Agriculture MREDI Grant
Quarter 6 Sub-project Reports

Research Center/MAES subproject of the Agriculture MREDI Grant
41W225 – Principal Investigator: Barry Jacobsen; Email: bjacobsen@montana.edu

Executive Summary

Significant progress was made in all sub-projects and this summary hits only the high points of work done in this 6th quarter . Detailed information can be found in each sub-project report. Of particular note was the pea research on variety yield, variety protein content, protein content measurement and water use efficiency. These factors are important to the local adoption in the various areas of MT and to development of a pea protein fractionation plant in MT. Of note is the variety Nette 2010 which was amongst the highest yielding at most test locations and had the highest water use efficiency. Both factors are critical to using peas in continuous cropping scenarios as compared to crop fallow. Research on determining pea protein content which will be important to pea producers delivering to a protein fractionation plant has shown that NIR equipment now used in many grain elevators can reliably determine protein content. Research in this project and in prior research has shown that protein content is related most strongly to location environmental factors and less so to genotype. However, nitrogen available from applied or residual fertilizer or from Rhizobial inoculants plays an important role. In this regard, the use of granular inoculants compared to peat –powder-based inoculants appear to be more effective in supplying nitrogen to the pea plant and thus supporting increased protein content. Adequate phosphorous and available sulfur fertility is also critical. Thus we are beginning to understand how to maximize both yield and protein content.

Also relating to nitrogen, work in the Peter's lab has progressed on the bacterium, Azotobacter vinelandii, which can fix N on non-legume crops. Work is progressing on both rhizosphere microflora associations and effects of various elements on pea yield. This work has been slowed be3cause of available $ and we have recently begun the process to reallocate unused $ from other projects to complete this work.

The cover crop project has yielded a wealth of data and while warm season crops may out yield cool season mixes, they use more water thus limiting their use in continuous cropping scenarios. The yield of the best cool season mixes which are terminated in July produced very high quality hay and forage and clearly demonstrated the ability to produce revenues of ~$120.00/A. Thus, these cool season cover crops could be used in continuous cropping scenarios without negatively affecting winter wheat yields. It should be noted that the completion of a 4-year experiment in part supported by this funding was done under normal annual rainfall condition but not drought.

The precision agriculture component of this project has made considerable progress on incorporating weed management (see data from Rew and Jha in this report) and in developing the software to exploit available yield, weed distribution, protein, environmental and other data and develop optimal net return models that producers can use. These models are based on machine learning and artificial intelligence and it is likely the software developed will have significant commercial value. Work on hyperspectral optics for use in detecting herbicide resistant weeds and in detecting and mapping various weeds in crops has continued cooperatively in the Jha and Shaw laboratories and a provisional patent application has been filed.

The durum breeding project is moving along as expected and the line MT112219 was identified a high yielding (higher than several commercially used varieties). While this line had lower protein than several commercially used varieties this line had the highest milling yield and was satisfactory in other pasta quality factors.

Work has continued on the effects of availability of pea or cover crop nectar to wheat sawfly parasites and their influence on sawfly damage. While data is still being developed it appears that nectar availability is important to sawfly parasitism it is not a reliable commercial control.

A 10-minute film detailing this project has been developed and can be viewed at https://youtu.be/7kvXqS8YiHo. Work on the participatory research networks and economic analysis is underway.

Hiring

- Employees at the CARC, NWARC and SARC have been reallocated to assist with projects at the centers this past quarter.

Expenditures

- Total Personnel Services: $186,132.24
- Total Operations: $12,807.19

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

Progress towards milestones

1. Multi-location pea variety evaluation for yield, water use, and water use efficiency

Precipitation amount and distribution have substantial influence on crop production particularly in dryland farming. Farmers in Montana practice summer fallow to recharge soil moisture for the next crop to minimize drought effect on yield due to erratic rainfall. Replacing summer fallow with dry pea can enhance economic and agronomic benefits from intensification. But some varieties of dry pea could deplete more soil moisture than others thus resulting less residual soil moisture in the profile for the next crop. Therefore, this project component evaluated six dry pea varieties across Montana to evaluate their water use and water use efficiency. Water used in this report is the sum of initial soil moisture at planting and precipitation received during the growing season minus the residual soil moisture that was measured immediately following harvesting. The soil moisture was measured to a depth of 36 inch. Precipitation received between the month of April and August including were considered as input. Then, water use efficiency was calculated as the amount of grain produced per unit of water used. All the water lost during the growing period are assumed as transpiration loss. This report presented water used (WU) and water use efficiency (WUE) of six dry pea varieties at diffident locations in Montana.

Table 1. Mean grain yield for each variety and locations in 2016 at different locations in Montana

Varieties	Location											
	Bozeman	Conrad	Corvallis irri.	Creston	Havre	Huntley dry	Huntley irri.	Moccasin	Sidney dry	Sidney irri.	Richland	Variety means
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 2. Analysis of variance table showing the effects of variety, location and their interaction on water use (WU) of six dry pea varieties.

Source	DF	Mean Square	F-Value	Pr > F
Replication	3	1.93192	2.72	0.0488
Variety	5	0.79715	1.12	0.3544
Location	5	614.809	864.37	<0.0001
Variety*Location	25	0.57595	0.81	0.7211

Table 3. Mean water used (WU) (in inch) for each variety at different locations in 2016

	Location/ WU					
Varieties	Sidney dry	Sidney Irri.	Creston*	Havre	Huntley dry	Conrad
Delta	10.45	17.98	4.43	17.37	9.87	9.39
Hampton	10.65	16.03	4.67	17.57	9.66	9.25
Jetset	11.00	17.94	4.60	17.78	9.73	9.74
Majoret	10.46	17.90	4.32	17.56	9.41	9.36
Navarro	10.77	18.19	4.42	17.27	9.45	9.82
Nette 2010	10.57	16.80	4.31	17.41	9.64	9.65
Location means	10.65	17.47	4.46	17.49	9.63	9.53
P-values	0.4187	0.2626	0.4987	0.7764	0.6972	0.6683
LSD (0.05)	NS	NS	NS	NS	NS	NS
CV (%)	3.83	8.19	6.80	2.96	3.94	6.11

Irri. = irrigated (the experiment was conducted with supplementary irrigation). NS = non-significant. *there was a underground channel that constantly supplied water, therefore, this site is abnormal

Table 4. Analysis of variance table showing the effects of variety, location and their interaction on water use efficiency (WUE) of six dry pea varieties.

Source	DF	Mean Square	F Value	Pr > F
Replication	3	26.0287	7.88	<0.0001
Variety	5	17.2997	5.24	0.0003
Location	5	1203.26	364.2	<0.0001
Variety*Location	25	8.64549	2.62	0.0004

Table 1 presents the yield of six pea varieties grown at 11 environments in 2016, which has been reported in Quarter 5 project report. Table 2 shows the statistical analysis for the effects of variety and location on water use of peas. Results indicate that total water use did not differed significantly among the six pea varieties (p=0.35). From the water use data in Table 3, we can see total water use varied greatly among the locations. Creston site only consumed ~4.5 inches water, yet produced over 5,000 lbs of peas. This site was abnormal, because there was an underground channel which provide constant below ground water supply, therefore, the pea yield was exceptionally high. Statistical analysis in Table 4, showing significant different among varieties and locations in water use efficiency (WUE). As mentioned above, Creston site is abnormal, therefore, the WUE is exceptionally high, while Huntley dryland site was hail damaged and produced very low yield, thus resulting very

low WUE. However, Havre site consumed similar amount of water as Sidney irrigated site, yet had much lower WUE than Sidney irrigated site. The reason is that Havre site received big rain storms in late growing season, which provide big amount of water but did not help pea yield (too late). Therefore, timely supply of water is critical for higher pea yield and WUE. The variety Nette 2010 resulted in the highest WUE than other varieties.

Table 5. Mean water use efficiency (WUE) (in bu/inch) for each variety at different locations in 2016.

	Location/ WUE					
Varieties	Sidney dry	Sidney Irri.	Creston*	Havre	Huntley dry*	Conrad
Delta	5.80	4.04	19.43	0.32	1.41	6.96
Hampton	5.69	4.44	18.23	0.41	1.34	7.09
Jetset	5.80	3.81	20.40	0.39	1.46	5.82
Majoret	6.07	4.11	19.45	0.36	1.23	4.24
Navarro	5.82	3.51	16.98	0.35	0.82	7.30
Nette 2010	6.36	4.47	26.90	0.37	1.02	9.29
Location means	5.92	4.06	20.23	0.37	1.21	6.78
P-values	0.5141	0.1730	0.0011	0.0056	0.3254	0.0213
LSD (0.05)	NS	NS	3.84	0.04	NS	2.61
CV (%)	8.91	13.45	12.58	7.59	30.82	25.55

Irri. = irrigated (the experiment was conducted with supplementary irrigation). NS= non-significant. *Creston site had a underground channel that provided water to the crop, and the Huntley dryland site was hail damaged.

Table 6. Analysis of variance showing the effects of variety, location and their interaction on residual soil moisture content of six dry pea varieties.

Source	DF	Mean Square	F Value	Pr > F
Replication	3	8.9	2.21	0.0913
Variety	5	5.2	1.29	0.2751
Location	5	552.8	137.69	<0.0001
Variety*Location	25	3.7	0.93	0.5712

Table 7. Mean residual soil moisture after harvesting (in inch) for each variety at different locations in 2016.

Varieties	Location/ Residual soil moisture after harvest (inch)						Variety means
	Sidney dry	Sidney Irri.	Creston	Havre	Huntley dry	Conrad	
Delta	4.5	8.5	5.0	6.1	4.0	4.2	5.4
Hampton	4.3	10.5	4.7	5.9	4.2	4.3	5.7
Jetset	3.9	8.6	4.8	5.7	4.1	3.9	5.2
Majoret	4.5	8.6	5.1	5.9	4.4	4.2	5.5
Navarro	4.2	8.3	5.0	6.2	4.4	3.8	5.3
Nette 2010	4.4	9.7	5.1	6.0	4.2	3.9	5.6
Location means	4.3	9.1	4.9	5.9	4.2	4.1	
P-values	0.4211	0.2619	0.5054	0.7733	0.6932	0.6689	
LSD (0.05)	NS	NS	NS	NS	NS	NS	
CV (%)	9.52	15.81	6.14	8.93	8.98	14.32	

The residual soil moisture left after harvesting was calculated for each variety and location. Analysis of variance showed that the soil moisture left after harvesting was statistically the same for all varieties since ANOVA did not show any significant differences (Table 6). The higher WUE recorded for some varieties (Table 5) without affecting the residual soil moisture is important findings in this study. The different locations differ in the residual soil

moisture content probably due to variation in precipitation or irrigation (Sidney Irri) received at each location before or after harvest (Table 7).

2. Using Vis-NIRS reflectance spectrum to predict protein of green and yellow peas (whole and powder) on spectra wiz spectrometer

Progress towards milestones

The yellow and green whole peas and powder collected from the experiments located at various research locations has been scanned through visual spectral (Figures 1-4).

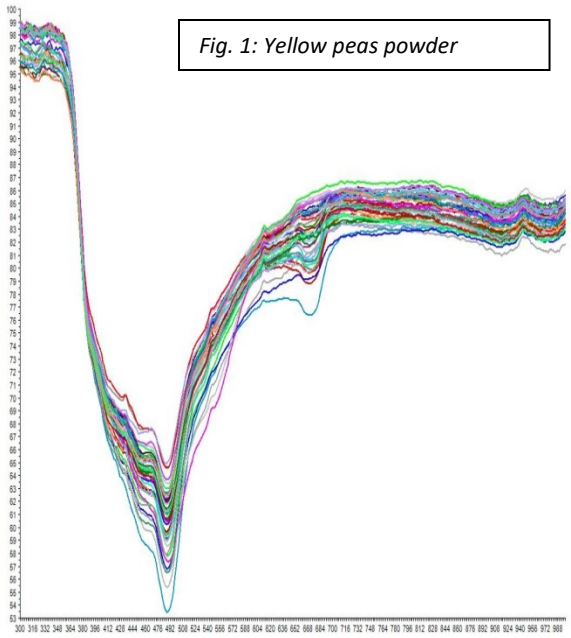

Fig. 1: Yellow peas powder

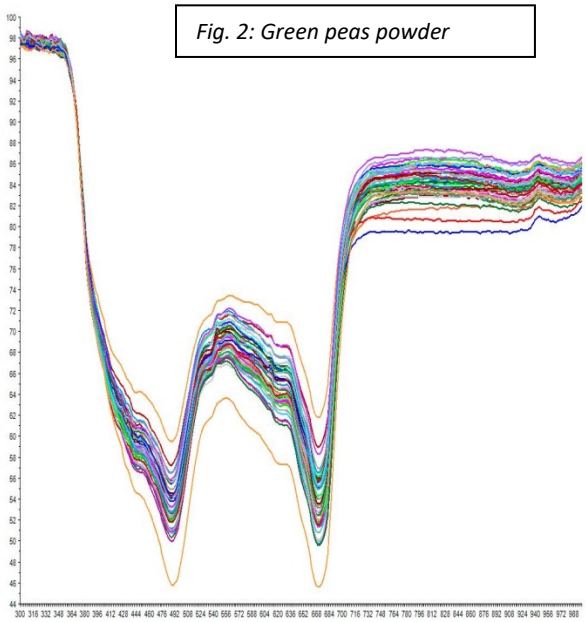

Fig. 2: Green peas powder

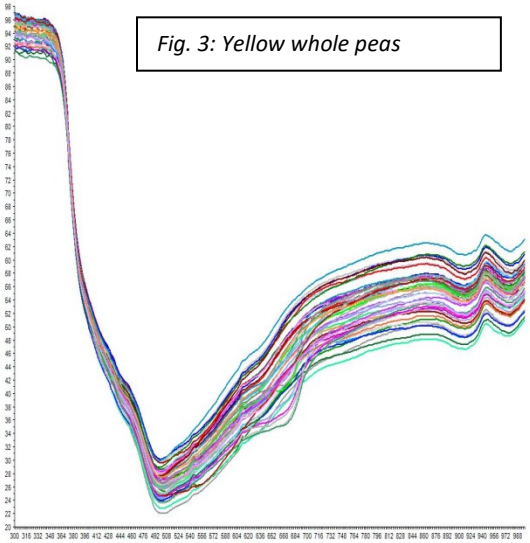

Fig. 3: Yellow whole peas

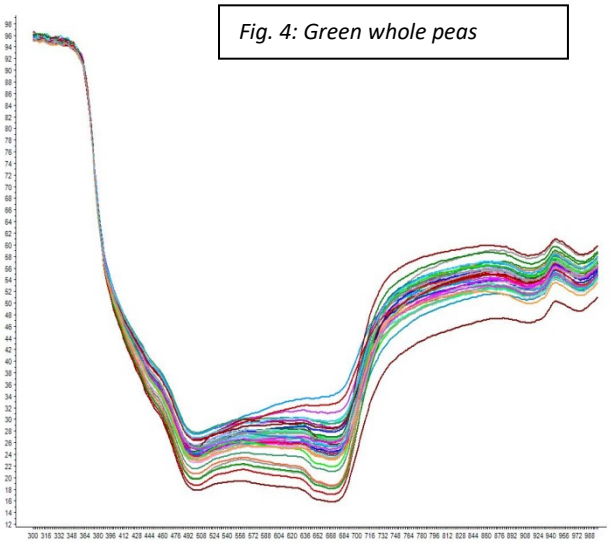

Fig. 4: Green whole peas

Those pea samples were also run through LICO Nitrogen Analyzer to analyze nitrogen contents.

Future Work: The data will be analyzed and a prediction model will be built to predict protein content for green and yellow peas. One manuscript will be written on protein concentration prediction by visible and near infrared reflectance spectroscopy (Vis-NIRS) in whole and ground peas.

Hiring

- No additional hires in Quarter 6.

Expenditures

- Total Personnel Services: $70,801.90
- Total Operations: $19,416.43

Soil Microbiology and Pea Protein subproject of the Agriculture MREDI Grant

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

Progress towards milestones

The following progress has been made:

A. Further investigation is underway to determine if protein variation is influenced by sample size and to determine if protein variation is similar in yellow pea and wheat.
B. A review has been written emphasizing how management factors common across Montana have influenced pea protein.
C. Yellow pea samples from the 2016 growing season are now being acquired.

A. ***Protein variation in yellow pea vs. wheat and effect of sample size on protein measurements***

When validating the factory calibration on the FOSS Infratec 1241, we found that the average difference between measured and predicted protein content was 1.02 % for ***whole-seed*** samples. Other studies have shown that the standard error of prediction between measured and predicted protein content of whole pea seeds with NIR range from 0.938% (Arganosa et al., 2006) to 1.341% (Tkachuk et al., 1987). Conversely, in wheat, standard error of prediction between measured and predicted protein content of seeds has ranged from 0.24% (Williams and Sobering, 1993) to 0.48% (Williams et al., 1985).

Similarly, our preliminary NIR calibration results show that the average difference between measured and predicted protein content with **pea flour** is ~ 0.8 %. Other studies have shown that the standard error of prediction between measured and predicted protein content of pea flour with NIR ranged from 0.33% (Tkachuk et al., 1987) to 1.19% (Arganosa et al., 2006). Conversely, with wheat flour, standard error of prediction between measured and predicted protein content ranged from 0.40% (Sorvaniemi et al., 1993) to 0.53% (Manley et al., 2002).

Because seed lots are split into respective subsamples for protein measurements and NIR calibration points, seed lots with low protein variation will theoretically provide better NIR calibration. It is therefore possible that wheat seed lots have less protein variation than pea because they tend to produce better NIR calibration fits. Likewise, it is possible that sample size may affect variation in protein measurements. For instance, protein has varied by greater than 10% depending on nodal position and variety in pea (Atta et al., 2004), meaning there is potential for small subsamples could have greater protein variation than large subsamples.

Accordingly, we have designed and are conducting two experiments to test how crop type (e.g. pea vs. wheat) and sample size affects protein variation in both **whole-seeds** and **grain flour**. See below for experimental methods and design.

Wheat and Pea Whole Seed Experiment Outline

Scope and Objective

When validating the factory calibration on the FOSS Infratec 1241, we found that the average difference between measured and predicted protein content was 1.02 %. Other studies have shown that the standard error

of prediction between measured and predicted protein content with NIR range from 0.938% (Arganosa et al., 2006) to 1.341% (Tkachuk et al., 1987). Conversely, in wheat, standard error of prediction between measured and predicted protein content has ranged from 0.24% (Williams and Sobering, 1993) to 0.48% (Williams et al., 1985).

Tighter NIR calibration for wheat may be due to more homogenous protein content within a wheat bulk sample. For instance, protein has varied by > 10 % depending on nodal position and variety in pea (Atta et al., 2004), whereas little information is available regarding seed position and protein variation in wheat. In other words, it is possible that wheat samples may have more uniform protein content relative to pea samples, and samples with uniform protein content should theoretically result in tighter NIR calibration.

Sample volume could also affect protein variation. That is, protein content may be more uniform if a larger sample volume is tested compared to a smaller sample volume taken from the same seed lot. Sample volume may be particularly relevant in pea where there is potential for high protein variation on the seed level. Hence the goals of this study are to test:

1. If greater protein variation exists whole-seed pea or wheat samples.
2. If sample volume influences protein variation differently between pea and wheat samples.

Methods and Statistical Analysis

Four spring wheat and four yellow pea bulk samples will randomly be obtained from Montana farms. From each bulk sample, four subsample volumes of 1, 2, and 3 tablespoons will be ground in a Udy mill with a 1-mm screen. Each subsample volume will then be run for total nitrogen using LECO combustion analysis. Protein will then be determined by multiplying total nitrogen by 5.70 and 6.25 for wheat and pea respectively.

The response of interest (y_{ijkl}) is the absolute difference in measured protein for two subsampled pairs corresponding to each crop type by sample volume combination. To test the effects of crop type (β) and sample volume (α) on absolute differences between protein measurements, ANOVA will be run using the following three-staged nested model with crossed factors:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \tau_k + \alpha\tau_{ik(j)} + \varepsilon_{ijkl} \quad \text{Eq. 1.}$$

where μ is the grand mean of all protein responses, τ_k is the seed lot corresponding to each bulk sample, and ε_{ijkl} is the error term assuming model residuals are $N(0,\sigma^2)$. Homogeneity of variance assumptions will be verified using a Levene's test, and post hoc multiple comparisons will be made if treatments are significant at the α=0.05 level. Statistical analysis will be run using the PROC GLM procedure in SAS (SAS Institute Inc., 2012).

Wheat and Pea Flour Seed Experiment Outline

Scope and Objective

Our preliminary NIR calibration results show that the average difference between measured and predicted protein content with pea flour is ~ 0.8 %. Other studies have shown that the standard error of prediction between measured and predicted protein content with NIR range from 0.33% (Tkachuk et al., 1987) to 1.19% (Arganosa et al., 2006). Conversely, with wheat, standard error of prediction between measured and predicted protein content has ranged from 0.40% (Sorvaniemi et al., 1993) to 0.53% (Manley et al., 2002).

