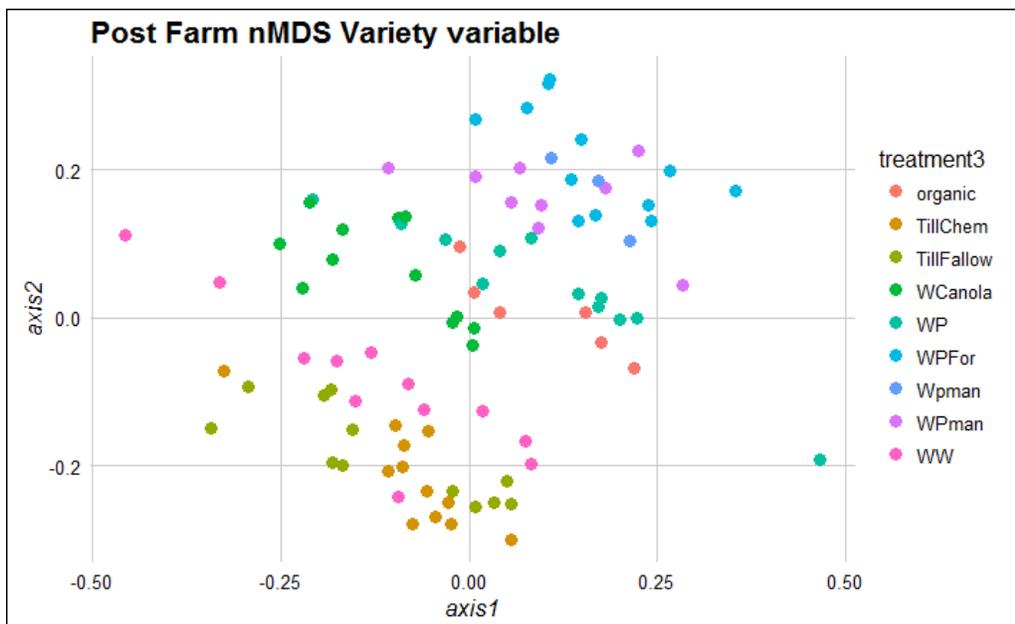


Figure 11: nMDS plot showing treatment variables (colors) grouping with each other. The plot shows that certain groups have similar or dissimilar community structure present. Treatment such as amount of nitrogen added (fertilizer) and previous crops growth (winter wheat) effects the microbial community structure.

Hiring

- No additional hires in Quarter 5.

Expenditures

- Total Personnel Services: \$56,213.07
- Total Operations: \$18,659.88

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

Progress towards milestones

The bioreactor to run the animal trial was delivered shortly after Quarter 4 report was submitted allowing the animal trial for this portion of the project to be completed. Although all the stats haven't been compiled, it looks like we have underestimated the natural ability of the sheep rumen microbes to reduce nitrate. We are thus contemplating trying an in vitro method with the collected samples, or otherwise may have to find funding to conduct a second trial.

Additionally, a paper has been published (online only at this point) acknowledging funding from MREDI. Although it isn't directly related to this part of the project, it fits well with aspects of the proposal and the projects overall goals - the reference is below:

Ishaq SL, Johnson SP, Miller ZJ, Lehnhoff EA, **Olivo S**, **Yeoman CJ**, Menalled FD. 2016. *Impact of cropping systems, soil inoculum, and plant species identity on soil bacterial community structure* as found at <http://link.springer.com/article/10.1007%2Fs00248-016-0861-2>. *Microbial Ecology In press* doi:10.1007/s00248-016-0861-2.

Hiring

- No additional hires in Quarter 5.

Equipment

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

Expenditures

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

Cover Crop/Grazing subproject of the Agriculture MREDI Grant

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

Progress towards milestones

The data in Figure 1 is from research prior to MREDI shows that producers using a cool season cover crop mix will achieve ~ \$100/A increased cash return compared to using crop fallow for either winter wheat or spring wheat. This data is from 4 years ago and was done when precipitation was <12 inches/year therefore this is a good proof of concept for integrating cover crops into annual cropping scenarios for most all locations in MT thus providing greater income potential for producers currently practicing crop fallow.

4 Year Cash Returns 2 Full Cycles Cover Crop/Wheat

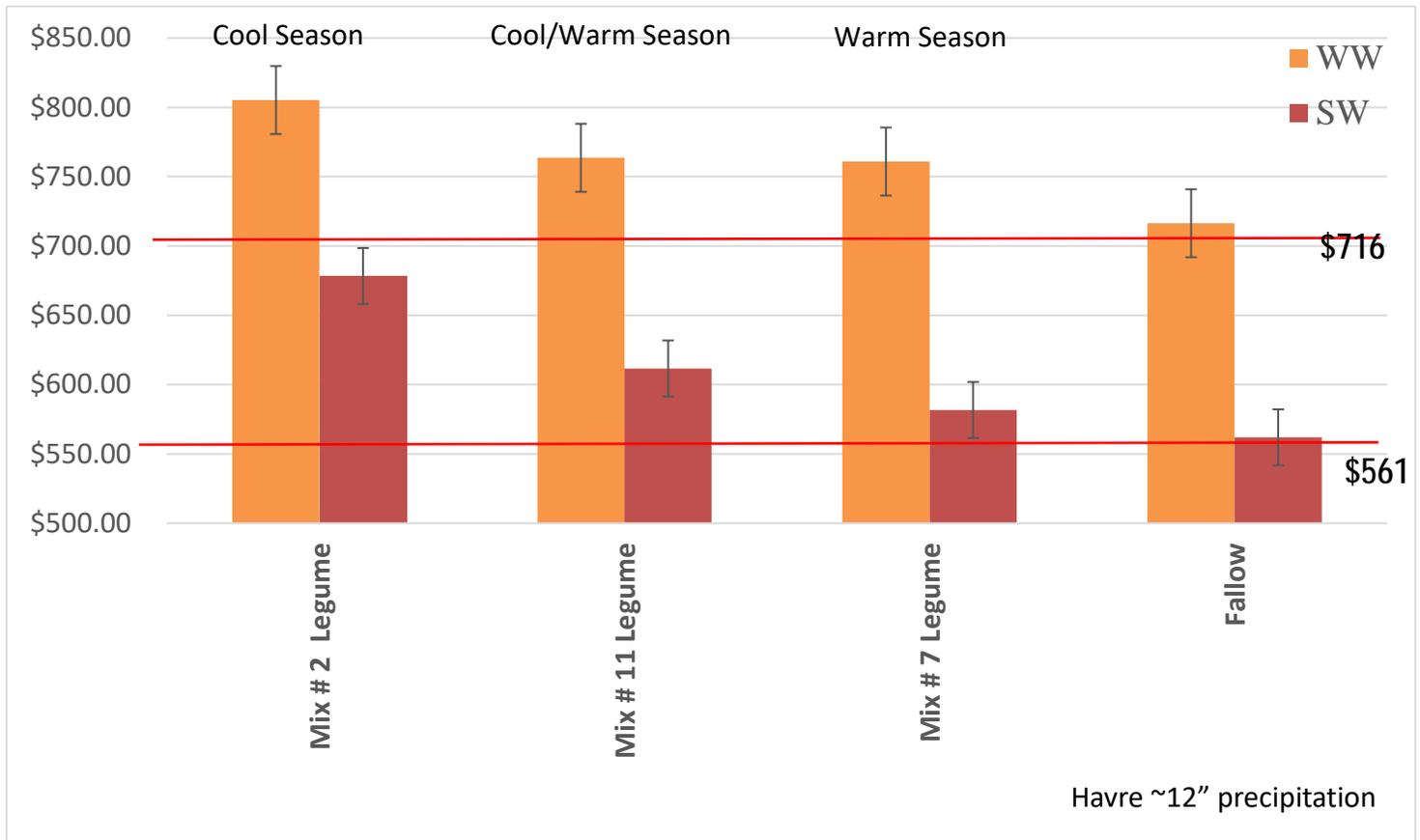


Figure 1. Cool season potential annual increase

Hiring

- No additional hires in Quarter 5.

Expenditures

- Total Personnel Services: \$16,523.26
- Total Operations: \$9,637.39

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

Progress towards milestones

Our group is finishing analysis and write-up for our first project which evaluated the grazing preferences of sheep on forage barley and oats. Those results are shown below. We are working on finishing up the nutrient analysis and interpretation of a warm-season grass variety trial, as well as a nutrient analysis and soil analysis on a long-term trial that was a part of Dr. Perry Miller's research program. In this study, we are evaluating not only the traditional nutrient contents of various cover crops, but we are also performing a complete micronutrient analysis on both plant tissue and soil. Our goal is to evaluate how much of the nutrients available in the soil actually reach the plant, as well as what nutrients are used to make up the plant "fingerprint". We hope that the analysis will be done and ready for interpretation by the end of this month.

Grazing preference trial

Materials and Methods: *All protocols were approved by the Montana State University Agriculture Animal Care and Use Committee (Protocol 2016-AA12).*

The fifteen cultivars were established on May 18, 2016 on Meadowcreek loam soils at the Bozeman Agricultural Research and Teaching Farm. The seedbed was prepared a year prior in 2015, and was rolled once more immediately prior to seeding. Species were established using a no-till drill, and seeded at a rate of approximately 73 kg ha⁻¹. Soil samples were taken prior to establishment, and fertilized appropriately. All species were grown in a dryland environment, with no supplemental irrigation supplied.

The study was planted as a randomized complete block design, with a total of three reps per cultivar. Individual plots measured 1.8 m x 4.6 m. Initial plant heights were taken in three locations within each plot across the diagonal using a meter stick. Initial herbage mass samples for all plots were taken on July 13, 2016, after a majority of the cultivars had begun heading. As all plots were uniform in growth, a 0.3 m x 0.3 m quadrat was randomly thrown into the middle of each plot, and samples were cut to a 2" height. Samples were immediately weighed for initial herbage mass, and placed in a 60°C oven for 72 hours for drying. Upon drying, samples were immediately reweighed in order to determine initial dry matter production. Initial plant heights were taken in three locations within each plot across the diagonal using a meter stick.

Each replication was fenced off individually using mesh nylon fencing. A solar-powered charger was used to electrify the fence. On July 18, 2016 at 0800, 8 Rambouillet rams (47.0 ± 8.3 kg) were placed into block 1 of treatments for a 24-h grazing period. Sheep were removed after 24-h and placed into block 2 for the second day of data collection. On day 3, sheep were moved for a final time into block 3 for the final 24-h grazing period. Sheep had access to *ad libitum* water at all times.

Residual herbage mass samples were taken each day immediately after sheep removal. Due to uneven grazing, two 0.3 m x 0.3 m quadrats were harvested from each plot to more accurately depict residual herbage mass. These samples were placed in a 60°C oven for 72 hours for drying. Herbage mass removal was calculated using the following equation:

$$\begin{aligned} \text{Herbage mass removal (g)} &= \text{initial DM herbage mass} - \text{residual DM herbage mass} \\ \text{Herbage mass removal (\%)} &= \frac{(\text{initial DM herbage mass} - \text{residual DM herbage mass})}{\text{initial DM herbage mass}} \end{aligned}$$

Samples were then ground using a Wiley mill (Thomas Scientific) with a 2 mm screen. Samples were thoroughly mixed, and subsamples were submitted for nutrient analysis. Acid detergent fiber (ADF), total digestible nutrients (TDN), crude protein (CP), and nitrate levels were all evaluated.

Data were analyzed using the Proc GLM procedure of SAS Version 9.4 (SAS Institute, Cary, NC). Plots were the experimental unit, and statistical significance was set at $P \leq 0.05$. Means are the least square means of the GLM procedure. Cultivar and replication were set as fixed effects. No interactions between cultivar and replication were found to be significant, or have a trend for significance ($P \geq 0.10$), and so those data are not shown.

