FY15 Specialty Crop Block Grant Program- Farm Bill

North Dakota Department of Agriculture

Final Performance Report
USDA Agreement # 15-SCBGP-ND-0026

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Project Title: Enhancing the Safe Use of Specialty Fruit and Vegetable Crops from Field to Table

NOGA# 15-317

Final Report

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Project Summary

This project aimed to enhance the utilization, safety and consumption of North Dakota-grown specialty crops (fruits and vegetables) through education and training. This project also increased the capacity of NDSU Extension personnel to deliver statewide programming in the area of food safety and nutrition education that included locally grown specialty crops. It was not part of a previously funded project.

The project addresses four key priorities of the North Dakota Department of Agriculture related to enhancing the competitiveness of specialty crops: 1) enhancing food safety, 2) assisting entities with developing Good Agricultural Practices; 3) increasing child and adult nutrition knowledge and consumption of specialty crops; and 4) developing local and regional food systems. Ensuring safe, nutritious food is critical not only for the consumer but also for the specialty crops industry as a whole. One foodborne illness outbreak could negatively impact the reputation of the entire food industry. This project continues to have potential outreach to a wide range of specialty crops in North Dakota, including apples, grapes, raspberries, strawberries, chickpeas, dry edible beans, onions, potatoes, pumpkins, leafy greens (spinach, leaf lettuce), snap beans, squash, sweet corn, tomatoes and others. This “field to table” project brought together a team including NDSU faculty/specialists in food science, nutrition, food safety, food law, horticulture, the network of Extension agents in at least 25 counties (nearly half the counties in North Dakota), with cooperation from state regulators and partners throughout North Dakota. This project promoted safe handling of fruits and vegetables among growers, small businesses, youth in 4-H clubs and classrooms and the end consumer, using both existing materials and materials developed during this project.

The U.S. Dietary Guidelines for Americans (2015) placed special emphasis on consuming more fruits and vegetables (with vegetables also including dry edible beans and pulse crops). According to the Centers for Disease Control and Prevention, about 14 percent of people meet the current recommendation for fruit and vegetable intake. Extension educators work with children and adults throughout North Dakota, including 4-H youth and children in hundreds of classrooms in schools, to teach them about current dietary recommendations in an engaging manner. The popularity of local foods represents a growing trend in North Dakota and throughout the U.S. as people want to "know their farmer" and "know their food." Members of the project team have worked closely with both the ND Departments of Agriculture and Health for many years. For example, the number of farmers markets selling locally grown foods in North Dakota is growing. According to the North Dakota Department of Agriculture, in 2004, North Dakota had only five farmers markets. In 2013, 46 farmers markets were in operation in North Dakota.

The objectives of the project were:

- To develop a project advisory group including Extension faculty, research faculty, Extension agents in agriculture and family and consumer sciences, growers and regulators.
- To increase awareness of existing Extension materials related to specialty crops (pulse education teaching kit, dry edible beans teaching kit, garden-to-table Extension publications, e.g. growing, preservation and use of grapes, berries, potatoes, tomatoes, etc.) through information releases, collaboration with stakeholders.
- To develop food safety demonstration training kits (pH meter, dehydrator, and canning equipment) to be housed in 25 counties and available for workshops and check-out.
- To create new educational materials related to specialty fruit and vegetable crops grown in North Dakota based on input from the advisory group, including online modules, fact sheets, information releases, Facebook posts and PowerPoint presentations.
- To increase knowledge and capacity related to food safety training through "train-the-trainer workshops" offered for Extension personnel using the newly developed materials.
- To offer face-to-face, webinar- and module-based training for growers and small food businesses related to food safety from field to table, including Good Agricultural Practices, FDA acidified foods/acidic food regulations and other topics of greatest need based on advisory group input.
- To increase knowledge of North Dakota specialty crops through the offering of classes for children in 4-H programs and schools related to North Dakota specialty crops.
- To evaluate use of resources, knowledge gain and changes in practices using survey methodology.

- ➢ Did the grantee provide a background for the initial purpose of the project, which includes the specific issue, problem, or need that was addressed by this project?
- ➢ Did the grantee establish the motivation for this project by presenting the importance and timeliness of the project?
- ➢ If the project built on a previously funded project with the SCBGP or SCBGP-FB, did the grantee describe how this project complimented and enhanced previously completed work?

Project Approach

In order to reach the objectives, these activities were performed:

- A project advisory group including a food scientist, a horticulture specialist, a food law expert, a marketing specialist, three Extension agents (two agriculture, one family and consumer science agent), continue to provide input on the educational tools, website development and/or have helped recruit participants to the webinars.
- We created 10 handouts, enlisting the aid of undergraduate students: Field to Fork: 1) Apples (FN1792); 2) Leafy Greens (FN1793); 3) Onions (FN1794); 4) Potatoes (FN1795); 5) Pumpkins (FN1796); 6) Raspberries (FN1797); 7) Snap Beans (FN1798); 8) Winter Squash (FN 1801); 9) Summer Squash (FN1837) and Sweet Corn (FN1799). All are available here: https://www.ag.ndsu.edu/fieldtofork/choose-your-crop
- We created additional "infographics" to share via social media and information releases to promote the "Wednesday Weekly Webinars" and the archives of the completed webinars.
- We created a series of information releases about specialty crops and these were provided to Extension agents and used in columns and blogs during the summer and fall of 2017; these materials were used in spring/summer 2018. The information releases are posted with the associated specialty crop at www.ag.ndsu.edu/fieldtofork/choose-your-crop
- Food safety demonstration training kits were disseminated to 25 counties. The kits include canning equipment and supplies, a binder of information and a pH meter with associated supplies. Ongoing training and support for the use of these kits is being provided.
- There is great interest in knowing more about preserving foods as the result of the Cottage Food Law enacted in North Dakota in August 2017, which allows home production and sales of North Dakota foods. The grant PI, a grant collaborator and an Extension agent all take part in the discussions related to food processing and selling foods at home; many of the foods of interest are ND specialty crops. A website was created by the North Dakota Department of Health, and many of the materials created by NDSU Extension are included on the "Cottage Food Resource List" (available at www.ndhealth.gov/FoodLodging/CottageFood.asp)
- A new partnership with North Central Region States has resulted in a new method to evaluate food preservation workshops, and the Institutional Review Board protocol was accepted by NDSU. To date, the tool has been used with more than 1,800 attendees in the North Central Region, including North Dakota. This will allow us to collate data across a wide range of programs. Data was entered related to food preservation workshops. Based on feedback from participants, the tool was updated in Spring/Winter 2018 and has been re-released for use in North Dakota as well as the North Central Region.
- Two courses on use of pH meters were provided for Extension staff. An update on the ND Cottage Food Law was held at the NDSU Extension Fall Conference in October 2017. Additional information was provided for Extension agents in May 2018.
- All 35 webinars offered from 2016 to 2018 are archived on the Field to Fork website, along with online modules, fact sheets and other items for broad use.
- A new "Eat Smart. Play Hard" magazine was released in May 2018. All of the recipes featured in the magazine (pages 21 to 25) include specialty crops grown in North Dakota. The magazine can be viewed at www.ndsu.edu/eatsmart or https://www.ag.ndsu.edu/food/health-and-

nutrition/eatsmart/eat-smart.-play-hard.-magazines-1/2018-19-eat-smart-play-hard-magazine-1/files/esph-magazine-2018-19.pdf