Because whole-seeds are ground and subsequently split into respective subsamples for protein measurements and NIR calibration points, potential for larger NIR calibration uncertainty for pea protein flour might be attributable to greater variation within a ground sample compared to wheat. For instance, protein has varied by > 10 % depending on nodal position and variety in pea (Atta et al., 2004), whereas little information is available regarding seed position and protein variation in wheat. In other words, it is possible that ground wheat samples

may have more uniform protein content relative to pea samples. Ground samples with uniform protein content should theoretically result in tighter NIR calibration.

Sample volume could also affect protein variation. That is, protein content may be more uniform if a larger sample volume is ground compared to smaller sample volume taken from the same seed lot. Sample volume may be particularly relevant in pea where there is potential for high protein variation on the seed level. Hence the goals of this study are to test:

1. If greater protein variation exists in ground pea or wheat samples.
2. If sample volume influences protein variation differently between pea and wheat samples.

Methods and Statistical Analysis
Four spring wheat and four yellow pea bulk samples will randomly be obtained from Montana farms. From each bulk sample, two subsample volumes of 1, 2, and 3 tablespoons will be ground in a Udy mill with a 1-mm screen. Two subsamples from each ground subsample volume will then be run for total nitrogen using LECO combustion analysis. Protein will then be determined by multiplying total nitrogen by 5.70 and 6.25 for wheat and pea respectively.

The response of interest (y_{ijkl}) is the absolute difference between protein measurements for each subsample pair. To test the effects of crop type (β) and sample volume (α) on absolute differences between subsampled protein measurements, ANOVA will be run using the following three-staged nested model with crossed factors:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \tau_k + \alpha\tau_{ik(j)} + \varepsilon_{ijkl} \quad \text{Eq. 1.}$$

where μ is the grand mean of all protein responses, τ_k is the seed lot corresponding to each bulk sample, and ε_{ijkl} is the error term assuming model residuals are $N(0,\sigma^2)$. Homogeneity of variance assumptions will be verified using a Levene's test, and post hoc multiple comparisons will be made if treatments are significant at the α=0.05 level. Statistical analysis will be run using the PROC GLM procedure in SAS (SAS Institute Inc., 2012).

B. Review emphasizing how current management could impact pea protein content

Based on more than 80 survey results to date, the most common yellow pea management practices across Montana are as follows:

1. Fertilizer—Approximately half of pea producers do not apply fertilizers whereas the remaining half apply various blends and rates of N-P-K-S fertilizers.
2. Inoculation type—Approximately half of pea producers use peat-based inoculant, and the remaining half use granular inoculant.
3. Legume\Rotation History—Producers growing peas for the first time may not have soil-resident rhizobia from growing pea or other legumes previously

A review has been written detailing how these management factors have impacted pea protein in growing climates similar to Montana (see information below).

Management Options for Boosting Protein in Field Pea in Montana
Mike Bestwick, Perry Miller and Clain Jones

Introduction
The pea protein market is expanding. In 2015, the pea protein market was valued at $22.8 M, and is projected to exceed $34.0 M by 2020 (MarketsandMarket, 2015). Market growth is being driven by consumer preference for

non-GMO and gluten-free sources of protein and has given pea a marketing advantage over dairy and soy-based protein sources. Further new processing technologies have made pea protein extraction efficient and affordable.

Examples of major food companies that use pea protein in their products include:

1. General Mills—Larabar ALT protein bar
2. Hampton Creek—Just Mayo
3. Now Sports—Pea Protein
4. Kirkland—Nature's Domain Dogfood
5. Barilla—Pasta noodles

Figure 1. Food products in which pea protein is a key ingredient.

Montana leads the nation in dry pea production with nearly 600,000 acres harvested in 2016 (NASS, 2016). If consistently high protein content can be maintained in Montana grown pea, the pea protein industry may target and pay more for Montana grown pea. High pea protein could increase producer revenues.

Protein content will hinge on growing conditions and management in Montana. Montana's major agricultural regions are characterized as semi-arid with highly variable precipitation and temperature patterns (Padbury et. al., 2002). Pea is typically seeded in April and terminal drought forces the crop to mature by July, which means drought will affect physiological processes related to pea protein formation. Management options may also affect protein formation, and those that are most pertinent to Montana include:

1. Fertilizer—Approximately half of pea producers do not apply fertilizers whereas the remaining half apply various blends and rates of N-P-K-S fertilizers.
2. Inoculation Type—Approximately half of pea producers use peat-based inoculant, and the remaining half use granular inoculant.
3. Legume\Rotation History—Producers growing peas for the first time may not have soil-resident rhizobia from growing pea or other legumes previously.

As the protein market continues to expand, studies addressing how management affects pea protein in drought-prone regions will become increasingly important in helping Montana producers decide how to manage pea for high protein. For instance, understanding how protein is influenced by different fertilizers, inoculant types, or crop rotations in a wet and dry growing season could help producers assess the financial risk of various management plans under climatic uncertainty. The objective of this document is to a) provide a basic physiological framework for protein formation in pea under ideal, drought, and nutrient-stressed growing conditions and b) highlight studies focused on how management has affected pea protein in drought-prone regions similar to Montana.

Protein Formation in Pea

Pea protein content is directly related to seed nitrogen (N) content—high seed N means high protein. At the plant level, pea protein depends on plant N uptake and remobilization of N to seeds. Under ideal growing conditions—meaning water and nutrients do not limit pea growth—soil nitrate (NO_3) is initially taken up by pea roots and stored in shoots and leaves until pods begin to fill. During pod-fill, roughly 60% of N stored in shoots

and leaves is remobilized to the seed, while around 50% of N taken up by roots is sent directly to the seeds (Schiltz et al., 2005) (Figure 2). Final seed N concentration is lastly determined by seed number—with fewer seeds per plant, final seed N is more concentrated and protein content is higher.

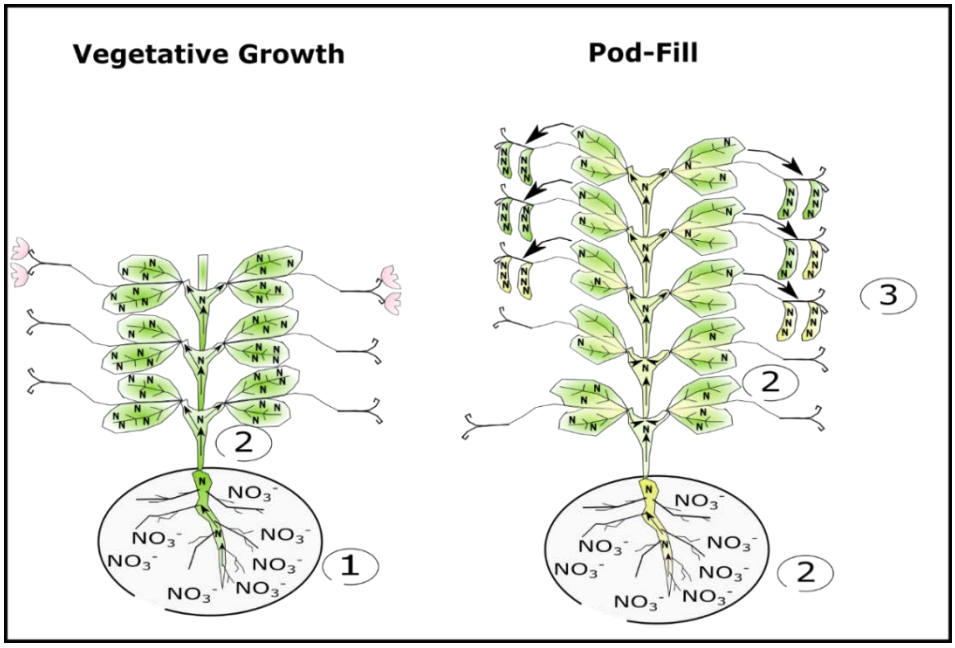

Figure 2. During vegetative growth, soil nitrate (NO_3^--N) is absorbed by roots and stored as N in pea roots, shoots, and leaves (1-2). During pod-fill, plant N stored in roots, shoots, and leaves are remobilized to the seed (2-3). The more N remobilized to the seed, the higher the protein content. This means plants with fewer seeds often have higher protein content.

Differences in plant genetics may play a role in dictating final seed protein. For instance, varieties that produce fewer seeds are more apt to produce high seed protein (Lhuiller-Soudele et. al., 1999). Similarly varieties that are more efficient at remobilizing shoot and leaf N to seeds during pod fill may produce higher protein compared to less efficient varieties (Larmure and Munier-Jolain, 2004). Because pea seeds successively develop from the lowest to highest nodes, individual seed protein can even be affected by nodal position and variety. Atta et al. (2004) observed that individual seed protein decreased from 29.8% in the first developed or lowest node to 24.9% in the tenth or highest node in the French variety L833 (Figure 3 A). Alternatively individual seed protein remained constant across nodes in the variety Colmo (Figure 3 B). Lower individual seed protein at upper nodes in L833 was attributed to less efficient N acquisition and remobilization to seeds when pods were filling in upper nodes compared to when seeds were developing in lower nodes. In Colmo, N acquisition and remobilization remained constant when seeds were developing at both upper and lower nodes.

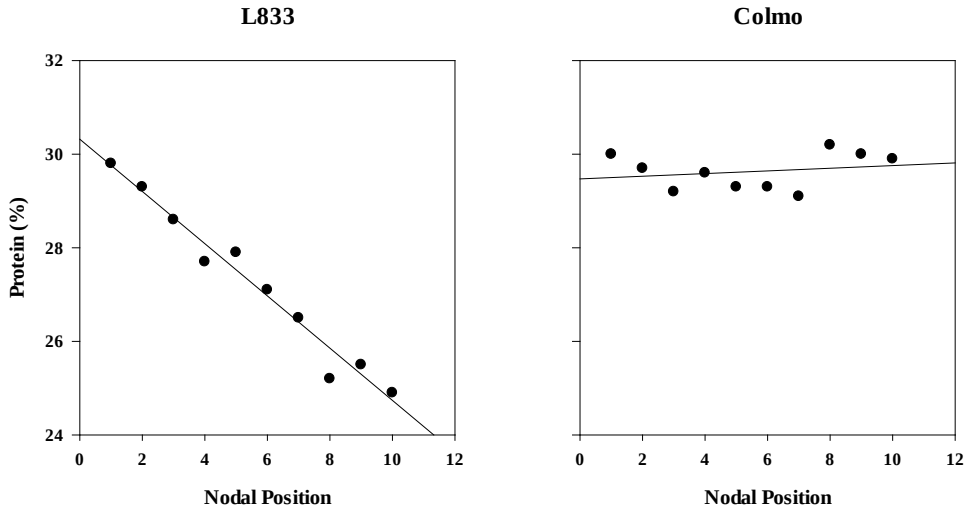

Figure 3. Individual seed protein as affected by nodal position and variety. Left). Individual seed protein decreases as nodal position increass in the French variety L833. Right). Individual seed protein remains constant across nodal position in the French variety Colmo. Figure adapted from Atta et al. 2004.

Despite the potential for variety to affect protein, environmental factors may have a greater effect on the physiological processes that affect protein formation for pea grown in Montana (Chen, unpublished data). For instance, drought stress may reduce seed number which in turn could affect final seed N concentration. Water, nutrient stress, or a combination of the two are common in Montana and are likely to affect protein for pea grown in Montana. More specifically, water and N stress are the best documented and are covered below.

Implications of Drought and Nitrogen (N) Stress on Protein

How could water stress affect pea protein?

Drought stress results from low or poorly timed precipitation relative to important crop growth stages. In pea, drought stress before flowering reduces vegetative biomass. Reduced vegetative biomass translates to less stored N in shoots in leaves, which means less potential for N to be remobilized to seeds during pod-fill (Lhuiller-Soudele et. al., 1999). Alternatively, drought stress incurred at any period over the crop cycle can reduce seed number (Guilioni et al., 2003). Reduced seed number has potential to boost protein since N is remobilized to fewer seeds. Depending on timing and severity of drought, it is possible that drought stress could increase, decrease, or have no effect on pea protein compared to a well-watered plant (Figure 4).

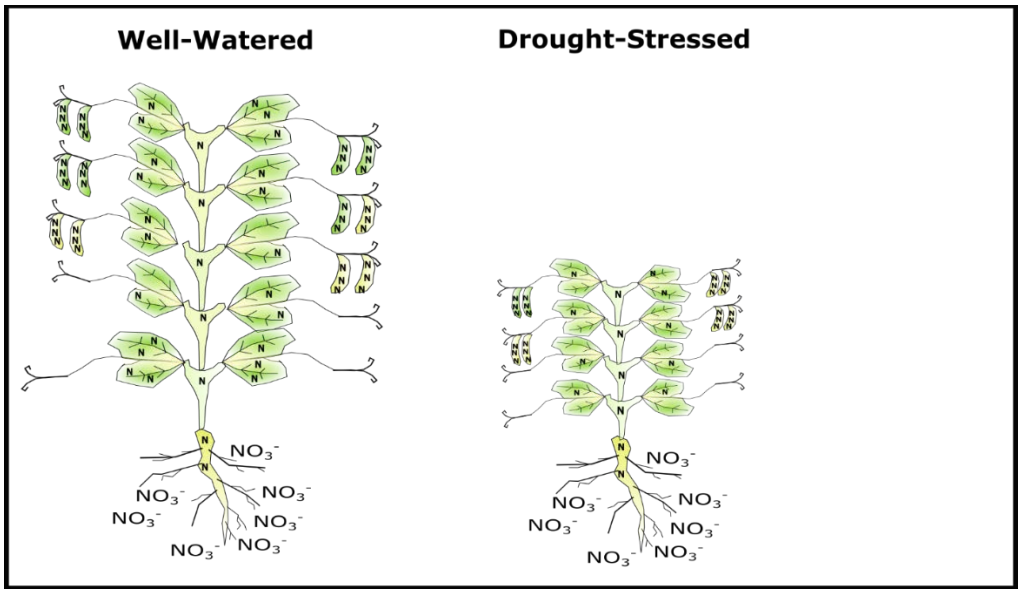

Figure 4. Compared to a well-watered plant, drought stress reduces biomass and seed number. Less biomass has potential to decrease protein since less N will be remobilized to the seed during pod-fill. Conversely fewer seeds may increase protein content on a per-seed basis. Drought stress has potential to increase, decrease, or have no effect on seed protein depending on timing and severity of occurrence.

How could nitrogen (N) stress affect pea protein?

Water availability is tightly related to N availability. Because no or low rates of fertilizer N are applied to pea, N becomes plant available primarily through decomposition of organic and crop residue and N_2 fixation. Under wet and warm growing conditions, soil microbial activity needed to decompose organic N into NO_3^- for plant uptake is high. Moist (but not saturated) soils also support a healthy environment for rhizobia populations which maintain nodule activity for effective N_2 fixation. Conversely decomposition and N_2 fixation rates are reduced when soils are dry or cool. This means more N will be made plant available through decomposition or N-fixation in a high rainfall year compared to drought conditions, but reductions in plant biomass and seed number from drought could also affect final protein (Figure 5).

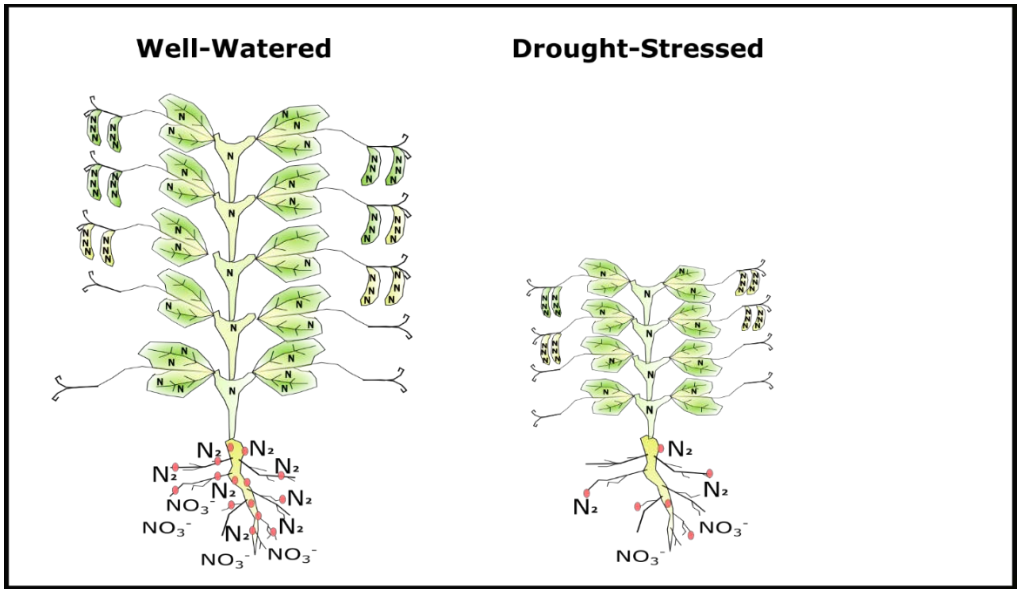

Figure 5. Compared to a well-watered plant, dry soils reduce the amount of soil nitrate (NO_3^-) absorbed by pea roots and atmospheric nitrogen (N_2) fixed in pea nodules (pink circles). While drought decreases the amount of N remobilized to seeds by reducing soil N uptake and vegetative N storage capacity, reduced seed number from drought stress may increase protein on a per seed basis. This means timing and severity of drought and N stress interactions will greatly affect final protein.

Nitrogen (N) Management and Pea Protein

Despite the complexity of how drought and N interactions may affect pea protein, a number of studies have addressed how protein responds to different N management strategies in semi-arid systems similar to Montana. Specific N management strategies include applying different N rates, use of inoculation, and inoculant type. The following highlights results from these studies.

How could high starter N rates affect pea protein?

Early studies conducted in the Canadian prairies suggest protein can be increased at high N-rates (Sosulski et al., 1974; Holl and Vose., 1980) regardless of wet or dry growing season conditions. For instance, Holl and Vose (1980) showed that N rates greater than 143 lbs/ac (160 kg/ha) increased protein by approximately six percent compared to an unfertilized control. This result was attributed to greater seed N accumulation during the pod fill period (Figure 6A). Likewise, McClean et. al. (1974) showed in a greenhouse study that seed protein increased from 20 to 30 % at N rates ranging from 0 to 293 lbs/ac (326 kg/ha) under high, medium, and low soil moisture levels (Figure 6B).

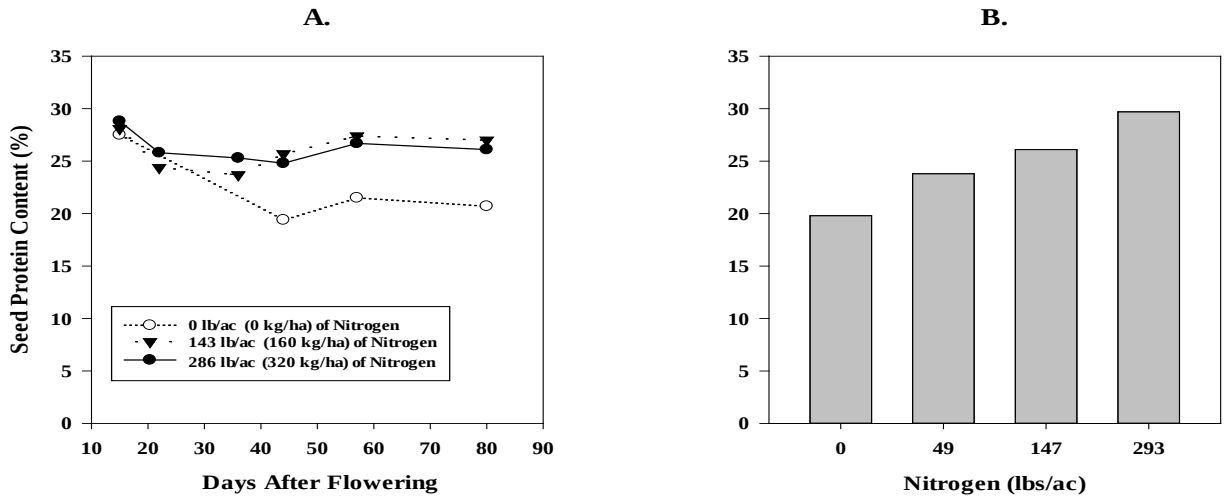

Figure 6. Seed protein content was observed to remain high over the pod-fill period when starter nitrogen (N) was applied at 286 and 143 lbs ac^{-1} compared to no starter N (Holl and Vose, 1980). B. Seed protein increased with starter N-rates of 0-0293 lbs/ac in a greenhouse experiment conducted by McClean et al., (1974).