Results and Discussion

Table 1. Morphological attributes of the fifteen cereal forage cultivars tested.

| Cultivar | Species | Initial | Initial | Residual | Herbage Mass Removal (%) | Initial | Residual | Visual Removal Estimation (%) |
|------------|---------|--|---|-------------------------------|-----------------------------------|-------------------------|-------------------------|--|
| | | DM Herbage Mass ¹ (kg) | DM Herbage Mass (kg ha ⁻¹) | DM Herbage Mass (kg) | | Plant Height (cm) | Plant Height (cm) | |
| Stampede | Oats | 1276.5 | 8014.1 | 586.6 | 53.6 | 43.4 | 14.5 | 86.7 ^a |
| Otana | Oats | 1822.32 | 11441.6 | 776.6 | 57.0 | 76.5 | 38.6 | 55.0 ^b |
| MT103038-6 | Barley | 1809.5 | 11362.0 | 818.6 | 54.3 | 45.0 | 25.7 | 56.7 ^b |
| Haxby | Barley | 1934.1 | 12144.0 | 915.7 | 52.5 | 62.2 | 36.3 | 55.0 ^b |
| Hays | Barley | 1605.2 | 10078.4 | 758.9 | 50.5 | 51.1 | 40.1 | 50.0 ^{bc} |
| Horsford | Barley | 1689.7 | 10609.1 | 806.8 | 51.2 | 56.4 | 42.4 | 48.3 ^{bcd} |
| MT103083 | Barley | 1542.0 | 9681.7 | 848.8 | 45.8 | 53.6 | 31.5 | 48.3 ^{bcd} |
| MT103038-4 | Barley | 1878.5 | 11796.0 | 1070.7 | 42.2 | 52.3 | 33.0 | 48.3 ^{bcd} |
| MT103089-3 | Barley | 1806.6 | 12044.1 | 813.9 | 57.0 | 61.0 | 34.8 | 41.7 ^{bcde} |
| MT103101-5 | Barley | 1461.1 | 9173.8 | 1000.0 | 38.3 | 64.3 | 40.6 | 46.7 ^{bcde} |
| MT10397-1 | Barley | 1896.9 | 11910.9 | 1023.6 | 44.9 | 68.6 | 41.0 | 45.0 ^{bcde} |
| Lavina | Barley | 2013.9 | 12645.8 | 1016.6 | 48.7 | 65.3 | 41.4 | 33.3 ^{def} |
| Haymaker | Barley | 1765.9 | 11088.2 | 874.4 | 50.0 | 59.2 | 32.3 | 36.7 ^{cdef} |
| Haybet | Barley | 1857.9 | 11665.0 | 1096.9 | 50.6 | 70.4 | 41.7 | 31.7 ^{ef} |
| Pronghorn | Barley | 1817.4 | 11410.5 | 831.0 | 53.4 | 89.4 | 66.3 | 21.7 ^f |

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

¹DM: dry matter

No differences were observed between cultivars in initial herbage mass (HM) production ($P = 0.38$), residual HM production ($P = 0.11$), initial plant height ($P = 0.38$), residual plant height ($P = 0.15$), and estimated HM removal ($P = 0.06$). There were cultivar differences observed in visual estimation of HM removal, with 'Stampede' showing considerably more HM removed by the grazing animals than any other cultivar entered. There were also differences noted in the forage barley varieties, with the new forage barley entries being significantly more preferred over some of the older varieties.

There was only a trend for significance in the measured removal of the plots, and it is believed that this difference is due to sampling method. As only two quadrats were removed and weighed per plot, we may have missed a significant portion of the plots, altering the analysis. Had we had the ability to sample a larger portion of the plot, the numbers may have been more reflective of the subjective visual observations. However, it was noted that each day the animals were turned into a new set of plots, all sheep went to the oat plots first before moving to the forage barley plots.

It is not surprising that there was no difference between the initial heights and HM production of the entries, as all entries appeared to be fairly similar in growth patterns. Some of the cultivars, particularly the two oat entries, did mature a little faster than the other entries in the trial, but the height was fairly similar among all plots.

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

| Cultivar | Species | CP | ADF | CF | TDN | NEm | Nitrate |
|----------|---------|------|--------|-----|------|------|---------|
| | | | | % | | | % NO3 |
| Haybet | Barley | 18.7 | 30.2a | 2.6 | 63.4 | 0.64 | 1.02 |
| Hays | Barley | 21.4 | 26.7b | 3.5 | 63.3 | 0.64 | 1.19 |
| Haymaker | Barley | 20.1 | 28.0ab | 2.5 | 62.9 | 0.64 | 0.98 |

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

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

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

There was no significant difference amongst cultivars in ADF ($P = 0.052$), CP ($P = 0.43$), and CF ($P = .97$), although there was a strong trend for significance in ADF. There was an effect of cultivar on TDN ($P = 0.036$), NEm ($P = 0.04$), and Nitrates ($P = 0.02$). There was also a significant effect of replication on NEm ($P < 0.01$), and TDN ($P < 0.01$), with all reps being significantly different from one another, and rep 3 having the highest values for both nutrients and rep 1 the lowest.

The cultivar with the highest TDN and NEm is part of a program breeding for improved digestibility and energy availability, so it is not surprising that it outranked some of the older varieties of forage barley and oats. The rankings found in this study are similar to another study conducted in Montana and Wyoming in 1994, where 'Horsford' and 'Haybet' barley were both found to have higher TDN and CP than 'Otana' and 'Stampede' oat varieties (Cash et al., 1997). The CP appears to be much higher than other reported values for similar cultivars, with our values ranging from 17.3- 21.5%, while other reports have CP values ranging from 8.6-15.3% when annual forages were grown in similar environments (Surber et al.,; Stamm et al., ; Todd et al.,). However, in these studies, the annual forages were being harvested for silage or hay, and were harvested at a later maturity than those harvested in our study. This is supported by our lower ADF values, which ranged from 26.5- 35%, while those in the aforementioned studies ranged from 18-46%. ADF is an indicator of digestibility in the forage, and as a forage matures, more fiber is accumulated and the digestibility is decreased. Also, many of our entries are newer varieties which have been bred for lower fiber and increased digestibility, resulting in the lower fiber values and elevated protein and energy values.

The oat entries had the highest levels of nitrates, which is in agreement with many other published reports. Oats tend to be the highest nitrate accumulators, in front of wheat and barley. It has also been found that barley and wheat varieties that have been bred for forage production tend to have lower nitrate risks than their cereal counterparts. While all of the entries tested reached at least a cautionary level of nitrates, where they would need to be limit-fed to pregnant animals, none of the forage barley entries reached a level close to the oat entries. However, we did not see any adverse effects in the grazing animals, and none of the four animals exhibited any signs of nitrate toxicity. This may be due to the short duration of grazing, as well as the fact that sheep appear to be slightly more resistant to elevated nitrate levels compared to other species of livestock and horses.



Hiring

- An undergraduate student has been hired to help finish the laboratory analysis and write-up findings for the remaining parts of the project.

Expenditures

- Total Personnel Services: \$3,776.86
- Total Operations: \$2,880.47

On-Farm Precision Experiment subproject of the Agriculture MREDI Grant

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

The OFPE team of PIs and key collaborators (farmers and industry representatives) meet every 2 weeks to discuss progress, data management and research approaches. See our website:

(<https://sites.google.com/site/ofpeframework/>) for detailed information about the project. No new hires were made during this quarter.

Progress towards milestones

2016 harvest data including yield and protein data from 4 farms and 8 fields has been cleaned and placed in the temporary database (MSU Box account). In addition, weed data, pre-harvest protein calibration and soil analysis information from 1 field per 4 farms has been stored and made available for analysis.

Yield and protein analysis and optimization for next winter wheat year's nitrogen fertilizer prescriptions has been conducted for 4 fields. We are creating nitrogen fertilizer application experiment prescriptions for at least 1 more field per farm (4 fields) this year. We are continuing with the study under the assumption that funding will be available in 2017 and 2018 to continue analysis and data management.

Yield and protein models were constructed for 1 field on each farm and used to find profit maximizing nitrogen fertilizer rate for side-dress application in each pixel (machine width size determination) in each field. The critical assumption is that the next time that the fertilizer will be applied to the same crop, the year (climate, costs and prices) will be exactly like 2016. Until we have multiple years of information from our experiments we will not be able to provide a probability of outcome, which is our main intent. Hence it is critical to continue this research.

Dr. Lisa Rew has created a preliminary predicted grass weed (cheatgrass) density map for a Broyles field (Sec35mid) which had the most data collected and has a reasonable range of weed densities. All other fields have too few data points and too low weed densities to produce a predicted map. In addition, the Broyles field is the only one where the data suggested a negative impact. The negative impact was interesting given that the entire field was treated with a herbicide that should have been effective.

Expenditures

- Total Personnel Services: \$121,419.57
- Total Operations: \$101,895.67
- Total Equipment: \$90,730.00

2) 41W226 – Principal Investigator: John Sheppard; Email: 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. Using the results from the prototype neural networks from last quarter as a guide, Janette Rounds (graduate student) has developed feed-forward neural networks that perform yield optimization. The networks developed during this quarter have the potential to exploit spatial information in the field in order to more accurately predict yield. Additionally, Ms. Rounds has developed feed-forward networks to optimize protein as well as yield.

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. The ability of the network to approximate the function is dependent on the number of layers in the network.

The networks were trained on data obtained from the 2016 harvest. Then a hypothetical net return optimization for 2016 was generated using the same harvest data. The optimization process also showed higher net return than the random variable nitrogen rate experiment we conducted this year. However, there are a few issues with the optimization as it currently stands. Other teams' analyses operated at a different scale than the neural network optimization, leading to the inability to compare our results to the optimizations produced by other teams. Additionally, the error for the prediction component was quite high, leading to some doubts about whether the optimization produced would have demonstrated the predicted improvement once it had been applied to the field. To address the scale issue, Ms. Rounds is making some adjustments to the network so that the scale of the input to the neural network matches the scale of the input to the other analyses. Additionally, Ms. Rounds is making some adjustments to the experimental design in order to account for the scale differences. In order to address the error issue, Ms. Rounds and Dr. Sheppard are exploring some alternative network architectures and alternative network training approaches.

Expenditures

- Total Personnel Services: \$35,306.19
- Total Operations: \$10,845.05

3) 41W228 – Principal Investigator: Clem Izurieta; Email: 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 (August-November). Undergraduate student Mike Trenk will change status to graduate student (M.S.) under the direction of Dr. Izurieta beginning in the Spring of 2017.

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. 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**

The abstract schema of the data model to provide new features necessary for data input, optimization, and prescription workflows (see figures 1 and 2) is completed. Minor modification of the base schema may occur in the future, if limitations of the original model are discovered. **[Done]**

Data Schema [Done]

- 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

Specific objects in the schema will continue to be added and evolve through the life of the project. The schema is designed to be flexible to allow new data types to be added, as required by new datasets or data relationships used in the agronomic models. For example, we are currently designing specific objects to handle collated data structures necessary to aggregate and collate data for controlling variables for the yield and protein agronomic models. **[Ongoing]**

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.
 - implemented and tested Oauth2 for possible incorporation
 - implemented authentication / authentication design for API
 - implemented basic API test (yield editor data) utilizing security design
 - worked on MongoDB configuration (with respect to security) including a Virtual Machine configuration

- 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**]
- Defining JSON spec for communication between server and client tools. [**In Progress**]
- Implementing OOEDS Java client, getting it working with OOEDS ReST API [**In Progress**]
- Developing data management tools for maintaining consistency in database when data model/schema changes, denormalization, etc. [**In Progress**]
- Prototyping high level query language for exporting data back out of database [**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**]
- Exploring the Open Ag Data Alliance standard for possible incorporation into API design. [**New**]
- Exploration on UI technologies for possible incorporation into UI design. [**New**]

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

3.1 Yield Editor Data Input [**In Progress**]

- 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. [**Done**]
 - Currently Integrating yield editor import python code into OOEDS rest api [**In Progress**]
- The re-definition of the OOEDS Library using Java is forcing additional testing/development. [**In Progress**]
- A prototype with a GUI was demonstrated to the greater team on Nov. 15, 2016.
 - 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 Data rectification workflow [**Planning**]

- We have recently identified a critical step in the process for preparing data for the optimization process. Data for independent variable need to be sampled at locations that correspond to the grain yield and protein response variables. The resulting collated datasets are then used to feed the model selection, calibration, and optimization steps of the optimization work flow. We are working on the software and data model additions necessary to import and query these collated datasets.