- A dietetic intern and agriculture Extension agent helped create two “workbooks” showing when to harvest specialty crop vegetables and fruits (with “life-size” photos of the items) and a weed identification guide showing typical weeds that can reduce productivity. Both of these items are posted on the website (www.ag.ndsu.edu/fieldtofork) and are being used to illustrate gardening principles to youth.
- Four videos were created based on safe home food preservation and released in May 2018. They are being shared on social media and will continue to be used in coming years, pending any changes in national recommendations.
- An online minicourse (“Selling Home-processed, Home-canned and Home Baked Foods Using Specialty Crops”) was created using “Versal” software that allows us to embed photos, quizzes and interactive quizzes. It is based on the guidelines of the North Dakota Department of Health and the Food and Drug Administration. The materials are housed at this website (www.ag.ndsu.edu/fieldtofork).
- The “Food Entrepreneur” website and “Local Foods” website are linked on the field to fork website to provide additional resources.
- A face-to-face growers workshop was held for 50 participants on Feb. 6, 2016 in Mandan, ND.
- Two food safety/preservation train-the-trainer workshops for a total of 50 Extension educators were held on May 3, 2016 and September 24, 2016.
- Three face-to-face grower workshops were held for about 75 total participants in Mandan on Feb. 4, 2017. The topics included two sessions that introduced the Food Safety Modernization Act (FSMA) (by Julie Garden-Robinson and collaborator, Holly Mawby from Dakota College at Bottineau) and an introduction to developing products using specialty crops (by Clifford Hall, Professor in Food Science).
- Julie Garden-Robinson provided two face-to-face gardening workshops for 60 people on Saturday, April 8, 2017 in Grand Forks on the topics of preserving and preparing produce.
- Julie Garden-Robinson provided a session on food fermentation of specialty crops for the North Dakota Department of Agriculture Local Foods conference, with 25 attendees, in Minot, ND on Feb. 2, 2018.
- Julie Garden-Robinson provided a food preservation workshop for the North Dakota Farm Bureau in Fargo, ND, with 20 attendees, on Nov. 17, 2018.

Project scope: We focused our efforts on developing materials based on specialty crops. We were able to “stretch” our resources by involving dietetic interns in helping develop educational materials at no cost to the grant. Funding was used to support the specialty crops described.

Project partners: Project partners outside of the Extension system included faculty from other disciplines (economics, food science, plant science, food law), teachers (high school, elementary), farmers market personnel, North Central Region food and nutrition specialists and agents, North Dakota Department of Health Division of Food and Lodging, North Dakota Department of Agriculture (local foods) and others. The partners assisted with marketing/dissemination of the educational materials, teaching and/or promoting webinars and sharing the information through social media. Perhaps the greatest outcome was enhanced communication among partners in North Dakota and the North Central Region of the U.S. to expand our capacity to provide relevant information to growers and small food processors related to many specialty crops.

- Were the activities and tasks performed during the entire grant period briefly summarized? This section should discuss the tasks provided in the Work Plan or the approved project proposal. This includes significant results, accomplishments, conclusions and recommendations, as well as favorable or unusual developments.
- If the overall scope of the project benefitted commodities other than specialty crops, did the grantee indicate how project staff ensured that funds were used to solely enhance the competitiveness of specialty crops?
- Did the grantee detail the significant contributions and role of project partners in the project?

Goals and Outcomes Achieved

The objectives and activities completed were listed in the previous section.

- We achieved all of our objectives in developing the new educational tools described previously. The tools included fact sheets, videos, webinars, modules, lessons and social media tools. We added "stretch goals" each year and developed additional items based on feedback from our workshops and webinars.
- We also evaluated outcomes of the education. On average, 40 people attended each of the webinars offered, and the participants include specialty crop growers, master gardeners, students and Extension agents/educators. To date, 1,045 people have completed the surveys delivered immediately after the webinar. A "post then pre" 5-point rating scale has been used for participants to rate knowledge prior to the webinar and knowledge after the webinar, and a significant change from an average rating of 2.61 (pre) to 4.10 (post) has been reported, and 55% of the respondents indicated they will change practices as a result of their participation. Most participants (59%) have been in the 41 to 64 age range, but participants have ranged from 18-25 to the 76 to 84 age range, and 88% of the participants are females. Most (59%) are home gardeners and/or educators (24%). Most people heard about the website through emails (63%) and news releases (9%). Many participants (150) have provided suggestions for future workshops and additional educational resources. We used the suggestions in the planning for the Winter 2017 and 2018 webinars.
- About 94% of the 33 teachers who have used the Specialty Crop educational magazine for elementary classrooms found that the reading level, social studies, languages arts and critical thinking activities were "right on target." About 97% of the teachers said the science activities were "right on target." Of those who rated the magazine, 73% were elementary teachers and 24% were associated with Extension (agents, 4-H volunteers). Of all the Ag Mags produced, the Fruits and Vegetables magazine has had the highest ratings for usefulness, with 92% rating it "very useful" and 8% rating it "moderately useful." The Specialty Crop Ag Mag received the 2017 First Place National Award from the National Federation of Press Women. Comments included: "The children's mag is full of challenging and interesting activities, during which the children learn about fruits and vegetables, often as secondary to learning math or language skills. It's an effective way to teaching about agriculture. It is a colorful publication, full of fun characters that

attract a child's interest. Teacher's guide is comprehensive and useful in guiding teachers in expanding activities and enhancing student understanding.

- According to survey evaluation of food preservation classes offered by Extension using the North Central Region tool, participants significantly increased their confidence in food preservation abilities, their understanding of the importance of following research-tested recipes, and their ability to identify and share with others trustworthy sources of information.
- Good analytics is the tool used to determine our reach and number of engagements on Facebook, Twitter and Instagram. From October 2016 to June 15, 2018, through Facebook our reach was 17,797 and the number of engagements was 931. Through Twitter, our reach was 12,138 and the number of engagements was 248. On Instagram, our reach was 1,452 and the number of engagements was 334.

- ➢ Did the grantee supply the activities that were completed in order to achieve the performance goals and measurable outcomes identified in the approved project proposal or subsequent amendments?
- ➢ If outcome measures were long term, was a summary of the progress made towards this achievement provided?
- ➢ Did the grantee provide a comparison of actual accomplishments with the goals established for the reporting period?
- ➢ Did the grantee clearly convey completion of achieving outcomes by illustrating baseline data that has been gathered to date and showing the progress toward achieving set targets?
- ➢ Did the grantee highlight the major successful outcomes of the project in quantifiable terms?

Beneficiaries

We reached specialty crop growers/farmers markets, consumers, small businesses/cottage food producers and Extension educators in Agriculture and Family and Consumer Sciences who used the developed resources to reach these target audiences. We also reached teachers who taught elementary-age students about "field to fork" specialty crops and their health. We reached 300 people directly through face-to-face workshops, 1,400 people through webinars and had more than 31,000 contacts through social media (Twitter, Facebook). We reached 25,000 people with recipes featuring specialty crops in an educational magazine. In addition, the handouts have been copied and distributed to reach thousands of additional people (and we have no way to count the additional contacts because anyone can print the materials). We engaged horticulture and food and nutrition specialists/agents to provide online workshops, which remain archived on YouTube for future viewing. We continued to work with farmers market personnel and cottage food producers, which is an area of continued growth. Forecasting potential economic results from our project was not an objective; however, we know on a national level that foodborne illness outbreaks cost the U.S. about $7 billion annually, and fruits and vegetables have been subject to numerous recalls in recent years.

- ➢ Did the grantee provide a description of the groups and other operations that benefited from the completion of this project's accomplishments?
- ➢ Did the grantee clearly state the number of beneficiaries affected by the project's

accomplishments and/or the potential economic impact of the project?

Lessons Learned

Overall, we reached or surpassed all of the objectives we set out to achieve. We initially planned to do more of our education "face to face" but in today's digital world, providing easy-to-access materials and training online became more relevant, and our webinars increased in popularity as the project progressed. We have reached a point where Wednesday Weekly Webinars are expected; a slate of 11 webinars will begin in February 2019. We had at least 1,400 participants in the online workshops.

Evaluation was sometimes a challenge, because people in general do not enjoy doing surveys after online or face-to-face workshops. To enhance the evaluation process, we made small "prizes" available (including fruit and vegetable cookbooks from another project) in drawings.

