Agriculture MREDI Grant
Quarter 3 Report
May 20, 2016

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

Progress towards milestones

This report will show substantial progress on objectives by all working groups. All field research dealing with pulse crops, cover crops and the On Farm Precision Agriculture Experiments (OFPE) are planted and data is being to be taken. Reports from Sheppard and Izurieta on data management and artificial intelligence demonstrate new technologies being brought into agriculture by this project. Information on this project and other MREDI components will be presented at eight MAES Field Days during June and July. Dates and locations are in Table 1 below. Field Days will also be scheduled for the four OFPE sites. In addition to addressing the objectives in the grant, the work by Drs. Jha and Shaw on detection of herbicide resistant weeds is a spin-off from the original objectives and promises to deliver an unexpected technology. Soil microbiology work by the Peters lab in conjunction with MAES Research Center personnel is providing new ecological information that will be critical to advancements in sustainable farming systems. All budgetary items are on target.

Table 1. MAES Field Day schedule

MAES Field Days	
CARC - Moccasin	June 21: 9:00 am
NARC - Havre	June 22: 4:00 pm
WTARC - Conrad	June 23: 8:00 am
SARC - Huntley	June 28: 9:00 am
EARC - Sidney	June 30: 9:00 am
Post Farm - Bozeman	July 7: 8:00 am
Hort Farm - Bozeman	July 7: 6:00 pm
NWARC - Creston	July 12: 2:00 pm
WARC - Corvallis	July 28: 4:00 pm

Hiring

- No additional hires in Quarter 3.

Expenditures

- Total Personnel Services: $48,919.88
- Total Operations: $1705.71

Pulse Crop Research subproject of the Agriculture MREDI Grant
41W211 – Principal Investigator: Chengci Chen; Email: cchen@montana.edu

Progress towards milestones

- Pea varieties were packaged and shipped to seven research centers and Bozeman and planted.
- Initial soil samples have been taken and shipped to the Microbiology Lab in Bozeman.
- Initial soil samples were taken to measure soil water contents and fertility.
- Cool season cover crop trial has been planted.

- Pea, lentil, and chickpea herbicide trials have been planted.

Hiring

- Dr. Maninder Walia started on April 21, 2016 to assist with the nitrogen fixation study.

Expenditures

- Total Personnel Services: None to date
- Total Operations: $2073.06

Soil Microbiology and Pea Protein subproject of the Agriculture MREDI Grant

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

Progress towards milestones

Project Scope and Objective

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

Currently little scientific information is available relating environment (e.g. soils and climate) and management (e.g. nutrient rates, inoculation, seeding date, etc.) to yellow pea protein. The **project objective** therefore is to identify how *management* is affecting yellow pea protein across Montana's diverse growing environments.

Methodology

The project requires participation from Montana yellow pea producers to both identify *management practices* and supply yellow pea samples for protein analysis. Hence the initial steps are to: a) identify potential sample streams, b) establish producer contact, c) obtain *field-specific* (to identify soil and weather inputs) yellow pea samples grown on Montana farms, and d) identify *management practices* for yellow pea across Montana.

Subsequent steps are to: e) conduct both combustion and NIR analysis on yellow pea samples to calibrate the NIR instruments located at the MSU campus and the Northern, Southern, and Eastern Agricultural research centers, and f) statistically analyze how standard management (M) practices and the Montana growing environment (E) affect crude protein content in yellow pea.

Progress regarding steps a – f are summarized below

Progress

Identifying Sample Stream and Establishing Producer Contact—steps a and b

We have followed various leads in hopes of efficiently sourcing pea samples from commercial sources or other labs who are already collecting pea samples for other purposes. Producer contact is established via flyers, direct producer contact, radio/web announcements, extension agents, industry, and presentations at state and regional pulse grower meetings to 'get the word out' on this project. These outreach efforts emphasize the potential for protein as a general marketing advantage and encourage yellow pea producers to submit samples from their farms over past and forthcoming (2016) growing seasons. Below is a list of the various and upcoming outreach efforts.

1. Flyers
    a. *Yellow Pea Protein Flyer* (Figure. 1)

--- PEA GROWERS, WE NEED YOUR HELP WITH A STATE-FUNDED PEA PROTEIN STUDY ---

What do we need? 1-lb (minimum) samples of yellow pea that we can pin to a field on a soils map, specific year it was grown, and your phone number or email so that we can follow up with a very short survey on pea management factors.

<u>More detail below and contact below:</u>
Market demand for yellow pea protein is rising and is helping to boost Montana/North Dakota yellow pea prices. Very little is known about how pea protein is influenced. How does agronomic management affect protein levels? Further, given the diversity in soils and climate across the state, evaluating how environment (e.g. soils and climate) interacts with management will be critical in raising protein.

Potential management factors that could impact pea protein include:

- Seeding date
- Nutrient Management (e.g. inoculant, N&P fertilizer rates)
- Rotation History (how many times peas or lentils grown in THAT field)
- Crop Interference Factors (weed, disease, hail, other)
- Variety

As a first step to improve local management for enhanced pea protein**,** the Montana Research and Economic Development Initiative (MREDI) has provided support to measure protein in **yellow** pea samples grown on Montana/North Dakota farms in past years and following harvest of 2016. We need field-representative 1-lb samples. These samples can be submitted to Michael Bestwick or Perry Miller by following the instructions on the attached sheet. Participants will be asked to fill out and submit a short survey regarding management factors (e.g. seeding dates, fertilizer etc.) and field location from where pea samples were collected. **We encourage participants to submit samples from multiple fields.**

By participating you will have your pea samples analyzed **free of charge**. Plus you will have more information on how different field locations, growing seasons, and potentially management has affected protein on your farm. Your contribution will further help identify how management and environment influences pea protein on a larger (Montana/Dakota) scale, and with more participants, stronger conclusions can be made. Producer engagement is therefore critical. Thanks for helping out!!

Any questions or concerns regarding project details, sample submission, or survey can be directed to:

Michael Bestwick
Research Associate
Phone: 406-249-1556
Email: Michael.bestwick@msu.montana.edu

Perry Miller
Professor – Sustainable Cropping Systems
406-994-5431
pmiller@montana.edu

Instruction for submitting yellow pea samples.

1. Fill a 1-quart Ziploc bag approximately 3/4 full with yellow pea seed.
2. Fill out the attached 'YELLOW PEA PROTEIN SURVEY'.
3. Place the 'YELLOW PEA PROTEIN SURVEY' (attached document) and seed samples in an appropriate sized box (minding that samples will not spill), and make out address to:
   ATTN: Michael Bestwick, MSU-LRES, 334 Leon Johnson Hall, Bozeman, MT 59717
4. ***If submitting samples from multiple fields (WE ENCOURAGE THIS), you will need to fill out the 'YELLOW PEA PROTEIN SURVEY' for each field. In this case, please make it clear which survey corresponds to its respective sample. We recommend placing each version of the survey within each Ziploc bag of the sample it pertains to, so we don't confuse them.

***We can cover shipping fees if you send the package with FED-EX Ground. Please contact Michael prior to sending peas, and he will provide you with details on obtaining a pre-paid shipping label ***

Figure 1. Sample Yellow Pea Protein Flyer

2. Direct Producer Contact
   a. More than 100 yellow pea producers have been contacted via phone and e-mail.
3. Radio/Web announcements
   a. Project background and sample submission details made available on project website at www.peaproteinproject.com
   b. Study announced on the Shelby Radio Minute—Monday January 18th, 2016

4. Extensions Agents
    a. Extension agents in counties with reported yellow pea acreage> 20,000 are promoting the study and distributing Yellow Pea Protein Flyer to producers in their counties.
    b. Specific Extension agents and respective counties are:
        i. *Bobbie Roos—Daniels County*
        ii. *Bruce Smith—Dawson County*
        iii. *Ken Nelson—McCone County*
        iv. *Marko Manoukian—Phillips County*
        v. *Jeff Chilson—Sheridan County*
        vi. *Colleen Buck—Sheridan County*
        vii. *Shelley Mills—Valley County*
5. Industry
    a. AGT Foods, Timeless Seeds, Pro-Coop, CG Ag Consulting, and Dry Fork Ag are encouraging their producer clientele to participate in the study.
6. Presentations at pulse grower meetings
    a. Montana Pulse Day—Dec 9th, 2015 Great Falls, MT
    b. Northern Pulse Grower Association Annual Trade Show and Convention—Jan 25th, 2016 Minot, ND
    c. MonDak Pulse Day—Feb 16th, 2016, Wolf Point, MT
7. Forthcoming Presentations
    a. 2016 MAES field days:
        i. CARC—Jun 21st, 2016, Moccasin, MT
        ii. NARC—Jun 22nd , 2016, Havre, MT
        iii. WTARC—Jun 23rd , 2016, Conrad, MT
        iv. SARC—Jun 28th, 2016, Huntley, MT
    b. MonDak Pulse Plot Tour—July 6th, 2016, Richland, MT

Sample Acquisition and Identifying Standard Management—steps c and d.
To date, 81 field samples from 48 independent farms over the 2013-2015 growing seasons have been acquired. Participating producers are asked to complete a survey regarding management of their peas when submitting their samples (attachment 2). This covers field legal location, seeding dates, nutrient management, and rotation history. Based on survey results, management variables that are consistent across farms are as follows:
1. Yellow pea is seeded in April
2. Yellow pea is grown with conventional fertilizer and pesticide inputs
3. Yellow pea is grown on no-till ground
4. Yellow peas are inoculated prior to seeding
5. Yellow peas are seeded following a cereal—generally spring wheat, winter wheat, or durum.

Management variables that vary across farms are as follows:
1. Variety selection varies both within and across farms and is biased regionally by location of seed suppliers. The three predominant varieties are CDC Meadow, CDC Treasure, and Delta. The CDC varieties tend to be associated with northeastern Montana, while Delta is associated with the north central Montana. These varieties constitute 27, 27, and 12 % of field samples respectively, while the remaining 34% are comprised of AC Agassiz, Montech 4152 and 4193, Mellow, Salamanca, Trapeze, Korando, Bridger, Spider, and Nette (n=81 total samples).
2. Forty-six percent of farms use granular inoculant and the remaining 54 % use peat-based inoculant (n=48 separate farms). Two farms have reported using liquid inoculant, and one farm did not use inoculant.
3. Nutrient management varies across farms (n=48 independent farms). Forty-eight percent of farms do not use starter fertilizer. Of the 52% of farms that ***do*** apply starter fertilizer (n=25 separate farms), the proportion that apply N-P-K and S are as follows.
    a. N: 84 %--Reported rates vary from 2 to 11 lbs ac^{-1}
    b. P_2O_5: 100 %--Reported rates vary from 15 to 52 lbs ac^{-1}
    c. K_2O: 20 %--Reported rates vary from 5-10 lbs ac^{-1}, and two producers have reported top dressing with *Sure K* at flowering.
    d. S: 64 %--Reported rates vary from 3-8 lbs ac^{-1}

Because variety selection, inoculation, and nutrient management represents the most variable management factors across farms, statistical analysis addressing management by environment (M x E) interactions (*see statistical analysis—step f.*) is focused on these management factors.

Combustion and NIR Analysis—step e.