3.3 Optimization [**In Progress**]

- 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.

- We have started implementation of a prototype for querying data for optimizations from the database in collated form (see above), and returning the results of optimization with provenance metadata back into the database.

3.4 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. 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]

5. Figures

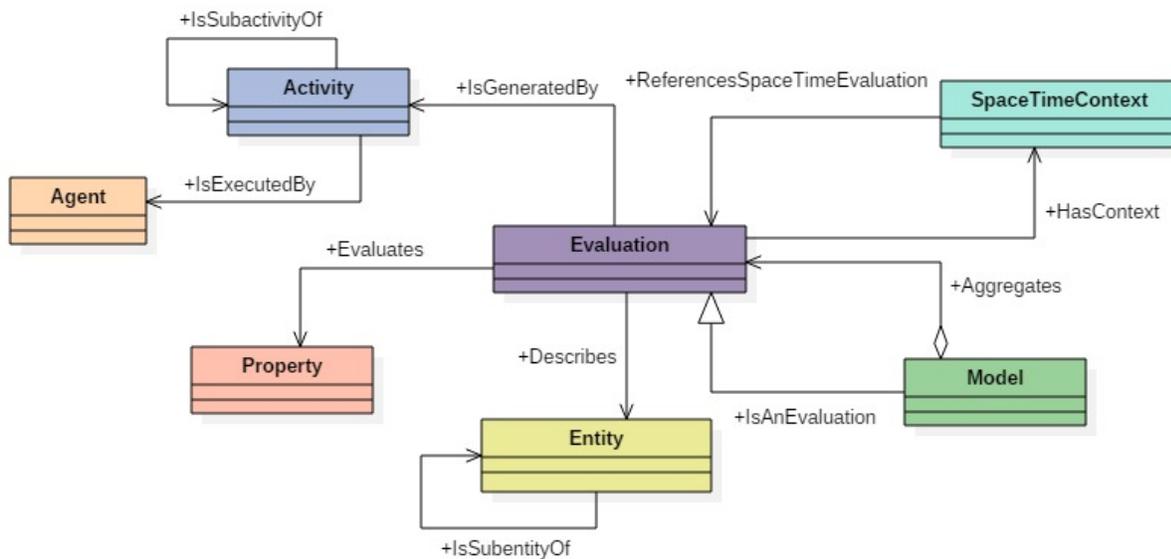


Figure 1. Executive Level Data Model

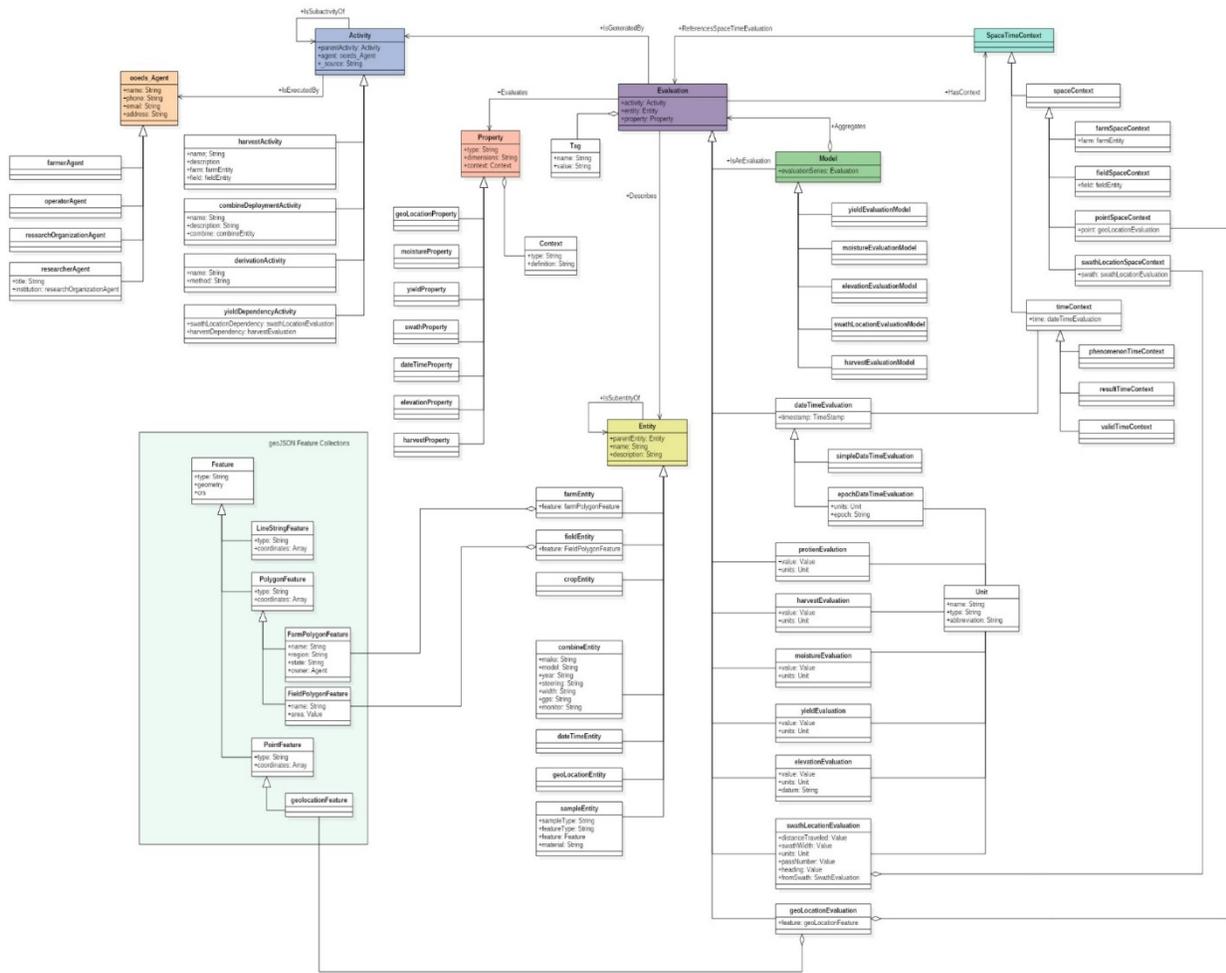


Figure 2. Each component describes an entire subsystem

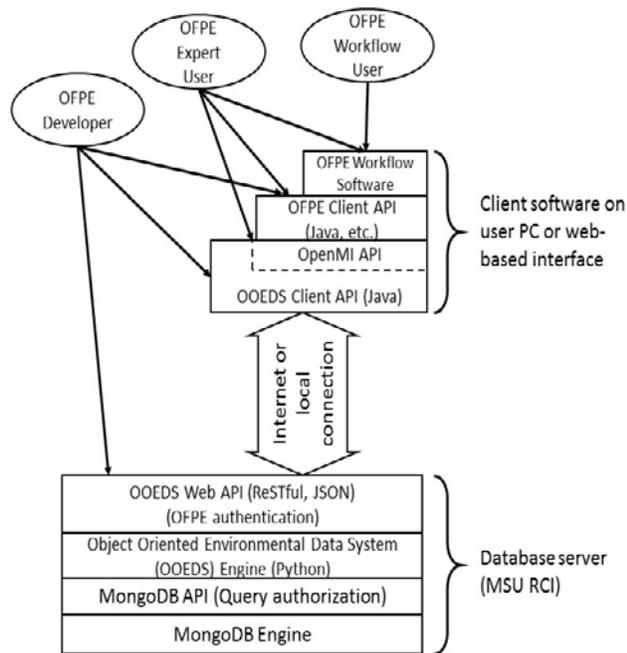


Figure 3. Software Architecture

Software functionality: the long term goal

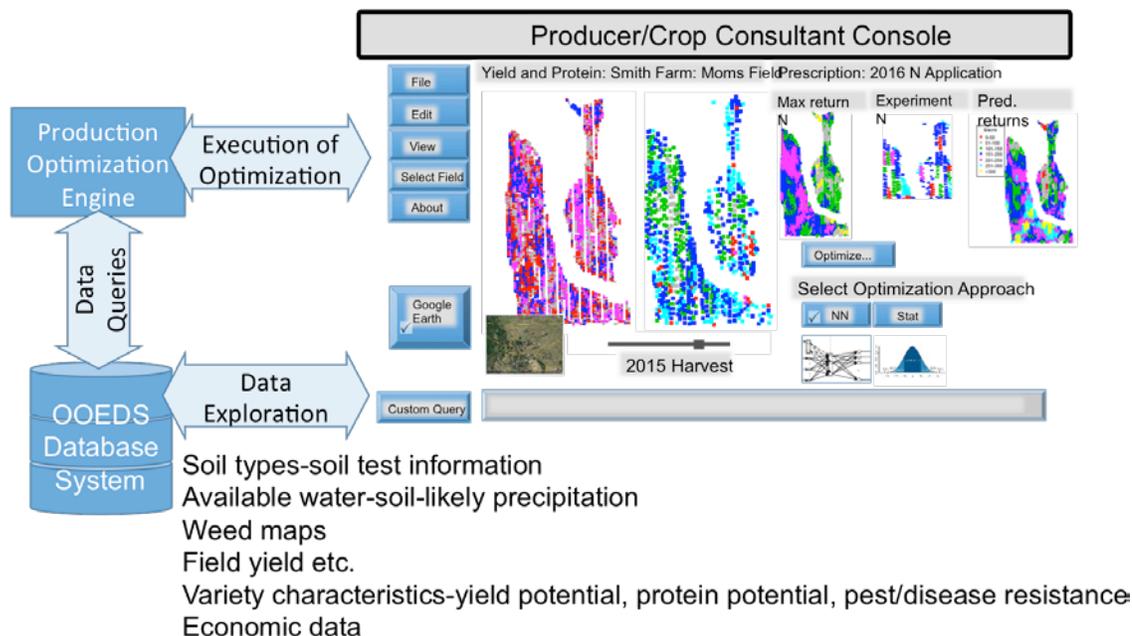


Figure 4. Example of potential GUI functionality

Expenditures

- Total Personnel: \$61,072.31
- Total Operations: \$33.56

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

Progress towards milestones

The Montana Climate office has installed weather station at 5 of the MAES Research Centers and real-time data is available online. Adaptive Hydrology has continued to install and maintain weather stations at the four OFPE participant farms. This includes the replacement of a faulty soil moisture sensor on the Broyles farm weather station. At this time, all weather stations are up and running and connected to online servers for data storage and viewing. Continued development with the Montana Climate Office on hosting and accessing weather data, and communications with participant farmers. The data are now fully accessible online and can be visualized and downloaded from the Montana Climate Office website. Adaptive Hydrology has also continued to support the acquisition and organization of remotely sensed field data using Google Earth Engine for use in predictive modeling. In addition, Adaptive Hydrology has supported the development of a non-linear statistical model and a Bayesian probabilistic statistical model. Finally, Adaptive Hydrology has continued to attend all meetings and presentations either in person or remotely via video conferencing.

Durum Quality subproject of the Agriculture MREDI project

41W221 – Principal Investigator: Mike Giroux; Email: mgiroux@montana.edu

Progress towards milestones

Our focus in this quarter has been on completing field trials, advancing breeding populations, and beginning to compile all 2016 agronomic and seed quality data. Our primary new breeding populations were created by crossing the top cultivars from across different durum growing regions of the U.S. in the previous quarter (Table 1). For each of the populations, approximately 750 F₂ seeds were planted in the

field this summer in Bozeman in spaced rows under irrigated conditions. A single head was harvested from 400 selected plants per population and the heads from each unique cross were bulk threshed. Seed was then sized using a standard US #4 sieve and 400-800 large seeds were planted in the greenhouse at a density of ~200 seeds per each 22" x 15" planting tray. Germination rates for all populations averaged ~ 90%. In December 2016 one head from each F₃ plant will be harvested and one seed from each head will be re-planted in the greenhouse to advance plants another generation. From each of these F₄ plants one head will be harvested in spring of 2017 and threshed independently. F₅ seeds from each selected head will be planted in a short row in Bozeman spring 2017. The rows with the best agronomic properties will be harvested and evaluated for protein and semolina color from which selections will be made for 2018 yield testing. Promising lines from 2018 field tests would then be tested in multiple locations in 2019 with high yielding lines also advanced to end product quality testing.