Is it worth applying high starter N rates to boost protein?

While these studies demonstrate the effectiveness of high fertilizer N-rates at increasing protein, it would not be practical to apply high doses of starter N on three accounts. First, nitrogen fertilizer is the greatest input cost for crop production in Montana, and it is unlikely that increased pea protein would offset fertilizer costs—particularly at N rates greater than 143 lbs/ac. Second, pea is marketed as being a low input crop in terms of nutrient requirements, so high rates of fertilizer N may deteriorate pea marketing potential. Third, improvements in seed inoculant has increased N-fixation potential (Rennie and Hynes, 1993) which may greatly reduce or even eliminate the need for fertilizer N. A recent Montana study found that inoculated pea could fix between 49 to 112 lbs/ac (54-124 kg/ha) depending on growing season conditions (McCauley et al., 2012). For these reasons, recent work has focused on how economical starter N rates and/or inoculant types (i.e., granular vs. peat) affect protein.

How could lower starter N rates affect protein and yield?

Starter N is most likely to be effective at boosting protein with low spring soil NO_3^- levels. (McKenzie et al., 2001 a.). A four year study conducted across Alberta showed that when spring soil NO_3^- was less than 18 lbs/ac (20 kg/ha), starter N rates of 18, 36, and 54 lbs/ac (20, 40, and 60 kg/ha) increased protein 33% of the time, decreased protein 25% of the time, and had no effect on protein 42% of the time. These increases in protein, however, were modest and did not increases protein by more than 0.8% relative to a non-fertilized control in any instances. When spring soil NO_3^- was greater than 18 lbs/ac (20 kg/ha), starter N generally did not benefit protein (Figure 7).

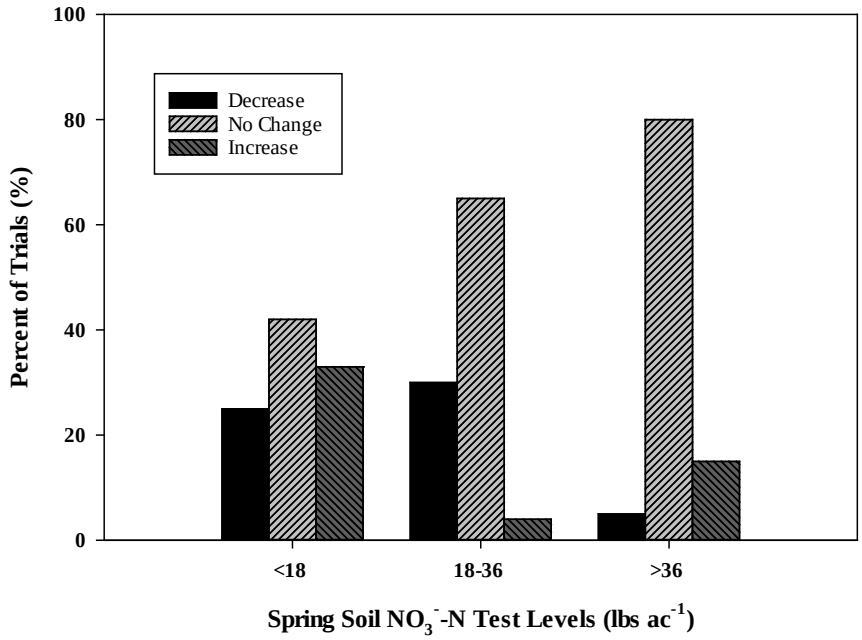

Figure 7. Percent of field trials in which the addition of starter nitrogen (N) fertilizer led to a decrease, did not change, or increased pea protein content at different spring soil nitrate (NO_3^-) test levels across Alberta. Figure adapted from McKenzie et al. (2001 a.).

The study also showed that starter N generally did not benefit yield. Application of starter N had no effect on yield in 73% of trials, increased yield in 24% of trials, and decreased yield in 3% of trials relative to an unfertilized control. In instances where starter N benefited yield, yields were increased by 8 and 12% at the 18 and 54 lbs/ac (20-60 kg/ha) N-rates respectively.

How could inoculation affect protein and yield?

Inoculant is most effective under low spring soil NO_3^- levels and in fields where legumes have never been grown. The aforementioned study in Alberta showed that inoculant led to a 4% increase in protein relative to a non-inoculated control when spring NO_3^- was less than 18 lbs ac^{-1} (20 kg ha^{-1}), but inoculant was less effective at soil N levels greater than 18 lbs/ac (Figure 8 A.). Likewise inoculant boosted protein in 64% of fields with no history

of legumes and in only 13% of fields with a history of legumes (Figure 8 B.). The effectiveness of inoculant in fields without legume history is often attributed to low soil rhizobia populations.

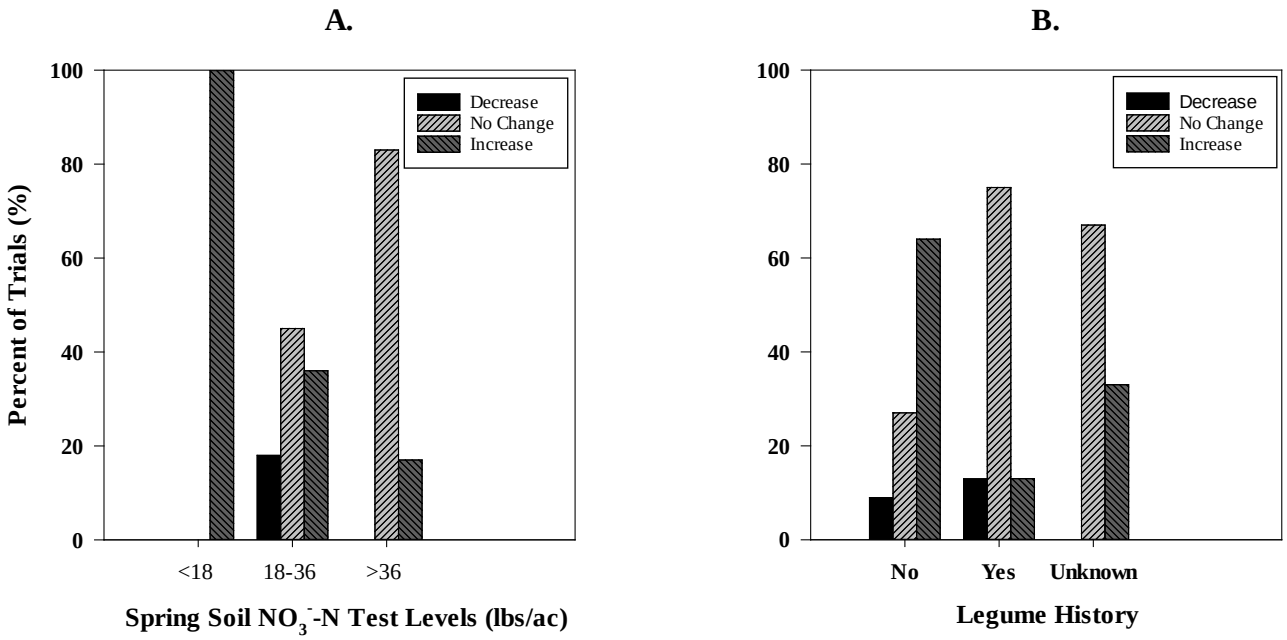

Figure 8. Percent of field trials in which the use of inoculation led to a decrease, did not change, or increased pea protein content at A) different spring soil nitrate (NO_3^-) test levels or B). on fields with different rotation histories of legumes across Alberta. Figure adapted from McKenzie et al. (2001 a.).

Yields also benefited from inoculant in 41% of trials with an average yield increase of 14% over a non-inoculated control. Notably inoculant did not increase yields in 55% of fields with no history of a legume crop. The lacking yield response, however, was attributed to indigenous populations of soil rhizobia widely present in Canadian prairie soils.

How could inoculant type affect N-fixation, protein, and yield?

Granular inoculant has been observed to be more effective at fixing N than peat-powder inoculant (Clayton et al., 2004b.). N-fixation is often superior with granular inoculant compared to peat-powder due to differences in application methods. Specifically, granular inoculant is applied to the soil at seeding, and peat-powder is applied directly on the seed. Soil-applied granular inoculant allows rhizobia populations to be evenly distributed on pea roots whereas rhizobia are clustered near the root crown with seed-applied peat-powder. Consequently nodules form on tap and lateral roots from granular inoculant, and larger nodules tend to cluster on the root crown with peat-powder (Figure 8 A). The widespread nodule distribution associated with granular inoculant has been attributed to increasing N-fixation.

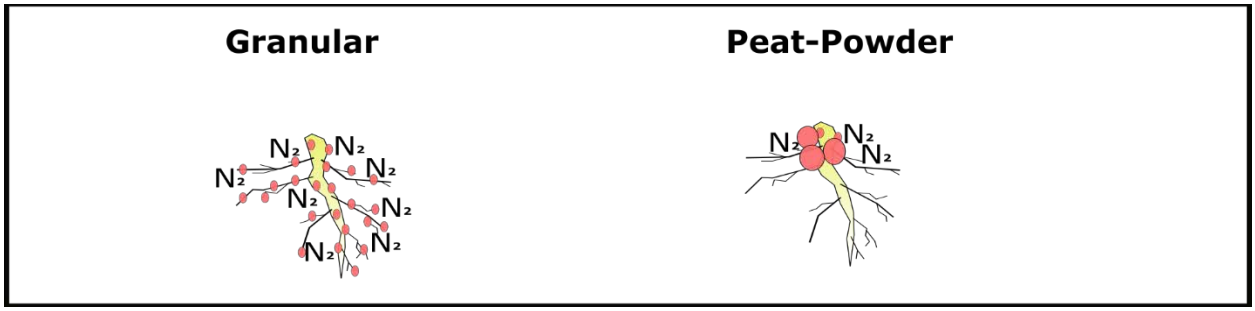

Figure 9. Nodules (pink circles) have been observed to be widely-distributed on pea roots from granular inoculant since granular inoculant is applied to the soil. Nodules from peat-powder are usually larger and clustered on the root crown because peat-powder is applied to the seed. Well-distributed nodules associated with granular inoculant have been attributed to greater N-fixation compared to peat-powder.

Increased N-fixation from granular inoculant has been shown to boost protein (Clayton et al., 2004a.). A two year study conducted at three locations in Alberta showed that seed protein averaged 19.4 and 17.6% using granular and peat based inoculant respectively (Figure 10). Starter N was applied at 0, 18, 56, and 72 lbs/ac (0, 20, 60 and 80 kg/ha) in this study but had little effect on protein content. It was therefore concluded that increased N-fixation from granular inoculant was mainly due to superior nodule distribution on pea roots, and greater N-fixation during the pod-fill period may have boosted final seed N and protein.

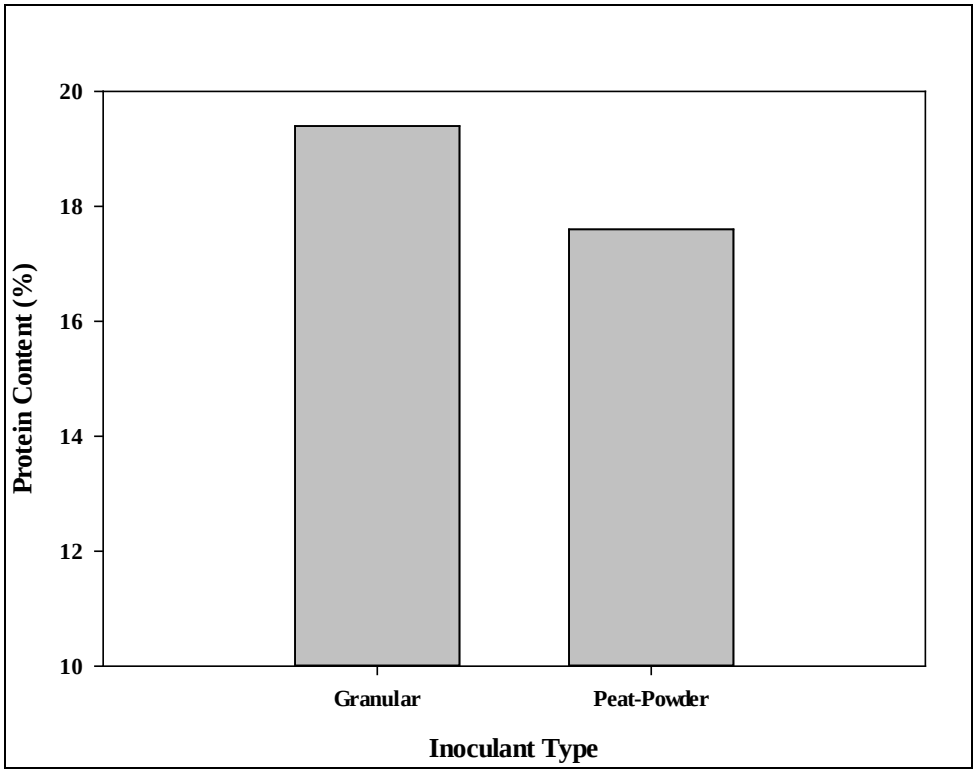

Figure 10. A two-year study conducted across three sites in Alberta showed that granular inoculant outperformed peat-powder at boosting protein. Granular was likely more effective due to better nodule distribution on pea roots. Figure adapted from Clayton et al., 2004 a.

Greater yield responses were also associated with granular inoculant relative to peat powder. Averaged across the six site and year combinations, seed yields were 630 lbs/ac (700 kg/ha) greater with granular inoculant than with peat-powder. Additionally application of fertilizer N did not benefit yields when granular inoculant was applied, suggesting that granular inoculant provided adequate N supply to meet yield potential.

Is it worth applying fertilizer N if inoculation fails?

Inoculant generally performs as well or better at maintaining high protein and yield compared to applying fertilizer N. However, with dry seedbed conditions, inoculant may fail to produce root nodules for effective N-fixation. In the case of inoculation failure, applying fertilizer N may be needed to provide pea with adequate N supply for protein and yield formation.

A two-year study conducted at two central Montana sites compared how pea protein and yield responded to a non-inoculated control (e.g. inoculation failure), granular inoculant, and fertilizer N applied at 0, 4, 6, and 8 weeks after seeding (McConnell et al., 2002). The results showed that protein and yields were consistently lowest for the non-inoculated control, but protein and yields were generally greatest with granular inoculant (Figure 11.). In a number of instances, granular inoculant and fertilizer N applied either 0, 4, or 6 weeks after seeding produced similar protein and yield. Hence, the conclusion from this study was that granular inoculant will provide adequate N supply to meet protein and yield potential under normal growing conditions, but under unusually dry spring conditions, it may be worth applying fertilizer N within 6 weeks after seeding to ensure adequate N supply.

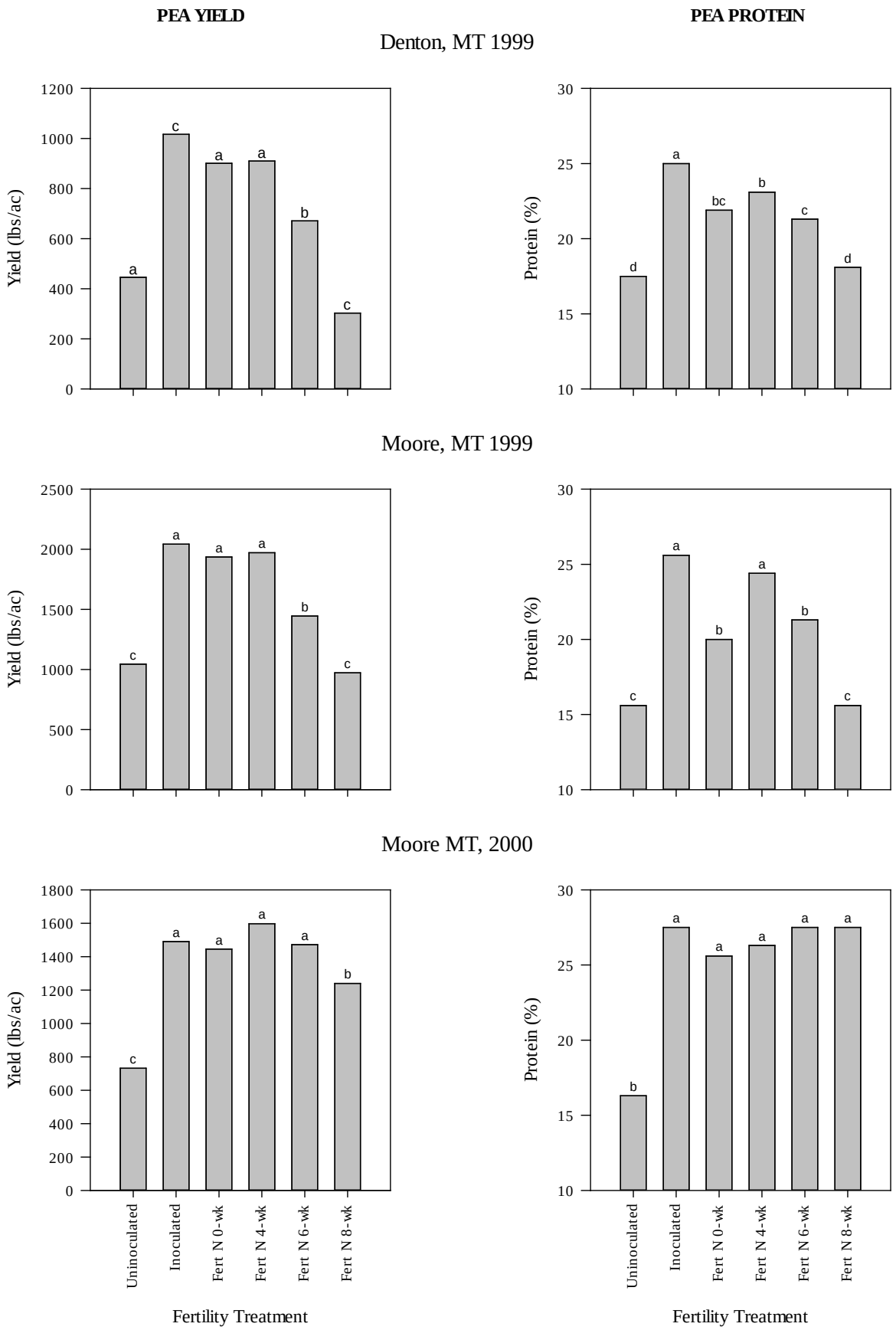

Figure 11. Effect of six fertility treatments on pea yield and protein at two Montana sites over the 1999-2000 growing seasons. Fertility treatments were no inoculation, granular inoculant, and broadcast fertilizer N applied at 0, 4, 6, and 8 weeks after seeding. Different letters above bars indicate statistical differences at the p>0.05 significance level.

What are the main points regarding the effects N management on pea protein and yield?
Combined, these studies indicate that protein can be boosted with high N-rates (>143 lbs/ac), but such high rates are non-economical and may not be necessary due to improvements in inoculant technologies. With lower starter N-rates, comparable or greater pea protein can be expected via N-fixation than through applying starter N. Nitrogen fixation will likely be greatest by using granular inoculant, but inoculation or starter N may show no benefit with high spring soil NO_3^- levels or in fields with a rotation history of legumes. Spring soil NO_3^- testing and knowledge of rotation history could therefore help in addressing the effectiveness of applying starter N and/or inoculant.

Application of starter N does not guarantee higher yields. Adequate N supply can likely be met by inoculation, and granular inoculant may be more effective than peat-powder. Fields with high of soil rhizobia populations may likewise eliminate the need for starter N or inoculant to meet yield potential, but under dry spring growing conditions when root nodules may not develop, it may be beneficial to apply starter N within 6 weeks after seeding.

Potential Implications of Phosphorus, Sulfur, and Potassium Management on Pea Protein

Although most studies have focused on the effects of N management on pea protein, phosphorus (P), sulfur (S) and potassium (K) are known to affect N-fixation and could therefore affect protein too. The next sections describe how P and S deficiency limit N-fixation and highlights how P, S, and K deficiencies have affected protein in the neighboring Canadian prairies.

How does P stress limit N-fixation?
Phosphorus indirectly affects N-fixation. Pea roots absorb P in the form of phosphate (HPO_4^{2-}). Plants deficient in phosphorus produce less green-leaf area and biomass which in turn reduces photosynthesis. Reduced photosynthesis limits the amount of atmospheric carbon (CO_2-C) assimilated by pea. Because rhizobia living in pea nodules use plant carbon (C) as an energy source to fix N, N-fixation can be reduced (Figure 12.) from P deficiency.