The aforementioned field samples (n=81) have been analyzed for protein content using combustion analysis (LECO analyzer). The specific method measures percent grain nitrogen (N) on a dry weight basis, and a multiplier of 6.25 is used to convert grain N to protein percentage. Each field sample has been run in duplicate (i.e. two separate subsamples) to account for within sample variation. The mean protein content and standard deviation for field samples are 23.4 and 1.95 % respectively, but more importantly, the mean range and standard deviation in subsampled duplicate pairs are -0.12 % and 1.49 % respectively (Fig 2.).

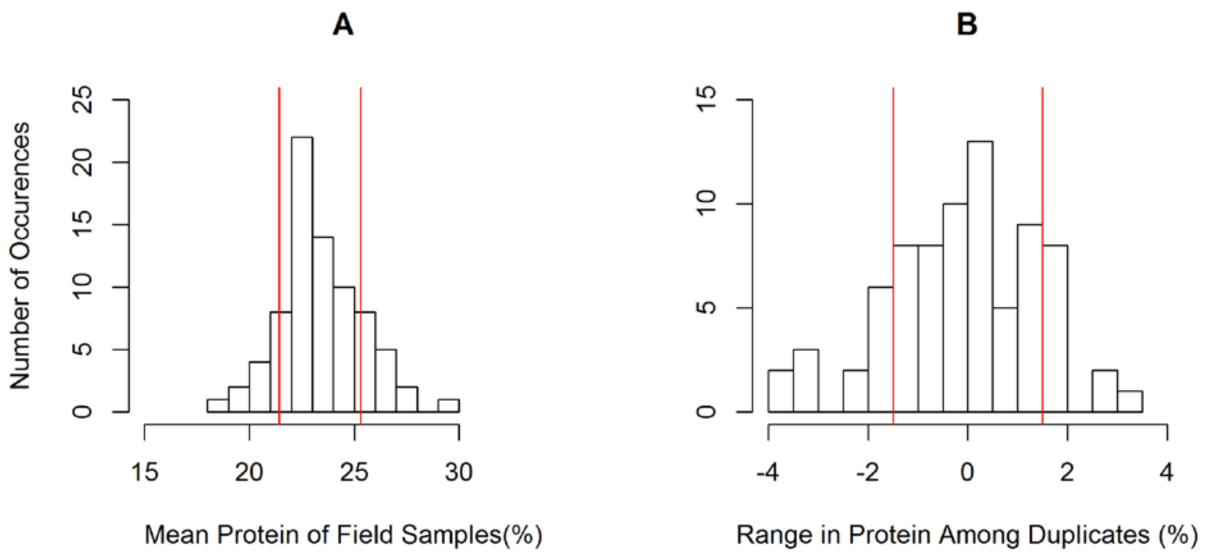


Fig 2. Distributions of mean yellow pea protein content of analyzed field samples (A) and range in subsampled duplicates from field samples (B). Vertical lines are ± 1 SD on the m

The high standard deviation (~1.49 %) in mean range among duplicates suggests that there is potential for high within sample variation. Within sample variation likely arises from both inter and intra-plant variation. All-Khan and Youngs (1973) observed protein could range to ~ 9% among plants, and Atta et. al. (2004) showed that intra-plant protein could range by ~10% depending on nodal position and variety. Such variability needs to be addressed for two reasons:

1. Mean protein responses produced from combustion analysis are used as points for NIR calibration curves. With potential for high variability among calibration points, NIR calibration will not be precise. If NIR becomes the standard for measuring yellow pea protein and awarding premiums on an industrial scale, it will be critical to ensure precise NIR calibration so that premiums are awarded fairly.

2. Large measurement uncertainty in protein response will reduce statistical power for identifying how M x E interactions affect protein content in pea.

Refinements to sampling technique are being conducted to reduce within sample variation. These refinements will ensure better NIR calibration and reduce error uncertainty when analyzing M x E interactions on protein content.

Statistical Analysis to Identify if M x E Interactions Affect Protein Content—step f.

Moisture is the limiting constraint on crop productivity in Montana, but it is unclear how moisture stress will affect pea protein. For instance, an early greenhouse study showed that if soil moisture dropped to near wilting point before supplemental irrigation was applied, protein increased by 1.4% compared to treatments where soil moisture was maintained at field capacity or dropped to 50% of field capacity (McLean et al., 1974). A recent greenhouse experiment showed no differences in protein across moisture regimes where plant available water was held at 90, 75, 60, 50, 40, 30 and 20% of field capacity during vegetative growth (Prudent et al., 2016). Plant available water will depend greatly on soils, climate, and seeding dates throughout Montana. We are currently combining reported seeding dates and field legal descriptions with gridded climate (Abatzoglou, 2013) and soils data (Soil Survey Staff, 2015) to explore broad-scale trends between moisture related variables and pea protein content. Examples of moisture related variables we are investigating are:

- Precipitation,
- Evapotranspiration
- Heat stress.

It is possible that moisture stress could have a canceling effect on protein formation. For instance, nitrogen (N) acquisition and remobilization from vegetative tissues to seeds for protein synthesis occurs simultaneously with seed formation. Consequently low moisture stress may increase N acquisition and remobilization to seeds, but low drought stress during flowering and pod stages could also increase seed number/size and diminish protein on a per-seed basis (Prudent et al., 2015). Alternatively high moisture stress during vegetative stages may reduce soil rhizobia activity required for N-fixation (McCauley et. al., 2012; Serraj et. al., 1999) and subsequent protein synthesis. Controlling for timing and severity of moisture stress is therefore critical for addressing how M x E interactions affect protein.

To account for timing and severity of moisture stress, individual drought stress patterns have been simulated based on producer survey, soils, and climate information, as well as basic growing degree models to estimate crop growth stages (Miller and Holmes, unpublished data 2004). Each line in Fig. 3-A. represents a simulated drought stress pattern derived from producer reported seeding dates and field legal locations, as well as soil and climate inputs. Drought intensity (y-axis) is expressed as a ratio of 0-1 with a value of 1 indicating extreme drought, and growing degree days from reported seeding dates are on the x-axis. Clustering techniques are being applied to group simulated drought stress patterns into similar drought classes. Currently three drought classes have been classified and can be interpreted as 'Favorable', 'Moderate', and 'Severe' drought classes based on relative timing and severity of drought intensity (Fig. 3-B.). Classified drought classes will ultimately be used to compare how different management factors affect pea protein in different growing environments. More specifically, drought classes will serve as the environment (E) component when addressing M x E interactions.

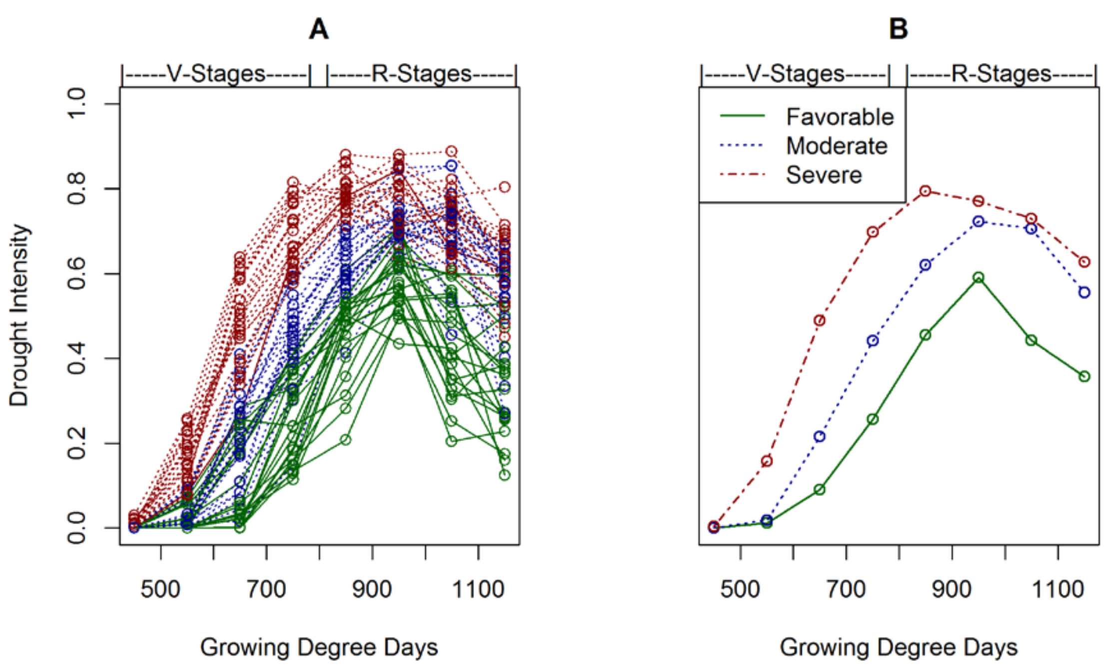


Figure 3. (A) Simulated drought stress patterns generated from field legal locations and seeding dates provided in producer surveys as well as gridded climate and soils data. (B) Classified drought classes based on similar drought patterns.

Based on management that varies across farms (*see section—Sample acquisition and Identifying Standard Management—steps c and d*) and literature review, possible M x E interactions that may influence protein which are prevalent to Montana producers include but are not limited to:

- **Drought class x Inoculant Type x N interactions**— Reports from the Canadian prairies have generally concluded that starter N has no effect on pea yield, but the effect on pea seed protein is less well known. Montana producers use either granular or peat-based inoculants and different blends and rates of starter N. Studies from Alberta reported granular inoculant was superior to peat based inoculant at boosting yield and protein (Clayton et al., 2004), yet early greenhouse studies showed that small doses of starter N coupled with inoculant can enhance nodulation and N-fixation (Mahon and Child 1979). A recent Canadian report also showed that starter N rates of 30 kg ha^{-1} boosted protein by ~0.30 % when yields were less than 3000 kg ha^{-1} (Grenkow et. al., 2014), while another Canadian study found inoculant and fertilizer N to be most effective when soil nitrate-N was less than 20 kg N ha^{-1} (McKenzie et. al., 2001).

- **Drought class x Starter Nutrient interactions**—Roughly 50 % of producers apply various blends and rates of starter fertilizer, but the overall effectiveness of starter nutrients may depend on nutrient type. Notable contrasts worth investigating are as follows.
    - **P vs. (-P) contrasts**—All producers that apply starter fertilizer use P in Montana. Phosphorous (P) promotes nodulation (Jakobsen, 1985), and early field studies in Saskatchewan have shown P applications up to 56 kg ha^{-1} may boost protein by 1.7% relative to no applied P (Sosulski et al., 1974). A more recent European study showed that that protein increased by ~ 3% at P-rates of 90 kg ha^{-1} over the 0 P control (Eman et. al., 2009).
    - **(P + S) vs. (P-S) and (S) vs. (-S)**—Two-thirds of producers that apply starter P also apply starter S. Potting experiments have shown sulfur deficient soils to halve N-fixation relative to sulfur sufficient soils (Zhao et. al., 1999).
- **Drought class x Variety interactions**—Producers have reported growing 13 different varieties. Chen (unpublished data, 2015) reported that environment had the greatest impact on protein relative to variety, but environment was defined by site x variety x year combinations. Newer techniques which classify environment based on drought patterns may reduce environmental variance, making it more probable to detect varietal differences while providing a physical interpretation of environment for breeders (Chenu et. al., 2011).

As more samples/surveys are collected, more drought classes may be identified, and more complex interactions can be analyzed. For example, it may be possible to address Variety x Inoculant Type x Drought Pattern interactions.