Table 1. Single cross durum breeding populations.

| Cross | Current Generation | Population Size |
|----------------------|---------------------------|------------------------|
| AC-Brigade X Alzada | F ₃ | 600 |
| Alkabo X AC-Brigade | F ₃ | 400 |
| Alkabo X Joppa | F ₃ | 400 |
| Alzada X Joppa | F ₃ | 600 |
| Carpio X AC-Brigade | F ₃ | 600 |
| Carpio X VT-Peak | F ₃ | 400 |
| Joppa X Orita | F ₃ | 400 |
| Joppa X Tioga | F ₃ | 600 |
| Joppa X VT-Peak | F ₂ | 400 |
| Mead X Joppa | F ₃ | 600 |
| Silver X Joppa | F ₃ | 400 |
| Strongfield X Alkabo | F ₃ | 400 |
| Strongfield X Divide | F ₃ | 400 |
| Strongfield X Tioga | F ₃ | 800 |
| VT-Peak X Alkabo | F ₃ | 400 |
| VT-Peak X Divide | F ₃ | 400 |

To develop additional breeding material with an emphasis on integrating the low cadmium trait, crosses were made between unique F₁'s from Table 1 or between F₁'s and the low cadmium parents Strongfield and AC-Brigade (Table 2). Resulting F₁'s were grown in the greenhouse over the summer and heads were bulk harvested for each cross. From each population, approximately 1,000 F₂ seeds were planted in Yuma, AZ by Northern Seed, LLC November 1st, 2016 in five 5'x40' plots. One head from selected plants will be harvested and brought back to Bozeman where they will be bulk threshed, seeds sieved to remove smalls, and planted in spaced rows in Bozeman spring 2017 for further selection.

Table 2. Double cross durum breeding populations with emphasis on integration of the low cadmium trait.

| Cross | Current Generation |
|--|---------------------------|
| Stongfield X Mead/Joppa F ₁ | F ₂ |
| Stongfield X Joppa/Alzada F ₁ | F ₂ |
| Strongfield X Alkabo/Joppa F ₁ | F ₂ |
| AC-Brigade X Stongfield/Tioga F ₁ | F ₂ |
| Strongfield /Tioga F ₁ X AC-Brigade | F ₂ |
| Strongfield/Tioga F ₁ X Mead/Joppa F ₁ | F ₂ |

| | |
|---|----------------|
| Strongfield/Tioga F ₁ X Alkabo/AC-Brigade F ₁ | F ₂ |
| Strongfield/Alkabo F ₁ X AC-Brigade/Carpio F ₁ | F ₂ |
| Strongfield/Divide F ₁ X Joppa/Tioga F ₁ | F ₂ |
| Alkabo/AC-Brigade F ₁ X Strongfield/Divide F ₁ | F ₂ |
| Alazada/Strongfield F ₁ X AC-Brigade/Carpio F ₁ | F ₂ |
| Mead/Joppa F ₁ X Strongfield/Alkabo F ₁ | F ₂ |
| Alazada/Havasus F ₁ X Strongfield/Tioga F ₁ | F ₂ |
| Alkabo/AC-Brigade F ₁ X Alzada/Strongfield F ₁ | F ₂ |
| AC-Brigade X Strongfield/Alkabo F ₁ | F ₂ |
| Strongfield/Tioga F ₁ X AC-Brigade/Carpio F ₁ | F ₂ |

2016 Experimental durum breeding material evaluation

Plots (4'x10') of durum populations (111 lines total) created at MSU were evaluated this summer in Bozeman, MT at the Post Farm under irrigated and rainfed conditions (3 replicates each environment) and in Conrad, MT (2 replicates) under rainfed conditions to determine the agronomic properties of lines in development relative to current elite cultivars. Populations were created by crossing relatively un-adapted genotypes containing mutations in starch synthase II (175 and 55) (Hogg et al. 2013) to the cultivars Divide and Mountrail which were then crossed again to either Divide or Mountrail (Table 3). Two of the populations have lines that segregated for low cadmium (Table 3) and all three segregated for mutations in a starch synthase II gene that increases pasta firmness, an important quality trait. The elite cultivars in the statewide durum nursery were included for comparison.

Table 3. Experimental Durum Trial Entries

| Cross | No. entries | Low Cd- segregation |
|-----------------------|--------------------|----------------------------|
| Divide//Mountrail/175 | 60 | Yes |
| Divide//Mountrail/55 | 30 | No |
| Mountrail//175/Divide | 12 | Yes |
| Elite cultivars | 9 | NA |

In all three locations, several lines yielded as well or better than the top cultivars currently grown in Montana (Figure 1, 2, 3). Some of the better yield performing experimental lines also had high protein as measured by NIR. The top 40 performing experimental lines will be further evaluated this winter for semolina quality and color. After further screening, the top performing experimental lines will then be grown in replicated plots at several locations in 2018.

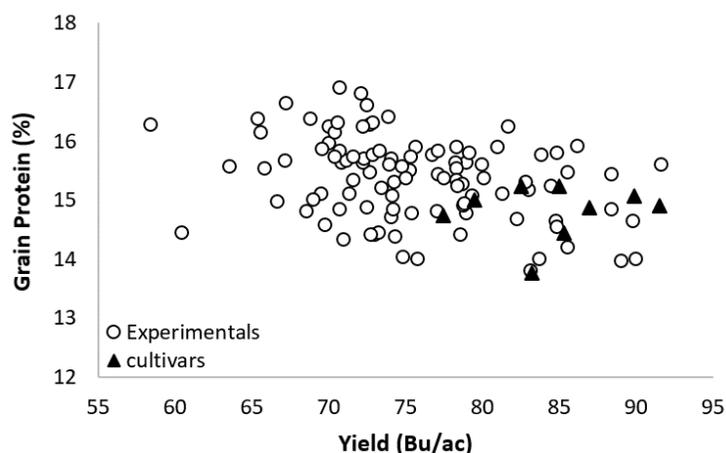


Figure 1. Yield and grain protein of experimental durum lines and top cultivars grown in Bozeman, MT under irrigated conditions.

*each data point represents the average of three reps.

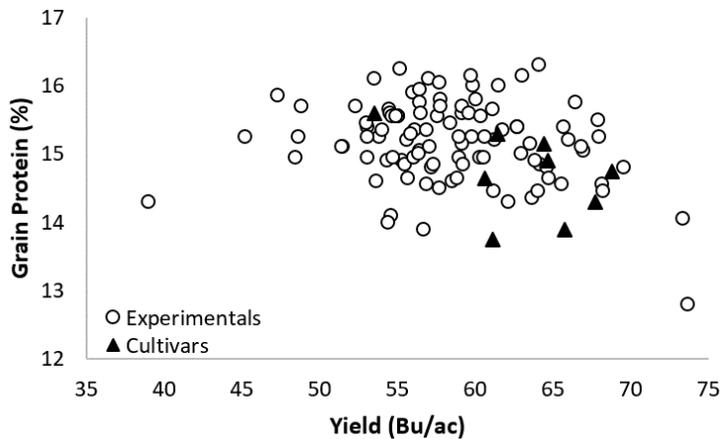


Figure 2. Yield and grain protein of experimental durum lines and top cultivars grown in Bozeman, MT under rainfed conditions.

*each point represents the average of three reps.

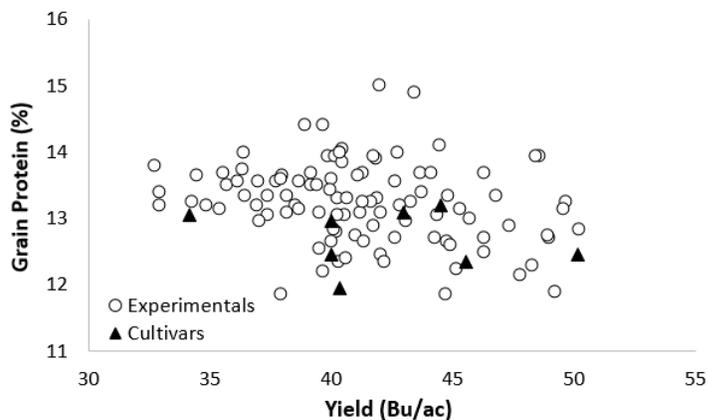


Figure 3. Yield and grain protein of experimental durum lines and top cultivars grown in Conrad, MT under rainfed conditions.

*each point represents the average of two reps.

State Durum Trial

An intrastate durum trial was conducted the summer of 2016 at Bozeman (Giroux), Churchill (Northern Seed, LLC), Conrad (Northern Seed, LLC and WTARC), Moccasin (CARC), Havre (NARC), and Sidney, MT (EARC). The trial encompassed 10 elite cultivars and 5 experimental lines developed at MSU. Yield and agronomic data was recorded for all stations and sub-samples have been sent to the USDA in Fargo, ND for quality analysis which will include all seed characteristics along with milling traits and semolina color traits. The full agronomic and seed quality report will be completed and appear in our next quarterly report.

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

Northern Seed is continuing to identify improved durum varieties 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 2nd Nature Research's (NR).

The 2016 Durum yield trials focused on the diverse germplasm sources mentioned above were harvested and analyzed based on yield, test weight, seed protein %, semolina sedimentation values and semolina color. Based on these evaluations 64 lines were selected for advancement and will be planted in Montana at multiple sites spring of 2017. Heads from these same selections were harvested from the 2016 Bozeman site and are currently being grown in Yuma, AZ to begin the purification process. These purifications will be harvested in April and planted in Montana spring of 2017.

Hiring

- No additional hires in Quarter 5.

Equipment

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

Expenditures

- Total Personnel: \$55,518.27
- Total Operations: \$13,565.17
- Total Equipment: \$70,994.00

Wheat Stem Sawfly subproject of the Agriculture MREDI project

41W222 – Principal Investigator: David Weaver; Email: weaver@montana.edu

Progress towards milestones

Previously we characterized the effect of sugar availability on the longevity of both species of native parasitoids of the wheat stem sawfly. Here we report on the effect of sugar on egg load dynamics for newly-emerged *Bracon cephi* females and *B. lissogaster* females. In Fig. 1 (below) the more abundant species, *B. cephi*, has greater retention of egg load when provisioned with a sucrose solution (indicated with blue bars) rather than with water only (black bars). This species resorbs eggs to live longer, which allows for more time to locate wheat stem sawfly larvae to parasitize. Thus, at 10 days the sucrose-provisioned females had eggs available to use, while those provided water only had exhausted their egg supply. In remarkable contrast, newly-emerged females of the rarer *B. lissogaster*, actually produce more eggs after emergence using existing metabolic reserves (black bars), but this is enhanced by the consumption of sucrose (Fig. 1).