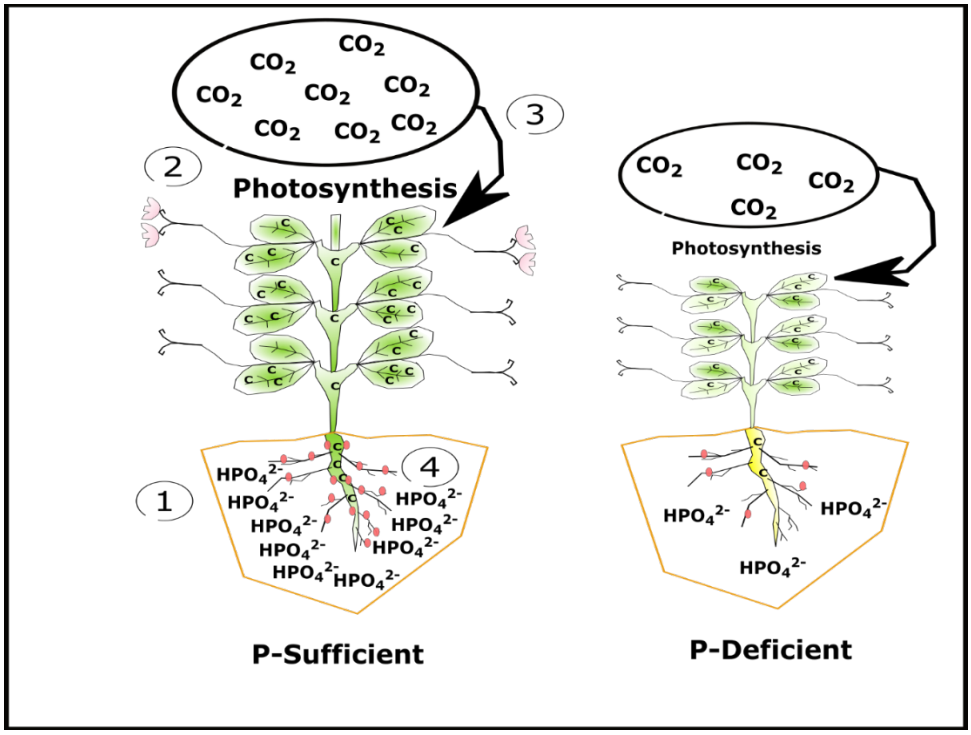

Figure 12. Phosphate (PO_4^{2-}) is absorbed by roots which builds green leaf area and biomass (1-2). Greater green leaf area and biomass allows the plant to convert atmospheric carbon dioxide (CO_2) to plant carbon (C) through photosynthesis (2-3). Stored plant carbon is used as an energy source for rhizobia populations living in pea nodules (pink circles) which fix nitrogen (3-4). Consequently low soil phosphate can indirectly inhibit N-fixation.

For instance, Jakobsen (1985) measured N_2-fixation in response to various P fertility rates 15-18 days after seedling emergence in a greenhouse experiment. His results showed that pea nodules had the greatest N-fixation rates at the highest P rates (Figure 12). Since starter P can increase N-fixation, starter P could also affect protein.

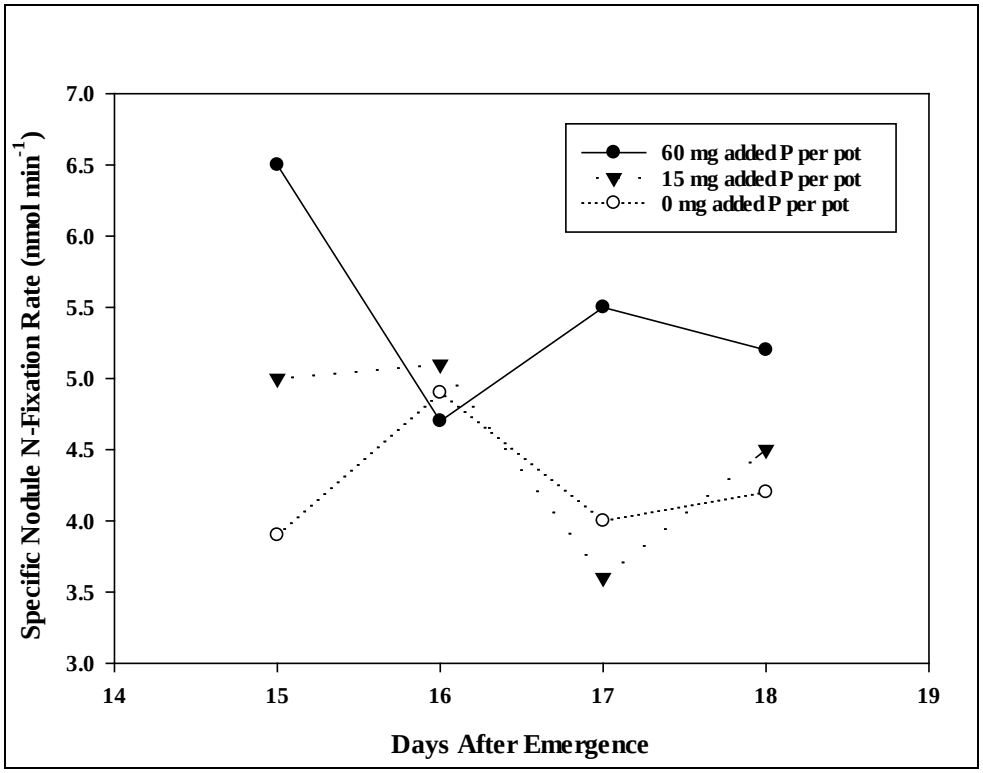

Figure 13. Nitrogen fixation was higher in pea plants that were dosed with 60 mg of phosphorus (P) per pot compared with plants that were given 0 and 15 mg of P per pot. Figure adapted from Jakobsen (1985).

How could starter P affect protein and yield?

An early study conducted in Saskatchewan showed that starter P boosted protein by 1.7% at rates up to 56 kg ha^{-1} compared to no applied starter P (Sosulski et al., 1974). A more recent province-wide study in Alberta showed that starter P at rates up to 26 kg ha^{-1} only increased protein on average by 0.2% across 52 sites (McKenzie et al., 2001b). At sites with spring soil P tests below 15 ppm in the top 6 inches (15 cm), the average increase in protein was 0.4% from starter P.

The latter study also showed that starter P could boost yields and was most likely to do so with low soil P test levels. Specifically, starter P boosted yields at 19 of 52 sites and at 16 of 30 sites with soil P test levels below 15 ppm. The average yield advantage was 7% over a non-fertilized control at sites where yield increases were observed. Combined, these studies suggest there is potential for slight increases in protein and yield from starter P.

How does S stress limit N-fixation?

Sulfur (S) directly affects N-fixation. Sulfur is absorbed by pea roots in the form of sulfate (SO_4^{2-}), and low soil SO_4^- reduces nodule formation on pea roots (Figure 14). Fewer nodules means less N-fixation.

Figure 14. The number of root nodules (pink circles) can be reduced if soils are deficient in sulfate (SO_4^{2-}). Reduced nodule number may reduce nitrogen fixation.

In a greenhouse study, Zhao et. al. (1999) compared N_2-fixation rates in pea between 0 and 50 mg of S per pot. Results showed that N_2-fixation was consistently higher with the treatment that included S at 28, 38, 56, and 73 days after sowing (Figure 15). Sulfur (S) could affect N-fixation and potentially protein.

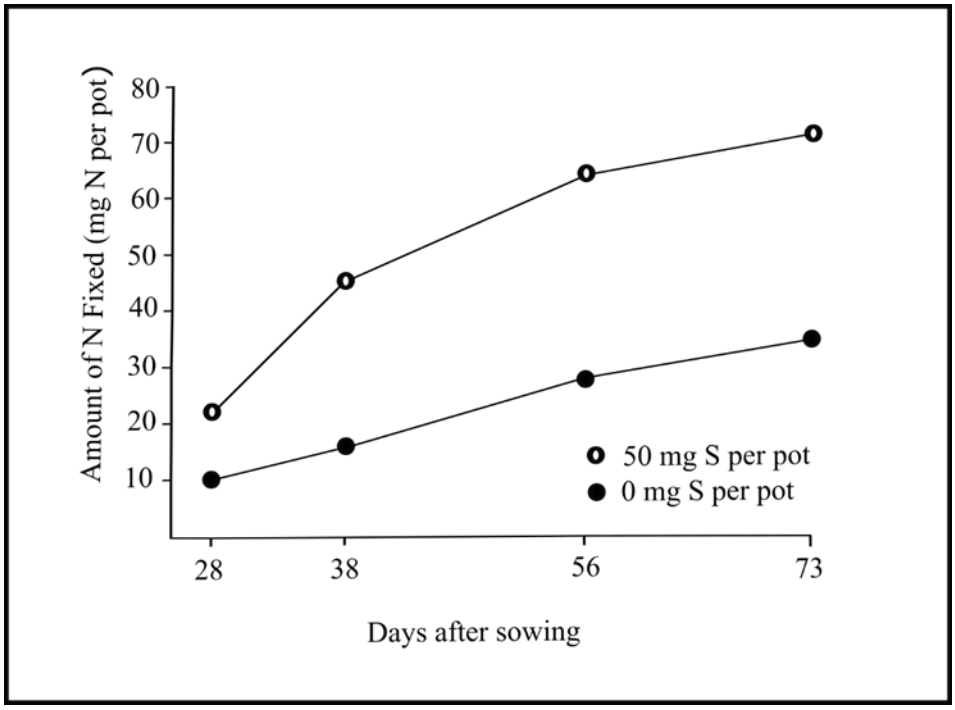

Figure 15. Addition of 50 mg per pot of starter sulfur (S) increased N-fixation over the first 73 days of the crop cycle in a greenhouse experiment. Figure adapted from Zhao et al. (1999).

How could starter S affect protein and yield?

Few field studies have addressed how starter S affects protein in pea. Across 52 sites in Alberta, increases in protein or yield were generally not observed between starter S at rates of 0 and 18 lbs/ac (20 kg/ha) (McKenzie et al., 2001 a). Spring soil S levels were not reported in this study, so it is possible that soil S was sufficient for protein. In Montana, notably, sulfur testing is somewhat unreliable due in part to high levels of gypsum in many soils, so on-farm experimentation may be the best method for Montana producers to test how starter S affects protein and yield in their fields.

What about potassium (K)?

Potassium (K) indirectly affects pea development and N-fixation similar to phosphorus (P). Montana soils are generally rich in K and on average exceed 250 ppm, though some ag soils have K levels as low as 70 ppm, especially in coarse, low pH soils (Clain Jones, personal communication, June 2016). With generally high K-levels, it is unlikely that K would inhibit protein formation or yield although this has not been explicitly tested in Montana.

What are the main points regarding P, K, and S management?
Soils deficient in P, S or K can reduce N-fixation. Reductions in N-fixation has potential to reduce protein and yield. A province-wide study in Alberta indicates that starter P can lead to modest increases in protein yield, and increases are more likely to be realized with when soil P tests are below 15 ppm. Alternatively starter S did not increase protein or yield in Alberta. Because most Montana soils are high in K, it is unlikely that additional K will boost protein and yield in most Montana soils.

Summary and Final Remarks

Pea protein depends on N uptake and remobilization of N to the seed. Both drought and management interactions will likely affect these processes in Montana. Drought may reduce protein by decreasing N uptake and remobilization of N to the seed, but drought may also increase protein by reducing seed number. Application of starter N and use of inoculant may increase soil N uptake and N-fixation, but these management options will be most effective with low spring soil nitrate (NO_3^-) tests, in fields where legumes have never been grown, and under dry seedbed conditions. Granular inoculant could be more effective than peat-powder at increasing N-fixation due to greater nodule distribution on tap and lateral roots. Starter P fertilizer may indirectly increase N-fixation and will most likely be effective if soil P tests are low. Starter S may directly enhance N-fixation, but soil S testing is unreliable in Montana, so on-farm experimentation and tissue S testing may be the best way to determine if starter S benefits protein. Due to high K levels in most Montana soils, it is unlikely that starter K will benefit protein on most fields, but might on K deficient fields (soil test K < 250 ppm).

Pea protein is a new study area for Montana. Results presented here have largely been extrapolated from Canadian studies which may have been conducted over more favorable growing conditions than what is typical in Montana. For instance, recent statewide variety testing in Montana showed mean yields ranged from (2243-2680 kg/ha) and growing season (April-August) precipitation ranged from 3.7-16.7 inches (94-424 mm) (Mohammed et al., 2016). The studies from the Alberta and Saskatchewan reviewed in this document seldom reported yields below 2700 lbs/ac (3000 kg/ha) and (McKenzie et al., 2001a.; Clayton et al., 2004a.), suggesting drought did not limit pea production to the extent that would be expected in Montana. It is possible that drought and management interactions could affect pea protein differently in Montana than in Canada. The information provided here should therefore be considered as a starting point for adapting management to maintain high pea protein.

C. Yellow pea sample collection from 2016 growing season

To date, 80 yellow pea samples and surveys have been collected from the 2013-2015 growing seasons, and 35 additional samples have been collected from the 2016 growing season. We anticipate to acquire 50-100 additional samples between now and April, 2017. Once all samples have been collected and tested for protein, management surveys will be sent to producers, and a formal analysis will completed to identify how both management and climate affect protein content in yellow pea.

Hiring

- No additional hires in Quarter 6.

Expenditures

- Total Personnel Services: $64,026.22
- Total Operations: $4,085.02

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

Progress towards milestones

This quarter has been focused on processing of samples and collection of data. New soil samples were received throughout the fifth and sixth quarters of 2016. These new samples were cataloged and stored at -20°C. Samples were separated for different analysis and shipped to proper locations.

1) Chemical testing
   a) The samples have been delivered to the University of Idaho for chemistry analysis which includes: extended Soil Fertility Test (pH, organic matter, ammonium, nitrate, available P and K, boron, and sulfate), moisture content test and Soil Trace Element (Metals) Screen. These result will be received by the beginning of March.

2) nifH Primer development
   a) Several nifH primers were made and tested against A*zotobacter vinelandii* for a control. The Universal F2 primer performed the best with the control DNA (Figure 1). All soil samples were then tested for the nifH as a control before being sent off for sequencing.
   b) A quote has been received from the company MR DNA to perform nifH targeted sequencing on our soil samples. The next generation sequencing methods will deliver the most accuracy and depth for our samples. We will be sending the soil DNA to the company after extraction and quality testing has been performed on all of the samples.

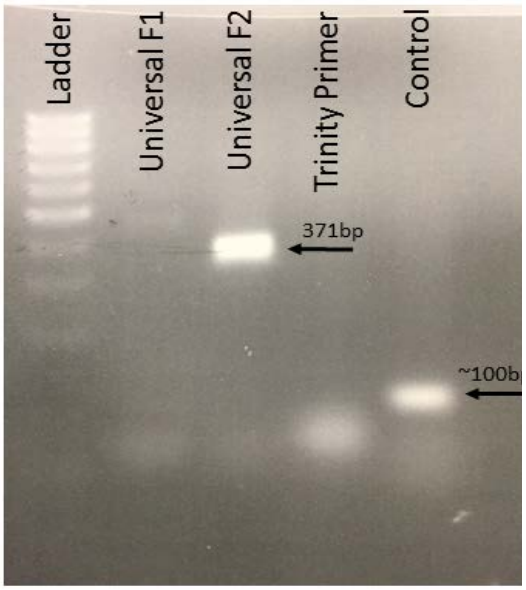

Figure 1: Test for control primers against azotobacter vinelandii genomic DNA. nifH primers universal F2 and universal R gave the brightest and largest band. The 371bp PCR product is a large enough cassette to determine diversity.

3) Soil Extraction
   a) We have refined a method for the extraction of DNA using the MolBio Power Soil kit and continuing the extraction from the spring and summer samples. After DNA extraction is complete we test our DNA against our primers for 16s DNA (533F, 805R) and for nifH (Table 1).

Table 1: Spring 2016 DNA extraction. A few examples of the DNA extraction process for all samples. DNA is extracted with the Molbio Powersoil Kit, quantification of DNA is done on nano-drop spectrometer. PCR is performed on all extracted DNA to test for the amplification of 16s and nifH genes. These genes will be re-amplified on site for targeted next generation sequencing analysis.

DNA code	Location	Depth	DNA Extracted (ng/mL)	16s primers	nifH primers
JZ_017	NARC	0-6"	18.1	x	x
JZ_018	NARC	12-24"	6.4	x	x
JZ_019	NARC	24-36"	4.4	x	x
JZ_029	NARC	6-12"	38.6	x	x
JZ_020	WARC	0-6"	n/a	n/a	n/a
JZ_021	WARC	12-24"	n/a	n/a	n/a
JZ_022	WARC	6-12"	19.4	x	x
JZ_023	EARC-Dry	0-12"	n/a	n/a	n/a
JZ_024	EARC-Richland	0-12"	27.2	x	x
JZ_025	EARC-Irr	0-12"	33.6	x	x

Timeline for future progress: After DNA extraction and PCR gene testing in the lab, DNA will be sent off for next generation sequencing at MR DNA. Once genomic data and chemical data is received (mid-March to mid-April) we will have a large data set that will encompass many variables including geography, nutrient application, irrigation methods and pea variety. A pipeline has already been refined in summer of 2016 for multivariate statistics to analyze this large data set. Data processing and hypothesis testing should take place over the summer of 2017 with the finalization of the work by Fall 2017.

Hiring

- No additional hires in Quarter 6.

Expenditures

- Total Personnel Services: $68,885.74
- Total Operations: $21,554.55

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

Progress towards milestones

The animal trial was inconclusive, so there is nothing to report on that. They have been planning an in-vitro experiment but do not have data from that yet.

Hiring

- No additional hires in Quarter 6.

Equipment

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

Expenditures

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

Cover Crop/Grazing subproject of the Agriculture MREDI Grant

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

Progress towards milestones

Statewide MREDI

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

In seven locations, the cool season species produced from 615 to 2267 pounds above ground biomass on a dry matter basis with oats being the greatest across all environments. With the polycultures being lower than the

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

Large Termination Cover Crop Project

In the large plot termination trial where alternative economics streams of cover crop usage were evaluated, there are some cover crops when used as either a dry forage (hay) or grazing that have shown a $100/acre return over what a transitional winter or spring wheat/fallow rotation. Uses current wheat, hay and grazing prices. There are also cover crops returning less that the traditional wheat/fallow rotations. It should be noted that the harvest date for the cover crops has average July 10 across the entire trial from 2012 to 2016 (Table 1). By harvesting the cover crops as either a dry forage (hay) or grazing at this time point, the deep soil moisture is protected for the following cash crops. No cover crop is allowed to produce viable seed, if at all possible, and it allows winter wheat to have a chance to be included in the rotation, since the cover crop is terminated after the grazing or haying to allow for fall planting of winter wheat should that be the desire. Over the duration of the trial there has been timely rains during the wheat years and both above and below in crop normal rainfall. There has not been a devastating drought or a below normal rainfall without timely rains during the wheat years. Therefore, no assumption can be made about the overall economic two-year rotation should a severe drought occur in this rotation.

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

Table 1. Cover crop above ground biomass and either winter or spring wheat yields across all years of large termination cover crop trial near Havre Montana. Crop years 2012 to 2016.