Moving Ahead
Obtaining conclusive results will require a) obtaining more field samples and b) reducing measurement uncertainty. It is unlikely that field samples will be obtained during the summer growing season due to producer workload, but upcoming outreach events directed at establishing producer contact will generate sample streams following harvest. Summer 2016 work will mainly be focused on identifying identify how bulk-averaged yellow pea protein measurements are affected by the number of subsamples run on LECO.

A final step is to produce a review emphasizing how E x M factors may affect yellow pea protein in Montana.

<u>Hiring</u>

- No additional hires in Quarter 3.

<u>Expenditures</u>

- Total Personnel Services: $15,448.58
- Total Operations: $1463.21

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

Progress towards milestones

We have sequenced soil samples for 16s rRNA and are in the process of analyzing them. 16s is a highly conserved gene found in all bacteria and archaea and is used to characterize community composition and phylogenetic diversity. We are using these sequences to obtain measures of genetic diversity and community structure for the Post Farm and statewide pea/trial. At the Post Farm preliminary analyses of alpha diversity, which is a measure of community richness, show no significant differences between sites, time points or fertilizer treatments (Figure 1). However, preliminary analysis at the phylum level on the statewide samples show changes in diversity geographically (Figure 2). We will continue to analyze these samples looking other metrics including beta diversity and phylogenetic structuring. All soil samples were also tested for a wide chemistry panel. Preliminary analysis of the statewide samples shows differences in meaningful metrics such as ammonia and nitrate-N+ nitrite-N (Figure 3). At the Post Farm we are interested in community structure and phylogenetic diversity differences between treatments and we will use multivariate statistics to understand how these differences are potentially shaped with the soil chemistry, fertilizer application, and plot treatments. With the statewide pea trial study, we are interested in what variables lead to higher pea yield. We will also use multivariate statistics looking at community structure, genetic diversity, and soil chemistry and their relationship to pea yield.

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

At the Post Farm we have also sampled wheat plants to obtain the rhizosphere. The rhizosphere is a narrow area of soil around plant roots that is directly affected by the plant. Microorganisms in the rhizosphere are different than from bulk soil and contribute to nutrient and mineral availability for plants and other microorganisms. At the Post Farm we will use 16s rRNA and NifH to look at differences in the microbial community and the nitrogen fixing community between different treatments and between the bulk soil sampled in 2015.

Evaluation of AZBB163

We designed and implemented a greenhouse experiment using the soil sampled from the Post Farm and spring wheat inoculated with an ammonia producing strain of *Azotobacter vinlandii* (AZBB163) (Figure 4). For this experiment we are measuring germination rate, sampled for rhizosphere microbiome analysis, will sample leaves to look at fixed nitrogen incorporation, and measure photosynthetic efficiency. This experiment will provide valuable information about the effectiveness of AZBB163 as an inoculant for wheat growth and yield.

Future Work

For the Post Farm and statewide pea trial, the goal in the next quarter is to finish the multivariate statistics and make conclusions about factors contributing to pea yield (statewide pea trial) and differences in diversity and phylogenetic structure between treatments (Post Farm). We are also in the process of sequencing NifH from all sites and 16s and NifH for the Post Farm rhizosphere. In the next quarter the goal is to complete sequencing and begin analysis. Understanding the NifH community and diversity will provide insight into the variability of soil chemistry at all study sites. We will also be able to better understand the impacts of fertilizer, different pea uses, and pea varieties on the nitrogen fixing community. At the Post Farm 16s and NifH sequencing of the rhizosphere when compared with bulk soil will likely show differences in diversity and community structure and lend insight into soil chemistry differences. At the Post Farm we will also look at changes in the microbial community and diversity between the pea treatments and wheat rotation to understand the nitrogen fixing community for both crops. We will continue the AZBB163 greenhouse experiment and analyze the results which can lead to key insights into using an ammonia excreting strain as a wheat seed inoculant. This project will ultimately give a big picture idea of the relationship between nitrogen from pea, nitrogen from fertilizer, crop productivity, soil chemistry and the microbial community. Understanding and interpreting this complex list of variables has the potential to be applicable to pea and wheat health and yield and contribute to more sustainable agriculture in Montana.

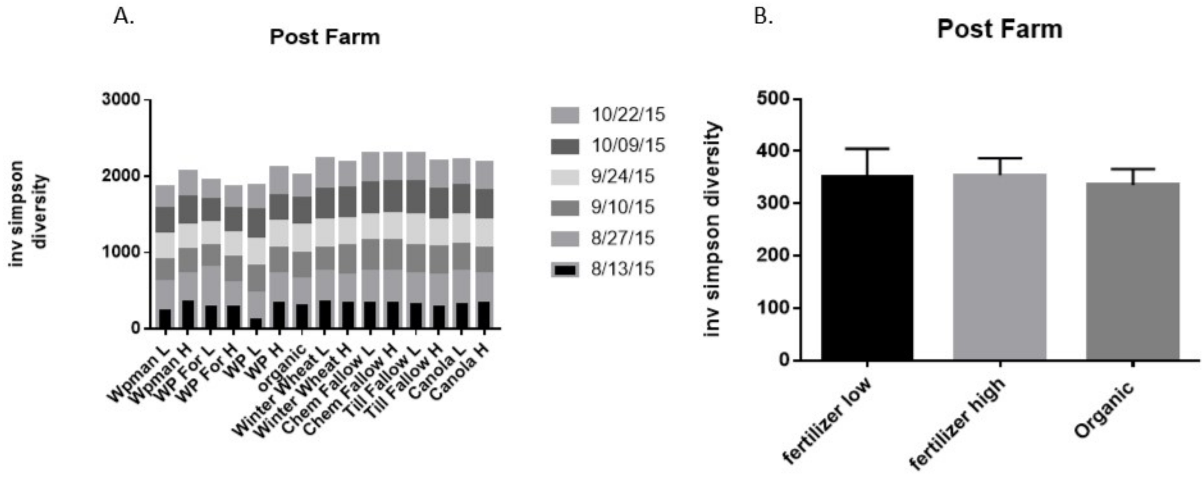


Figure 1. Alpha diversity was calculated for six time points at 15 plots. A) A two-way ANOVA with Tukey's multiple comparison test (p=0.1414) showed no significant differences between time points. Between sites there are significant differences. P=0.0011 which can be attributed to WP L from 8/13/15. B) There are no differences in alpha diversity between high fertilizer, low fertilizer, or organic sites.

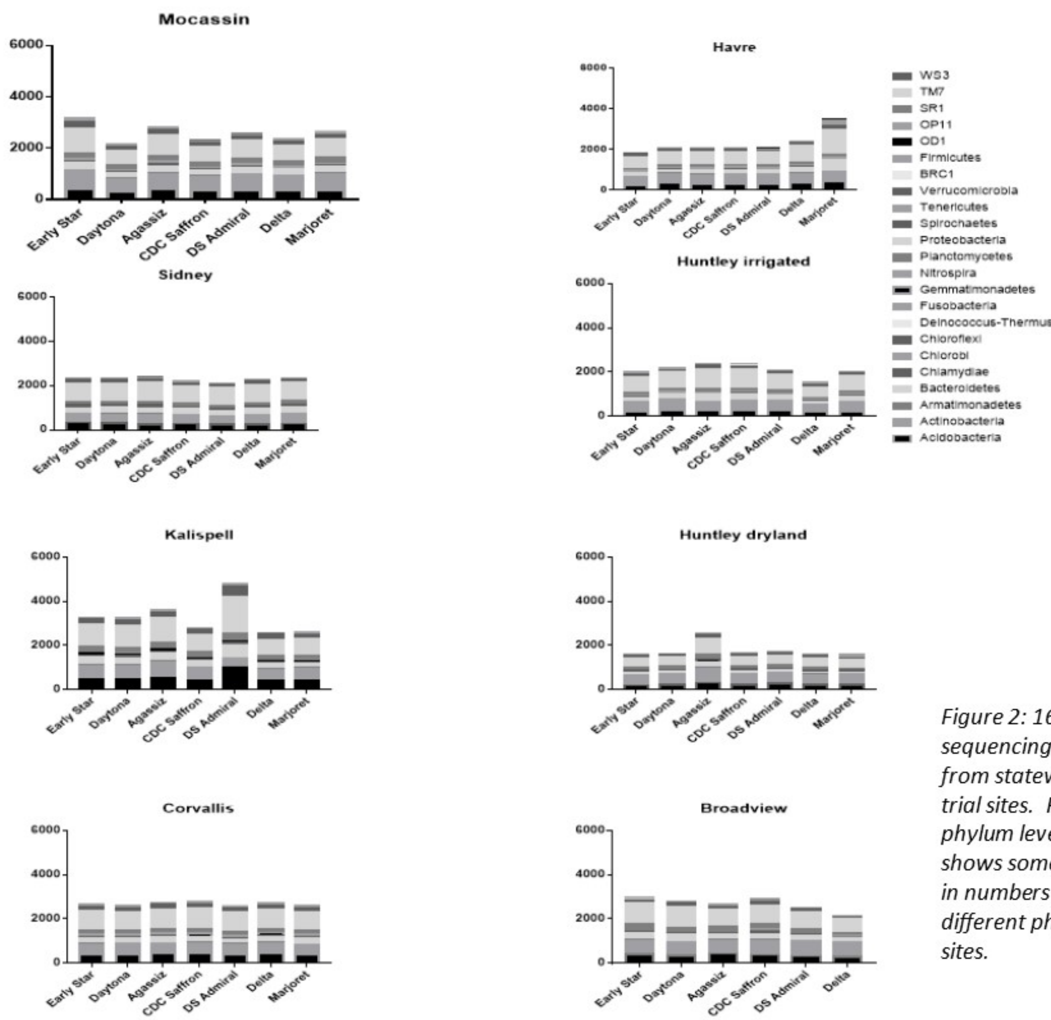


Figure 2: 16s rRNA sequencing of soil from statewide pea trial sites. Preliminary phylum level analysis shows some changes in numbers of OTUs of different phyla across sites.