As an unexpected outcome, we became increasingly involved on a state and regional level with personnel in health departments and Extension systems through other projects that were linked to the objectives we set out in this project. In this way, we avoiding "reinvention" of existing materials and also shared the materials and lessons we learned in this project.

- Did the grantee offer insight into the lessons learned by the project staff as a result of completing this project?
- Did the grantee provide any unexpected outcomes or results that were an effect of implementing this project?
- If goals or outcome measures were not achieved, did the grantee identify and share the lessons learned to help others expedite problem-solving?

Contact Information

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Additional Information

To view the materials, archived webinars, handouts and links to other information, please see www.ag.ndsu.edu/fieldtofork We compiled existing materials with new materials developed in this project to create a "one stop shop" to allow our users easy access to a variety of materials on the topics of specialty crops.

- Did the grantee provide any additional information available (i.e. publications, websites, photographs) that is not applicable to any of the prior sections?

Development of improved fungicide application strategies for managing Sclerotinia head rot in confection sunflowers

Final Report

Project Summary

This project evaluated drop nozzles mounted on a high-clearance sprayer for their ability to deliver fungicides to the front of confection sunflower heads for management of Sclerotinia head rot, an economically important disease of confection sunflowers for which no management tools are currently available. The disease is common and widespread; in a bi-annual survey of sunflower production fields conducted by university research and extension personnel, Sclerotinia head rot occurred in an average of 23 and 39 percent of sunflower fields in North Dakota and Minnesota, respectively, in the 5-year period from 2011 to 2015. In fields with the disease, an average of 5 percent (North Dakota) and 10 percent (Minnesota) of plants were diseased. Crop rotation is an ineffective management tool for the disease due to the causal pathogen's broad host range and persistence in the soil, confection sunflower hybrids with resistance to the disease are not available, and traditional methods of applying fungicides over the top of the crop canopy confer poor fungicide deposition to the front of sunflower heads where the disease is initiated and have not provided control of head rot. Sclerotinia head rot develops when the fungal pathogen *Sclerotinia sclerotiorum* infects sunflower florets, and management of the disease with fungicides requires fungicide deposition to the front of sunflower heads. The delivery of fungicides above the crop canopy through nozzles mounted directly on a high-clearance boom; the delivery of fungicides horizontally to the front of sunflower heads with drop nozzles mounted on a high-clearance boom; and a non-treated control were compared. The '360 Undercover' drop nozzle (360 Yield Center; Morton, IL) and the 'FK110 Plus 2' drop nozzle (Kuhn Landmaschinen AG; Dintikon, Switzerland) were evaluated, with multiple nozzle types and configurations tested on the '360 Undercover' drop nozzle. This was a new project that did not build upon any previous award from the Specialty Crop Block Grant program.

Project Approach

Project execution (tasks completed): Field trials were established in Carrington and Oakes, ND using a widely grown confection sunflower hybrid. A hail storm in early July caused significant damage to the field trial in Carrington, resulting in high variability in the height and placement (relative to the seeding row) of sunflower heads: plants exhibited varying degrees of stunting, secondary shoot development, and stems exhibiting crooked regrowth from the point of hail injury. In order to evaluate the fungicide deposition patterns that would be expected in sunflowers not damaged by hail, the plants most severely impacted by hail were eliminated from the study by clipping the heads shortly before fungicides were applied. Fungicides were applied at early to mid-bloom when 90 percent (Carrington) or 97 percent (Oakes) of plants had open ray flowers, and fungicide deposition to the front of sunflower heads was assessed with water-sensitive spray cards affixed to the front of sunflower heads. Across all treatments, the fungicide Proline (prothioconazole, 480 g ai/liter; Bayer CropScience) was applied at 5.7 fl oz/ac with 0.25% v/v Silkin (WinField Solutions, St. Paul, MN), a spreader-sticker adjuvant. Sunflowers were inoculated with laboratory-produced spores of *S. sclerotiorum*, and overhead irrigation delivered through micro-sprinklers was utilized to promote pathogen infection and disease development. Disease levels were assessed at or shortly before maturity, with sunflower rust – another common disease of sunflowers – assessed in addition to Sclerotinia head rot. Rust, which developed from natural inoculum, was present at economically important levels at both study locations. The trials were harvested, and seed yield and quality – including contamination of the harvested grain with sclerotia (resting structures of the Sclerotinia fungus) – were assessed. The water-sensitive cards utilized to assess spray deposition were scanned and assessed computationally for the percent of the card surface to which fungicides were deposited.

Major results and conclusions:

1. Relative to traditional fungicide applications over the top of the canopy, the 'Undercover 360' drop nozzle sharply increased fungicide deposition to the front of sunflower heads; the 'FK110 Plus 2' drop nozzle did not.
    - The 'Undercover 360' drop nozzle is equipped with a single set of nozzles targeted at sunflower heads, while the 'FK110 Plus 2' drop nozzle is equipped with pairs of nozzles targeted at the mid-canopy, upper canopy and sunflower heads.
2. The planting direction of sunflowers may influence the utility of drop nozzles.
    - In Oakes, where sunflowers were planted east-west, driving direction of the sprayer strongly influenced fungicide deposition conferred by drop nozzles and the corresponding sunflower yields under disease pressure.
    - In Carrington, where sunflowers were planted at northwest-southeast angle, fungicide deposition through drop nozzles and the corresponding sunflower yields under disease pressure were similar irrespective of sprayer driving direction.
3. The application of fungicides through the 'Undercover 360' drop nozzle sharply increased the yield response conferred by fungicides.
    - In Oakes, applications through drop nozzles (with the sprayer driving east) increased sunflower yields 71% (from 899 to 1535 lbs/ac, a statistically significant increase), while traditional fungicide applications increased yields 42% (to 1273 lbs/ac).
    - In Carrington, where disease pressure was lower, applications through drop nozzles (irrespective of driving direction) increased sunflower yields 19% (from 0.24 to 0.28 lbs/plant), while traditional fungicide applications increased yields 8% (to 0.26 lbs/plant).
4. The mechanism for the yield increase associated with drop nozzles was unclear: Control of sunflower rust was nearly identical irrespective of fungicide application method, and the use of drop nozzles did not reduce end-of-season levels of Sclerotinia head rot relative to traditional fungicide application methods.

If fungicide applications suppressed Sclerotinia head rot, delaying disease development, the observed result – sunflower yield responses that correspond to increases in fungicide deposition despite similar end-of-season disease levels – would be expected. When head rot disease development is delayed, increased proportion of the kernels on diseased heads are able to complete seed development, and the frequency with which diseased heads shatter and fall to the ground prior to harvest is reduced. In this project, disease was only assessed at or near sunflower maturity in this project, and it is unclear whether differences in disease progression occurred. In follow-up research, additional disease assessments will be added in order to permit an assessment of disease progression.

Role of project partners: Co-investigator Kelly Cooper and his staff in Oakes managed the research trial in Oakes, conducting the general agronomics, irrigation, and pathogen inoculations.

Ensuring that the project funds only benefitted specialty crops: In addition to confection sunflowers, Sclerotinia head rot also affects oilseed sunflowers (not considered a specialty crop). However, commercial hybrids with high levels of resistance to head rot are available for oilseed sunflowers, and resistant hybrids are the preferred management strategy for this disease in oilseed sunflower production. Fungicides are of interest to producers of confection sunflowers due to the absence of commercial hybrids with resistance to this disease.

Goals and Outcomes Achieved

Level of disease pressure and consistency of disease pressure across plots within field trials.