	2012	2013		2014			2015			2016		
	7.33 IC	18.46 CY/13.28 IC			12.03 CY/ 4.87 IC			12.05 CY/ 7.52 IC			18.86 CY/ 12.21 IC	
	CC, lb/ac	WW bu/ac	SW bu/ac	CC, lb/ac	WW bu/ac	SW bu/ac	CC, lb/ac	WW bu/ac	SW bu/ac	CC, lb/ac	WW bu/ac	SW bu/ac
Barley	909.2 defgh	47.0 h	43.4 efg	10026.0 a	33.3 bc	29.6 abcdef	6176.7 a	42.4 ab	26.2 bcde	6697.5 a	80.4 abcd	26.1 b
Mix 1	2069.6 a	50.5 gh	44.9 cdef	4629.9 fg	31.6 bc	26.7 def	4019.4 cd	45.4 ab	27.2 bcd	3001.9 de	78.8 abcd	30.7 a
Mix 2	1498.5 abcd	57.1 efg	45.1 cde	5780.4 cd	28.6 c	26.4 f	4538.0 bc	45.3 ab	26.0 bcde	4603.9 b	77.7 abcd	31.9 a
Mix 3	1413.8 abcde	56.3 efg	39.2 g	5448.2 de	32.1 bc	30.0 abcdef	2858.4 ef	42.4 ab	25.9 bcde	3264.3 de	82.0 abc	32.3 a
Mix 4	1834.1 ab	56.9 efg	42.7 efg	6504.2 b	34.1 abc	28.5 bcdef	3450.8 de	42.6 ab	22.2 e	4006.1 bc	78.1 abcd	31.4 a
Mix 5	862.5 defgh	55.3 fg	44.5 cdef	4506.2 fgh	29.6 bc	26.4 ef	2361.9 fghi	42.8 ab	25.6 bcde	1347.1 ghij	84.4 a	31.6 a
Mix 6	690.1 ghi	60.5 def	45.8 bcde	713.1 qrs	35.7 ab	32.1 abc	1430.5 jk	46.0 ab	26.7 bcde	985.9 hijk	76.0 cd	31.7 a
Mix 7	1770.1 ab	74.1 a	50.8 a	482.1 rs	34.8 abc	30.8 abcde	886.8 k	48.4 a	26.7 bcd	791.2 hijk	77.2 bcd	30.3 ab
Mix 8	919.1 defgh	60.7 def	45.2 cde	1070.9 pqrs	34.1 abc	30.8 abcdef	1072.2 k	44.2 ab	25.5 bcde	1250.5 ghij	76.8 bcd	30.4 ab
Mix 9	683.9 ghi	65.5 bcd	48.3 abc	3054.9 klmn	34.5 abc	29.8 abcdef	2063.7 ghij	45.8 ab	24.9 cde	1993.6 f	83.8 ab	33.3 a
Mix 10	1386.1 abcdef	70.9 ab	49.7 ab	3291.6 jklm	31.7 bc	28.7 bcdef	1486.8 jk	46.3 ab	27.4 bc	853.5 hijk	80.6 abcd	31.1 a
Mix 11	1452.0 abcde	61.3 cdef	44.4 cdef	3036.3 klmn	33.8 abc	28.7 bcdef	3444.7 de	42.0 ab	25.8 bcde	3576.2 cd	79.8 abcd	31.0 a
Mix 12	1452.0 abcde	56.0 efg	40.6 fg	4397.4 fgh	34.5 abc	28.7 bcdef	4828.3 b	41.2 b	26.3 bcde	3998.0 bc	73.6 d	31.8 a
Mix 13	1643.1 abc	65.2 bcd	45.1 cde	3954.5 ghij	33.8 abc	27.9 cdef	2018.5 hij	40.4 b	22.9 de	1421.8 fgh	76.8 bcd	32.7 a
Mix 14	390.4 hi	62.4 cde	43.9 cdef	3850.5 hij	36.3 ab	30.9 abcd	2468.7 fgh	45.9 ab	25.8 bcde	950.4 hijk	77.8 abcd	34.3 a
Mix 15	972.9 cdefgh	61.7 cdef	43.6 defg	2333.6 o	33.6 abc	30.6 abcdef	2728.3 fg	46.4 ab	26.0 bcde	1246.4 ghij	80.9 abc	32.5 a
Fallow	0.0 i	68.3 abc	47.9 abcd	0.0 s	34.1 abc	29.2 abcdef	0.0 l	46.0 ab	31.9 a	0.0 l	77.5 abcd	30.0 ab
Std Err:	CC = 273.44 lb/ac; WW = 2.24 bu/ac; SW 1.54 bu/ac											
Numbers within column with different superscript differ by p = 0.05												
CY = Crop Year; inches of moisture measured Sept - August												
IC = In Crop; inches of moisture measured April - July												
Cover Crop in 2012 and 2013 were planted on fallow ground												

Hiring

- No additional hires in Quarter 6.

Expenditures

- Total Personnel Services: $16,523.26
- Total Operations: $5,397.91

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

Progress towards milestones

The manuscript “Preference and forage quality of 13 cultivars of forage barley and 2 cultivars of oats when grazed by sheep” was published in the American Journal of Experimental Agriculture. It is currently available online. Dr. Glunk also has given several presentations using this information, as well as preliminary information from the larger cover crop trial through the MREDI project. This includes presentations in St. George, UT, as well as Shelby, Bozeman, Plentywood, and Scobey, MT.

Glunk’s group, alongside Dr. Tony Hartshorn’s lab, is still working on finishing-up soil and forage quality analyses. Additionally, Dr. Glunk is working with several faculty on developing cover crop fact sheets and Extension modules, which will include information obtained from this project.

Hiring

- No additional hires in Quarter 6.

Expenditures

- Total Personnel Services: $7,572.42
- Total Operations: $8,648.73

On-Farm Precision Experiment subproject of the Agriculture MREDI Grant

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

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

Progress towards milestones

PI Maxwell and Technician Davis (MSU LRES)

Phil Davis has been in contact with our farmer cooperators and we fully intend to carry out On-Farm Precision Experiments on at least 1 field on each farm in 2017. We have already constructed or in the process of creating variable nitrogen fertilizer rate prescriptions based on previous year yields and in some cases protein. We are continuing with the study under the assumption that funding will be available in 2017 and 2018 to continue analysis and data management. We have applied for grants with the Montana Fertilizer Advisory Committee to at least fund Phil Davis our field technician to assure delivery and cleaning of data from the combine instruments following harvest. We unsuccessfully applied to the Western Regional USDA SARE program, the USDA NIFA foundational grant program and the USDA OREI grant program to fund our study. While some of these programs were encouraging we have since learned of some other USDA programs to apply to in 2017.

We are in the full depths of analysis of 2016 data.

Table 1: OFPE fields and analysis.

We have analyzed the data to compare the economic performance of a set of different nitrogen fertilizer application strategies under different ways to characterize the relationship between nitrogen top-dress rate and grain yield and percent protein. We compared 4 different strategies: 1) The site specific optimum (profit maximizing) nitrogen application rate at each yield point in the field, 2) no nitrogen applied, 3) the farmer selected rate to be uniformly applied to the field if we were not doing our experiment, and 4) the profit maximizing uniform rate of nitrogen (Figure 1).

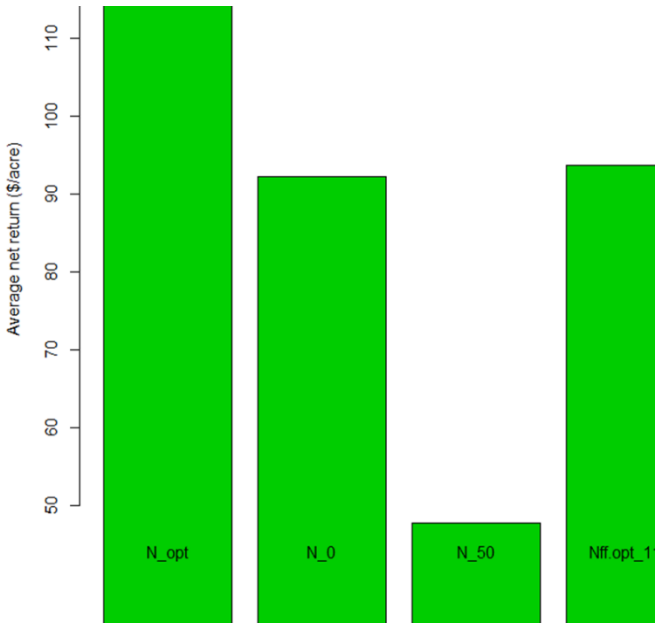

Figure 1. The calculated average net return per acre using different top-dress nitrogen fertilizer application strategies based on the 2016 yield and protein response in the winter wheat crop on the sec35mid field.

Clearly the site-specific optimized rate approach (N_opt) has higher net returns than the 0 nitrogen rate (N_0), 50 lb/acre rate that the farmer would have applied (N_50) and the full-field uniform application to get maximum net return (Nff_opt). The average net returns shown in Figure 1 are unrealistically high because some of the fixed costs for this farm are unknown, but because those would be constant over the treatments the relative outcome would be the same.

Co-PI Rew (MSU LRES)

Rew and Maxwell analyzed weed cell data collected 14 days following herbicide application on Broyles field (Sec35mid) that was infested with cheatgrass. A cell was a 10 m by 10 m area that was sampled with a 0.1 m frame 10 times to estimate the average density of the grass and broadleaf weeds. The total cells visited was 266. Yield monitor points were assigned to each weed density cell. There was a negative relationship between grass weed density and winter wheat yield as expected (Figure 2).

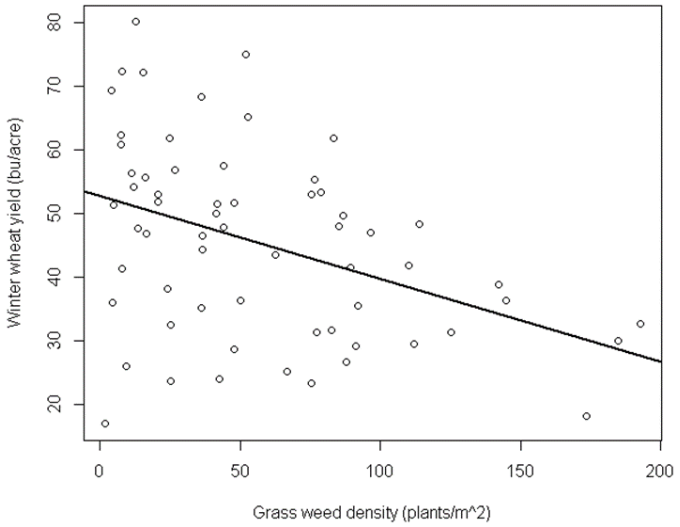

Figure 2. The relationship between grass weed density and winter wheat yield at selected points (cells) in the field.

There was also a significant negative impact of grass weed density on winter wheat protein content (%) in the same field (Figure 3). There was no relationship between broadleaf weed density and crop yield or percent protein. The grass weed impact was convincing evidence that we should construct a weed density map predicted from geographic variables (Figure 4).

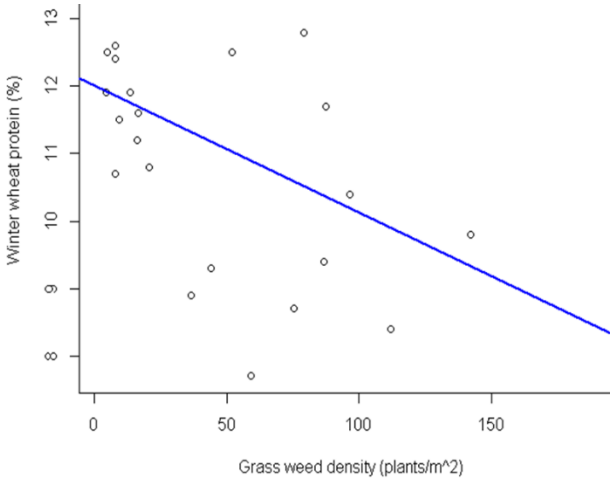

Figure 3. The relationship between winter wheat protein content (%) and weed density.

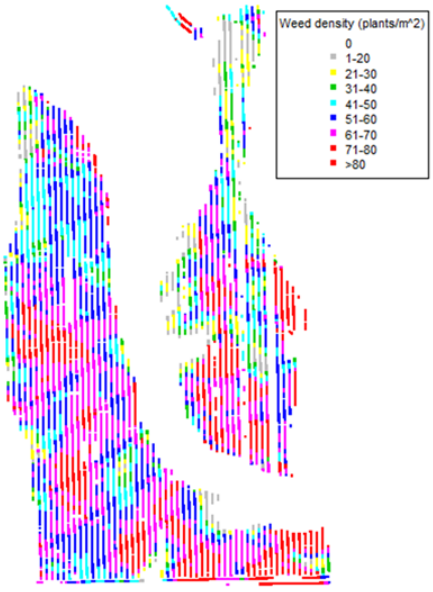

Figure 4. Predicted weed density map.

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 on the dominant grass weed (cheatgrass). The weed analysis aspect of this study was presented by Dr. Maxwell as an invited speaker in the Weed Science Society of America annual meeting Symposium on Precision Agriculture.

Hiring

- No additional hires in Quarter 6.

Expenditures

- Total Personnel Services: $146,076.58
- Total Operations: $135,679.72
- 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. A graduate student, Amy Peerlink, has been brought on to assist with completing and improving the usability of the optimization software component. Janette Rounds (graduate student) is continuing to analyze and develop the optimization software component.

Ms. Rounds and Ms. Peerlink are expanding the nitrogen prescription generating process to handle protein inputs as well as yield inputs. Currently, the nitrogen prescription process takes in the previous year's yield, groups the yield into bins and then assigns a nitrogen rate using one of a set of random methods developed in the early months of 2016. The particular random method used in the OFPE experiments was one that minimized the jumps between different nitrogen rates. In other words, while still maintaining an element of randomness, this method reduced the number of times the applied nitrogen rate jumped suddenly from a low rate to a high rate and vice versa. We wanted to minimize these jumps in order to minimize the stress on farmer's machines. However, we used the random element in this method to try and capture data for a variety of conditions on the field. However, since this process does not also take previous year's protein into account, it is clear that we cannot assess many of the conditions on the field. We are extending the method so that protein variations are taken into account. This will allow us to collect more detailed information about a field. It is our hope that we can model the field more accurately using more detailed prescriptions. The downside to this, however, is that taking protein into account will reduce the number of times a particular condition is assigned the same nitrogen rate, reducing replication. Additionally, this will involve making the current prescription generation code more robust to input errors, and improving the current code. Finally, we hope to add a graphical user interface so as to improve usability.

Ms. Rounds is developing a Deep Learning approach to optimizing yield. Deep Learning is a set of machine learning algorithms that attempt to model high level abstractions in a set of data. Deep Learning may take the form of a neural network, but it tends to be more involved, adding more layers and more calculations. A traditional neural network can only have about three layers before the training methods start to fail, and as such is limited in the abstraction it can model. A Deep Learning network (usually called a deep network) can have as many layers as we need.

The particular Deep Learning method we intend to use involves using stacked auto-encoders. An example of this network is seen in Figure 5. Auto-encoders take in a set of inputs and transform that input, usually reducing dimensions. In the training process, we then attempt to convert the input back to its original form and use the

difference between the original and de-transformed inputs to modify the transformation. After training, when we use the auto-encoder, only the input-to-transformed input part is used. When we stack the auto-encoders, higher auto-encoders in the stack tend to produce more abstracted output. For example, if we have an image that is being fed through the stacked auto-encoders, the first auto-encoder might identify lines in the image. The next auto-encoder might identify shapes and the auto-encoder after that might identify relationships between shapes. This makes stacked auto-encoders a powerful tool for accurate prediction in very complex systems. As we will discuss later, traditional neural networks can predict protein with an accuracy of about 40% in the best case. It is out hope that stacked auto-encoders will provide much higher accuracy. Another reason to use a stacked auto-encoder is that they are actually a form of neural networks, thus so we can re-use much, though not all, of the code for the traditional neural network, speeding up development time.

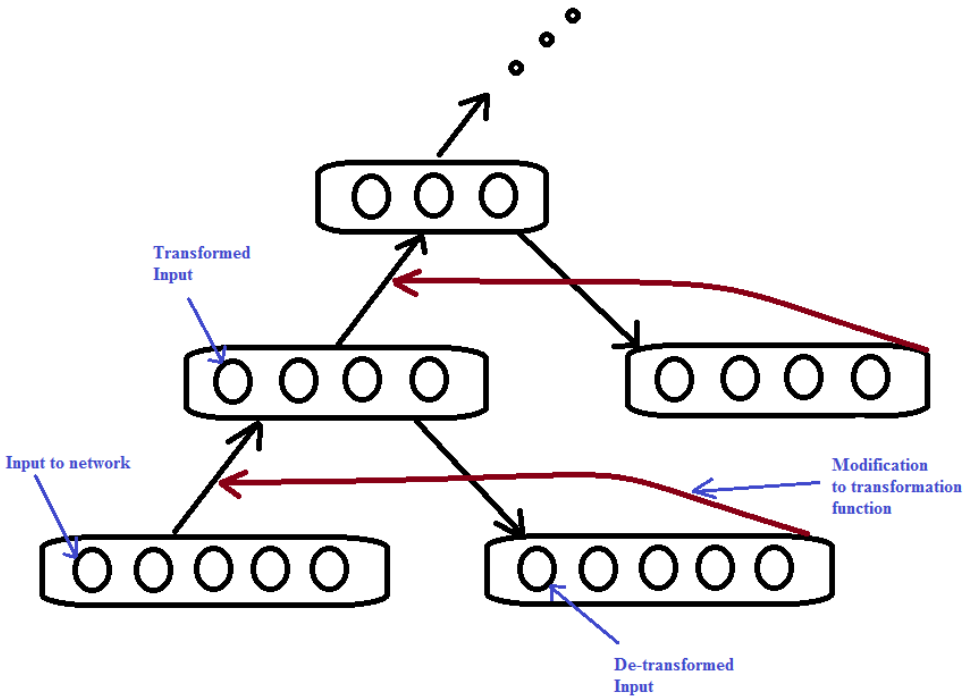

Figure 5: Example of a stacked auto-encoder. Inputs feed into a layer, and get transformed as they go into the next layer (also reducing the dimensions). Then, in order to make sure the transformation works the way we want, we try to reconstruct the original input. The difference between the original and reconstructed input is used to modify the transformation and make it better.

One of the other issues we have faced is multi-scale spatial data. For example, if there are 800 locations in a field where protein data is collected, and 14,000 locations where yield data is collected, there is no one-to-one relationship between yield and protein data collection locations.

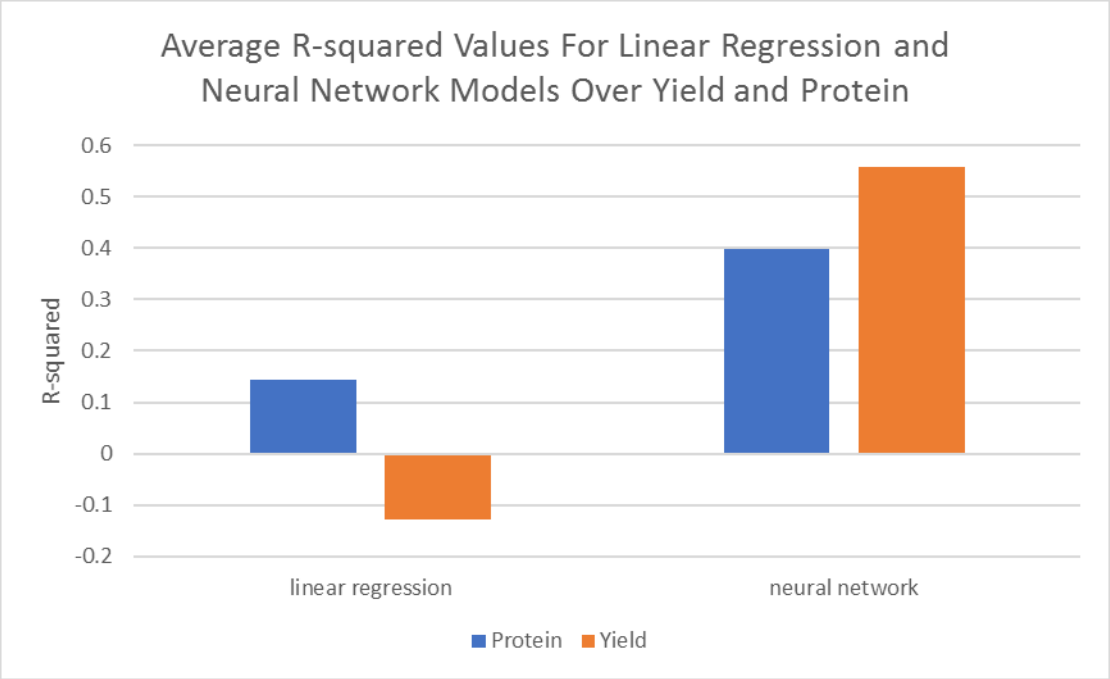

Figure 6: Average R-squared values for different network types. Protein and Yield networks take in different amounts of information and must be trained differently, although we plan to modify this in the future.

However, Dr. Sheppard and Ms. Rounds are developing a method of sampling points that will allow us to deal with this type of data. This method groups yield and protein data into cells. Then for each yield or protein point in a cell, a point in another cell is selected (there are a number of ways to select points that Dr. Sheppard and Ms. Rounds are developing) and used as one of the inputs for the original cell in either the traditional neural network or the stacked auto-encoders. This allows us to both capture information about the space surrounding a point, and allows us to relate points with different information that occur in different densities across a field. It could also allow us to increase the amount of data available for constructing and assessing networks.

Finally, using the traditional neural network developed last quarter, Ms. Rounds has run computation experiments to identify whether a neural network outperforms a linear regression strategy for yield optimization. The results show that a neural network is far more accurate at predicting yield than a linear regression approach, and usually recommended applying more nitrogen than the linear regression approach.

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 developed during this quarter have the potential to exploit spatial information in the field in order to more accurately predict yield.

In our analysis, we compared our neural network approach to a linear regression model. To compare linear regression and neural network models, we used a measure called the Coefficient of Determination (or R^2). This measure represents the amount of variation in the data that can be explained by a particular model. We selected the neural network with the best average R^2 values for protein, as protein R^2 values were universally lower than yield R^2 values. The network that we selected had an R^2 value for protein of 13.3%.