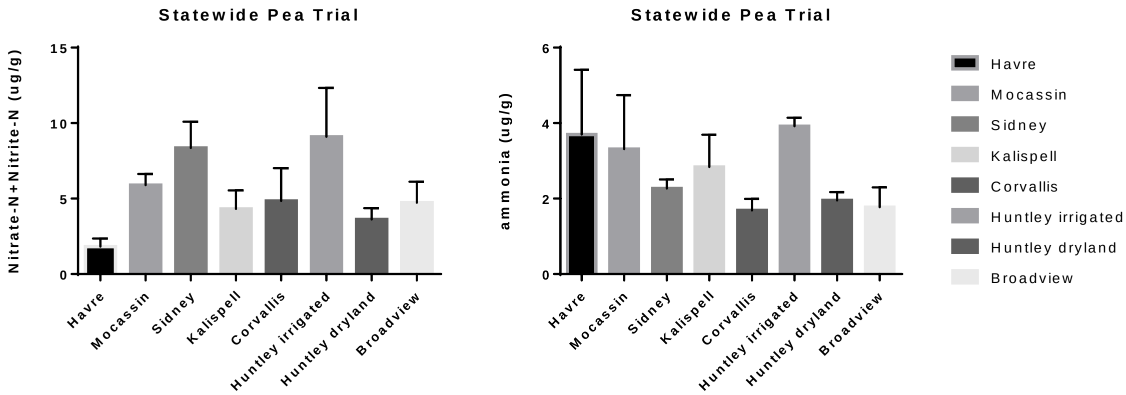


Figure 3. Preliminary soil chemistry results for the statewide pea trial. Graphs indicate some differences between ammonia and nitrate-N+nitrite-N between agriculture stations

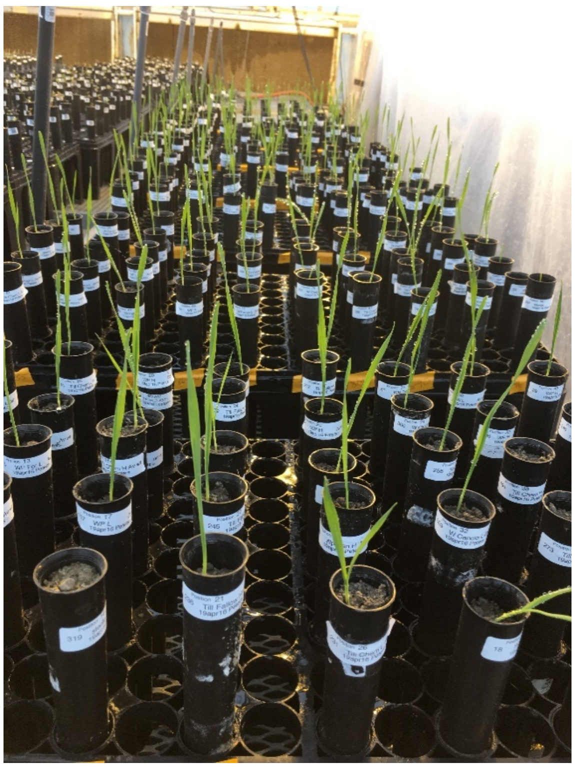


Figure 4. Greenhouse experiment set up with germinating wheat.

Hiring

- No additional hires in Quarter 3.

Expenditures

- Total Personnel Services: $38,614.04
- Total Operations: $14,662.09

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

Progress towards milestones

Progress continues to optimize the growth and characterization of cultures obtained from rumens of several species. The process for the bioreactor purchase has begun as additional funds from Dr. Barry Jacobsen, Dr. Charles Boyer and Dr. Renee Pera have been identified to help cover the price differential between the quotes received for the bioreactor and current purchase price.

Hiring

- No additional hires in Quarter 3.

Equipment Purchased

- The bioreactor purchase is in the beginning purchase stage.

Expenditures

- Total Personnel Services: $30261.30
- Total Operations: $14,830.14

Cover Crop/Grazing subproject of the Agriculture MREDI Grant

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

Progress towards milestones

The cover crop statewide trial has been established at all seven research centers. The trial was seeded at two different times; a time that would be appropriate for planting cool season species and a time that would be appropriate for warm season species at each location. There were four polyculture (cocktails) mixes selected for the trial.

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

Each of the poly cultures were planted at each seeding time point. For the remainder of the study, each individual monoculture that makes up the poly cultures was planted alone, thereby allowing the comparison of how each competes in the mix and how each contributes to the polyculture. This will be done for above ground biomass, forage quality (CP, ADF and DM) and nitrate level of the harvested forage.

The large multiyear trial has been established for the cool, cool/warm and warm species mixes. Infiltration rates were taken prior to seeding the cover crops. Emergence is taking place as this update is being written. The last seeding date Montana PBS was out recording footage with both a hand held camera and MSUN with a rotorcraft drone. Prior to establishing the cover crops, 306 soil samples were taken, to evaluate both the physical and chemical properties of the long term cover crop wheat trial. Normal soil tests will be evaluated along with collaboration with ARS scientist Dr. Rick Haney's new test along with CO2 respiration. Bacterial population will also be compared in Dr. Carl Yeoman's lab. Expected haying and grazing of the above ground biomass will occur in early July. Winter and spring wheat have been established on field two with previous year's cover crop. Access tubes are being placed in several selected plots to evaluate root morphology, and density on this current years cover crops. We will be able to document and evaluate root structure under different poly and monocultures and if how the cover crops were terminated in the past if root structure of current cover crop is affected.

Hiring

- Roger Hybner, who had been funded under 41W225, is now funded from this budget as a Research Associate on this project.

Expenditures

- Total Personnel Services: None to date
- Total Operations: None to date

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

Progress towards milestones

Annual forage plots will soon be planted in Bozeman for a sheep preference (grazing) trial that will be incorporated into the MREDI grant as addition to the large cover crop variety trial that is going in at the Research Centers.

Hiring

- None to date

Expenditures

- Total Personnel Services: None to date
- Total Operations: None to date

On-Farm Precision Experiment subproject of the Agriculture MREDI Grant

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

Progress towards milestones

The On-Farm Precision Experiment (OFPE) Group meets biweekly to discuss progress and implementation of our project. The OFPE Team met and presented a project overview to Matt Clancy, managing director of Next Instruments the manufacturer of our on-combine Crop Scan protein analyzer. The meeting was also attended by Delmna Heiken from Triangle Agriculture in Fort Benton who is the distributor for the Crop Scan instrument. Matt agreed to give us access to absorbance raw data files that can allow us to look for further grain qualities than just protein.

Phil Davis, along with Pat Carr, Ken Kephart, Roger Ondoua, Bruce Maxwell and a few field assistants have collected soil samples on 4 fields prior to fertilizer application. Samples were prepared for analysis and are waiting to be shipped to AgVize as well as the MSU Analytical Lab for a full compliment of analysis.

Phil Davis and Janette Rounds have successfully produced fertilizer prescription maps for all fields on cooperator farms and top-dressed fertilizer was applied on all but the Van Dyke fields, which will be occurring in the near future. The treatment responses are already clearly visible (Figure 1).

Figure 1. Nitrogen fertilizer treatment responses on winter wheat at the Wood farm near Fort Benton.

Applying fertilizer driven by our GIS files was more difficult than anticipated because different brands of machinery have different specifications. We are indebted to Travis Anderson (John Deere dealership in Billings) for putting significant effort into the application process. Phil Davis, Bruce Maxwell, Pat Carr, Lisa Rew and Nick Silverman along with a couple of undergraduate students from MSU have been busy mapping weeds in two fields and are schedules to continue creating weed maps for 4 fields, one on each cooperator farm. Bruce Maxwell has continued to focus on developing a statistical model to optimize N-fertilizer application to maximize net return between the experimental treatments in each field based on previous yield data and previous off-site experiments.

Hiring

No new hires were made in this quarter for any of the aspects of the OFPE Project. Maxwell has also recruited undergraduate students (Paul Hegedus) funded by a fellowship from the Weed Science Society of America to work on aspects of precision weed management associated with the OFPE, Kendall Franks who is one of Maxwell's Advisees and doing an internship with Western Triangle Research Center and Laura Ippolito undergraduate advisee of Maxwell funded by the Montana Climate Assessment.

Expenditures

- Total Personnel Services: $55,920.46
- Total Operations: $35,423.71
- Total Equipment: $45,680.00

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. Over the past quarter Janette Rounds (graduate student) has, in conjunction with Phil Davis, generated randomized fertilizer application prescription maps for all fields in the study. These maps were generated by dividing a field into "plots" based on the size of the fertilizer application equipment, finding the average yield from the previous year for that plot, and then assigning a yield bin (either "High", "Medium", or "Low") based on the average yield within that plot. Next, each plot was assigned a fertilizer application rate such that all yield bins had approximately equal numbers of each fertilizer application rate. Based on input from the farmers and more detailed equipment specifications, the size and location of the plots in the prescription maps were modified so that the prescription maps and the as-applied maps would more closely align. Software is also under development that will maximize the amount of a field covered by plots, so as to maximize the experimental and optimization space within each field.

The OFPE optimization team has also identified a recurrent neural network as the first new optimization strategy to be implemented. A recurrent neural network was chosen because the spatial and temporal dependence properties of the fertilizer application problem can be exploited by the recurrent neural network for information. An appropriate architecture for the recurrent neural network is currently under development. It is anticipated that this approach to optimization will be compared to a previous method based on Bayesian optimization and logistic regression.

Neural networks are a biologically inspired machine learning technique with applications to classification, regression, and optimization. A neural network generally consists of a set of nodes that often come in three types: input, output, and hidden. These nodes are organized into "layers" as seen in Figure 2. Each output and hidden node calculates the weighted sum of its inputs and then uses the weighted sum as the inputs to an "activation function." The activation function can be one of many different kinds including piecewise linear, logistic, or Gaussian. If the node is a hidden node, the output of the activation function then becomes the inputs to the next set of nodes. The input nodes simply feed inputs into the first hidden layer without modification. There can be as many hidden layers as necessary, especially since more hidden layers allow one to model more complex and nonlinear concepts. When links are permitted to feed back to prior layers, we say the resulting network is "recurrent." The advantage to using recurrent networks is that such networks naturally incorporate memory and temporal characteristics into their predictions. The major process in building a neural network is selecting the weights on the inputs to the nodes. This is called "training" the weights, and it is performed through a variety of means. In the vast majority of cases, humans do not select the weights.

One of the main reasons we chose a neural network is because it is a natural extension of Dr. Patrick Lawrence's model described in his dissertation on this subject. The goal of our optimization process is to maximize the net income for a field. Dr. Lawrence started with the premise that maximizing the yield for the field would maximize the income. Dr. Lawrence selected the model with the lowest error in generating the yield from a set of linear and non-linear models with a range of inputs, including the amount of fertilizer applied, precipitation, and electrical conductivity. Other inputs were available; however, the model selection process identified the previously mentioned inputs as the most relevant ones. The model that was selected was a logistic function that requires the definition of seven parameters. Most of these parameters acted as coefficients for the inputs.

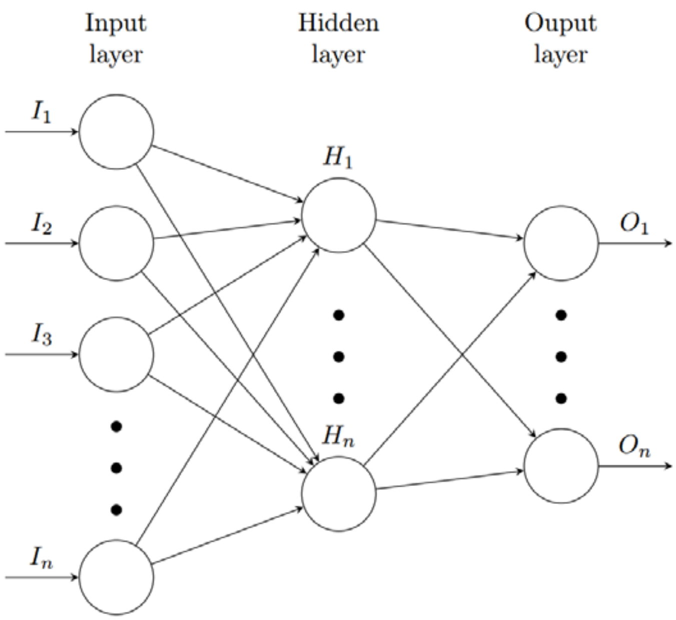


Figure 2: Neural network example with a single hidden layer

Using a neural network will allow us to use all available inputs and to capture nonlinear interactions between these inputs. The training process will emphasize those inputs that reduce the error in the final output. Additionally, the parameters that needed to be specified in Dr. Lawrence's model would not need to be specified in the neural network. The training process will identify a set of weights to act as coefficients for each input.