- The goal of achieving end-of-season Sclerotinia head rot severity index levels of at least 20% in the non-treated control was met in Oakes (76% severity index) but not Carrington (8% severity index). More aggressive irrigation was needed in Carrington.
- The goal of achieving a coefficient of variation (a statistical measure of the consistency of results) below 25 for the head rot severity index was met in Oakes (CV = 15.4) but not Carrington (CV = 66.7). The high variability in disease results was in Carrington was caused by the low numbers of plants per plot resulting from hail damage.
- The goal of achieving a coefficient of variation below 15 for the yield data was met in Carrington (CV = 14.1) but not in Oakes (CV = 32.8). The high variability in yield data in Oakes was caused by a late-season wind storm which resulted in significant lodging and shelling of heads.

1. Disease control achieved with fungicides.
   - The target of achieving at least 50% control of Sclerotinia head rot was not achieved.
     - The best fungicide application strategy reduced end-of-season Sclerotinia head rot severity index by 21% at one testing location (Oakes).
     - A fungicide application strategy was identified that nearly doubled the yield response to utilizing fungicides in confection sunflowers under disease pressure: Relative to traditional fungicide applications over the top of the canopy, applying fungicides with the ‘Undercover 360’ drop nozzle equipped with a pair of hollow-cone nozzles (on side ports) increased the yield response to fungicides from 0.02 to 0.04 lb/plant (Carrington; non-treated control = 0.24 lb/plant, drop nozzle = 0.28 lb/plant) and from 374 to 719 lbs/ac (Oakes; non-treated control = 899 lbs/ac, drop nozzle = 1618 lbs/ac).
     - The poor relationship between the observed yield response and end-of-season disease levels will be addressed in follow-up research.
2. Dissemination of research findings to stakeholders
   - Approximately 300 stakeholders (primarily crop advisors and industry agronomists, but also producers, extension personnel and researchers) were directly reached through talks given at research and outreach events. This exceeded the goal of directly reaching 100 stakeholders through talks.
   - Because of unresolved questions regarding what drove the observed yield responses to applying fungicides through drop nozzles, publishing results from this project will be delayed until follow-up research is completed. However, the total outreach goal of reaching 200 stakeholders (100 through talks and 100 through results published online) was exceeded given the high number of stakeholders reached directly through talks.
3. Adoption of research findings by producers.

- The goal of at least five stakeholders adopting findings from this research to improve their disease management decisions was almost certainly met. The poor fungicide deposition associated with traditional application methods documented in this project was of met with high interest by the crop advisors and industry agronomists attending NDSU's Western Crop and Pest Management School, where key results from this project were presented. The talk was very well received and was the highest-rated talk at this major extension event, which draws it audience primarily from the major sunflower production region in North Dakota. In follow-up conversations, crop advisors attending the event indicated that this information would make them less likely to recommend the use of fungicides to control Sclerotinia head rot with traditional application methods.

Beneficiaries

This project is expected to benefit the nearly 300 farmers producing confectionary sunflowers on approximately 100,000 acres in North Dakota and Minnesota (2012 USDA Census of the Agriculture). The use of fungicides to control Sclerotinia head rot is periodically promoted by fungicide registrants (DuPont, for instance, has promoted is fungicide Vertisan for head rot control). While follow-up research will be needed before recommendations can be made relative to the application of fungicides through drop nozzles, at a minimum, this research indicates that the application of fungicides with traditional application methods (over the top of the canopy) is of limited value for controlling Sclerotinia head rot. If this information results in avoiding unnecessary fungicide applications to 5% of the acreage grown to confection sunflowers (a conservative estimate if conditions are cool and wet during bloom), this would represent savings of approximately $150,000/year assuming costs of $30/acre (fungicide product plus application cost).

Lessons Learned

Major lessons learned from this project are that (1) fungicide applications directed horizontally at sunflower heads can confer equivalent control of rust in confection sunflowers as traditional fungicide applications over the top of the sunflower canopy; (2) sharp increases in fungicide deposition to the front of sunflower heads can be achieved with the use of drop nozzles; and (3) assessments of Sclerotinia head rot disease progression may be needed to properly explain the yield responses to fungicide applications in sunflowers.

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Pea and Lentil Market Analysis

Final Report

Project Summary

There has been an increasing demand for plant protein in the United States. Specifically, manufacturers have been interested in replacing traditional (animal) protein with plant protein due to *IgE* mediated allergies, increasing costs and changing consumer preferences (e.g., vegan preferences). Legumes, including peas, lentils and other pulses, are an important source of protein in addition to carbohydrates and fiber. In light of changing consumer preference, interest in a healthy diet and increasing demand for plant-based protein, it is important to explore and quantify the market potential for pulses, especially peas and lentils, as food ingredients.

This report consists of pea and lentil market analysis, specifically focusing on two markets, including the ingredient market and commodity market. The ingredient market analysis consists of analyzing current trends, major market categories in which pea and lentil are used as main ingredients, and identifying the manufacturers and/or distributors of the products. After identifying the trends in the ingredient market, we forecast the major market growth categories in the ingredient market using autoregressive integrated moving average (ARIMA) models. The pea and lentil commodity market analysis considers supply and demand, including the identification of export markets for U.S. peas and lentils. Similarly, after identifying the trends in commodity markets, we use both the ARIMA model and neural network auto regression (NNAR) to forecast production, prices, and utilization in the United States.

The results of the ingredient market analysis indicate that the top market categories for peas include frozen food, cereal bars, savory snacks, and canned food. The top market categories for lentils include soups, savory snacks, dried food/pasta/noodles/pizza, crackers, and sauce dips. We also identified some of the market categories that manufacturers need to avoid because of their diminishing growth. Ingredient market categories that show a decreasing trend include chilled meats, artisanal bread rolls, breakfast cereals, and cookies (sweet biscuits). The results of the commodity market analysis for peas and lentils indicate that production of both peas and lentils is highly volatile, and show a decreasing trend during 2017–2020. The pea price forecast shows an increasing trend during 2017–2020, while the lentil price forecast shows a decreasing trend during the same period. Results of ingredient market analysis coupled with commodity market analysis help pea and lentil growers, processors of North Dakota, and the Northern Pulse Growers Association with the information about manufacturers and/or distributors of products containing peas and lentils as ingredients as well as the general market structure of peas and lentils.

This project did not build on a previously funded project with the SCBGP.

Project Approach

Project approach consists of introduction, literature review, econometric analysis, discussion of the results, and finally conclusions and recommendations. All these sections were addressed in detail in the project final report included in the additional information section.

Results of the project including the list of manufacturers and/or distributors of products containing peas and lentils are provided, which help to improve the competitiveness of the peas and lentils for North Dakota growers. For example, the growers or the association representing the growers can contact directly the manufacturers or distributors to enhance the market accessibility for their produce.

Travel to the International Food Technologists (IFT) industry conference in Chicago to disseminate results was completed in July 2016.

Few unfavorable developments have occurred during the analysis. For example, the domestic food utilization data are available at an aggregated level including both peas and lentils. We wish to have the food domestic utilization data at a disaggregated level to understand peas and lentil consumption at the individual level.

I thank Dr. Frayne Olson, and Dr. David Ripplinger for their help since the start of this project. I also thank them for their productive comments in the earlier drafts of the final project report. I thank Dr. Clifford Hall for his contribution to the review of abstract submitted to IFT conference. I thank Dr. Aaron DeLarporte for his productive comments in earlier drafts of the grant report. Last but not the least, I thank Shannon Berndt for helping us find the information as well as reviewing our work all along this project period.

Goals and Outcomes Achieved

Project report consists of goals and outcomes achieved. For example, goal 1, which is to determine pea and lentil market trends are analyzed as mentioned in the final report. Also, goal 2, which is identification of market potential using the econometric analysis of forecasting the major growth categories in which peas and lentils are used as ingredients. In total, we identified eight market categories in the ingredient excluding the pet food market. We also analyzed the commodity market analysis of production, price, and domestic food utilization of peas and lentils. We highlighted the results/outcomes of the project in the final report in the additional outcomes section.