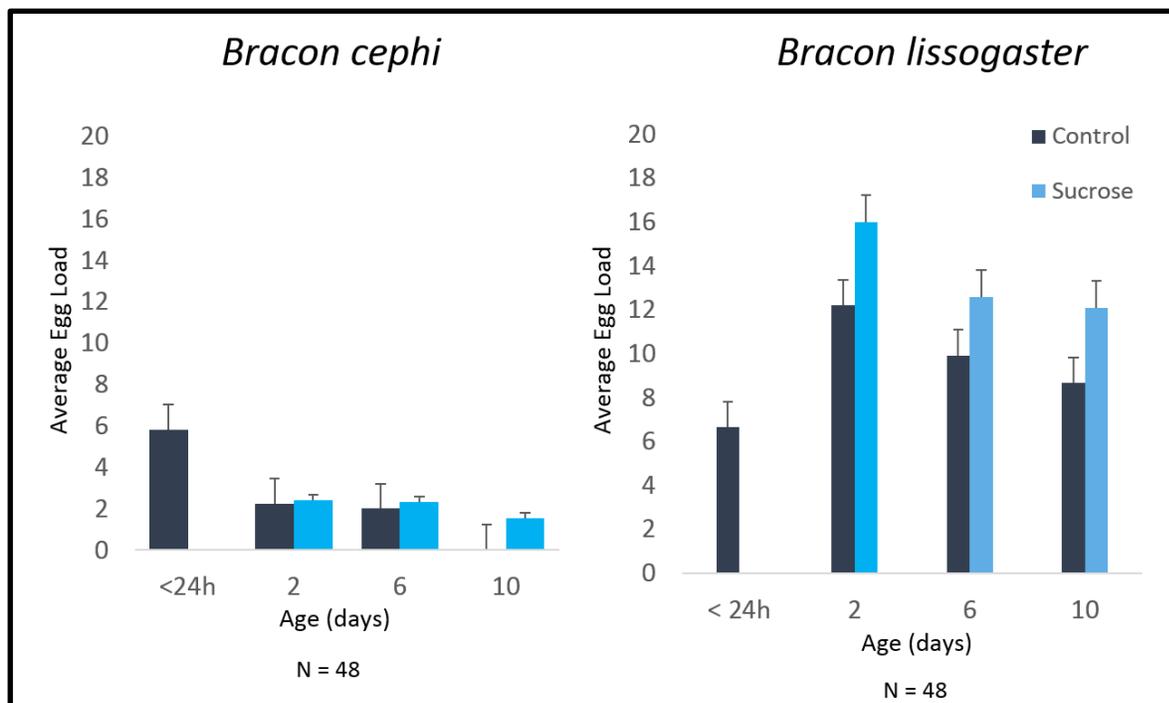


Figure 1. Mean egg load over time for newly-emerged females of *Bracon cephi* (left) and *B. lissogaster* (right). Black bars are females provisioned with water only and the blue bars are for females provisioned with an aqueous solution of 1 mol. sucrose.

These results are only for newly-emerged first generation females of both species. We have not completed the experiments assessing the role of sugar availability on the egg load dynamics of the second and more abundant generation of either species.

In the field we have continued to monitor the populations of wheat stem sawfly parasitoids after the 2016 wheat harvest. We are using the same paired fields of: 1) cover crop bordering wheat matched with 2) fallow bordering wheat, as well as: 3) the same paired fields of pulse crops bordering wheat matched with 4) fallow bordering wheat. This required the collection of postharvest wheat stubble and dissection of the overwintering structures of both the pest wheat stem sawflies and the two parasitoid species. The overwintering stages of the hosts and thus, the parasitoids are constrained by the food availability within the developing wheat stem. The more robust (and higher yielding) wheat stems will produce larger wheat stem sawfly larvae which should, in turn, produce larger parasitoids.

Our preliminary results indicate that there may be differences in the average size of the parasitoids (Figure 2a) relative to the average size of the wheat stem sawflies (Figure 2b). This is too preliminary to discuss further. It is quite complicated, because the only reason for a different size of the second generation of parasitoids in wheat adjacent to flowering species would be that the eggs from the first generation parasitoids were different than for the first generation in wheat adjacent to fallow. Current data suggests that parasitoids from overwintering structures can weigh from 24 – 54% of the weight of the wheat stem sawflies from the same field, but is not complete (Figure 2).

This project will fully support 2 graduate students beginning January 1, 2017. Dayane Reis will be conducting the laboratory and greenhouse component of this project, while Ben Fischer will continue to monitor the field populations of wheat stem sawflies and parasitoids at our 12 locations.

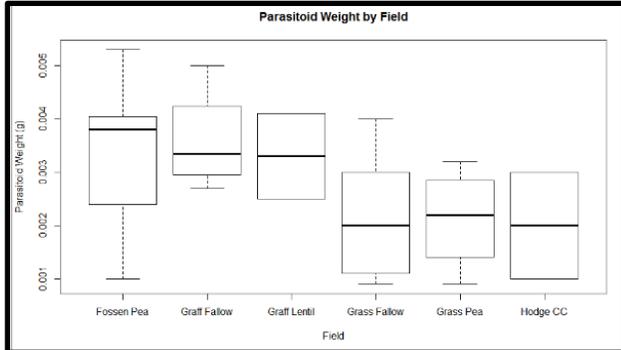


Figure 2a. Parasitoid size

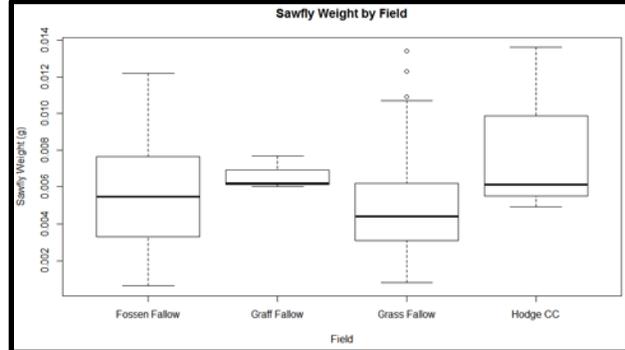


Figure 2b. Sawfly size

Hiring

- No additional hires in Quarter 5.

Expenditures

- Total Personnel: \$6,918.26
- Total Operations: None to date

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

1) **41W217 – Principal Investigator:** Prashant Jha; Email: pjha@montana.edu

PULSE CROP HERBICIDE EVALUATION

Progress towards milestones

The ultimate goal of this project is to investigate potential new weed control options in pulse crops and mitigate herbicide carry-over concerns in wheat-pulse rotation to enhance the profitability and sustainability of pulse production in Montana.

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

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 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 in 2016, pulse crops will be planted-back in spring of 2017 to determine herbicide carry-over potential and pulse crop safety and yields).

Objective 1

Effect of Fall-Applied Herbicides on Lentil. In general, lentil was the most susceptible among the three pulse crops to the soil-residual herbicides across all four Montana locations (Huntley, Sidney, Havre, and Moccasin). The hierarchy of crop susceptibility was lentil > pea > chickpea. The carryover effects of few herbicide treatments were dose-dependent and varied among locations depending on soil-type and environmental conditions (Tables 1 to 4).

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 did not cause any significant injury (0 to 5%) at any of the two rating dates and were safe to lentils during the entire growing season (Table 1).

An unacceptable level of injury (35 to 60%) was observed at the first rating date (early spring 2016) with Corvus applied alone in the previous fall at 4 to 8 oz/A or tank-mixed with Sencor (4 or 8 oz/a). Almost similar injury response (30 to 52%) was observed with Corvus-based herbicide treatments at the subsequent rating date later in the season. The visually-assessed injury symptoms included chlorosis, stunting, plant deformation, reduction in plant height, and stand loss. Spartan charge (6 to 12 oz/A) and Authority MTZ (8 to 16 oz/A) also caused significant injury (ranging from 15 to 60%) to lentils early in the season. The injury symptoms from Spartan charge and Authority MTZ included chlorosis, stunting, leaf burn, and necrosis. However, injury from these two herbicides declined at the subsequent evaluation date, only 5% at the low rates; which suggests that the carry-over concerns to sensitive pulse crops like lentil can possibly be mitigated when these soil-residuals are applied in the fall after wheat harvest.

Consistent with injury ratings, herbicide programs including Sencor, Valor, Anthem Flex, and Prowl plus Outlook applied at both rates provided the highest lentil grain yield (>1200 lbs acre⁻¹). A significant reduction in lentil yield was observed with Spartan charge, Authority MTZ, and Corvus-based herbicide programs (Table 1). Among all treatments, Corvus applied at 8 oz/a had the least (89 lbs acre⁻¹) lentil yield, which was unacceptable.

Table 1. Influence of fall-applied soil-residual herbicides on visual injury (%) and grain yield (lb/a) of pulse crops (lentil, pea, and chickpea) during 2016 growing season at Huntley, MT. ^{a, b}

| Herbicide(s) | Rate (oz/a) | 13-Jun 2016 | | | 14-Jul 2016 | | | Grain yield | | | | | |
|-----------------|-------------|-------------|-------|----------|-------------|------|----------|-------------|-----|----------|---|--------|----|
| | | Lentil | Pea | Chickpea | Lentil | Pea | Chickpea | Lentil | Pea | Chickpea | | | |
| | | % injury | | | % injury | | | lbs/a | | | | | |
| Sencor | 4 | 0 g | 0 d | 0 d | 0 e | 0 e | 0 d | 1234.9 | ab | 1144.6 | a | 2465.6 | a |
| Sencor | 8 | 0 g | 0 d | 0 d | 0 e | 0 e | 0 d | 1205.3 | ab | 1106.3 | a | 2496.3 | a |
| Anthem Flex | 3.64 | 0 g | 0 d | 0 d | 0 e | 0 e | 0 d | 1263.4 | ab | 1082.3 | a | 2525.7 | a |
| Anthem Flex | 7.28 | 5 fg | 2 cd | 0 d | 0 e | 0 e | 0 d | 1339.6 | a | 1038.6 | a | 2466.6 | a |
| Prowl + Outlook | 16 + 18 | 0 g | 0 d | 0 d | 0 e | 0 e | 0 d | 1210.9 | ab | 1131.8 | a | 2587.6 | a |
| Prowl + Outlook | 32 + 36 | 0 g | 0 d | 0 d | 0 e | 0 e | 0 d | 1208.5 | ab | 1176.1 | a | 2453.7 | a |
| Valor | 3 | 1 g | 0 d | 0 d | 0 e | 0 e | 0 d | 1240.1 | ab | 1118.0 | a | 2339.8 | ab |
| Valor | 6 | 4 fg | 3 cd | 0 d | 0 e | 0 e | 0 d | 1281.3 | ab | 1143.9 | a | 2333.4 | ab |
| Spartan Charge | 6 | 25 de | 0 d | 0 d | 5 e | 0 e | 0 d | 931.9 | cd | 1082.4 | a | 2747.3 | a |
| Spartan Charge | 12 | 67 a | 3 cd | 0 d | 40 b | 0 e | 0 d | 598.4 | ef | 1068.3 | a | 2834.7 | a |
| Authority MTZ | 8 | 15 ef | 3 cd | 0 d | 5 e | 0 e | 0 d | 1042.5 | bc | 1120.2 | a | 2808.7 | a |
| Authority MTZ | 16 | 55 ab | 2 cd | 2 d | 22 d | 0 e | 0 d | 847.8 | cde | 1077.0 | a | 2610.6 | a |
| Corvus | 4 | 40 c | 13 cd | 20 bc | 38 b | 10 c | 5 c | 500.5 | fg | 733.2 | b | 1908.1 | bc |
| Corvus | 8 | 60 ab | 42 a | 35 ab | 52 a | 34 a | 15 b | 89.5 | h | 330.2 | c | 1499.7 | c |
| Corvus + Sencor | 3 + 4 | 35 d | 7 cd | 18 c | 30 c | 6 d | 15 b | 771.8 | de | 799.4 | b | 1824.4 | bc |
| Corvus + Sencor | 6 + 8 | 58 b | 32 b | 29 ab | 55 a | 22 b | 20 a | 321.3 | gh | 638.2 | b | 1568.4 | c |

^a Herbicide treatments were applied postharvest in wheat stubble in fall 2015 and pulse crops planted-back in spring 2016.

^b Means within a column followed by similar letters are not significantly different based on Fisher's protected LSD test at P < 0.05.

Sidney site: The injury ratings and symptomology on lentil with a majority of the herbicide treatments were consistent with those observed at the Huntley location. *Sencor*, *Anthem Flex*, *Prowl plus Outlook* treatments did not cause any injury symptoms and were safe to lentils (Table 2). Unlike the Huntley site, Valor at 3 to 6 oz/a caused 23 to 47% injury at the first rating date, which might be attributed to differences in soil and environmental conditions at Huntley vs. Sidney site. A significantly high level of injury to lentil was observed early in the season with Corvus-based treatments. Injury from all other herbicide treatments was also significantly higher at the first rating, but declined later in the season.

Lentil grain yield at this site was higher (>1350 lbs acre⁻¹) with *Sencor*, *Anthem Flex*, or *Prowl plus Outlook* treatment. Consistent with injury, a significant reduction in lentil yield was observed with all other treatments. Similar to the Huntley location, the lowest yield (181 lbs acre⁻¹) of lentil at the Sidney site was observed with Corvus applied at 8 oz/a.