The incorporation of the optimization component into the larger workflow is a long-running goal. This quarter, the Boost geometry libraries were identified as a major requirement of the optimization component. The OFPE optimization team has identified a RESTful interface as a means of importing and exporting data in between the optimization component and the database. This RESTful interface allows for the modification of either the database or the optimization component.

Expenditures

- Total Personnel Services: $12,063.90
- Total Operations: $6897.15

3) 41W228 – Principal Investigator: Clem Izurieta; Email: clem.izurieta@gmail.com

Progress towards milestones

Payn and Izurieta are managing the team focused on design and implementation of the data management and workflow technology. The underlying software for data management has been named the Object Oriented Environmental Data System (OOEDS). The system is based on state-of-the-art “NoSQL” database technologies, and will handle transfer and storage of digital information for the data import, model calibration, experimental design, yield optimization, and application prescription phases of OFPE process. There have been no new hires to the team managed by Payn and Izurieta during the past quarter, and undergraduate student Jenna Lipscomb has left the team.

The larger team, including Pol Llovet, Thomas Heetderks, Seth Kurt-Mason, Michael Trenk, and Melissa Dale, (and occasionally Nick Silverman and Phillip Davis), have been meeting every other week to track project progress and address the shifting priorities inherent in a research and development project. A flexible “kanban” style project management system is being used to track project milestones and the tasks necessary to accomplish each milestone. MSU’s “Box” cloud service is being used as a central document repository for the project, and a “Github” service is being employed to provide centralized management of code organization and revision during software development.

The last quarter saw progress on the following activities:

OOEDS Data Model

- We continue to revise and extend the schema of the data model to provide new features necessary for data input, optimization, and prescription workflows (see below).
- Our design products in development of the data model provide a valuable contribution to the environmental data management literature, we are revising key figures and outlines for a manuscript targeting one of the environmental modeling journals.
- For both manuscript preparation and executive level documentation, we are developing simplified schema of the data model. These schema represent the modeled domain (not the software) and are intended to communicate the data management concepts to a more general audience.

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.
- As driven by features needed for workflow development (see below), steady progress continues on the OOEDS implementation of the web interface (RESTful API).

Workflow software products (in order of current priority):

Yield Editor Data Input

- Based on the data input files from the Yield Editor software, we have defined the structure of the configuration file necessary to input data to the database, and implementation and testing of the code is well under way.
- We expect to complete the initial version of the software for this workflow next quarter.

Optimization

- The fundamental activities and sequences to support the workflow have been defined in design documentation.
- Activity in the next quarter will be to implement the design for querying data for optimizations from the database, and returning the results of optimization back into the database.

Prescription

- 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.

Expenditures

- Total Personnel: $30,746.60
- Total Operations: $33.56

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

Progress towards milestones

- Dr. Silverman has installed 4 Davis weather stations at the four primary cooperator farms. Three of the weather stations are connected to the internet and available online (Table 1). The fourth station (Broyles) is not connected but is successfully logging data. We are in discussions with Davis on how to get the Broyles' station connected. At this point, all four farms have stations installed and are recording data. The links are provided below:

Farm:	Station Link
Jess Wood	Davis | Wood Station
Chuck Merja	Davis | Merja Station
Mark Van Dyke	Davis | VanDyke Station
Gary Broyles	Davis | Broyles Station

Table 1. Weather station links to observe real-time conditions updated every hour.

- A 5th weather station was set up at the Missoula Airport to compare with the National Weather Service station and two other commercial grade weather stations (FarmHub and Decagon). The preliminary results of this comparison indicate that they are all performing consistently for temperature, relative humidity and precipitation (graphics available upon request).
- Dr. Silverman has been actively assisting in weed mapping, flux tower installation, and aerial imagery collection.
- He has worked with participant farmers to help forecast spring conditions and other climate relationships.
- He is continuing to develop a platform for serving weather station data from the Climate Office to the OFPE database. This platform will also be available for any stakeholder to use to access weather, climate, and soil moisture data across the state.

Dr. Silverman has been developing relationships between wheat yield and remotely sensed variables to support the OFPE software development and make early season predictions. He is developing this platform using the Google Earth Engine so that it can be spatially distributed across the state.

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

Progress towards milestones

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

Table 1. 2016 Statewide Durum Nursery Cooperators

Research center	Location	Contact	email
MSU-CARC	Moccasin, MT	David Wichman	dwichman@montana.edu
MSU-EARC	Sidney, MT	Chengci Chen	cchen@montana.edu
MSU-Bozeman	Bozeman, MT	Mike Giroux	mgiroux@montana.edu
		Andy Hogg	ahogg@montana.edu
MSU-NARC	Havre, MT	Darrin Boss	dboss@montana.edu
Northern Seed	Bozeman, MT	Craig Cook	ccook@northernseedmontana.com
		Dale Clark	dclark@northernseedmontana.com
USDA-ARS	Fargo, ND	Linda Dykes	linda.dykes@ars.usda.gov
NSDU-WREC	Williston, ND	Guatam Pradhan	guatam.pradhan@ndsu.edu
MSU-WTARC	Conrad, MT	Gadi Reddy	reddy@montana.edu

Table 2. 2016 Statewide Durum Nursery Entries and Replication

Line/variety	Source	entry
Mountrail	NDSU	1
Divide	NDSU	2
Alkabo	NDSU	3
Grenora	NDSU	4
Tioga	NDSU	5
Carpio	NDSU	6
Joppa	NDSU	7

Silver	NARC-Turner	8
Alzada	Northern Seed	9
MT101717	NARC-Turner	10
MT101694	NARC-Turner	11
MT112434	NARC-Turner	12
MT112444	NARC-Turner	13
MT112463	NARC-Turner	14
MT112219	NARC-Turner	15

Table 3. 2016 Statewide Durum Nursery

Cooperator	Location	Environment	Entries	Exp #	# reps
MSU-Giroux/Hogg	Bozeman, MT	Irrigated	15	D1601	3
Northern Seed	Bozeman, MT	Irrigated	15	D1602	3
MSU-NARC	Havre, MT	Rainfed	15	D1603	3
MSU-EARC	Sidney, MT	Rainfed	15	D1604	3
MSU-EARC	Sidney, MT	Irrigated	15	D1605	3
MSU- WTARC	Conrad, MT	Rainfed	15	D1606	3
Northern Seed	Conrad, MT	Irrigated	15	D1607	3
MSU-CARC	Moccasin, MT	Rainfed	15	D1608	3
NDSU-WREC	Williston, ND	Rainfed	15	D1609	3

Durum Breeding Populations

For each of the unique durum crosses (Table 1, Q2 report) 750 F_2 seeds were space planted at the MSU Post Farm in Bozeman on April 20th with the goal of obtaining seed from the 250-500 most desirable plants per population. Plants will be selected for traits such as plant height, head size, agronomic adaptability and maturity date. Lines will then be further screened by seed morphology and seed color.

To diversify and enrich populations for the low Cadmium trait (Cd-) a second round of crossing was performed this winter (Table 4). F_1's from the first round of crossing (see Quearter2where one of the parents were Cd- were crossed to either Strongfield or AC-Brigade (both Cd-), or to another F_1 that also is heterozygous for Cd^-. The F_1 seed from these second crosses is currently being advanced in the greenhouse and the F_2 populations from these crosses will be grown in the greenhouse.

Table 4. Further development of new durum breeding material with emphasis on low Cadmium lines

Cross	Generation	Unique cross #
Stongfield X Mead/Joppa F_1	F_1	1
Stongfield X Joppa/Alzada F_1	F_1	2
Strongfield X Alkabo/Joppa F_1	F_1	3
AC-AC-Brigade X Stongfield/Tioga F_1	F_1	4
Strongfield /Tioga F_1 X AC-AC-Brigade	F_1	5
Strongfield/Tioga F_1 X Mead/Joppa F_1	F_1	6
Strongfield/Tioga F_1 X Alkabo/AC-Brigade F_1	F_1	7
Strongfield/Alkabo F_1 X AC-Brigade/Carpio F_1	F_1	8
Strongfield/Divide F_1 X Joppa/Tioga F_1	F_1	9
Alkabo/AC-Brigade F_1 X Strongfield/Divide F_1	F_1	10
Alazada/Strongfield F_1 X AC-Brigade/Carpio F_1	F_1	11
Mead/Joppa F_1 X Strongfield/Alkabo F_1	F_1	12
Alazada/Havasu F_1 X Strongfield/Tioga F_1	F_1	13

Alkabo/AC-Brigade F_1 X Alzada/Strongfield F_1	F_1	14
AC-AC-Brigade X Strongfield/Alkabo F_1	F_1	15
Strongfield/Tioga F_1 X AC-Brigade/Carpio F_1	F_1	16

2016 Experimental durum breeding material evaluation

Durum populations created at MSU are also being evaluated to determine if any of the material has advantages over current elite cultivars. Populations were created by crossing EMS induced lines (175 and 55) (Hogg et al. 2013) to the cultivars Divide and Mountrail which were then crossed again to either Divide or Mountrail (Table 5). The top 102 $F_{5:8}$ lines from the three populations were selected based on several agronomic and seed characteristics such as yield, protein, plant height, seed color, and seed size. Two of the populations have lines that segregated for Cd^- (Table 6; Figure 2 Q2 report) and all three segregated for a gene that effects pasta firmness, an important quality trait. The elite cultivars in the statewide durum nursery were included for comparison.

Short plots of 203 experimental lines, including the 102 described above, were grown in Yuma, AZ this winter in cooperation with Northern Seed. Seed received back will be assessed for yield, seed size and protein content.

Table 5. Experimental Durum Trial Entries

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

Table 6. Experimental Durum Trials

Cooperator	Location	Environment	Reps.	Plot length (ft)	No. Rows
Northern Seed	Conrad, MT	Rainfed	2	20	7
MSU-Giroux/Hogg	Bozeman, MT	Irrigated	3	10	4
MSU-Giroux/Hogg	Bozeman, MT	Rainfed	2	10	4

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

Northern Seed is seeking to improve the durum varieties available to the Montana grower through a few different avenues. The first of these is to evaluate the current germplasm that Northern Seed acquired from Montana State University (Joyce Eckhoff's breeding program (JE)). The second is to continue cooperative research with Dr. Mike Giroux through his testing and quality improvement program. The third is to closely work with 2nd Nature Research's extensive durum breeding program to select varieties for Montana. (This program was formerly the WestBred high quality Desert durum program, and is now owned by Barkley Ag of Yuma, AZ.)

Northern Seed has been in the process of selecting MSU JE germplasm to fit the Montana growing conditions. Various generations (F_5 to F_{11}) of this material were grown in Yuma, AZ this past winter. Selections were made from both the single plot and single row entries that would more likely fit the agronomic conditions of Montana. The 156 selections are being grown in replicated plots this summer at Bozeman. General agronomic traits, along with yield and quality of these lines will be evaluated and acceptable lines will be tested in replicated trials across the state at 5 locations next year. At the same time, head row purification will begin on those lines showing the most promise. An additional plot, other than that planted for yield, was planted so heads could be selected from those that appear to be the best at harvest time. After we receive the quality information on the Bozeman harvest, heads of the best lines will be planted in Yuma, AZ this fall and harvested next April to bring back to Bozeman to begin the production of Breeder Seed.