I did go to the International Food Technologists (IFT) industry conference to disseminate the results at the conference to the food professionals. In the conference, I also interacted with about 10 ingredient companies and learnt how they have been using peas and lentils in developing their products. I did not get a chance to go to the NPGA annual convention meeting at Minot, due to unforeseen circumstances. But, we are in contact with Shannon Berndt of the NPGA to discuss ways to disseminate the results of the final project report to the growers in North Dakota. The NPGA has the contact information of the interest groups including growers, and processors of pulses to which we decided to send the final report or highlights of the report directly to them or upload the final report onto the website of the NPGA. In this way, we plan to achieve the dissemination of the results to growers as promised in the proposal.

Additionally, I analyzed and estimated a non-linear model which is Neural Networks Autoregression (NNAR) in addition to linear model, Autoregressive Integrated Moving Average (ARIMA) model. Originally, in the proposal, we promised an econometric analysis using only ARIMA/VAR model. However, we thought it would be the best if we compare two different models in order to get the best of the results. Therefore, we estimated NNAR model in addition to ARIMA model.

Finally, we also analyzed global market for U.S. peas and lentils. We analyzed the global market for U.S. peas and lentils along with NNAR model using the funds diverted from market data section to salary section.

Beneficiaries

Beneficiaries include North Dakota pea and lentil growers, the Northern Pulse Growers Association (NPGA), and North Dakota processers. Approximately, there are 839 growers who cultivate peas and lentils along with other pulses in the region including North Dakota, Montana, and South Dakota. However, about half of the total growers cultivate peas and lentils in fewer proportions compared to other pulses. According to the NPGA, about 66 pulse processors are present in the region. The results of ingredient market analysis will benefit about 420 growers, and 66 processors in the region. For example, the list of the manufacturers and distributors would help growers as well as processors about the potential market information for their product.

Lessons Learned

I enjoyed working on this project. However, there are few lessons I learnt while working on this project. First, availability of the data is challenging. Fortunately, we worked closely with the Northern Crops Institute in understanding the ingredient market in detail. Shannon Berndt from the NPGA also helped us with the project whenever we need more information about the commodity data sources and review of the poster presented at the IFT conference. Second, when analyzing the commodity market analysis, I learnt that analysis of a linear model (ARIMA) coupled with a non-liner model (NNAR) model helped us in comparing the results of two models to substantiate our claims in the project.

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Additional Information

Please find the poster attached to the email. The poster was presented at the IFT conference in July 2016 at Chicago, USA. Attached is also the final Pea and Lentil Market Analysis report that includes all data.

Optimizing agronomic practices for faba bean production in North Dakota

Final Report

Project Summary

The objectives of this project were to:

1. Determine the optimal planting date, planting rate, and varietal adaptation for faba bean in western and eastern ND.
2. Identify diseases that may attack faba bean in ND and identify the most cost-effective fungicides and their proper timing.
3. Evaluate faba bean tolerance to preemergence- and postemergence-applied herbicides.
4. Identify the need, proper timing, and effectiveness of pre-harvest desiccation.

Project Approach

- The following studies were conducted at two locations: Seeding date, seeding rate, variety trial, herbicide tolerance, crop desiccation, and fungicide efficacy.
- All studies were conducted using traditional small plot field methods.
- All studies were considered successful except for studies conducted at Carrington that were severely damaged by hail.
- This information was presented at multiple grower meetings in Jan-Mar 2017 and summer 2017.

Goals and Outcomes Achieved

Field studies:

- Seeding date study:
  Minot: Faba beans are a cool season legume that are known to tolerate cold soils and frost. The objectives of this trial were to observe and document agronomic characteristics, seed quality and seed yield of two varieties that were planted at 2 week intervals over a period of a month and a half. As would be expected, statistically significant genetic by environmental interactions were recorded on most characteristics observed with the exception of lodging and grain protein. Although seedling emergence took 17 days for the first seeding date, this delay did not result in any more seed mortality compared to other seeding dates. Plants tended to initiate flowering sooner as seeding date was delayed and had a shorter duration of flowering, which probably contributed to the declining yield trend. Plants tended to grow taller as planting dates were delayed and the first seed pod was higher off the ground as well. Statistical differences between seeding dates were also noted for test weight and kernel weight, but there was not a clear trend for these characteristics. There was a declining trend for seed yield with delayed seeding although the first three dates produced statistically similar yields.

  Langdon: Faba Beans are a long-maturing, cool season, annual legume that grows best under moist conditions which needs to be planted early to obtain higher yields. The objective of this trials was to determine the optimum seeding date for our region. Differences occurred between the two varieties for many agronomic traits. Boxer had a higher yield than Tabasco. Both varieties responded similarly to seeding date for most agronomic traits and this resulted in few variety x date interactions. The June 6 seeding date had the lowest yield for both varieties. The May 3 seeding date was optimum for both varieties, but differences were not always significant compared to the May 3 and 23 seeding date.

- <u>Seeding rate study</u>:

  Minot: Summary: Faba beans are large seeded crop, requiring a large volume of seed to be planted, thus restricting the planting speed and number of acres that can be planted in a day. Results of this trial would indicate that higher seeding rates do not have a direct impact on seed yields. Small but statistically significant interactions between seeding rates and the number of days to the end of flowering and days to mature were observed.

  Langdon: Faba beans are a long-maturing, cool season, annual legume that grow best under moist conditions. The current recommended seeding rate for faba beans is 4 seeds per square foot. Faba beans are very large seeds often requiring a seeding rate of 3-4 bushels per acre, depending on the seed size. The research objective of this trial was to help determine the optimum seeding rate for our region. There were no significant differences in yield between the seeding rate of 3 to 6 seeds/ft^2 for the variety Boxer, whereas the variety Tabasco had a significantly lower yield at the lowest seeding rate. Most of the other significant differences between agronomic traits occurred between varieties.

- <u>Variety trial</u>:

  A faba bean variety trial was conducted at Minot and Langdon. Yields ranged from 4415-6100 lb/A at Minot, and 2238-6052 lb/A at Langdon.

- <u>Herbicide tolerance study</u>:

  Minot: The objective of the study was to evaluate faba bean tolerance to preemergence (PRE) and postemergence (POST) herbicides. Faba beans were planted May 2. PRE and POST treatments were applied May 4 and June 7, respectively. Faba beans were 4-5 inches tall at the POST application. Basagran caused only slight crop injury soon after application. Raptor applied alone caused moderate to severe stunting, although plants recovered somewhat over time. In contrast, only slight injury was observed where Raptor was tank mixed with Basagran. Raptor applied alone resulted in reduced crop yield. Herbicides that caused little injury included Sharpen, Spartan, Metribuzin, Authority MTZ, Spartan Elite, Prowl, Valor, and Fierce. Reflex applied POST caused excessive crop injury.

  Carrington: Very little crop injury was observed with any herbicide treatment. Yield data were affected by hail during the season resulting in a high coefficient of variation for yield. Thus, the yield data for 2016 at Carrington are not reliable.

- <u>Herbicide desiccation study</u>:

Minot: Reglone desiccated leaves, pods, and stems faster than Glyphosate or Glyphosate + Sharpen. Faba beans harvested 7 days after treatment yielded more than faba beans harvested 14 days after treatment.

Carrington: Data are not reliable due to hail damage.

- Fungicide efficacy study:
  Minot: Dry conditions and low disease pressure resulted in inconclusive results for fungicide timing or efficacy.

  Carrington: Data are unreliable due to hail damage.

- Research results were presented at winter and summer meetings in Williston, Wildrose, Crosby, Minot, Underwood, Langdon, and Carrington. Approximately 500 growers and industry personnel attended these meetings. This included meetings sponsored by the Northern Pulse Growers Association.