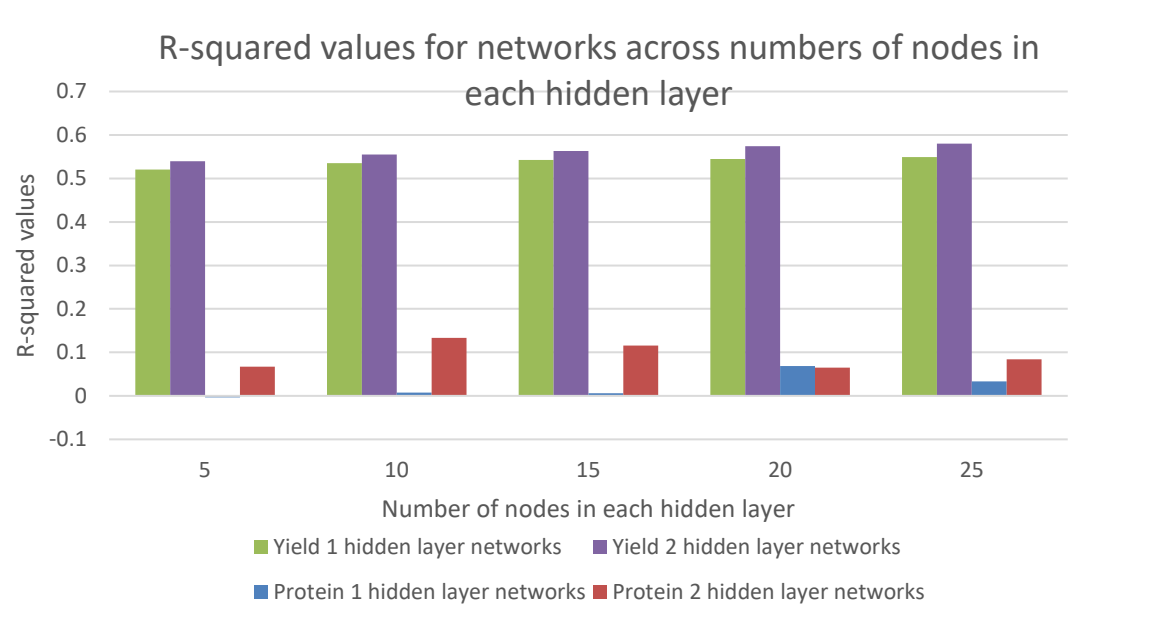

Figure 7: R-squared values for linear regression and a neural network. Negative R-squared values mean that the model explains less variation than a model that only outputs the average value (in other words, a model that fits a horizontal line).

Once we had selected a neural network model, we then compared the results to the linear regression model using a different field. Interestingly, on a different data set (different field, different farm), the R-squared values for the neural network for protein were much higher, with an average R^2 value of 39.8%. This is almost three times the initial R^2 values. The neural network always had higher R^2 values than the linear regression model. Once we had compared the prediction accuracy of each model, we also used the models to predict what would have happened if different nitrogen rates had been applied. All other variables were kept constant. We also examined the amount of nitrogen that each method recommended based on a linear representation of the expected price of winter wheat. The linear regression model always selected applying no nitrogen. The neural network often applied fairly low amounts of nitrogen in general, but over much of the field, the network recommended applying no nitrogen. Both of these models were compared to the actual average nitrogen applied this year and a constant nitrogen application rate of 45 pounds of nitrogen per acre.

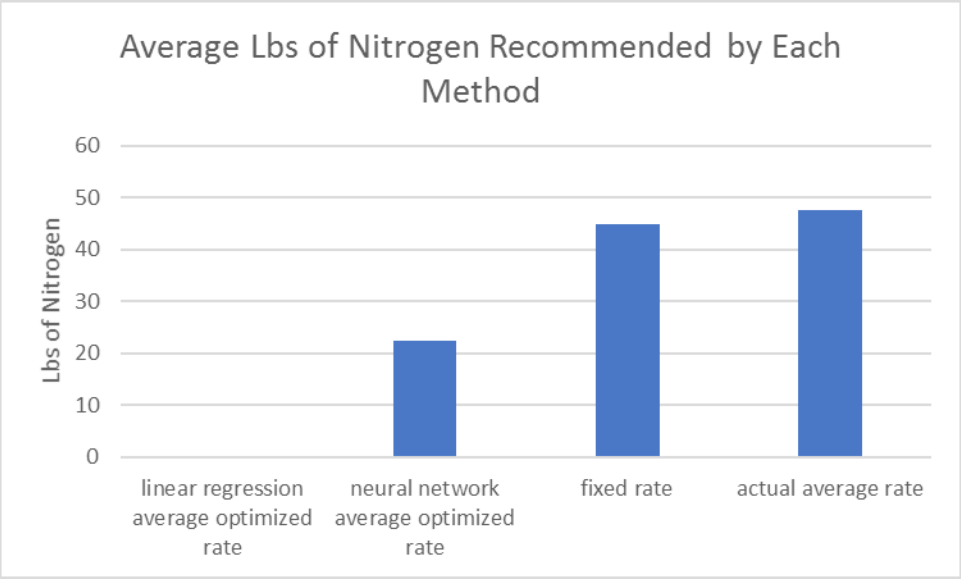

Figure 8: Average amount of nitrogen recommended by each method.

Finally, we compared the net return for the field, given a linear representation of winter wheat prices. We found that the linear regression model reported much higher average net returns than the neural network model. This is potentially due to the lower accuracy of the linear regression model, which appears to overestimate yield for a field. The neural network, although far more accurate, reported slightly lower average yield than the actual yield. The net return from the nitrogen optimizations from both the neural network model and the linear regression model was higher than the net return from the fixed rate method of nitrogen application. This means that although the linear regression model and the neural network model both misestimate the net return for a given field, both methods suggest that variable rate nitrogen application improves net return.

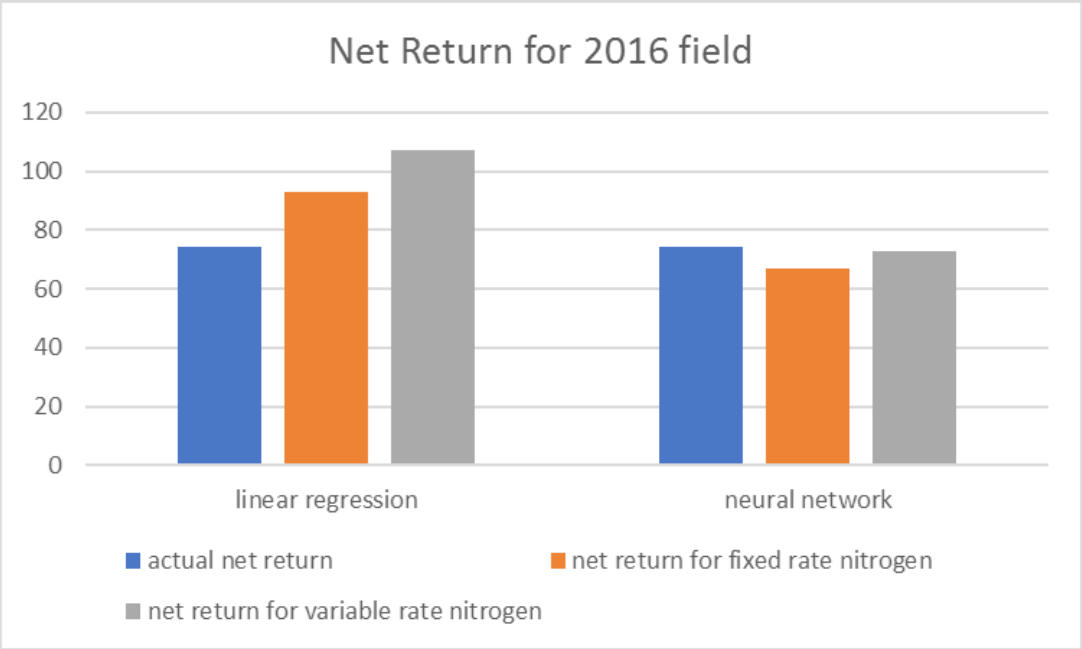

Figure 9: Average predicted net return for a field.

Hiring

- No additional hires in Quarter 6.

Expenditures

- Total Personnel Services: $41,372.75
- Total Operations: $10,845.05

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

Progress towards milestones

Payn and Izurieta are managing the team focused on design and implementation of the data management and workflow technology. The underlying software for data management has been named the Object Oriented Environmental Data System (OOEDS). The system is based on state-of-the-art "NoSQL" database technologies, and will handle transfer and storage of digital information for the data import, model calibration, experimental design, yield optimization, and application prescription phases of OFPE process.

There has been one new hire to the team managed by Payn and Izurieta during the past quarter (November-January). Undergraduate student Louis Solana was hired to document the existing technology and API to facilitate its transition at a latter point in time. Further, the documentation will have a tutorial component to aid developers of the OOEDS API. Mike Trenk continues to work as a classified employee pending his official admission into the CS MS program.

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**]

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**]

Data Schema

- Refactored references to geoJSON features from Entities to bring the way we store attribute information for those features into better alignment with the way we do it elsewhere in the data model.
- Changed where *measurementContext* information (average, maximum, minimum) is stored. Previously, *measurementContext* was an attribute of Properties. Now, it is associated with Evaluations in much the same way that *SpaceTimeContext* information is. Each Evaluation has a *measurementContext*.
- Added several Model subclasses to accommodate different data types. Subclasses now include: featureModels, timeSeriesModels, and rasterModels. The model type helps describe the logical grouping of the data into forms typically encountered in the OFPE project.
- Adding models required additional evolution and property types for fertilizer costs, soil types, NDVI data, weeds occurrence mapping data, photo points, crop sales, and farm operations costs.
- Added a *versionTimeContext* to keep track of quality assurance/quality control (QAQC) activities that result in adjustments to metadata.
- Added several Activity subclasses required to generate the new Evaluation types discussed above: *terrainAnalysis*, *weedMapping*, *nitrogenTreatment, instrumentCalibration, farmOperations, QAQC.*
- Added two new sub-classes of Agent: manufacturer and laboratory

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 work on fine-tuning configuration on MongoDB cluser
  - flask/nginx SW configuration on mredi-api server
  - security & api design & development
  - prepared & presented PI demonstration
  - currently researching web UI technologies for OFPE client use
  - working on installing and configuring SW on MREDI servers

- working on building OFPE/OOEDS API
- working on building OFPE client

- Defining JSON spec for communication between server and client tools. [**Done**]
- Implementing OOEDS Java (and JavaScript) client, getting it working with OOEDS ReST API. These are being pulled into two separate ReST Layers [**In Progress**]
- Prototyping high level query language for exporting data back out of database [**In Progress**]
- Redesigning OOEDS Rest API to be a one-to-one implementation of the data model. (All classes have a corresponding rest endpoint) [**In Progress**]
- Moving security into OFPE Layer, which acts as a proxy to the OOEDS Rest API. [**In Progress**]
- Began the development of the database agnostic OOEDS Java Library. This library is an important component for organizing our code base, and will allow for much more rapid development of software using the OOEDS data model in the future. [**New**]
- 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. <u>Workflow software products (in order of current priority):</u>

<u>3.1 Yield Editor Data Input [**In Progress**]</u>

- 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 will be demoed to the greater team [**In Progress**]
  [**Done**]
    - 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.

<u>3.2 Data rectification workflow [**Planning**]</u>

- We have recently identified a critical step in the process for preparing data for the optimization process. Data for independent variables 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.

<u>3.3 Optimization [**In Progress**]</u>

- 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

We are actively working on developing a manuscript for an environmental informatics journal (e.g. Environmental Monitoring and Software). The topic of the manuscript will be to introduce an extensive objective oriented data model suitable for storing environmental data in NoSQL (or object-oriented) databases. Our goal is to have a manuscript ready for submission by the end of Spring semester. Rob Payn, Seth Mason and Clem Izurieta are meeting on a bi-weekly basis to develop this manuscript. [**In Progress**]

Figures

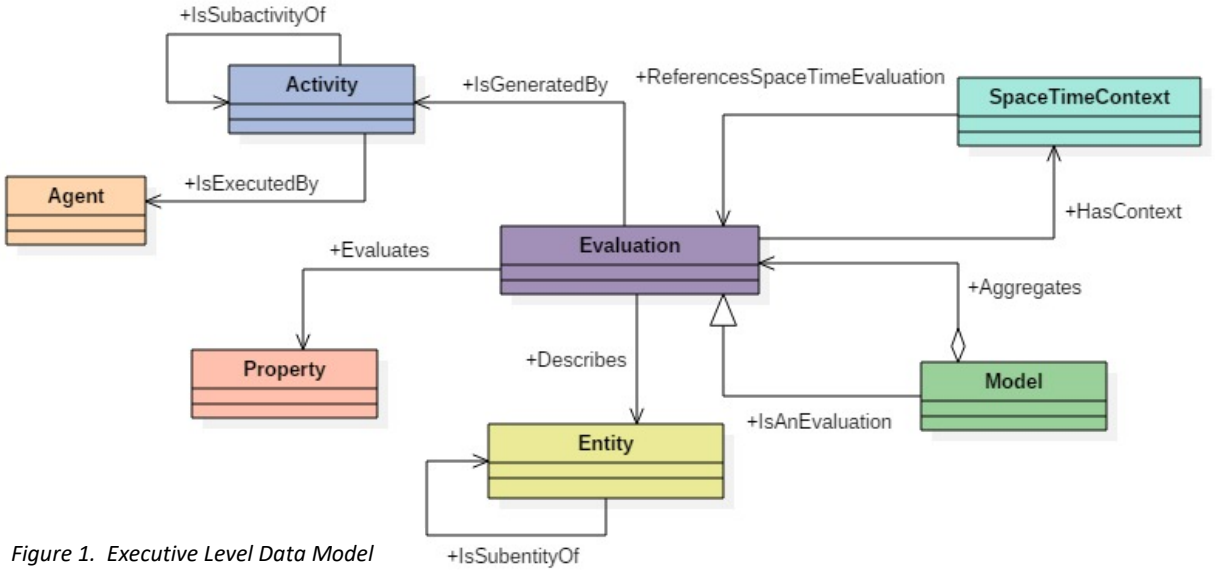

Figure 1. Executive Level Data Model

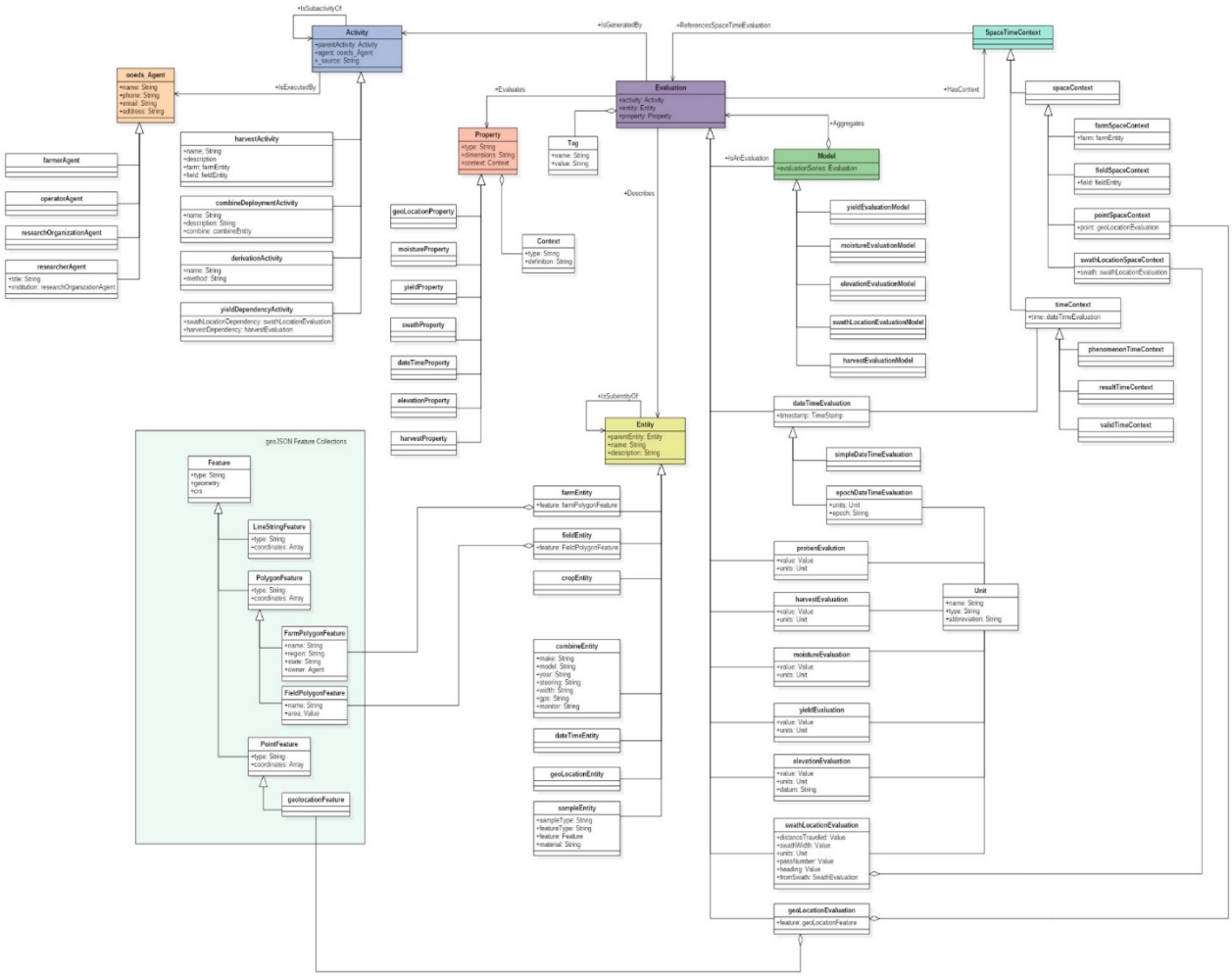

Figure2. Each component describes an entire subsystem

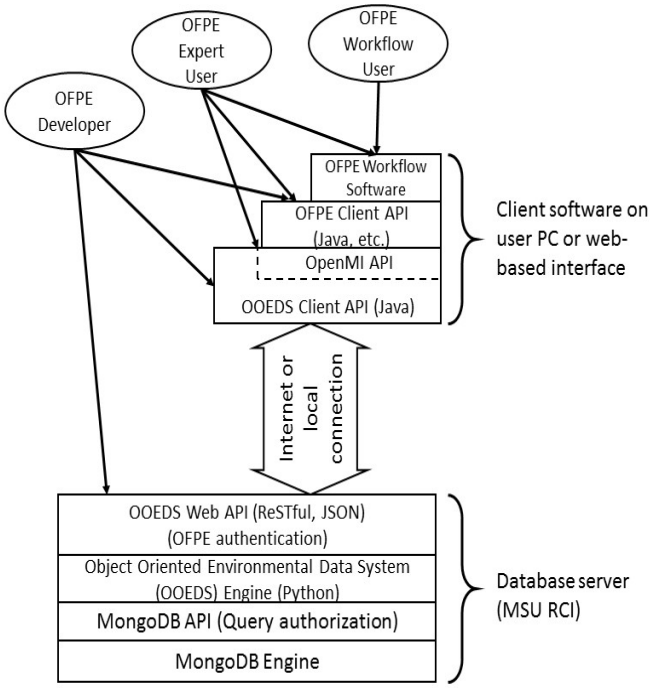

Figure 3. Software Architecture

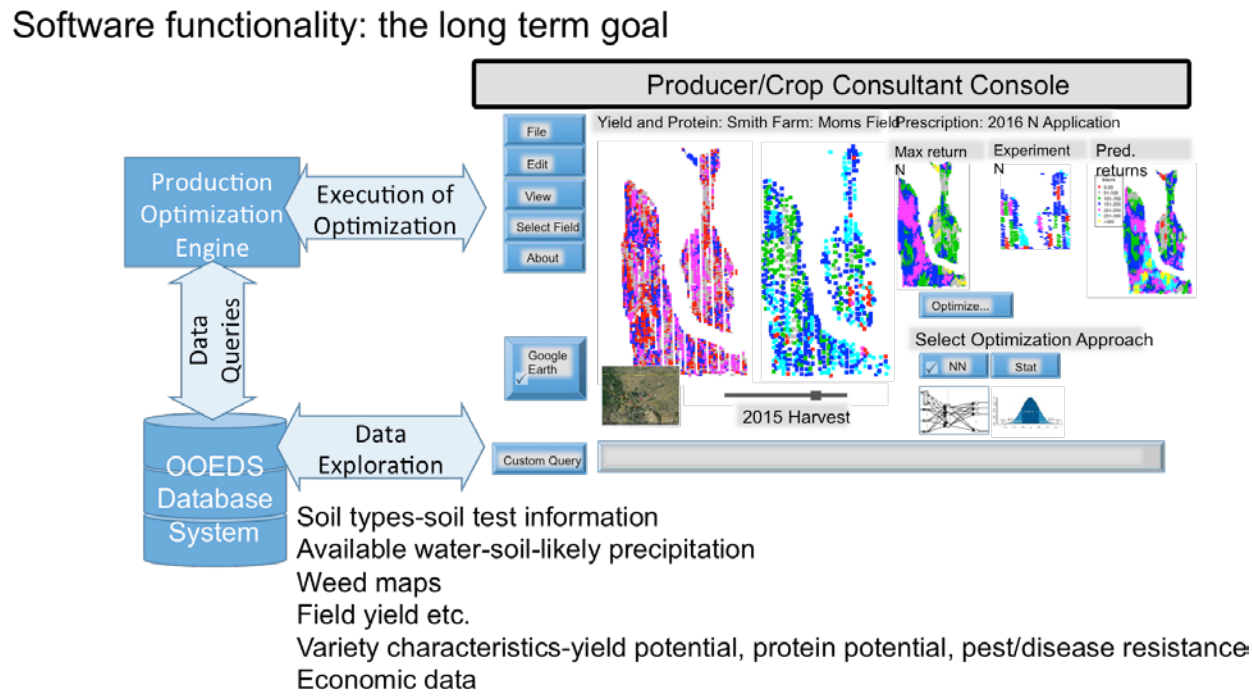

Figure 4. Example of potential GUI functionality

Hiring

- No additional hires in Quarter 6.