The 2nd Nature Research durum program has been a very successful program over many decades. High quality, low Cadmium Desert Durum's and the successful Montana variety, Alzada, were developed from this program. The Desert

Durum germplasm has been used by many northern breeding programs over the years as a source of not only high quality but also as a source of heat and drought tolerance. (Although this program focuses on irrigated varieties, the SW Desert can experience some fairly extreme temperatures during the grain filling process.) Northern Seed has planted over 4000 plots for this cooperative effort, including replicated yield trials in Scobey, Conrad and Fort Benton.

Through these combined efforts, Northern Seed feels confident that they will have a new variety for the Montana growers very soon.

Hiring

- No additional hires in Quarter 3.

Equipment

- The Perten Glutomatic and the Brabender Quadrumat Jr. Durum mill both arrived and we have become actively using both for analysis of durum quality. However the Brabender Quadrumat Durum mill that arrived did not in fact meet the specifications of what was ordered resulting in our semolina yields and ash being too high.
- We do not anticipate ordering any additional equipment for this project.

Expenditures

- Total Personnel: $16,887.39
- Total Operations: $8168.75
- Total Equipment: $71,824.58

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

Progress towards milestones

Analysis is being conducted on flowering crop species grown in the greenhouse and measurements and data analysis on field-collected wheat stem sawfly parasitoids continue to be performed from last quarter.

Hiring

- No additional hires in Quarter 3.

Expenditures

- Total Personnel: $28.56
- 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
HERBICIDE CARRY-OVER STUDIES FOR SUCCESSFUL INTEGRATION OF PULSE CROPS IN CEREAL-BASED CROPPING SYSTEMS OF MONTANA

Progress towards milestones

The field studies have been initiated across multiple locations: Huntley, Moccasin, Havre, and Sidney, MT, starting September 20, 2015. There are 3 major objectives of these field studies:

1. Effect of fall-applied soil residual herbicide programs on pea, lentil, and chickpea tolerance and weed control (emphasis on kochia and Russian thistle control) in the following year (*plots established*)
2. Effect of Group 2 Sulfonylurea herbicides applied in the fall PRE and spring POST in winter wheat (including Clearfield wheat varieties) and carry-over to pea, lentil, and chickpea (*plots established*).
3. Spring-applied PRE/POST herbicide tolerance (variety response) and weed control in pea, lentil, and chickpea.

For objectives 1 and 2, herbicide treatments were applied in the fall of 2015. All the sites-Huntley, Moccasin, Havre, and Sidney have been planted to pea, lentil, and chickpea this spring (2016). All crops have successfully emerged. Crop safety and weed controls will be initiated and the yields will be determined at harvest. List of treatments applied in fall of 2015 are shown below in the table below.

Trt No.	Treatment Name	Form Conc	Form Type	Other Rate	Other Rate Unit	Appl Code	Appl Description	Rep 1	2	3
1	METRIBUZIN 75 DF	75	DF		4 oz/a	A	FALL-APPLIED	101	217	303
2	METRIBUZIN 75 DF	75	DF		8 oz/a	A	FALL-APPLIED	102	216	314
3	SPARTAN CHARGE	4	SL		6 oz/a	A	FALL-APPLIED	103	213	311
4	SPARTAN CHARGE	4	SL		12 oz/a	A	FALL-APPLIED	104	209	307
5	VALOR SX	51	WG		3 oz/a	A	FALL-APPLIED	105	210	317
6	VALOR SX	51	WG		6 oz/a	A	FALL-APPLIED	106	212	302
7	CORVUS	2.63	SC		4 oz/a	A	FALL-APPLIED	107	211	304
8	CORVUS	2.63	SC		8 oz/a	A	FALL-APPLIED	108	201	313
9	AUTHORITY MTZ	45	DF		8 oz/a	A	FALL-APPLIED	109	204	301
10	AUTHORITY MTZ	45	DF		16 oz/a	A	FALL-APPLIED	110	215	310
11	CORVUS	2.63	SC		3 oz/a	A	FALL-APPLIED	111	214	316
	METRIBUZIN 75 DF	75	DF		4 oz/a	A	FALL-APPLIED			
12	CORVUS	2.63	SC		6 oz/a	A	FALL-APPLIED	112	207	308
	METRIBUZIN 75 DF	75	DF		8 oz/a	A	FALL-APPLIED			
13	ANTHEM FLEX	4.3	SC		3.64 fl oz/a	A	FALL-APPLIED	113	205	309
14	ANTHEM FLEX	4.3	SC		7.28 fl oz/a	A	FALL-APPLIED	114	208	315
15	PROWL H2O	3.8	SC		16 fl oz/a	A	FALL-APPLIED	115	206	305
	OUTLOOK	6	EC		18 fl oz/a	A	FALL-APPLIED			
16	PROWL H2O	3.8	SC		32 fl oz/a	A	FALL-APPLIED	116	203	312
	OUTLOOK	6	EC		36 fl oz/a	A	FALL-APPLIED			
17	NONTREATED							117	202	306

Figure 1: Pea, lentil, and chickpea planted in spring 2016 on fall (2015)-applied soil-residual herbicide plots established in wheat stubble at MSU- SARC, Huntley, MT; Photo: P. Jha

<u>Hiring</u>

- Anjani Jha and Shane Leland have been hired as research assistants to this project.

<u>Equipment</u>

- The environmentally-controlled growth chamber at SARC, Huntley, has not been purchased yet.

<u>Expenditures</u>

- Total Personnel: None to date
- Total Operations: None to date

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

<u>Progress towards milestones</u>

***Precision herbicide application (spot-spray system) for weed control in fallow*:**
Field studies on light-activated sensor controlled (LASC) precision spot spray system (WeedSeeker) were conducted in summer of 2015 and will be continued in 2016. During 2016, we will test the efficacy of WeedSeeker technology using a 30 feet, tractor-mounted precision sprayer (30 sensor units spaced 12 inches apart) in large-scale plots in fallow. Demonstration plots for growers will also be established across Montana during various MAES/ Research Centers' field days. Based on our research over the last 3 years, the WeedSeeker technology has shown up to 70% reductions in the herbicide use to manage weeds in chemical fallow.

***Hyperspectral imaging to detect herbicide-resistant weeds in crop fields*:**
This project is focused on the development of detection methods to distinguish between herbicide-resistant and susceptible weeds. We have been focusing on the identification of glyphosate- and dicamba-resistant kochia biotypes from MT, which poses an economically significant for Montana wheat growers. Initial tests in Q2 of this project showed the possibility of a distinguishing between the susceptible and resistant kochia plants by observing the plants' reflectance (color) with a hyperspectral imager from Resonon, Inc. (a local Bozeman company). Further testing has taken place during Q3 of the project at the MSU Southern Agricultural Research Station in Huntley, MT to help refine our understanding of these spectral differences. Our goal was to image the plants in controlled conditions (greenhouse) and analyze the spectral difference between the three resistance classes.

Implementation of machine learning classifiers

By utilizing standard machine-learning algorithms, classifiers have been built to successfully distinguish dicamba-resistant and glyphosate-resistant kochia from the susceptible kochia (collected from wheat-fallow fields in northern Montana).

Imaging Setup

When we imaged the kochia plants, we used two types of illumination: diffuse natural sunlight, and artificial sunlight provided by a solar simulator.

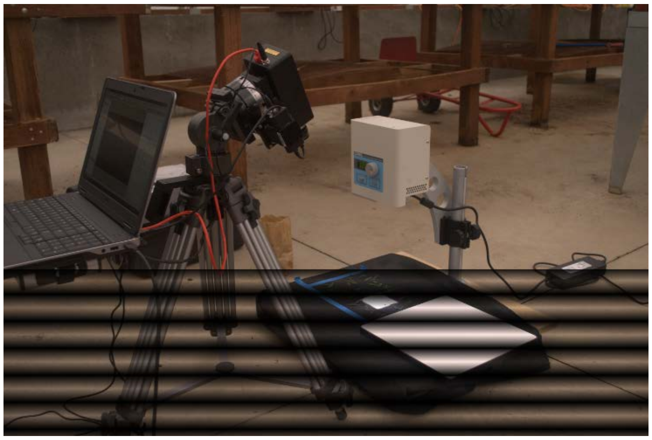
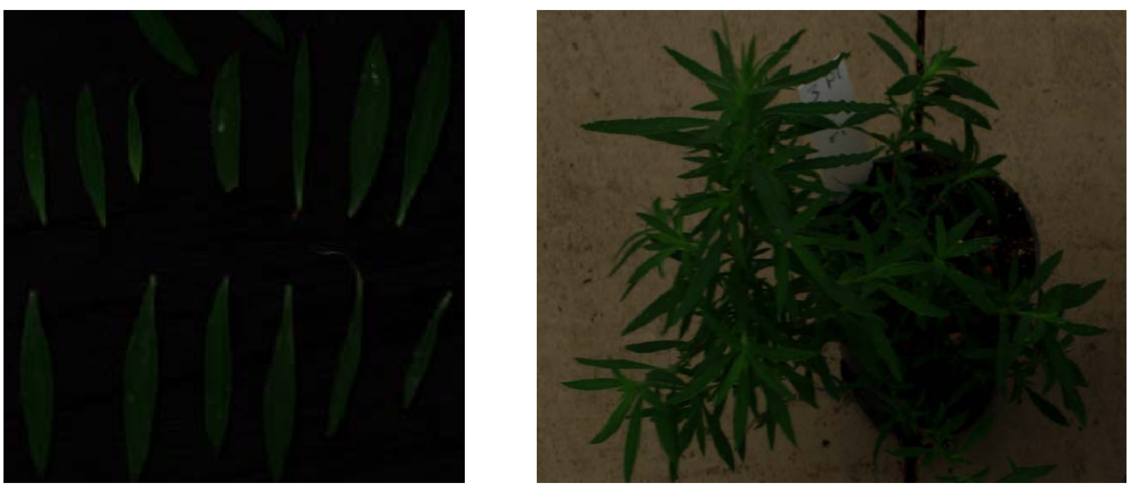


Figure 1: Artificial sunlight imaging set up at MSU-SARC Greenhouse, April 2016

For each herbicide resistance class, we imaged three configurations of the plants: clusters of whole plants, individual plants, and individual leaves from a specific plant. We imaged several dozen plants, along with hundreds of individual leaves, providing us with a substantial data set to develop a classification system. Figure 2 illustrates the different imaging configurations.



Figures 2: Examples of the different imaging configurations used in the April data collection

Data Pre-processing

Once we had the data collected, we had to extract the data relevant to the resistance classification problem.

1. Identify the spectra associated with each leaf imaged in a single data cube.
2. With user input, label each of these spectra as one of the three resistance classes (glyphosate-resistant, GR; dicamba-resistant, DR; or susceptible, SUS)
3. Separate the labeled spectra into training and testing data sets. We used approximately 70% of the labeled data as training data, and the remaining 30% as testing.

Analysis and Results

Using the training data extracted in the preprocessing step, we were able to perform analyses to examine the spectral differences between resistance classes. The first test we conducted was to take the mean of all the spectra collected for each of the three resistance classes and compare them. Ideally, we should see a clear difference between the mean spectra from the resistance classes. The comparison is shown in Figures 3 and 4 below.