Beneficiaries

- At least 500 growers will benefit from this information if they choose to grow faba beans. Approximately 500 growers and industry personnel benefitted by attending the winter and summer meetings.

Lessons Learned

- Earlier planting dates tended to favor higher faba bean yield.
- Seeding rate of 6 plants/ft2 did not differ significantly from 4 or 5 plants/ft2.
- Varieties yielded over 6,000 lb/A in the variety trials.
- Faba beans were tolerant to several preemergence and postemergence herbicides. Thus, adequate control of many common weeds should be achievable once the herbicides are labeled.
- Diquat appears to provide adequate crop desiccation.
- In general, faba beans appear to grow well in eastern and western ND.

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Optimizing fungicide application strategies for improved management of Sclerotinia in dry edible beans

Final Report

Project Summary

Sclerotinia (white mold) is a critical production constraint for dry beans in North Dakota, causing significant economic losses when cool, wet weather occurs during bloom. In annual surveys of dry bean producers conducted by the NDSU Extension Service in coordination with the Northarvest Bean Growers Association, 61 percent and 46 percent, respectively, of dry bean producers in North Dakota and Minnesota identified white mold as their worst disease problem from 2013 to 2016, the four most recent years for which data are available. Fungicides were widely utilized to manage the disease; the fungicides thiophanate-methyl and boscalid – products with poor efficacy against other dry bean diseases that are utilized almost exclusively for Sclerotinia control – were applied to an average of 50 percent (thiophante-methyl) and 32 percent (boscalid) of the dry bean acreage across both states during the same period. Fungicides applications are often made with a poor understanding of the optimal timing and optimal number of fungicide applications relative to canopy closure and environmental conditions. The goal of this project was to provide rigorous, research-based recommendations on fungicide usage with the goal of reducing production costs by eliminating unnecessary applications.

This project was designed to expand on a Specialty Crop Block Grant awarded in 2014 by (1) testing how the timing and duration of conditions favorable for Sclerotinia influences optimal fungicide application timing; (2) assessing how the profitability of a second fungicide application is influenced by the row spacing in which dry beans are seeded; (3) evaluating the impact of precipitation patterns on optimal fungicide use within a context that can be adopted by irrigated producers to optimize irrigation scheduling where Sclerotinia is a limiting factor; and (4) expanding field trials to locations beyond Carrington.

A severe hail storm in Carrington caused moderate to severe defoliation of the dry beans at bloom initiation, precluding the ability to address the original research objectives, and the studies in Carrington were repurposed to address related research objectives.

Project Approach

Project execution (tasks completed): Field trials were established in Carrington, Oakes, and Hofflund, ND in accordance to the work plan. Field trials were planted into conventionally tilled fields, with agronomics were conducted in accordance with recommended practices.

- To evaluate optimal <u>fungicide application timing</u>, two pinto bean varieties differing in plant architecture were seeded to wide and narrow rows, and micro-sprinkler systems were established to deliver irrigation over 3-week periods beginning at late vegetative growth (V4), full bloom (R2), or mid-pod development (R4). The hail storm in early July at bloom initiation (R1) caused nearly complete defoliation of the dry beans, precluding white mold disease development and the ability to address the original research questions. At late pod-fill, when the dry bean canopy had mostly recovered, the plots with nearly complete canopy closure were utilized to evaluate the impact of fungicide application methods (nozzle spray pattern, application pressure, droplet size, spray volume, and boom height) on fungicide deposition within the dry bean canopy. Fungicide deposition was assessed with water-sensitive spray cards placed within the crop canopy; the cards were scanned and assessed computationally for the percent of the card surface to which fungicides were deposited.

- To evaluate the returns to applying fungicides once versus twice, two pinto bean varieties differing in plant architecture were seeded to wide and narrow rows and subjected to one of three irrigation regimes differing in the soil moisture level at which irrigation was delivered. Testing was conducted in Oakes and Hofflund with irrigation delivered via a linear irrigator and in Carrington with irrigation delivered via micro-sprinklers. The Carrington study, which suffered moderate hail damage at bloom initiation, was repurposed to test application rates of a commonly employed fungicide, with a single fungicide application made at the R3 growth stage when the canopy began to recover.

Significant results:

1. Optimizing fungicide deposition. Assessments of fungicide application timing were precluded by severe hail damage, and the plots that had recovered from the hail storm by late pod-fill were utilized to evaluate application methods to optimize fungicide deposition to the lower canopy where most Sclerotinia infections occur.
   - Boom height had little impact on fungicide deposition to the lower canopy. Instead of setting the boom at the height above the canopy recommended by spray nozzle manufacturers, some producers lower the boom when targeting Sclerotinia, hoping to force additional fungicide into the lower canopy. Lowering the spray boom such that the recommended distance was measured from the canopy mid-point or three-quarters of the canopy height had no effect on fungicide coverage or estimated spray volume deposition to lower leaves and stems.
   - When targeting lower leaves, spray volume was optimized at 15 gal/ac. Increasing spray volume from 10 to 15 gal/ac (an increase of 50%) resulted in 147% increase in spray coverage and 162% increase in estimated fungicide deposition to lower leaves. Increasing spray volume from 15 to 20 gal/ac (an increase of 33%), had no impact on spray coverage or estimated spray deposition to lower leaves.
   - When targeting the main stem at mid-canopy height, spray volume was optimized at 20 gal/ac. Increasing spray volume from 10 to 15 gal/ac conferred a 95% increase in spray coverage and a 68% increase in estimated fungicide deposition to stem tissue at mid-canopy height, and increasing spray volume from 15 to 20 gal/ac conferred an additional 169% increase in spray coverage and 250% increase in estimated fungicide deposition.
   - Flat-fan nozzles delivering medium-coarse to coarse droplets optimized fungicide deposition to lower leaves and stems. Flat-fan nozzles delivering fine to fine-medium droplets were less effective, and twin-jet nozzles were less effective across the full droplet spectrum.
2. Optimizing application rates of the fungicide thiophanate-methyl. Hail damage in Carrington precluded assessment of the returns to one versus two fungicide applications at that study location, and the returns to applying 30 vs. 40 fl oz/ac of Topsin-M FL (the most commonly utilized commercial formulation of the fungicide thiophanate-methyl) were evaluated with a single application at the R3 growth stage when the canopy began to recover. In both ‘Stampede’ and ‘Lariat’ pintos seeded to both narrow and wide rows, the high application rate improved disease control and yield and was more profitable than the low application rate. Averaged across both varieties and both row spacings, the low application rate reduce disease levels (the percent of the canopy diseased) from 15% (non-treated) to 11% and increased yield from 2,295 to 2,411 lbs/ac.

3. Returns to one versus two fungicide applications. Applying Topsin-M at 30 fl oz/ac at early bloom reduced the percent of canopy diseased from 31 to 23%; when a second fungicide application (Endura at 8 oz/ac) was made 2 weeks later, disease severity was reduced to 16%. However, disease development occurred late in crop development when yield potential was already set, and none of the fungicide applications impacted yield. The response to fungicides could not be rigorously assessed in Hofflund due to low disease pressure (2% in the non-treated control).
4. Impact of row spacing. Planting pinto beans to wide (28- or 30-inch) rows had little impact on Sclerotinia but reduced yields relative to narrow (14- or 15-inch) rows.
    - Averaged across fungicide treatments, wide row spacing decreased end-of-season Sclerotinia (percent of canopy exhibiting white mold) from 13.8 to 13.6% ('Lariat' pintos, Carrington); 10 to 8% ('Stampede' pintos, Carrington); 24 to 23% ('Lariat' pintos, Oakes); and 2 to 1% ('Stampede' pintos, Hofflund).
    - Averaged across fungicide treatments, wide row spacing reduced yield from 2,449 to 2,119 lbs/ac ('Lariat', Carrington); from 2,660 to 2,433 lbs/ac ('Stampede', Carrington); 3,065 to 2,650 lbs/ac ('Lariat', Oakes); and 3,500 to 3,175 lbs/ac ('Stampede', Hofflund).
5. Irrigation scheduling. Moderate deviations from the standard recommendation of irrigating when soils drop below 50% volumetric water content had no impact on dry bean yield, white mold severity, or returns to fungicides (one vs. two applications or low vs high application rates) at any of the study locations (Carrington, Oakes, or Hofflund).