Expenditures

- Total Personnel: $87,952.63
- 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 continues to monitor weather data coming in from the MSU Research Centers. Adaptive Hydrology has continued to maintain and monitor weather stations at the four OFPE participant farms to determine their ability to sustain data output through winter conditions. 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 analyzing field trial data from the 2016 durum trials conducted across the state and of preliminary yield trials. The primary new breeding populations listed previously were advanced

another generation in the greenhouse and are on track to be in the field in 2017. These populations were created by crossing the top cultivars from across different durum growing regions. We are currently growing F_4 plants and one head from each plant will be harvested in spring of 2017 and threshed independently. F_5 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 single row 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 product quality testing.

Our additional breeding materials that emphasize integration of the low cadmium trait are currently growing in AZ. We will travel to AZ when they are mature and do head selections. Selected heads 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.

2016 Experimental durum breeding material evaluation and selection

As we reported in our last quarterly report, we identified a small number of durum lines from crosses with adapted and unadapted parents that individually have high yield, increased pasta firmness and the low cadmium trait. We will choose a small subset of these lines to be grown in the MT intrastate trial in 2017.

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 9 elite cultivars and 6 experimental lines developed at MSU. Yield and agronomic data was recorded for all stations and sub-samples were sent to the USDA in Fargo for quality analysis, which included all seed characteristics along with milling traits and semolina color traits.

METHODS:

Six advanced experimental durum lines and nine elite durum varieties were tested at five Montana State University Agricultural Experiment Centers, two locations maintained by Northern Seed, LLC, and the North Dakota State University Williston Research Extension Center. Rainfed experiments were grown in Havre, MT (MSU-NARC), Sidney, MT (MSU-EARC), Conrad, MT (MSU-WTARC and Northern Seed, LLC), Moccasin, MT (MSU-CARC), and Williston, ND (NDSU-WREC). Irrigated trials were grown in Bozeman, MT (MSU-Post Agronomy Farm), Churchill, MT (Northern Seed, LLC) and Sidney, MT (MSU-EARC). There were three replicates of each line/variety grown at each location, all seed was treated with CruiserMaxx Vibrance for Cereals (Syngenta) (5 fl oz/100 lb), and Mountrail was considered the check variety. The individual research centers/cooperators provided agronomic data and grain sub-samples from the three replicates per line per location were bulked and submitted to Linda Dykes (USDA-ARS, Fargo, ND) for analysis of seed traits, milling and semolina quality, and mixing strength. Overall statewide agronomic performance can be found in Table 1.

AGRONOMIC RESULTS SUMMARY:

Across all nine locations, encompassing both irrigated and rainfed trials, there was no statistical significant differences for all traits based on ANOVA. However, numerically the highest yielding line was MT112219 (67.0 bu/ac) which also had the highest test weight (60.2 lb/bu). Mountrail had the lowest yield (58.0 bu/ac) and test weight (55.5 lb/bu) (Table 1). MT112219 and MT101717 were the shortest lines (27.2 and 27.9 in, respectively) and had the lowest grain protein (both 13.8 %), while Tioga had the highest protein (15.1 %) and was the tallest (32.7 in.).

Under irrigated conditions at three locations there were no significant differences observed for any measured trait, however MT112219 numerically was the second highest yielding line (99.3 bu/ac) behind Carpio (101.9 bu/ac), had the highest test weight (61.3 lb/bu), and was the shortest (31.3 in) (Table 3). Under irrigation,

MT112434 ranked the lowest for yield (86.6 bu/ac) and test weight (59.4 lb/bu) but the highest for protein (14.2 %), while MT101717 ranked the lowest for protein (12.7 %).

Under rainfed conditions no significant differences were observed between lines tested for heading date, yield, test weight, or protein content, however MT112219 ranked the highest for yield (50.8 bu/ac) and test weight (59.6 lb/bu) but the lowest for protein (14.1 %). In 2015, MT112219 was also the top yielding line under rainfed conditions. MT112219 was the shortest line (25.2 in) and was significantly shorter than the check Mountrail (29.3 in). The lowest yielding line under rainfed conditions in 2016 was Mountrail (41.1 bu/ac) which also had the lowest test weight (53.3 lb/bu). Tioga was the tallest cultivar (30.7 in) with the highest protein (15.5 %) under rainfed condition.

QUALITY RESULTS SUMMARY:

Grain quality results supplied by the USDA-ARS showed no significant difference for test weight, individual kernel weight, grain hardness, or grain protein based off ANOVA (Table 2). Significant differences did exist for percent large kernels, percent small kernels, and kernel diameter. Overall, MT101717 and MT112219 again had the largest test weights (61.2 and 60.9 lb/bu, respectively) while Mountrail had the lowest test weight (58.7 bu/ac), though not significantly. MT101717 had the smallest individual kernel weight (37.3 mg) which resulted in it having the largest percent of small kernels (13.8%) along with Mountrail (13.8 %). Alzada had the largest individual kernel weight (44.2 mg) and kernel diameter (3.0 mm) which equated to it having the greatest percent of large kernels (77.0 %) and lowest percent of small kernels (5.1 %). MT101717 and MT112219 had the lowest grain protein (13.8 % and 14.0 % respectively) while line MT112434 had the highest grain protein content (14.9%), although not significantly.

After milling, no significant differences were detected for semolina milling yield, semolina brightness (L*), whole grain ash, semolina protein, or falling number (Table 3). Significant differences did exist for semolina yellow color (b*), mixograph pattern, and semolina ash (Table 15). MT112219 had the highest milling yield (63.7%) with its semolina having the lowest protein content (12.6%) and lowest brightness score (83.5) along with Alzada (83.5). MT101717 had the lowest whole grain (1.4 %) and semolina ash content (0.57%) and the second lowest semolina protein (12.7 %). Grenora had the lowest milling yield (61.7%), while Mountrail had the brightest (84.5) semolina and highest semolina protein (13.7%), though not significantly. Mountrail had significantly the least yellow semolina (24.9) while Joppa had the most yellow semolina (30.3). All MT lines except MT101694 had significantly more yellow (27.2-28.7) semolina than Mountrail. Mountrail had significantly the lowest mixograph pattern score reflecting weak gluten with a score of 3.0 while Alzada had the highest mixograph pattern score of 6.9. Mixograph pattern scores for all the experimental MT lines except MT101694 were significantly higher than Mountrail.

Table 1. Agronomic means from 2016 intrastate durum trials all locations (n=9) and conditions.

Line/Variety	Heading (Julian)[1]	Flowering (Julian)[2]	Plant Height (in)	Yield (bu/ac)[3]	Test Weight (lb/bu)	Protein (%)[3]
Alkabo	174.7	188.8	32.1	59.7	57.9	14.7
Alzada	172.6	188.0	28.4	62.7	58.2	14.8
Carpio	176.3	188.8	32.3	65.7	59.0	14.8
Divide	174.8	188.4	32.2	64.5	57.8	14.7
Grenora	174.5	188.0	30.8	63.7	57.1	14.8
Joppa	175.8	188.1	31.9	61.5	57.5	14.6
Mountrail	175.3	187.7	31.4	58.0	55.5	14.9
Silver	172.8	187.3	29.5	62.1	57.6	14.6
Tioga	175.2	187.3	32.7	61.2	58.4	15.1
MT101694	174.5	189.3	31.2	61.8	58.8	14.4

MT101717	173.5	188.8	27.9	65.4	60.0	13.8
MT112219	172.7	189.8	27.2	67.0	60.2	13.8
MT112434	174.4	189.7	31.3	59.9	58.1	14.9
MT112444	173.3	188.4	30.9	63.1	57.8	14.5
MT112463	173.1	188.2	28.6	62.6	57.5	14.3
Grand Mean	174.2	188.4	30.6	62.6	58.1	14.6
CV (%)	3.0	3.6	15.4	41.6	9.3	15.2
LSD (0.05)	7.1	13.8	4.3	25.5	5.2	2.1
P-value	NS	NS	NS	NS	NS	NS

[1]Data for five locations
[2]Data for two locations
[3]Reported on a 12% moisture basis
NS = No significant difference based on ANOVA p<0.05
Underline = Highest and lowest values

Table 2. USDA-ARS seed quality means from all locations for 2016 intrastate durum trial.

Line/Variety	Test Weight (lb/bu)[1]	Kernel Weight (mg)	Large Kernels (%)	Small Kernels (%)	Kernel diameter (mm)	Hardness	Protein (%)[1]
Alkabo	59.4	40.5	57.0	10.8	2.8	74.7	14.6
Alzada	59.2	44.2	77.0	5.1	3.0	76.4	14.8
Carpio	59.9	40.8	64.8	8.1	2.8	77.3	14.7
Divide	59.5	39.9	59.3	9.2	2.8	75.4	14.8
Grenora	58.9	40.0	56.9	9.0	2.8	79.1	14.6
Joppa	59.3	38.8	45.7	12.8	2.7	79.3	14.5
Mountrail	58.7	39.3	45.9	13.8	2.8	74.8	14.5
Silver	59.4	39.2	55.9	9.7	2.8	76.1	14.6
Tioga	59.5	42.6	67.1	7.4	2.9	74.4	14.8
MT101694	60.1	38.1	51.4	12.1	2.8	80.7	14.2
MT101717	61.2	37.3	47.0	13.8	2.8	82.6	13.8
MT112219	60.9	40.5	58.1	9.6	2.9	77.6	14.0
MT112434	59.3	41.6	64.1	8.3	2.9	73.8	14.9
MT112444	58.8	39.5	60.2	9.2	2.9	75.3	14.4
MT112463	58.8	38.0	64.1	9.4	2.9	76.0	14.3
Grand Mean	59.5	40.0	58.3	9.9	2.8	76.9	14.5
CV (%)	3.1	10.9	34.5	45.8	5.7	8.9	12.4
LSD (0.05)	1.7	3.9	18.1	3.8	0.2	6.3	1.7
P-value	NS	NS	0.048	<0.001	0.031	NS	NS

[1]Reported on a 12% moisture basis
NS = No significant difference based on ANOVA p<0.05
Underline = Highest and lowest values

Table 3. USDA-ARS semolina quality means from all locations for 2016 intrastate durum trial.

Line/Variety	Milling Yield (%)	Brightness (L*)	Yellowness (b*)	Mixograph pattern	Whole grain ash (%)[1]	Falling Number (sec)	Semolina protein (%)[2]	Semolina ash (%)[1]
Alkabo	62.8	84.2	28.8	4.4	1.5	422.6	13.5	0.6
Alzada	62.5	83.5	30.2	6.9	1.5	443.7	13.6	0.7

Carpio	63.1	84.1	30.3	6.8	1.5	426.3	13.6	0.6
Divide	62.9	84.5	27.0	4.7	1.5	429.9	13.5	0.6
Grenora	61.7	84.4	28.5	4.8	1.5	430.3	13.5	0.6
Joppa	62.5	84.0	30.3	6.4	1.5	422.3	13.3	0.6
Mountrail	62.4	84.5	24.9	3.0	1.5	420.3	13.7	0.6
Silver	62.9	83.9	26.5	5.4	1.5	416.6	13.5	0.6
Tioga	63.7	84.2	28.7	5.6	1.5	418.1	13.6	0.6
MT101694	61.7	83.7	26.5	4.0	1.4	412.4	13.2	0.6
MT101717	61.8	83.7	28.7	4.8	1.4	440.7	12.7	0.6
MT112219	63.7	83.5	27.2	5.4	1.5	422.3	12.6	0.7
MT112434	62.5	83.9	28.1	5.9	1.5	437.8	13.7	0.6
MT112444	61.9	83.6	28.7	6.1	1.5	442.6	13.3	0.7
MT112463	62.0	83.6	28.3	6.7	1.6	435.9	12.9	0.7
Grand Mean	62.5	84.0	28.2	5.4	1.5	428.1	13.4	0.6
CV (%)	3.5	1.1	8.1	35.1	11.1	7.5	15.5	10.5
LSD (0.05)	2.1	0.9	1.7	1.5	0.2	30.3	2.0	0.1
P-value	NS	NS	<0.001	<0.001	NS	NS	NS	0.002

[1]Reported on a 14% moisture basis

[2]Reported on a 12% moisture basis

NS = No significant difference based on ANOVA p<0.05

Underline = Highest and lowest values

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

We are currently packaging seed for the N17 spring planting yield trials which will be planted at 5 locations in Montana (Bozeman, Conrad, Ft. Benton, Havre, and Scobey). The purifications planted near Yuma in November are progressing nicely and the anticipated harvest is mid to late April. The material harvested in Yuma will be cleaned, packaged and planted near Bozeman in early May to provide pure seed which will be the starting basis for variety increase and release over the next 2 years.

Hiring

- No additional hires in Quarter 6.

Equipment

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

Expenditures

- Total Personnel: $73,680.36
- Total Operations: $34,423.20
- Total Equipment: $70,994.00

Wheat Stem Sawfly subproject of the Agriculture MREDI project

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

Progress towards milestones

Bracon cephi. Previously, we characterized the effect of sugar availability on the longevity and egg load dynamics of this species. Here we report on the effect of flowering plant species on egg size in both species. As for responses to pure carbohydrates, the two native parasitoid species responded different to flower access. In Fig. 1 (below) the more abundant species, *B. cephi*, has eggs that are not statistically different in size at 10 days for

provisioned sucrose and flax, buckwheat and pea flowers. As previously reported this species resorbs eggs to live longer, which allows for more time to locate wheat stem sawfly larvae to parasitize. In Figure 1, there were no eggs left at 10 days for parasitoids that could access flowering wheat or alfalfa. This is a bit surprising because the largest eggs are associated with peas, which may provide other sources of nutrients, but does not actually have nectar that is accessible for these small parasitoids. The fact that the eggs are exhausted in wheat and alfalfa is also unexpected. Neither would provide nectar, but these data suggest that this species must forage for carbohydrates in the field during their search for wheat stem sawfly larvae to parasitize in wheat. If they did not, they would be ecologically unsuccessful rather than being the more commonly encountered of the two species in wheat fields.

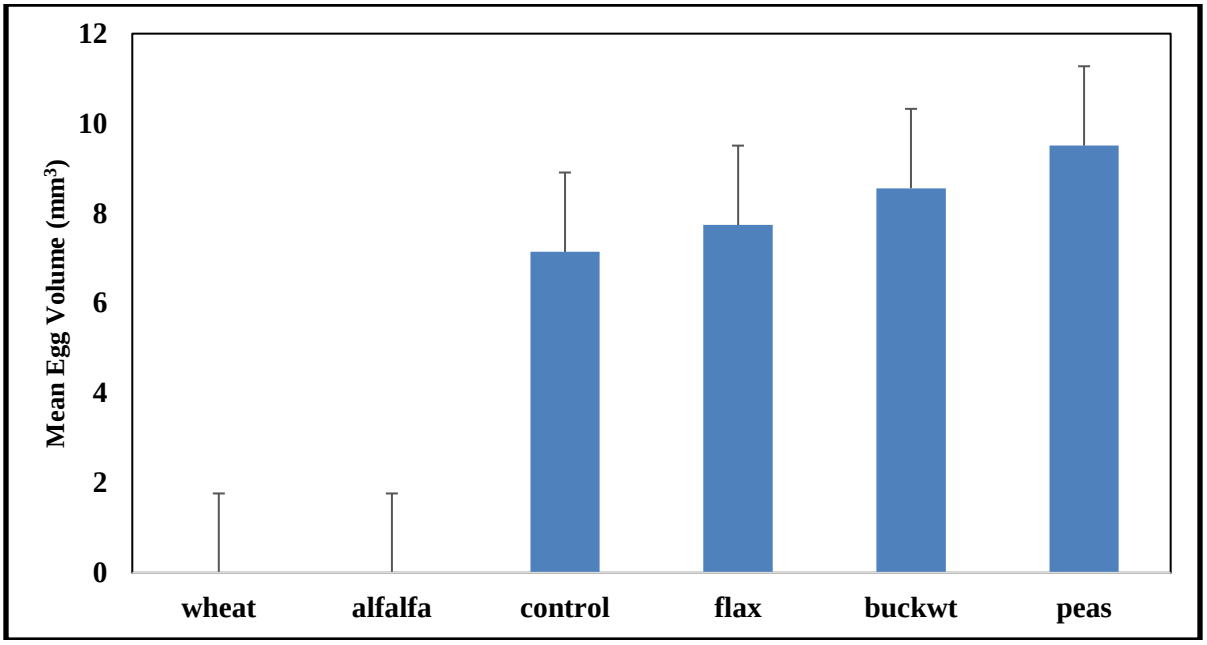

Figure 1. Mean egg size (volume) at 10 days for Bracon cephi. All plant species were presented at the flowering stage. The control is a 1 molar sucrose solution provisioned daily.

Bracon lissogaster. Previously, we also characterized the effect of sugar availability on the longevity and egg load dynamics of this species. Adding to the remarkable contrast to *B. cephi,* 10 day old females of *B. lissogaster* had the largest eggs when feeding on the control 1 molar sucrose, access to flowering alfalfa and flax produced slightly smaller individuals, while access to flowering wheat, peas and buckwheat actually produce the smallest eggs. Although these sizes are different, there is less than 1mm^3 difference between the mean values for egg size for the control and the mean values for buckwheat (Fig. 2). The large egg for the control appear smaller than the large eggs from peas for *B. cephi*.

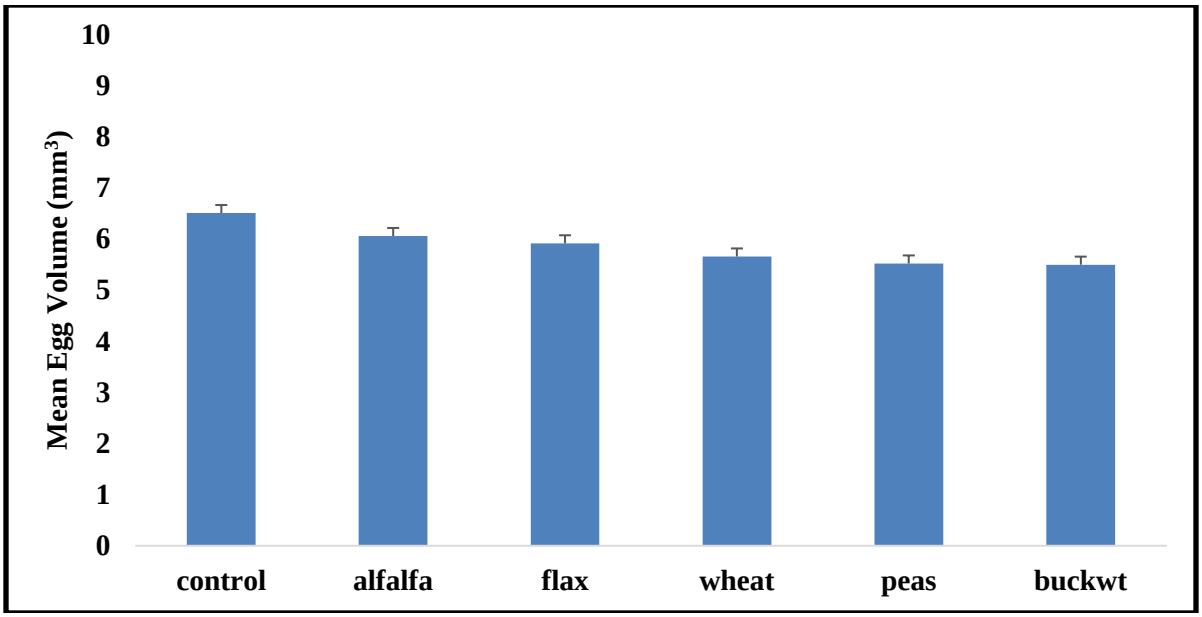

Figure 2. Mean egg size (volume) at 10 days for Bracon lissogaster. All plant species were presented at the flowering stage. The control is a 1 molar sucrose solution provisioned daily.