Table 2. Influence of fall-applied soil-residual herbicides on visual injury (%) and grain yield (lb/a) of pulse crops (lentil, pea, and chickpea) during 2016 growing season at Sidney, MT. ^{a, b}

| Herbicide(s) | Rate (oz/a) | 13-Jun 2016 | | | 18-Jul 2016 | | | Grain yield | | | | | |
|-----------------|-------------|-------------|------|----------|-------------|------|----------|-------------|-----|----------|------|--------|-----|
| | | Lentil | Pea | Chickpea | Lentil | Pea | Chickpea | Lentil | Pea | Chickpea | | | |
| | | % injury | | | % injury | | | lbs/a | | | | | |
| Sencor | 4 | 0 f | 0 d | 0 D | 0 g | 0 d | 0 b | 1523.8 | a | 1972.8 | cde | 2104.5 | a |
| Sencor | 8 | 0 f | 0 d | 0 D | 0 g | 0 d | 0 b | 1595.5 | a | 2072.2 | bcd | 2095.2 | a |
| Anthem Flex | 3.64 | 0 f | 0 d | 0 D | 0 g | 0 d | 0 b | 1358.8 | a | 2132.3 | bcd | 1969.5 | abc |
| Anthem Flex | 7.28 | 0 f | 0 d | 0 D | 0 g | 0 d | 0 b | 1386.1 | a | 2113.3 | bcd | 1930.4 | abc |
| Prowl + Outlook | 16 + 18 | 0 f | 0 d | 0 D | 0 g | 0 d | 0 b | 1569.7 | a | 2330.7 | abcd | 2067.4 | ab |
| Prowl + Outlook | 32 + 36 | 0 f | 0 d | 0 D | 0 g | 0 d | 0 b | 1550.2 | a | 2255.9 | abcd | 2173.9 | a |
| Valor | 3 | 23 e | 0 d | 0 D | 12 f | 0 d | 0 b | 836.5 | bc | 2149.5 | abcd | 1910.4 | abc |
| Valor | 6 | 47 c | 0 d | 0 D | 28 e | 0 d | 0 b | 665.8 | bc | 2399.6 | abcd | 1933.2 | abc |
| Spartan Charge | 6 | 22 e | 0 d | 0 D | 17 f | 0 d | 0 b | 899.1 | b | 2485.4 | abc | 2200.2 | a |
| Spartan Charge | 12 | 67 b | 0 d | 0 D | 53 b | 0 d | 0 b | 622.7 | bcd | 2685.3 | a | 2352.4 | a |
| Authority MTZ | 8 | 18 e | 0 d | 0 D | 12 f | 0 d | 0 b | 787.6 | bc | 2585.6 | ab | 1891.2 | abc |
| Authority MTZ | 16 | 40 d | 0 d | 0 D | 35 d | 0 d | 0 b | 293.1 | de | 2549.9 | ab | 1903.0 | abc |
| Corvus | 4 | 65 b | 12 c | 10 C | 32 de | 5 c | 0 b | 538.7 | cd | 1912.6 | de | 1977.4 | abc |
| Corvus | 8 | 83 a | 67 a | 22 A | 68 a | 40 a | 5 a | 181.5 | e | 771.0 | g | 1499.4 | c |
| Corvus + Sencor | 3 + 4 | 70 b | 13 c | 12 C | 35 d | 5 c | 3 a | 500.1 | cde | 1482.1 | ef | 1546.5 | bc |
| Corvus + Sencor | 6 + 8 | 78 a | 50 b | 18 B | 45 c | 30 b | 3 a | 300.8 | de | 1150.5 | fg | 1535.1 | bc |

^a Herbicide treatments were applied in wheat stubbles during fall 2015 across all four locations.

^b Means within a column followed by similar letters are not significantly different based on Fisher's protected LSD test at P < 0.05.

Havre site: Among all treatments, no significant injury was observed with Sencor or Prowl plus Outlook treatments. Treatments including Anthem Flex or Valor provided slight injury (3 to 10%) to lentil at the first rating date, however, the injury declined to less than 3% at subsequent rating dates later in the season (Table 3). In contrast, a considerable visual injury to lentil was noticed with Spartan charge, Authority MTZ, or Corvus-based programs. However, there was a greater recovery of lentil plants from the injury caused by these herbicides at this site compared to other sites.

The lentil grain yield response was consistent with injury observed for the majority of herbicides. For example, herbicide treatments including Sencor, Valor, and Prowl plus Outlook at both rates and Anthem Flex at 3.64 oz/a rate had higher grain yields (>1356 lbs acre⁻¹). All other treatments caused considerable reduction in lentil yield.

Table 3. Influence of fall-applied soil-residual herbicides on visual injury (%) and grain yield (lb/a) of pulse crops (lentil, pea, and chickpea) during 2016 growing season at Havre, MT.

| Herbicide(s) | Rate (oz/a) | 9-Jun 2016 | | | 19-Jul 2016 | | | Grain yield | | | | | | |
|-----------------|-------------|------------|------|----------|-------------|------|----------|-------------|--------|----------|--------|--------|--------|----|
| | | Lentil | Pea | Chickpea | Lentil | Pea | Chickpea | Lentil | Pea | Chickpea | | | | |
| | | % injury | | | % injury | | | lbs/a | | | | | | |
| Sencor | 4 | 0 h | 0 d | 0 d | 0 e | 0 c | 0 c | 1506.8 | a | 1430.6 | cd | 1435.4 | d | |
| Sencor | 8 | 0 h | 0 d | 0 d | 0 e | 0 c | 0 c | 1427.4 | ab | 1430.2 | cd | 1473.4 | cd | |
| Anthem Flex | 3.64 | 0 h | 0 d | 0 d | 0 e | 0 c | 0 c | 1313.5 | bcd | 1443.6 | cd | 1664.7 | a | |
| Anthem Flex | 7.28 | 10 | efg | 0 d | 2 d | 3 e | 0 c | 0 c | 1356.9 | abc | 1451.2 | cd | 1632.2 | ab |
| Prowl + Outlook | 16 + 18 | 2 h | 0 d | 0 d | 0 e | 0 c | 0 c | 1360.4 | abc | 1485.5 | c | 1620.7 | abc | |
| Prowl + Outlook | 32 + 36 | 0 h | 0 d | 0 d | 0 e | 0 c | 0 c | 1509.2 | a | 1666.6 | a | 1631.0 | ab | |
| Valor | 3 | 3 gh | 0 d | 2 d | 0 e | 0 c | 0 c | 1486.1 | ab | 1479.1 | c | 1659.1 | a | |
| Valor | 6 | 7 fgh | 5 bc | 2 d | 2 e | 3 ab | 0 c | 731.8 | f | 1628.7 | ab | 1652.0 | ab | |
| Spartan Charge | 6 | 33 c | 0 d | 0 d | 18 cd | 0 c | 0 c | 191.5 | h | 1510.3 | bc | 1593.5 | abc | |
| Spartan Charge | 12 | 75 a | 0 d | 3 d | 63 a | 0 c | 0 c | 1194.8 | cd | 1444.0 | cd | 1549.7 | abcd | |
| Authority MTZ | 8 | 17 de | 3 cd | 0 d | 2 e | 0 c | 0 c | 540.8 | g | 1225.3 | e | 1524.9 | abcd | |
| Authority MTZ | 16 | 55 b | 8 b | 0 d | 40 b | 3 ab | 0 c | 1161.2 | d | 1246.6 | e | 1500.3 | bcd | |
| Corvus | 4 | 20 d | 5 bc | 10 c | 3 e | 3 ab | 3 b | 867.4 | ef | 1050.1 | f | 1238.3 | e | |
| Corvus | 8 | 37 c | 13 a | 28 a | 13 d | 5 ab | 3 b | 1207.4 | cd | 1336.1 | de | 1055.5 | f | |
| Corvus + Sencor | 3 + 4 | 12 ef | 0 d | 10 c | 2 e | 0 c | 0 c | 972.9 | e | 1091.5 | f | 1253.9 | e | |
| Corvus + Sencor | 6 + 8 | 40 c | 5 bc | 17 b | 23 c | 2 bc | 7 a | 1427.4 | ab | 1430.2 | cd | 1129.7 | ef | |

^a Herbicide treatments were applied postharvest in wheat stubble in fall 2015 and pulse crops planted-back in spring 2016.

^b Means within a column followed by similar letters are not significantly different based on Fisher's protected LSD test at P < 0.05.

Moccasin site: The visual injury with the majority of the herbicide treatments were consistent with other locations. A considerable decline in injury was observed at the second rating date (Table 4). The grain yield response was also consistent with other locations, with some variation in the magnitude of yield reduction. Among all treatments, Prowl plus Outlook provided the highest grain yield of lentil (~ 1400 lbs acre⁻¹). Grain yields with Sencor, Anthem Flex, and Valor at both rates and Corvus at 3 oz/a plus Sencor at 4 oz/a treatments were comparable (maximum yield of 1139 lbs acre⁻¹). Unacceptable lentil yield reductions were observed with other herbicide treatments tested (Table 4).

Table 4. Influence of fall-applied soil-residual herbicides on visual injury (%) and grain yield (lb/a) of pulse crops (lentil, pea, and chickpea) during 2016 growing season at Moccasin, MT.^{a, b}

| Herbicide(s) | Rate (oz/a) | 9-Jun 2016 | | | 19-Jul 2016 | | | Grain yield | | | | | |
|-----------------|-------------|------------|------|----------|-------------|-----|----------|-------------|-----|----------|-----|-------|------|
| | | Lentil | Pea | Chickpea | Lentil | Pea | Chickpea | Lentil | Pea | Chickpea | | | |
| | | % injury | | | % injury | | | lbs/a | | | | | |
| Sencor | 4 | 0 g | 0 d | 0 d | 0 d | 0 c | 0 b | 1043.8 | bc | 1703.6 | abc | 752.2 | cde |
| Sencor | 8 | 0 g | 0 d | 0 d | 0 d | 0 c | 0 b | 1042.8 | bc | 1701.3 | abc | 777.4 | bcde |
| Anthem Flex | 3.64 | 0 g | 0 d | 0 d | 0 d | 0 c | 0 b | 1112.6 | bc | 1767.1 | abc | 826.7 | bcd |
| Anthem Flex | 7.28 | 7 f | 5 c | 0 d | 0 d | 0 c | 0 b | 1080.1 | bc | 1605.5 | bc | 833.8 | bcd |
| Prowl + Outlook | 16 + 18 | 0 g | 0 d | 0 d | 0 d | 0 c | 0 b | 1399.0 | a | 1833.2 | abc | 792.6 | bcd |
| Prowl + Outlook | 32 + 36 | 0 g | 0 d | 0 d | 0 d | 0 c | 0 b | 1393.8 | a | 1825.4 | abc | 795.8 | bcd |
| Valor | 3 | 0 g | 0 d | 0 d | 0 d | 0 c | 0 b | 1139.1 | b | 1835.3 | abc | 870.8 | ab |
| Valor | 6 | 3 fg | 3 cd | 0 d | 0 d | 0 c | 0 b | 1082.5 | bc | 1690.2 | abc | 851.2 | abc |
| Spartan Charge | 6 | 28 d | 0 d | 0 d | 12 b | 0 c | 0 b | 601.7 | de | 1679.7 | abc | 810.6 | bcd |

| | | | | | | | | | | | | | | | | | | | |
|-----------------|-------|----|----|----|---|----|----|----|---|----|---|---|---|--------|-----|--------|-----|-------|-----|
| Spartan Charge | 12 | 57 | a | 5 | c | 2 | cd | 30 | a | 0 | c | 0 | b | 459.1 | ef | 1617.9 | bc | 791.1 | bcd |
| Authority MTZ | 8 | 12 | e | 0 | d | 0 | d | 0 | d | 0 | c | 0 | b | 942.8 | c | 1954.8 | a | 953.0 | a |
| Authority MTZ | 16 | 52 | b | 0 | d | 3 | bc | 30 | a | 0 | c | 0 | b | 398.0 | f | 1912.8 | ab | 797.0 | bcd |
| Corvus | 4 | 25 | d | 5 | c | 5 | b | 5 | c | 0 | c | 0 | b | 644.9 | de | 1566.7 | c | 677.4 | e |
| Corvus | 8 | 55 | ab | 20 | a | 12 | a | 15 | b | 10 | a | 5 | a | 473.5 | def | 1249.1 | d | 497.6 | f |
| Corvus + Sencor | 3 + 4 | 13 | e | 5 | c | 3 | bc | 0 | d | 0 | c | 0 | b | 1057.6 | bc | 1790.1 | abc | 810.5 | bcd |
| Corvus + Sencor | 6 + 8 | 33 | c | 13 | b | 12 | a | 5 | c | 5 | b | 0 | b | 650.0 | d | 1614.2 | bc | 733.7 | de |

^a Herbicide treatments were applied postharvest in wheat stubble in fall 2015 and pulse crops planted-back in spring 2016.