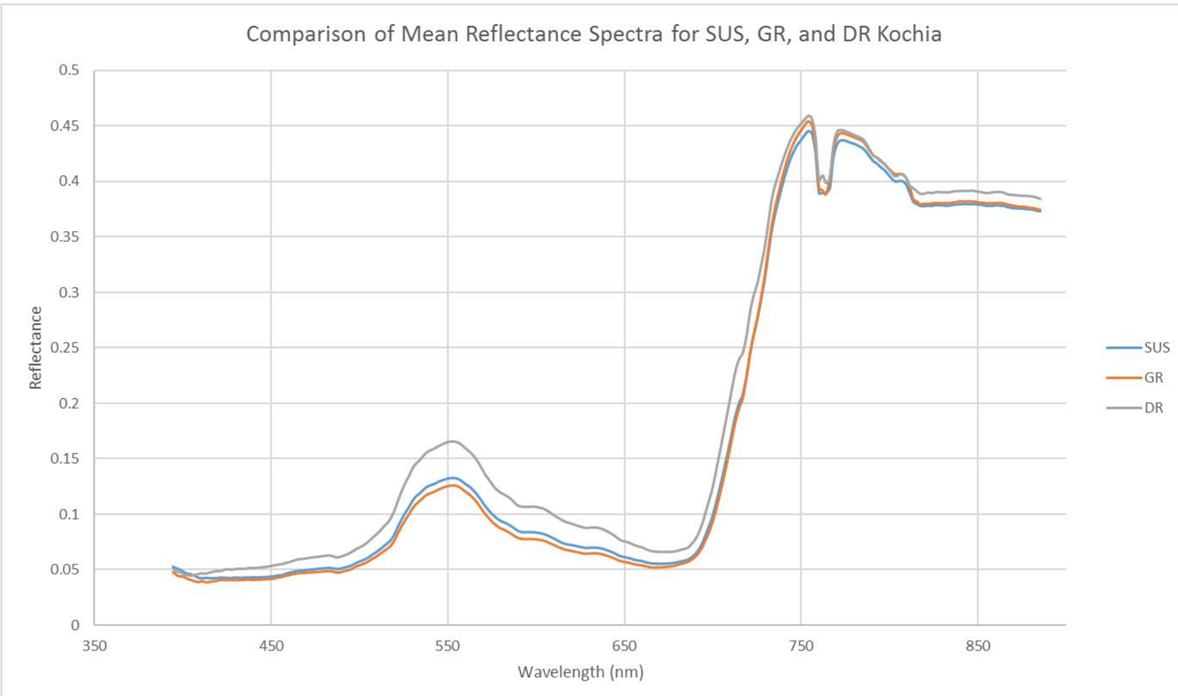


Figure 3: Mean reflectance spectra for each of the three resistance classes

As evident from this figure, there is a slight separation between all three resistance classes. The most notable difference is between GR and DR/SUS between 450 and 650 nm. We also applied other visualization techniques to determine if it is possible to separate the three resistance classes. In one technique, we selected three wavelengths of interest and plotted against one another for three wavelengths: 520, 745, and 813 nm. If the classes are separable, we should see distinct clusters of GR, DR, and SUS spectra in the scatter plot. That is exactly what we do see:

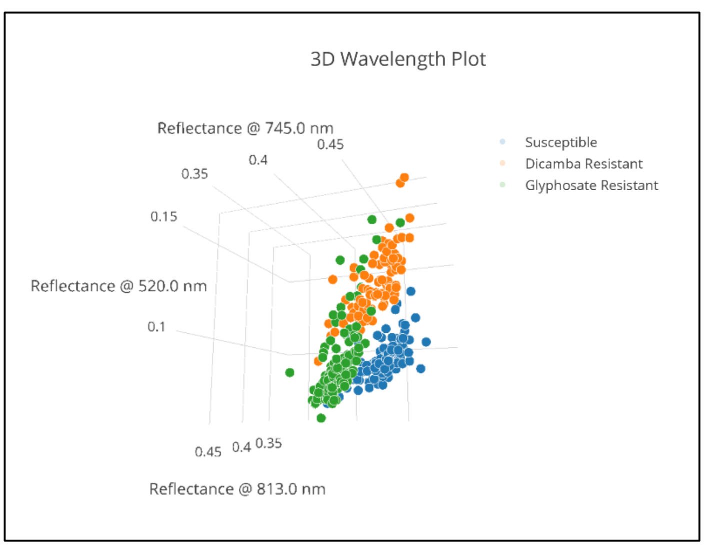


Figure 4: Reflectance values plotted against one another for three wavelengths: 520, 745, and 813 nm

We also used principle component analysis (PCA) to reduce the dimensionality of the data from 240 dimensions to three. Once the dimensionality has been reduced, we can create a scatter-plot of the 3D points, and we should expect to see behavior similar to that shown in Figure 5. We do see several nice clusters once again:

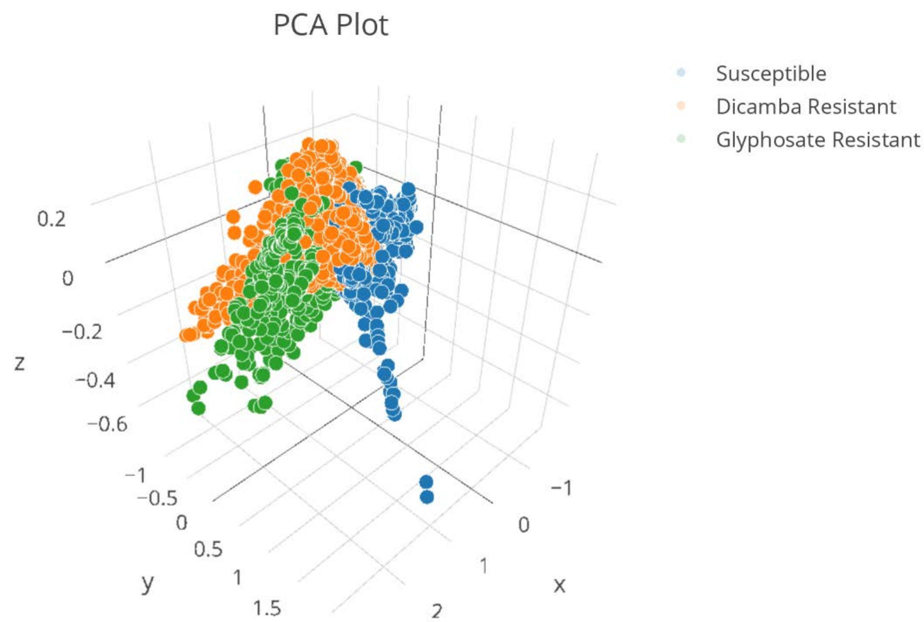


Figure 5: Principle component analysis of the three resistance classes

Since there appeared to be differences among the resistance classes, we used the <u>reflectance spectra we collected in the greenhouse to build resistance or susceptibility prediction models</u>. To create these models, we used standard machine learning techniques. The following table summarizes the training and testing accuracies for six common models.

Table 1: Training and testing accuracies for a variety of machine learning models

Machine Learning Model	**Training Accuracy (%)**	**Testing Accuracy (%)**
Decision Tree	100	87.5
Random Forest	100	93.9
Extra Trees	100	93.6
k-Nearest Neighbors (k = 5)	96.9	91.8
Adaboost	91.1	87.6
Gradient Boosting	97.5	93.8

<u>These results indicate many of these models are performing quite well on the given problem, which means they are able to find differences in the spectra between the resistance classes</u>. Additionally, using the random forest and extra trees models, we were able to examine the importance of each of the features (wavelengths) used to make a classification. Table 2 shows the two most important wavelength ranges in classifying a spectra as DR, GR, or SUS.

Table 2: Important wavelength ranges for classifying the resistance classes

Importance	**Lower Wavelength (nm)**	**Upper Wavelength (nm)**
1	739	756
2	518	524

Finally, we used the trained models to classify spectra of leaves with known susceptibility or resistance, but which had not been used to train the models. Figures 6 –8 show typical results of applying a trained classifier (random forest) to a set of imaged leaves. Each figure contains leaves of only one resistance or susceptibility. It is important to note that these leaves were not used in the training data for the model; all spectra classified in these images were new to the model.

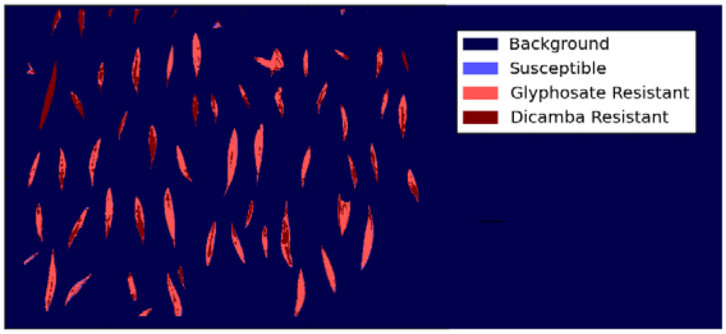


Figure 6: Example classification of GR Kochia illuminated in diffuse sunlight

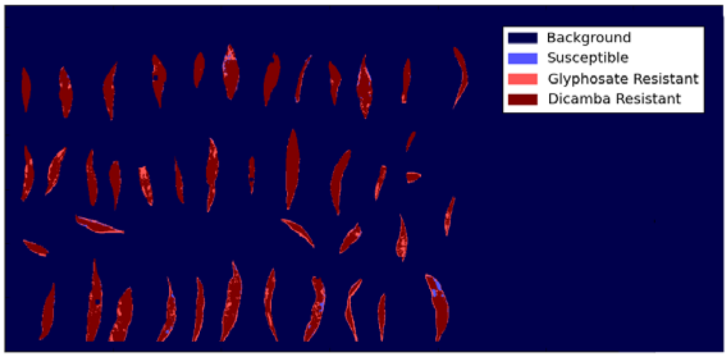


Figure 7: Example classification of DR Kochia illuminated in diffuse sunlight

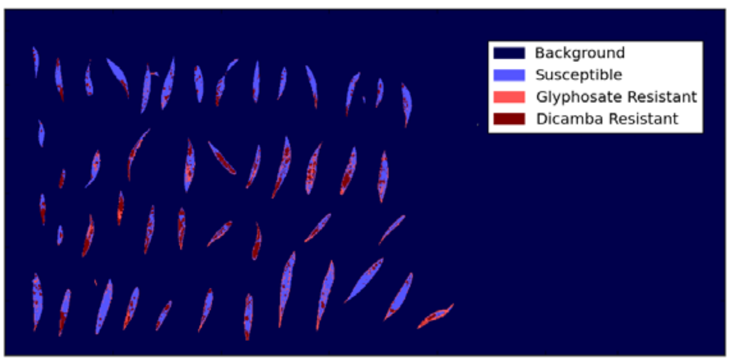


Figure 8: Example classification of SUS Kochia illuminated in diffuse sunlight

As we can see, the model for the most part, was quite accurate. *Together, these results indicate that there are significant spectral differences between the three herbicide resistance classes. Therefore, we can conclude that this approach has significant potential for dynamically determining herbicide resistance or susceptibility*.

Future Work

An important limitation to be aware of is that all of the GR kochia we imaged in April, 2016 were clones of a single plant. We would like to see more diverse populations of the glyphosate- resistant kochia so that we can be sure the difference we are seeing are actually due to the herbicide resistance, and not just a trait specific to that one plant, which is likely not the case.