It is apparent that there is some greater metabolic stability provided by body reserves in the egg dynamics of the rarer *B. lissogaster*. There is no exhaustion of eggs on wheat or alfalfa like for *B. cephi.*

Field data. From the field, have completed our assessment of weight of both the wheat stem sawfly and overwintering parasitoids from samples 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. Postharvest wheat stubble was dissected to determine the presence of wheat stem sawfly and then the overwintering structures of both the pest wheat stem sawflies and the two parasitoid species were also dissected and weighed. The overwintering stages of the hosts and thus, the parasitoids are constrained by the food availability within the developing wheat stem. As stated previously, the more robust (and higher yielding) wheat stems will produce larger wheat stem sawfly larvae which should, in turn, produce larger parasitoids.

The weight of the overwintering stage of the wheat stem sawflies varied across location and also across adjacent field type, either cover crop, pulse or fallow (Figure 3). There were a small number of very large overwintering stage wheat stem sawflies adjacent to pulse at one location but weights of overwintering wheat stem sawflies for other pulse locations or other types of adjacent fields were variable and showed no consistent pattern. The weight of the overwintering stage of the braconid parasitoids also varied across location and across adjacent field type, either cover crop, pulse or fallow (Figure 4).

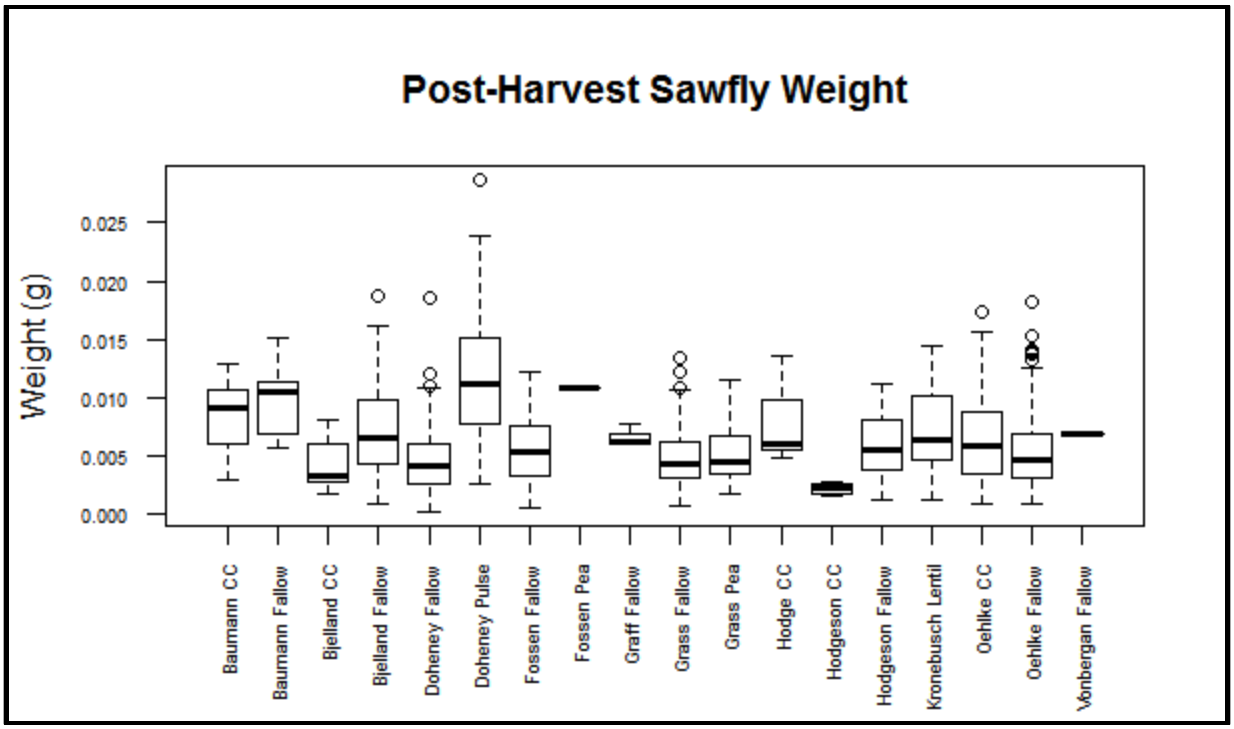

Figure 3. Box plots of weights of the wheat stem sawfly overwintering stage for all locations.

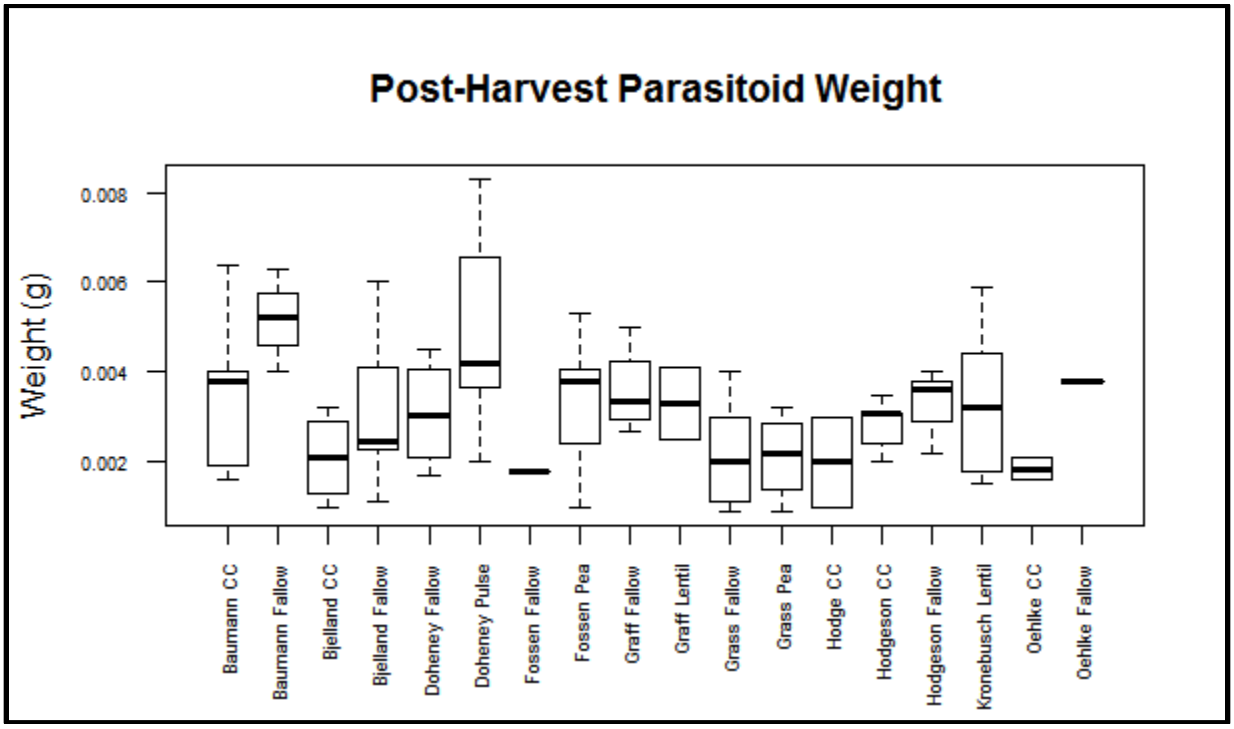

Figure 4. Box plots of weights of the overwintering stages of the parasitoid for all locations.

These same dissections also provided data on wheat stem sawfly and parasitoid numbers. There were seven out of 11 fields where wheat stem sawfly infestation was greater than a mean of 15%, with the greatest infestation of approximately 60%. In five of these seven fields wheat stem sawfly infestation was greater adjacent to the fallow fields. In the other two, infestation was greater than adjacent to fallow for one cover crop field and one field of pea. Of these locations mean parasitism was greater at 4 locations which were characterized by noticeable differences, and by as much as means of 17% adjacent to flowering pea versus 0.5% adjacent to the paired fallow field or 6% adjacent to a cover crop versus 0.8% adjacent to the paired fallow field. In the other

pairings, mean parasitism was too low to make meaningful comparisons. This means that infestation and losses due to wheat stem sawfly can also be quite high in some locations, despite the presence of the flowering crop. This suggests that the populations of parasitoids could increase in these areas, but it also indicates that planting flowering crops does not guarantee that parasitoids will impact wheat stem sawfly numbers, nor are wheat stem sawfly populations reduced by simply breaking the wheat monoculture by incorporating rotations or cover crops.

In late March, the fields will be sampled again to collect stubble for dissection to assess changes in overall mortality and size for both the wheat stem sawflies and the parasitoids. Parasitoids overwinter above the soil surface in the standing residue and invest significant body reserves in production of anti-freeze proteins and glycerol. The wheat stem sawfly is associated with the crown of the wheat stubble and does not produce as much cryoprotectant material. We expect changes in mean weight before metamorphosis to be quite informative.

Hiring

- No additional hires in Quarter 6.

Expenditures

- Total Personnel: $17,294.38
- Total Operations: None to date

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

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

Progress towards milestones

1. Weed Control in Pulse Crops

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

2. Light Activated Sensor Controlled (LASC) WeedSeeker Sprayer for Precision Weed Control

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

Figure 1: WeedSeeker sprayer with 16 LASC units for precision weed control in chemical fallow (2016).

Based on our field evaluations (2015-2016) of LASC technology (using a 16 feet ATV-mounted sprayer with 16 sensor units) in no-till fallow and post-harvest wheat stubble, weed control efficacy with LASC sprayer was consistent with the conventional broadcast sprayer. LASC sprayer reduced the herbicide (plus adjuvant) usage by up to 70% of the amounts used with a conventional broadcast sprayer. The herbicide savings were mainly due to savings in the spray volume using LASC sprayer vs. broadcast application. Based on results from the field research in 2015-2016, use of LASC sprayer reduced herbicide costs per acre by up to 70% compared with the conventional broadcast application for the herbicide programs tested in chemical-fallow/wheat stubble. This technology has proven accuracy in weed detection/sensitivity and spot spray (weed heights from 1 to 6 inches) at operating speeds of 10-12 mph.

I continue to collaborate with Dr. Joe Shaw (MSU Optics, Department of Electrical Engineering) on the hyperspectral imaging (MREDI subproject) to detect herbicide-resistant weeds in-crop (report presented by Dr. Joe Shaw).

Educational Activities

- Presentation on hyperspectral imaging to detect herbicide-resistant weeds in-crop. Weed Science Society of America Annual Meeting, February 6-9, 2017.
- Presentation on advanced optical sensor-based hyperspectral imaging and spot spray technologies for precision weed control. Malt Barley and Sugar beet Symposium, Billings, MT, January 10-11, 2017.
- Presentation on mitigating herbicide carryover and introducing new weed control options in pulse crops in Montana. CHS Grower Meeting, Malta, MT, January 9, 2017.
- Presentation on precision weed control technologies in Montana agriculture. MSU-Extension Crop and Pest Management Convention, Bozeman, MT, January 3, 2017.
- Presentation and demonstration on precision weed control technologies, MSU-SARC, Field Day, Huntley, MT, June 28, 2016.
- Presentation on weed control options for herbicide resistance management in pulse crops in eastern MT, MSU Eastern Agricultural Research Center Field Day, Sidney MT, June 24, 2016.
- Presentation on fall-applied soil residual herbicides in wheat stubble and rotational crop safety and weed control in pulse crops, Northern Agricultural Research Center Field Day DRC-NARC, Havre, MT, June 22, 2016.
- Presentation on management of glyphosate-resistant weeds in wheat-pulse rotation, Divide County Crop Improvement Meeting, Crosby, ND, December, 2016.

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

Hiring
The following people continue to work on this project:

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

Equipment

- The purchase of a growth chamber is under process and will be completed by the end of this month.

Expenditures

- Total Personnel: $46,156.76
- 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 6, we successfully addressed milestone #4, to submit a proposal with an industry partner for technology commercialization. We now have formal approval to proceed with NWB Sensors, Inc., a Bozeman company, for a study of commercialization paths for the hyperspectral weed-discrimination study reported here.
Hyperspectral weed imaging
Development on a machine-learning classification algorithm to distinguish between dicamba-resistant and susceptible Kochia is progressing. The resistant Kochia strains have been identified in Montana fields and pose a significant and potentially expensive problem for Montana farmers. The current tests are based on hyperspectral data of different strains of weeds placed amid crops during lighting conditions ranging from direct sunlight to overcast diffuse light.

To implement machine learning, different spectral features and combinations of these spectral features are being tested to map the locations of different strains of Kochia. Currently, the parameters include the NDVI of each pixel, nine selected wavelengths from previous greenhouse studies and parameters to fits of specific features of the spectra.

Figure 1 shows a sample spectrum of barley with a skewed Gaussian fit applied between 510 and 660 nm and an arctangent fit applied between 660 and 790 nm. Figure 2 shows the gradient of the same spectrum, a skewed Gaussian fit between 475 and 565 nm, a skewed Gaussian fit between 580 and 655 nm and a Voigt profile fit between 665 and 785 nm. In each case, parameters such as height, width and center of the fit (in nm) are used as parameters for the machine-learning classifiers. The different fits and spectral ranges were chosen based on a hyper-parameter search to minimize the chi-square values over distinct features in the spectra.

Lastly, we have begun exploring principal component analysis to reduce each spectra from 240 dimensions to 6 to examine information useful for separating the Kochia strains, which may be hidden in the dimensionality of the data.

Commercialization and Dissemination

The principal investigator of this subproject gave an invited presentation at the annual meeting of the Montana Grain Growers Association at Great Falls, Montana, in early December 2016. This presentation was extremely well received and generated significant discussion that provided useful insight into how growers would like to use the technology. Just before this presentation, a disclosure of the weed-discrimination technology was submitted to the Montana State University Technology Licensing Office and submitted by that office as a provisional patent application involving co-inventors from the MSU Colleges of Engineering and Agriculture. Related to this step, formal approval was gained to proceed with a commercialization study with NWB Sensors, Inc., a Bozeman, Montana company. Efforts are underway to launch that study during the upcoming quarter.

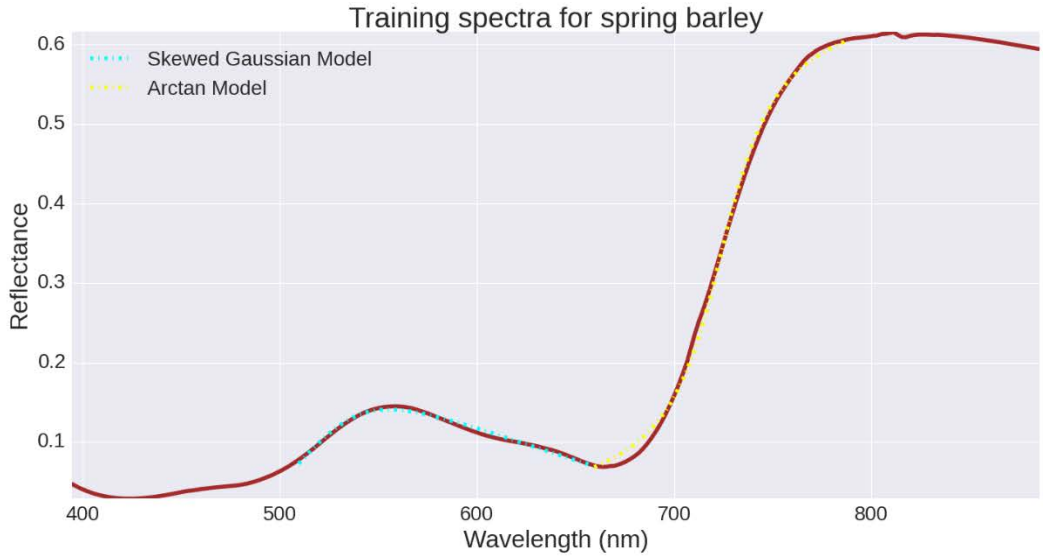

Figure 1. Barley reflectance spectrum with skewed Gaussian fit for 510-660 nm and arctangent fit for 660-790 nm.

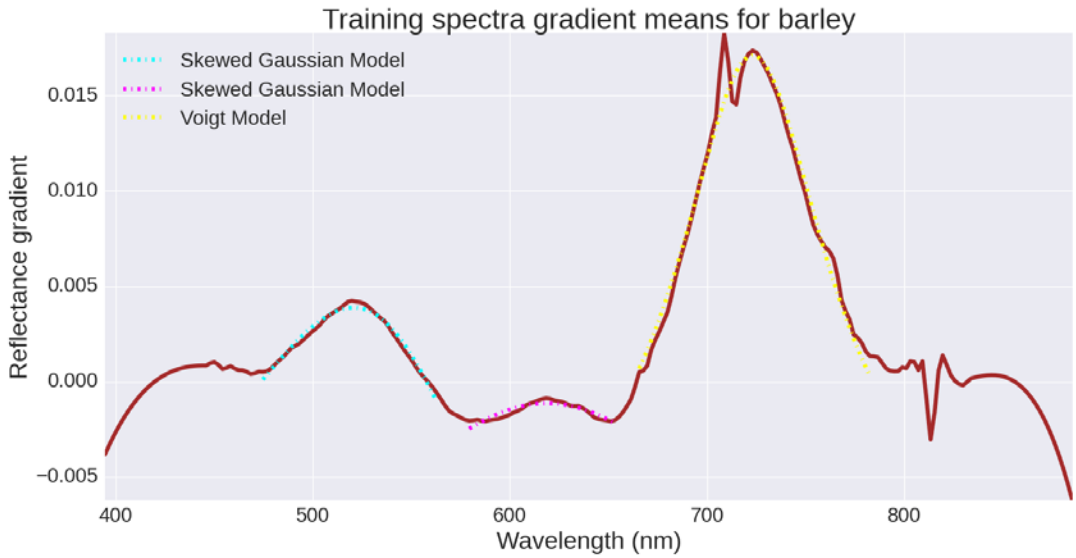

Figure 2. Gradient of the barley spectrum with a skewed Gaussian fit for 475-565 nm, a skewed Gaussian fit for 580-655 nm and a Voigt profile fit for 665-785 nm.

<u>Hiring</u>

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

- Dr. Joseph Shaw: subproject director (receiving partial summer salary)

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

Equipment Procurement

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

Expenditures

- Total Personnel: $25,869.69
- Total Operations: $9,718.34
- Total Equipment: $16,716.00

Film Production for the Agriculture MREDI Grant
41W218 – Organizer: Eric Hyyppa; Email: eric_hyppa@montanapbs.org

Progress towards milestones

Montana PBS filmed additional interviews and pulse crop seedlings/fields in December. They began editing and color correcting the footage and developed a short video that can be viewed at https://youtu.be/7kvXqS8YiHo.

Equipment Procurement

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

Expenditures

- Total Personnel: $6,617.87
- Total Operations: $7,283.95
- Total Equipment: $7,999.00

Economic analysis subproject of the Agriculture MREDI project
41W219 – Principal Investigator: Anton Bekkerman; Email: anton.bekkerman@montana.edu

Progress towards milestones

None to report in Quarter 6.

Hiring

- No additional hires in Quarter 6.

Expenditures

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

Participatory research network subproject of the Agriculture MREDI project
1) 41W224 – Principal Investigator: George Haynes; Email: haynes@montana.edu

Progress towards milestones

Dr. Haynes and his cohorts are currently scheduling interviews with collaborators for early March.

Hiring

- No additional hires in Quarter 6.

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

The past quarter has largely been devoted to analysis of the interview data we collected with 37 producers from throughout the state. We have hired an undergraduate research assistant to help with data management. She is also being trained in qualitative data analysis.

While findings are still very preliminary, it is clear that those interviewed for this project hold a mix of positive and negative attitudes towards MSU research and technology development. Negative attitudes are clustered around the perceptions that MSU faculty and research are *disconnected* from the practical needs of producers, *not locally applicable* to producers' immediate climate, and that proposed technologies and techniques do *not adequately address real-world concerns*.

While participants were critical of MSU, data also indicated that the university enjoys a strong overall reputation. Participants expressed overwhelmingly positive attitudes towards the university as a whole and towards specific faculty members who were praised for their accessibility and willingness to help producers address concerns.

As discussed in our presentation during the *Celebrate Agriculture* conference, hosted by the Department of Agricultural Economics and Economics, our data suggest a more collaborative approach to technology development would increase stakeholder interest in adoption.

Hiring

- Greer Wagner, undergraduate research assistant

Expenditures

- Total Personnel: $249.42
- Total Operations: $7,429.53