^b Means within a column followed by similar letters are not significantly different based on Fisher's protected LSD test at $P < 0.05$.

Effect of Fall-applied Herbicides on Pea. Across all four locations, fall-applied Sencor, Valor Anthem Flex, Prowl plus Outlook, Authority MTZ at 8 oz/a, Spartan charge 6 to 12 oz/a were safe to pea (0 to 5% visual injury at the first rating and 0 to 3% at the second rating date). Authority MTZ 16 oz/a caused 8% injury to pea at the Havre site; however, no significant injury (0 to 3%) was observed with this treatment at other three locations.

Corvus applied alone or tank-mixed with Sencor 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 at 8 oz/a ranged from 34 to 42% at Huntley, 40 to 67% at Sidney, 10 to 20% at Moccasin, and less than 15% at Havre during the 2016 growing season.

The grain yields of pea were consistent with the injury from the majority of the herbicide tested. For example, higher grain yields were observed with Sencor, Anthem Flex, Valor, Prowl plus Outlook, Spartan Charge, and Authority MTZ treatments, and varied from 1038 and 1144 lbs acre⁻¹ at the Huntley site. The least grain yield (330 lbs acre⁻¹) was observed with Corvus at 8 oz/a. At other three sites, the pea yield response with a majority of herbicide treatments was similar to the Huntley location. However, the magnitude of yield reduction was significantly different among locations (Tables 1 to 4), depending on the soil and environmental conditions.

Effect of Fall-applied Herbicides on Chickpea. Among all three pulse crops, chickpea was the most tolerant to the soil-residual herbicides. 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 chickpea (no significant injury was observed at first and second ratings). At Huntley, the maximum injury (20-35%) to chickpea was observed with Corvus-based herbicide programs; however, injury did not exceed 15% by the second rating date. Furthermore, the injury to chickpea was less than that in lentil and pea.

Except Corvus-based programs causing significant yield reductions, all other herbicide treatments were safe and did not cause chickpea grain yield reductions. Some yield variations were observed among the sites (soil and environmental conditions). For example, herbicide treatments, including Sencor, Anthem Flex, Prowl plus Outlook, Valor, Spartan Charge, and Authority MTZ had grain yields of 2333 to 2834 lbs acre⁻¹ at Huntley; the same treatments yielded 1910 to 2352 lbs acre⁻¹ at Sidney, 1435 to 1664 lbs acre⁻¹ at Havre, and 752 to 953 lbs acre⁻¹ chickpea at the Moccasin site.

Effect of Fall-applied Herbicides on In-Crop Residual Weed Control. Herbicide treatments including 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 control of kochia and Russian thistle (>90%) throughout the growing season in pulse crops in 2016, across all four locations (weed control data shown in Table 5; 9-month after herbicide treatment). Kochia and Russian thistle control with Sencor (4 to 8 oz/a), Anthem flex (3.6 to 7.2 oz/a) and Prowl + Outlook (32 + 36 oz/a) were also adequate (75 to 85%) during the growing season across all four locations.

Implications: These fall-applied soil-residual herbicides with safety to pulse crops as demonstrated by this multi-location field research conducted in 2015/2016 can be potentially utilized by pulse producers

with rotational flexibility to winter or spring wheat. This research will allow product registration for some of these relatively new products that will add more tools for weed control in pulse crops. The added benefits of these fall-applied soil residual products in wheat-pulse rotation are: 1) controlling glyphosate-resistant kochia, Russian thistle, and marehail which are an increasing problem for Montana producers; and 2) reducing glyphosate-induced selection pressure for further development and spread of glyphosate-resistant weeds in wheat-pulse rotation in Montana.

Table 5. Kochia and Russian thistle control (%) 9 months after treatment (MAT) with various fall applied soil-residual herbicides averaged across four locations in MT.^{a, b}

| Herbicide(s) | Rate (oz/a) | Kocia | | Russian thistle | |
|-----------------|----------------|-----------------------|----|-----------------|------|
| | | ----- % control ----- | | ----- | |
| Sencor | 4 | 77 | h | 84 | Bc |
| Sencor | 8 | 84 | de | 81 | Cdef |
| Anthem Flex | 3.64 | 81 | fg | 76 | Efg |
| Anthem Flex | 7.28 | 86 | cd | 83 | Bcd |
| Prowl + Outlook | 16 + 18 | 76 | h | 73 | G |
| Prowl + Outlook | 32 + 36 | 78 | h | 76 | Fg |
| Valor | 3 | 95 | b | 93 | A |
| Valor | 6 | 97 | ab | 94 | A |
| Spartan Charge | 6 | 96 | ab | 92 | A |
| Spartan Charge | 12 | 98 | a | 94 | A |
| Authority MTZ | 8 | 97 | ab | 93 | A |
| Authority MTZ | 16 | 97 | ab | 94 | A |
| Corvus | 4 | 80 | g | 84 | Bc |
| Corvus | 8 | 84 | de | 86 | B |
| Corvus + Sencor | 3 + 4 | 83 | ef | 78 | Def |
| Corvus + Sencor | 6 + 8 | 87 | c | 81 | Bcde |

^a Herbicide treatments were applied in wheat stubbles during fall 2015 across all four locations

^b Means within a column followed by similar letters are not significantly different based on Fisher's protected LSD test at P < 0.05.

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 by December 2016.

Expenditures

- Total Personnel: \$26,798.66
- Total Operations: \$105.26

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

PRECISION WEED CONTROL USING ADVANCED OPTICS AND SENSOR-BASED TECHNOLOGIES

Progress towards milestones

During this quarter we completed milestone #3, to complete collection of field data by 30 September 2016. Our next milestone is to prepare a plan for technology transfer and commercialization by December 31, 2016. Accordingly, our primary activities this quarter were acquiring, compiling, and processing data from hyperspectral weed and crop imaging experiments.

Hyperspectral weed imaging

After gathering data from the Huntley field station in July, we have been developing an algorithm to distinguish between dicamba-resistant susceptible Kochia in the field based on the measured spectral differences. This resistance has been identified in Kochia growing in Montana fields and poses a significant and potentially expensive problem for Montana farmers. Initial tests in greenhouses and controlled outside settings have been successful. The latest work looks at different strains of weeds placed in the midst of crops, viewed under lighting conditions ranging from direct sunlight to overcast diffuse light.

The algorithms being developed are based on machine learning classifiers, where various spectral features are used to map the locations of the different strains of Kochia. Figure 1 shows example reflectance spectra obtained from hyperspectral images of different varieties of Kochia surrounded by sugar beet plants in the field at Huntley, Montana. Figure 2 is a photograph of the plants.

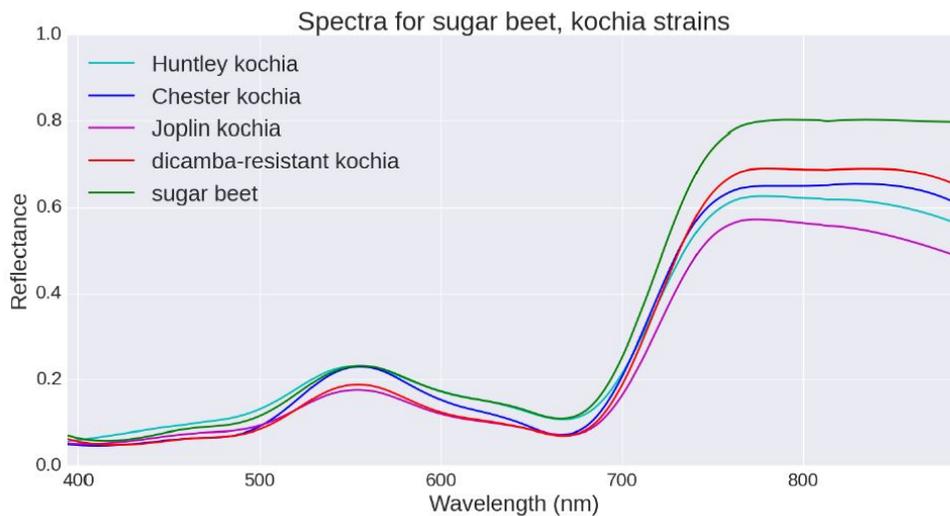


Figure 1. Reflectance spectra of sugar beets and different strains of Kochia.



Figure 2. Different strains of potted weeds positioned among sugar beet plants for hyperspectral imaging experiments.

The early image processing algorithms required the presence of a reflective calibration panel in the image, near the plants being imaged. However, for more effective hyperspectral imaging for weed remote sensing over larger areas, we are exploring methods of self-calibration. These include normalizing spectra, working with median spectra, or adjusting reflectance spectra based on a single calibration and subsequent measurements of parameters such as solar irradiance. This study is in a preliminary stage, so preliminary results will be shown in a later report.

Dissemination

The principal investigator of this subproject has been invited to give a presentation at the upcoming annual meeting of the Montana Grain Growers Association. Additionally, a poster summarizing this subproject was presented at the annual Optical Technology Center conference, held in October 2016 at Montana State University – Bozeman. Finally, the hyperspectral imaging work was demonstrated and summarized in the MREDI tour attended by political and business leaders from around Montana in September 2016. Figure 3 is a photograph from this event, showing visitors speaking to MSU students and researchers about hyperspectral agricultural imaging.



Figure 3. MSU student and research engineer visiting with Montana political, educational, and business leaders during MREDI tour in September 2016.

Hiring

The following people continue to work on this project:

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

Equipment Procurement

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

Expenditures

- Total Personnel: \$25,335.53
- Total Operations: \$8,729.04
- Total Equipment: \$16,716.00

Film Production for the Agriculture MREDI Grant

41W218 – Organizer: Eric Hyypa; Email: eric_hyypa@montanapbs.org

Progress towards milestones

Montana PBS has filmed additional interviews, pulse crop seedlings/fields and will continue to film into December. They have also begun editing and color correcting the footage.

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

Progress towards milestones

Significant progress has been made on evaluating the state-wide impacts of the pulse acreage expansion in Montana. Dr. Bekkerman has developed a theoretical model of the price dynamics associated with the expansion to correctly represent the capacity constraints in Montana's grain handling infrastructure. Specifically, because Montana's grain handling infrastructure was developed around the traditional wheat–fallow cropping system, the rapid and large increase in pulse production is likely to create grain volumes that will exceed the handling capabilities of Montana's existing infrastructure.

One consequence of this capacity constraint is elevators' use of price bids to reduce incentives for farmers to deliver both wheat and pulse crops. This is primarily done by reducing price bids for both of the crops. To test this hypothesis and estimate the potential price impact of the emerging pulse industry, wheat price data from four Montana regions where pulse crops have most prominently entered into the agricultural landscape has been gathered daily: Northeastern region, north-central region, Southwest and Billings region, and the Golden Triangle region. The data span the period 1998–2016, representing both periods of low and high pulse production in Montana and, thus, providing an empirical opportunity to measure price dynamics resulting from increase pulse production.

The results of the regression analysis of daily wheat basis show that after 2009—that is, after the most rapid and largest expansion of pulse acreage and production occurred—there's, on average, a 25% reduction in basis for wheat. Moreover, as Figure 1 shows, the greatest reductions occur during months immediately at or after harvest, indicating that the influx of supply that triggers Montana's grain handling capacity constraint occurs when farmers attempt to deliver their pulses immediately after harvest. These results provide empirical evidence that price dynamics are likely to exist and continue until Montana's grain handling infrastructure appropriately adjusts to handle the higher volume of agricultural products.

The estimated price dynamic effects were then incorporated into a simulation model that is intended to model the potential returns to additional pulse acres over the next five years. The model is based on a comparison of farm-level net returns for wheat–fallow, wheat–pea, and wheat–pulse cropping systems. That is, the model evaluates potential additional value added from producing pea or lentils relative to fallow.