Additionally, although our accuracies shown in Table 1 are quite impressive, our prediction models currently depend on the entire reflectance spectra to produce an accurate classification. It would be beneficial to develop methods to reduce the amount of data needed to train the model. Some ideas include only training on a subset of the wavelengths, utilizing principle component analysis.

Finally, all of this work was conducted in a greenhouse under fairly controlled circumstances (spatially and temporally consistent lighting, no wind, stationary imaging system, etc.). An important part of the path forward will be developing techniques to handle the more challenging conditions in the field, particularly the expected inconsistent lighting.

Moving outside the greenhouse: detecting herbicide-resistant weeds in-crop

Our milestone for April 1, 2016 was to develop an advanced-optics based weed sensor system for deployment on a spray boom. With the focus on the herbicide-resistant weeds, we will not be placing the sensors on spray booms. This is in part due to the restriction that we do not want to plant the resistant weeds in non-infected fields or regions. However, the ability to identify these resistant plants in controlled greenhouse experiments is very different from being able to identify them in uncontrolled field settings. Therefore, we will be moving to outdoor real-world experiments using potted herbicide- resistant kochia plants placed into a variety of monocot and dicot crops, some of which will contain planted susceptible kochia. These field experiments were conducted in April 2016 and will be continued in summer 2016.

During the Q3 experiments at the MSU Southern Agricultural Research Center, Huntley, MT, a preliminary outdoor field experiment was conducted. Potted susceptible kochia, glyphosate-resistant kochia, and dicamba-resistant kochia were placed between the rows of a winter wheat field. To allow for calibration a spectralon-reflectance panel in the field next to plants, similar to the controlled greenhouse experiments, 9 shows these plants and the calibration panel. A tray of dicamba-resistant kochia can be seen on the left side of the image. Currently these tests grouped the glyphosate-resistant and -susceptible kochia, but future testing hopes to allow separation of these types.

Figure 9. The potted kochia plants placed into a winter wheat field. A tray of dicamba resistant kochia can be seen on the left side of the image. Glyphosate and susceptible kochia are placed between the rows

The Resonon hyperspectral imager imaged all of these plants, and calibrated reflectance spectra were extracted. Our work had shown that in addition to the calibrated reflectance spectra, the slope of the reflectance spectra could be used to identify these plants, this slope is the derivative in the wavelength dimension of the image. For every pixel in the image both the reflectance and reflectance slope were derived, examples of these spectra are shown in Figure 10 with (a) showing reflectance, and (b) showing reflectance slope. This figure contains the reflectance of winter wheat, herbicide-susceptible kochia (Kochia), dicamba-resistant kochia (Kochia DR) and the median plant reflectance observed in the image. Plants were identified as pixels with a NDVI greater than 0.5.

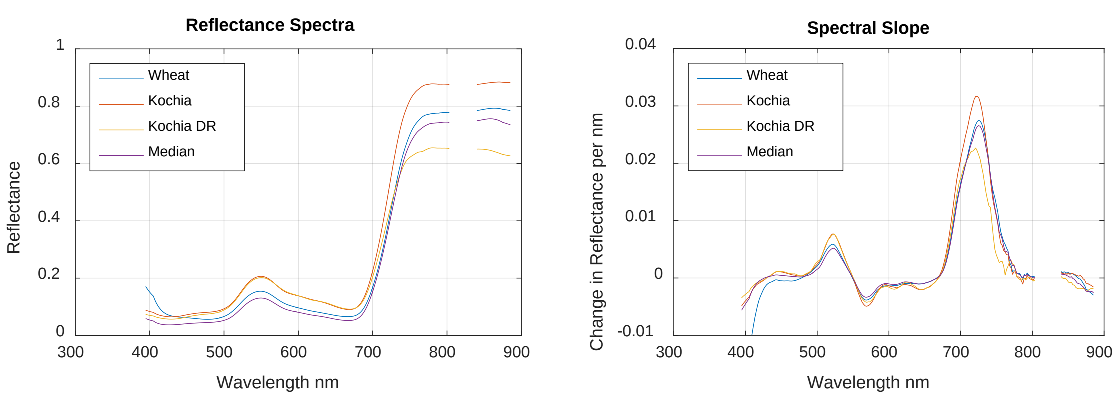


Figure 10. Reflectance spectra (a) and spectra slope (b) for three plant types in the images and the median reflectance spectra

Just by observing these plots, there are differences between the different plant types (crop and weed), most strongly present in the near infrared (NIR), but also in the visible as well. To obtain these reflectance, a calibration process had to be applied to the data that used the reflectance panel to compensate for the spectrum of the incident light. When moving to a realistic field situation, it cannot be expected that a calibration panel will be present in most images, and alternative calibration techniques were explored.

The technique that we are currently implementing is a multi-step process that uses signals of the image itself to act as normalization. In this process first each pixel is normalized by its total brightness so that the integral of the reflectance spectra would equal 1. Then these normalized spectra are divided by the median spectrum. In the spectral slope, a similar process is used to calculate a spectral difference from the median spectrum. Examples of the signals that result from this process are shown in Figure 111 (a, b).

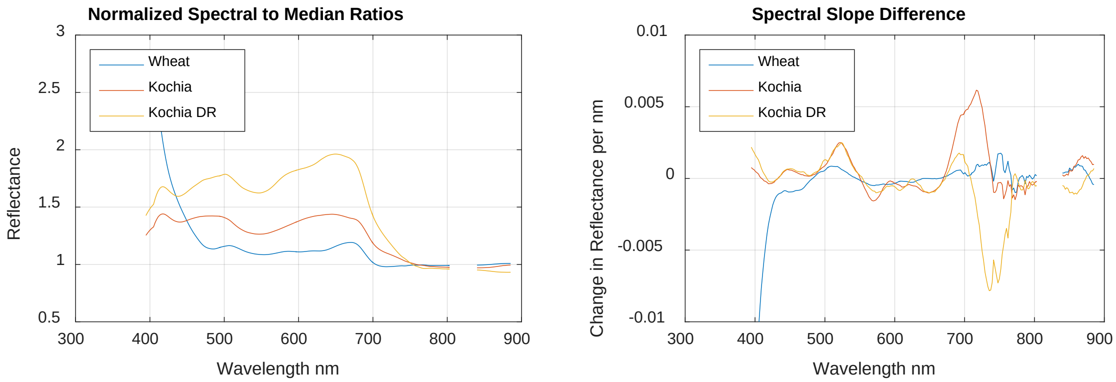


Figure 11. Normalized reflectance spectra (a) and spectral slope difference (b) for three plant types in the images and the median reflectance spectra

It is interesting to note that the normalization processes enhances the differences in the visible, but suppresses the differences in the NIR region of the reflectance, however this is not true for the spectral slope differences.Once these data were obtained, classifiers were trained to identify the different plant types, dicamba- resistant kochia, kochia (susceptible and glyphosate-resistant) and winter wheat. This classification was based on the image-normalized parameter, and not on the parameters that required calibration with the known reflectance panel. An RGB image of the scene and a classification image are shown in Figure 12. In the bottom classification image, winter wheat is shown in green, kochia is in red, and dicamba resistant kochia is in blue. This image still contains the spectralon reflectance panel but this panel is not required of the data processing.

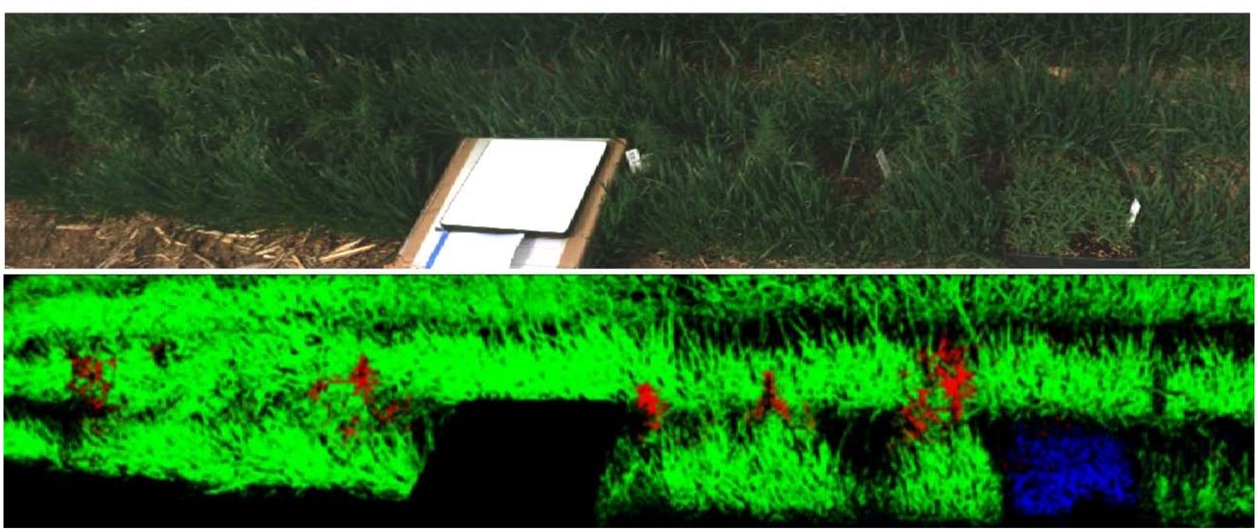


Figure 12. An RGB image of the scene (top) and the classification image (bottom) where winter wheat is in green, kochia is in red, and dicamba resistant kochia is in blue

This analysis is still in its early stages. It does however, show that there is a possible path forward to a real-world field situation where we do not have control over the lighting and lighting can change rapidly. This will be a novel technology, with wide-scale agricultural implications for precision weed control.

Hiring

The following people continue to work on this project:

- Dr. Joseph Shaw: subproject director (to receive partial summer salary only)
- Mr. Paul Nugent: Research Engineer and Ph.D. student (partial academic year salary)

- Mr. Andrew Donelick: Ph.D. student

Equipment Procurement

- No additional equipment was purchased during Quarter 3.

Expenditures

- Total Personnel: $1822.15
- Total Operations: $6157.62
- Total Equipment: $16,716.00

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

Progress towards milestones

Continues to be in the pre-production phase until Summer 2016.

Equipment Procurement

- Nothing additional to date

Expenditures

- Total Personnel: $0.00
- Total Operations: $6607.37
- Total Equipment: $7999.00

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

Progress towards milestones

A graduate student continues to collect the estimates from the literature and necessary data to estimate an elasticity value in case it is not found in the literature. Part of these data include elevator-level prices in Montana and fertilizer prices, both of which have been acquired using grant funds. By the next reporting date, I will be able parametrize the model.

Hiring

- No additional hires

Expenditures

- Total Personnel: $38,614.04
- Total Operations: $14,662.09

Participatory research network subproject of the Agriculture MREDI project

Progress towards milestones

Focus group files are currently being transcribed and preliminary data analysis will begin shortly. Plans are being made to collect additional data with organic producers at extension field days in June along with individual interviews. A conference abstract has been accepted to the Rural Sociological Society's annual conference. Colter Ellis and George Haynes continue to train Tom Woods, a graduate student from Political Science, who is an author on the presentation. George Haynes has begun discussions with MREDI team partners about data needs for the farm level cost:benefit analysis.

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

Hiring

- No additional hires beyond graduate student, Tom Woods.

Expenditures

- Total Personnel: $8420.91
- Total Operations: None to date

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

Hiring

- None to date

Expenditures

- Total Personnel: None to date
- Total Operations: $531.51