Role or project partners: Co-investigator Kelly Cooper and his staff in Oakes conducted all agronomic management of the dry beans in Oakes and applied fungicide and irrigation treatments; co-investigators Tyler Tjelde and Audrey Kalil and their staff in Williston conducted all aspects of the field trial in Hofflund except seed procurement and completion of reports.

Impact on non-specialty crops: The research conducted in this project was specific to dry edible bean production and cannot be directly applied to other crops. Fungicide application timing and frequency recommendations are often crop-specific, and fungicide application strategies that are optimal for Sclerotinia control on dry beans are unlikely to be optimal for Sclerotinia control on other crops such as soybeans. Likewise, fungicide recommendations are largely irrelevant for dry beans sold at farmers markets or locally through CSAs, as dry edible beans sold through such outlets are often exclusively or nearly exclusively organic.

Goals and Outcomes Achieved

1. Level of disease pressure and consistency of disease pressure across plots within field trials:
    - The target of 20% disease severity (percent of the canopy diseased) in the non-treated controls was met in the study conducted in Oakes (31% of canopy diseased). The levels of disease in the study in Carrington that was not lost to hail were close to this target (11% in the 'Stampede' pintos and 18% in the 'Lariat' pintos) and were sufficient for reaching rigorous conclusions. There was insufficient disease pressure in Hofflund (2% in the non-treated control) to reach rigorous conclusions relative to disease management.
    - In all of the field trials, the target for consistency of disease pressure across treatments (maximum CV of 25) was met for three of the six field trials; where it was exceeded, results were still sufficiently consistent across replicates to permit rigorous, statistical separation of treatments.

- In the two field trials in which yield could be assessed, the target for consistency of field pea yields (maximum CV of 15) was met in all of the field trials. Coefficients of variation for yield data ranged from 6.4 to 10.7.

2. Successful outcomes:
   - This is the first study to document the impact of nozzle spray pattern and droplet size on fungicide deposition to the lower canopy of pinto beans. The finding that flat-fan nozzles may facilitate better spray deposition to the lower canopy than twin-jet nozzles is consistent with previous research conducted in soybeans, but the finding that medium-coarse to coarse spray droplets facilitated better spray coverage in the lower canopy differs from research conducted in soybeans, where medium-fine droplets were optimal.
   - This study demonstrated that seeding pinto beans to wide (28-inch) rows can sometimes result in very limited gains in disease management while resulting in a strong yield penalty even under high white mold disease pressure.
3. Dissemination of research findings to stakeholders:
   - Major findings from this study were communicated directly to approximately 135 stakeholders (primarily producers, crop advisors and industry agronomists, but also extension personnel and researchers) through talks given at research and outreach events in 2017 and will be communicated to an estimated additional 400 stakeholders at scheduled talks at major extension events in North Dakota in early 2018. This exceeds the goal of directly reaching 400 stakeholders through talks.
   - Due to the hail damage in Carrington and low disease pressure in Hofflund, none of the research questions addressed in this project were evaluated at more than one study location in 2016, and publishing results from this project will be delayed until follow-up research is completed. However, the total outreach goal of reaching 500 stakeholders (400 through talks and 100 through results published online) will be met through direct contact with stakeholders through outreach talks.
4. Adoption of research findings by producers.
   - The goal of at least 100 stakeholders modifying their production practices on the basis of the findings from this study is likely to be met once follow-up research is completed and rigorous conclusions can be reached.

Beneficiaries

This research findings generated in this project will primarily benefit the approx. 3,400 farmers producing dry beans in North Dakota, Michigan and Minnesota, where cool, wet weather favorable for Sclerotinia disease development often occurs when dry edible beans are in bloom.

Lessons Learned

- The severe hail storm in Carrington in early July that precluded assessment of the original research objectives at the Carrington location, but – with some creativity – the field studies were able to be redirected to address related research questions. Very little information was available on the response to increasing the application rate of thiophanate-methyl to the high end of the labeled rate, and a rigorous data set comparing the intermediate and high application rates of this fungicide was assembled. No information was available on the impacts of droplet size and nozzle spray pattern on fungicide deposition within pinto bean canopies, and preliminary data were collected suggesting that flat-fan nozzles emitting medium to coarse droplets may optimize fungicide deposition to the lower canopy. This experience demonstrates that, with some flexibility and creativity, unexpected challenges can provide unique opportunities to address research needs.

- The strong agronomic performance of pinto beans in narrow (14-inch) rows under significant white mold pressure is contrary to conventional wisdom; most producers concerned about white mold adopt wide row spacing. The strong yield advantage and modest differences in disease severity observed in this project are consistent with findings from previous research, and follow-up studies to rigorously assess the impact of row spacing on dry bean agronomic performance would be of value.

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<u>Project Title:</u> Enhancing Tree Selection and Evaluating Tree Species in Western ND

<u>NOGA#:</u> 15-SCBGP-ND-0026, 15-322

Final Report: *2018*

<u>Partner Organization:</u> North Dakota State University

Project Summary

North Dakota is a diverse intercontinental environment with limited woody plant species that have been evaluated for use in USDA hardiness zones 3 – 4. The North Dakota State University Woody Plant Improvement Program was developed in the early 1970s focusing on plants with increased disease tolerance and winter hardiness for North Dakota landscapes (limited release list can be found at http://ndsuresearchfoundation.org/horticulture). This program has been essential in assisting nursery producers with growing proper planting material to be utilized by city foresters, landscapers and homeowners across ND. Most of the research and planting evaluations have occurred at the NDSU Dale E. Herman Research Arboretum located near Absaraka, ND (Cass County) and are not representative of growing conditions for Western ND. Currently available nursery stock is being shipped and utilized in Western ND with limited growth evaluation based on soil and environmental conditions.

Participants included four North Dakota city partners (Bismarck – Greg Smith, Bismarck Parks and Recreation District Operations Director; Dickinson – Gary Zuroff, Public Works Director; Minot – Brian Johnson, City Forester; Williston – Bruce Johnson, City Forester) as well as the ND Agriculture Experiment Station (NDAES) – Williston Research Extension Center (REC) and NDSU Dale E. Herman Research Arboretum. Non-city boulevard planting trials were conducted at the NDAES – Williston REC and the NDSU Dale E. Herman Research Arboretum located near Absaraka ND. The two sites, NDAES – Williston REC and the NDSU Dale E. Herman Research Arboretum, will provide a comparison of Eastern ND climate/soils to Western ND climate/soils as well as hardiness differences. These two locations were utilized for more control plantings of material, which is limited in city plantings (recreational park or city boulevard). Specific woody plant cultivar evaluations were conducted in Williston, Minot, Dickinson, and Bismarck, ND within city plantings and/or recreational park with replicated plantings located at NDAES – Williston REC and the NDSU Dale E. Herman Research Arboretum. With a minimum of 20 different woody accessions of species/cultivars (trees and shrubs) planted per year for evaluation with a minimum replication of four trees per location. With the approved grant extension, a third year (not in the original proposal) of planting was able to be conducted with the associated partners to increase the tree trial size and diversity of species and/or cultivar use.

This project was important and timely because it addresses a significant gap in tree diversity information for proper tree selection in Western ND. Besides lack of diversity, there are also many disease and pest issues related to many of the urban and landscape tree and shrub ‘standards’ such as Dutch Elm Disease (DED) (infects American Elm, confirmed in ND), the threat of Emerald Ash Borer (EAB) (infests Ash species, confirmed in MN), and Spruce Needle Cast diseases (infests Colorado Blue Spruce and Black Hills Spruce, confirmed in ND).