The simulation model makes a number of other assumptions:

- Annually, 10% of fallow acres are converted into pea or lentil production.
- There are decreasing productivity returns to additional land added to production. That is, farmers will convert the more productive land first and less productive land subsequently.
- Converted land is allocated proportionally to peas and lentils in accordance to historical regional allocations to the two crops (e.g., 70% to peas and 30% to lentils).
- The model simulates assumes two pulse yield scenarios:
 - Scenario 1 assumes that pulse yields are represented by historical average yields.
 - Scenario 2 assumes that pulse yields are 0.5 standard deviations above the historical average yields, characterizing potential yield increases due to research related to the MREDI project.

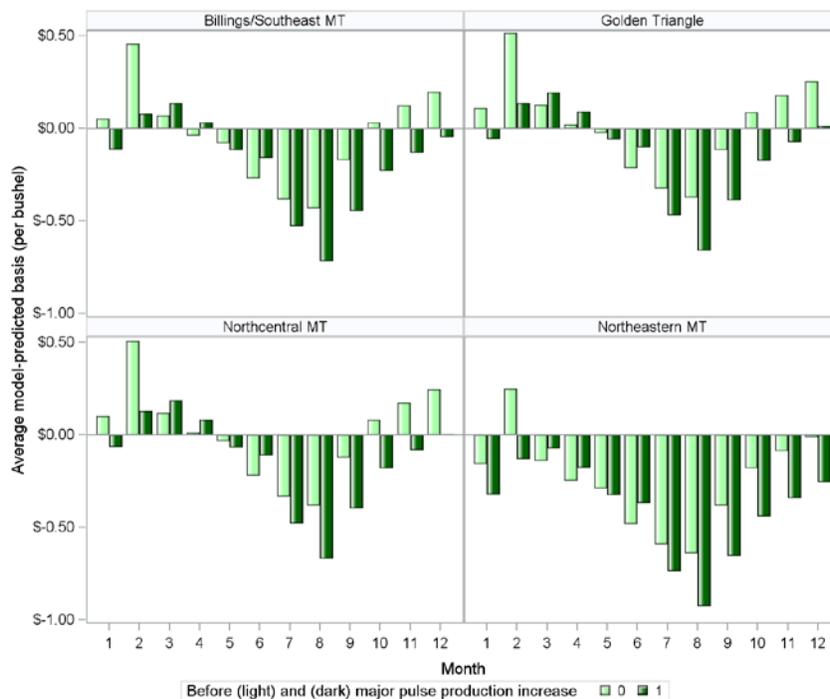


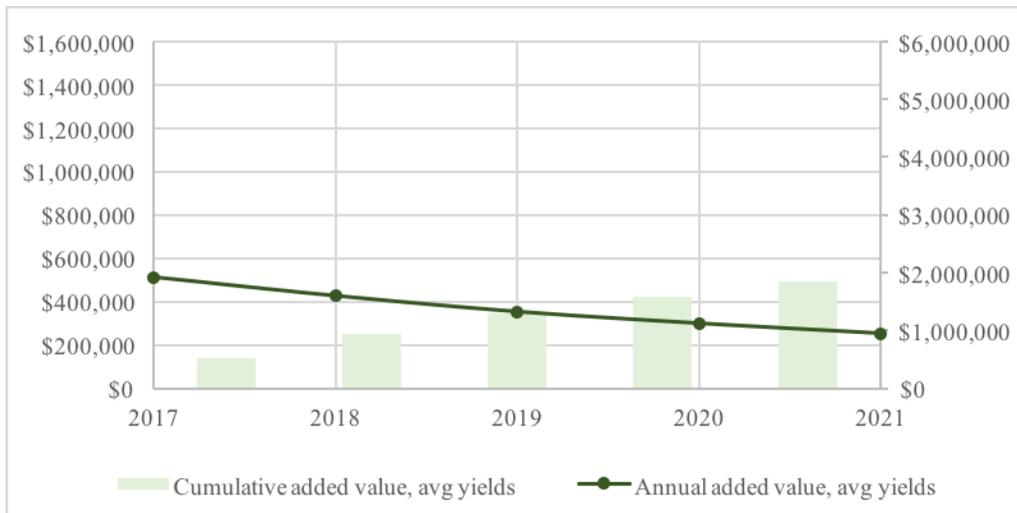
Figure 1: Estimated Basis in Montana Regions Before and After 2009

Notes: Data represent harvest-period 14% protein level spring wheat daily basis between 1998 and 2016. Data are made available by the USDA Agricultural Marketing Service.

The model is then simulated using current constrained grain handling infrastructure conditions and under the assumption that the grain handling infrastructure can handle all of the crop volume. The preliminary results are presented in Figures 2 and 3. The simulation analysis shows that under the constrained grain handling conditions and average pulse yield assumptions, the aggregate five-year state-wide return to increased pulse production is approximately \$1.75 million. However, if better yielding pulse varieties are used, the returns are likely to be closer to \$3.0 million. Moreover, as grain handling capacity is added to handle more pulse crops, returns are likely to be somewhere in between those shown in the unconstrained model and the constrained one.

Going forward, the simulation model will be checked for robustness and fine-tuned to examine the sensitivity of results to alternative market conditions.

(a) Constrained grain handling infrastructure



(b) Unconstrained grain handling infrastructure

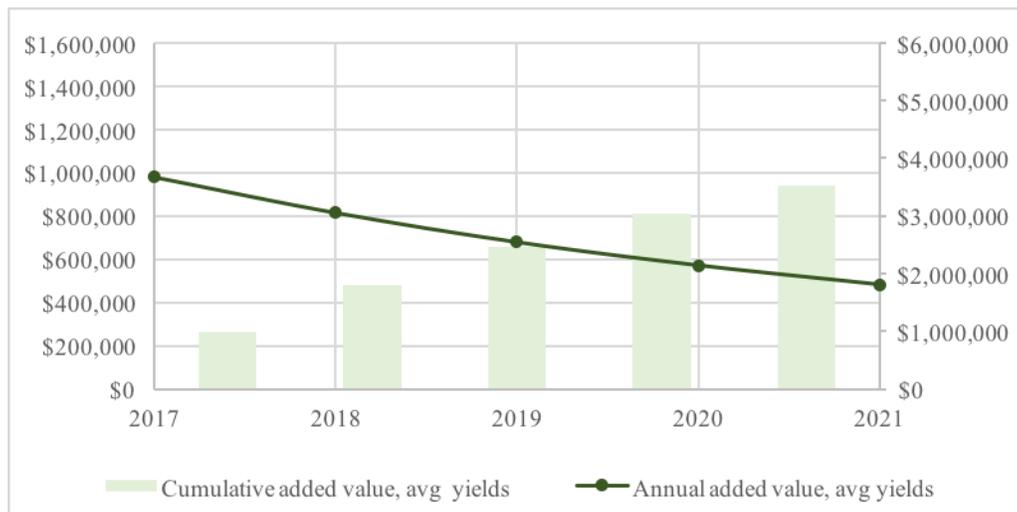
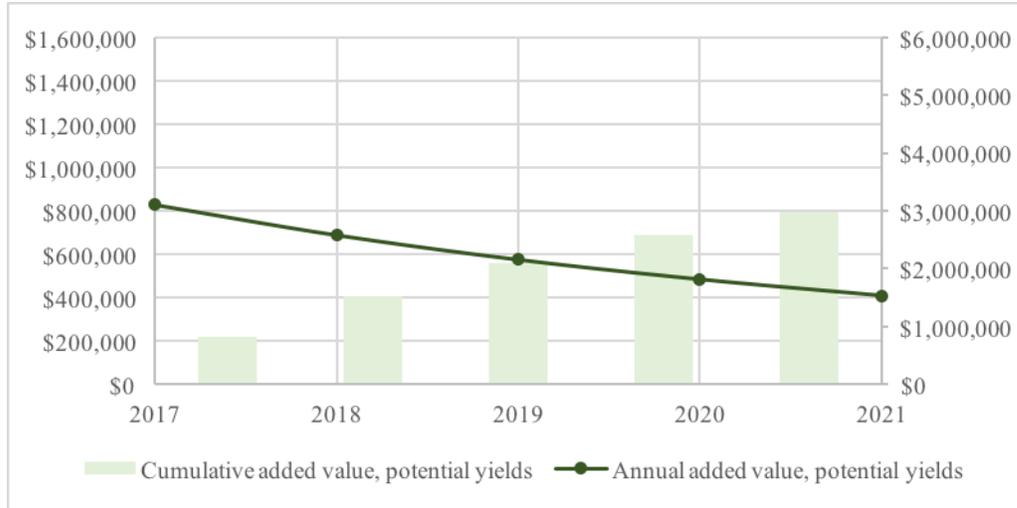


Figure 2. Simulated state-wide additional returns to converting to pulse cropping systems, average pulse yields are assumed

(a) Constrained grain handling infrastructure



(b) Unconstrained grain handling infrastructure

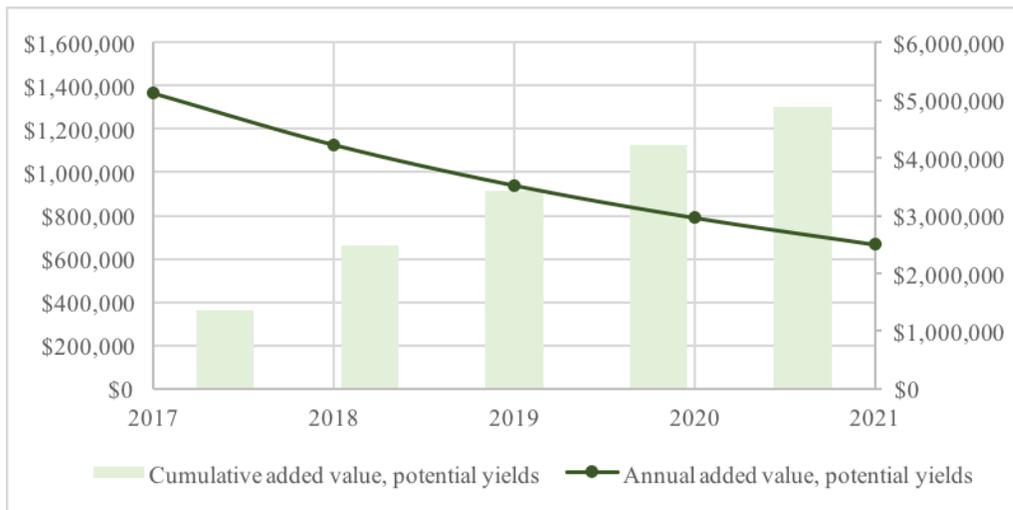


Figure 3. Simulated state-wide additional returns to converting to pulse cropping systems, potential higher pulse yields are assumed

Hiring

- No additional hires in Quarter 5.

Expenditures

- Total Personnel: \$38,519.51
- Total Operations: \$11,613.00

Participatory research network subproject of the Agriculture MREDI project

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

Progress towards milestones

The farm level cost:benefit analysis project is in the process of collecting data from the four precision agriculture collaborators and other farmers producing pulse and cover crops. The preliminary results from the precision

agriculture innovation project were presented at the Department of Agricultural Economics and Economics annual agricultural economics conference on November 11.

Hiring

- No additional hires in Quarter 5.

Expenditures

- Total Personnel: \$14,887.67
- Total Operations: None to date

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

Progress towards milestones

This quarter we completed transcriptions for all the audio recordings of our five focus groups and twenty individual interviews. In total, sixty-nine producers participated in this study. The systematic analysis of the 444 pages of single-spaced transcriptions, representing just under 30 hours of recorded time, will take several months.

Preliminary results were presented at the 10th annual Agricultural Economics Outlook Conference on November 11th. The presentation, titled “‘It’s Missing the Dirt’ Farmer Perceptions of Ag Research” gave a project overview, some initial findings, and served as an opportunity to receive feedback from producers. Over the next few months, these preliminary findings will be refined and further tested against the whole of the interview data.

Hiring

- None to date

Expenditures

- Total Personnel: None to date
- Total Operations: \$5,101.53