The ND Tree Selector Program is underutilized because of the limited associated database. If this database was increased, key species and/or cultivar information could be utilized by ND nursery crop producers/retailers and ND residents. The database allows for a user to input desired tree parameters and the website suggests trees for those set parameters. Having more of a selection within the database will assist in selling more tree species and/or cultivars in ND.

This project did not build upon previously funded projects with the SCBGP or SCBGP-FB.

<u>Project Approach</u>

Objectives of this project included:

1. Evaluate tree plantings of candidate species and/or cultivars to determine establishment, winter and drought hardiness, soil adaptation, pest susceptibility, aesthetic characteristics and survival in Western North Dakota.
2. Enhance and expand the ND Tree Selector website (http://www.ag.ndsu.edu/tree-selector/) for use by ND nursery commercial producers, city foresters, commercial landscape companies and ND residents.

There were two goals associated with the expected measurable outcomes of this project. Goal 1. was to evaluate tree plantings of candidate species and/or cultivars to determine establishment, winter and drought hardiness, soil adaptation, pest susceptibility, aesthetic characteristics and survival in Western North Dakota and goal 2. Was to Enhance and expand the ND Tree Selector website (http://www.ag.ndsu.edu/tree-selector/) developed by Dr. Joseph Zeleznik (NDSU Extension Forester) for use by commercial ND nursery crop producers/retailers, city foresters, commercial landscape companies and ND residents.

Trees were planted at all six sites in 2016 (19 different species and/or cultivars), 2017 (22 different species and/or cultivars) and 2018 (9 different species and/or cultivars). There was a total of 50 different species and/or cultivars planted with this project with 200 trees planted at each evaluation site for a total of 1200 trees. Project partners assisted with the planting and with gathering initial survival data. Evaluation of this project will be conducted long-term and beyond the limited time of this funding. These trees will be evaluated on a biannual basis for a minimum of 10 more years spanning into 2026 through 2028. Table 1. summarizes the six different evaluation sites and presents initial results on survivability of planted species/cultivars. These preliminary results allow for some initial recommendations to be made to growers and landscapers in Western North Dakota. With long-term evaluation, more recommendations will be made available to growers and landscapers. Initial results indicate that crabapple (*Malus* spp.) cultivars (Gladiator, Marilee®, Pink Spires and Red Baron), Prairie Sentinel® hackberry (*Celtis occidentalis* ‘JFS-KSU1’), Prairie Expedition® elm (*Ulmus americana* ‘Lewis and Clark’), thornless honeylocust (*Gleditsia triacanthos* var. *inermis*) cultivars (Northern Acclaim® and Street Keeper®), Sutherland caragana (*Caragana arborescens* ‘Sutherland’) and Ivory Pilar™ Japanese tree lilac (*Syringa reticulata* ‘Willamete’) all performed very well at each of the six experimental trial locations (Table 1.). While American hophornbeam (Ironwood, Ostrya virginiana), Prairie Stature® hybrid oak (*Quercus* x*bimundorum* ‘Midwest’), and Chinkapin oak (*Quercus muehlenbergii*) performed poorly at each of the six sites (Table 1.). Most of the species and cultivars performed differently at each of the six evaluation sites and recommendations can be made for a specific region of ND based on these results.

Overall, the Tree Selector portion of the project was not as successful as we had hoped it would be. The graduate student assigned to the project did not work out and was let go after two semesters. Only seven new species were added to the Tree Selector, bringing the total to 104 species/cultivars. New/updated materials were developed for 56 of the species/cultivars. An additional 31 new species/cultivars were identified as being good candidates for inclusion into the program. Results from the current trial are still too new to give firm recommendations; three years is simply not enough time to evaluate trees. Ideally, we prefer 10 years' worth of data before having enough confidence to make recommendations. This project is still ongoing with undergraduate students working on preparing the additional 31 new species/cultivars to the database.

Goals and Outcomes Achieved

There were two goals for this project.
Goal 1:

- Performance Measure: Evaluations for establishment, winter and drought hardiness, soil adaptation, pest and susceptibility, aesthetic characteristics and pruning requirements will be conducted on tree species and/or cultivars for Western ND city boulevard and recreational park plantings.
- Benchmark: Tree recommendations for North Dakota are outdated and limited especially for Western ND.
- Target: Increased information available to ND Commercial nursery plant producers/retailers, city foresters, commercial landscapers and ND residents for proper tree selection.
- Performance Monitoring Plan: Evaluations will be conducted and analyzed with information being presented to specialty crop beneficiaries.

The performance measure for this goal was completed. Preliminary information has been presented at NDSU Research Field Days at Williston and Absaraka, ND. Initial data be compiled and printed in the ND Nursery, Greenhouse and Landscape Association (NDNGLA) and ND Urban and Community Forestry Association (NDUCFA) newsletters. Information will also be provided at the annual NDNGLA and NDUCFA expos. This will allow members of the associated which are the specialty crop beneficiaries to be able to see the initial project results and use this information for crop growing, planning and planting. The target results of this project are long-term but short-term initial information is valuable to specialty crop beneficiaries.

Goal 2:

- Performance Measure: The ND Tree Selector website database will be updated by a minimum of 25% with added species and/or cultivars.
- Benchmark: Tree recommendations for North Dakota are outdated and limited especially for Western ND. Currently, the ND Tree Selector website has limited information for species and/or cultivars within the database and no information on the drier western part of the state.
- Target: Increased information included within the ND Tree Selector website database made available to ND commercial nursery producers, city foresters, commercial landscapers and ND residents.
- Performance Monitoring Plan: Online tree selector website will be updated and enhanced with new information on species and/or cultivars suitable for use in ND. Data collected from Western ND tree evaluation will be incorporated into the database for tree selection.

The performance measure for this goal has not been reached. As a result of graduate student complications, this portion of the project had a significant delay. The ND Tree Selector website database was redesigned during this project which will allow for more efficient addition of new species and cultivars resulting from data collected in Goal 1. Updates will be made public with notifications made to the ND Nursery, Greenhouse and Landscape Association for publishing in the newsletter as well as information made available at the annual expo.

Beneficiaries

The number of direct specialty crop beneficiaries are approximately 250 based on membership within the ND Nursery, Greenhouse and Landscape Association (NDNGLA) and ND Urban & Community Forestry Association (NDUCFA). Members of NDNGLA include specialty crop growers in ND, MN and Canada. Members of NDUCFA include specialty crop users such as city foresters, arborists, and landscapers. This research will have a significant impact on decision making of the direct and indirect specialty crop growers and specialty crop users in ND with providing up-to-date species/cultivar evaluation information for Western ND.

Results could have a national impact with gaining information on northern extremes of evaluated materials. This project could have multi-state benefits with potential to benefit Eastern Montana.

Lessons Learned

One key lesson learned was that having a paid city forester or dedicated employee at the evaluation site was critical. Each location assisted with planting arrangements and with yearly planting except for the Dickinson site. Shortly after receiving funding, the city forester for Dickinson resigned and left an opening in this position during the three planting seasons during this project. It was difficult to coordinate planting efforts with other city employees that were not dedicated to this project. This caused a significant delay in spring planting at the Dickinson location and at other subsequent planting sites.

With Goal 2., the performance measure was not initially achieved during this project period. A more efficient method of entering species/cultivars into the database should have been utilized. Unfortunately, the situation with this project and with not having the graduate student work out and being let go after two semesters put this portion of the project into jeopardy with respect to achieving the performance measure in a timely manner. This resulting in nearly half of the graduate student salary associated with this project being spent on an individual and not being able to get a new graduate student to work on the project because of not having the necessary guaranteed funding for another two years.

To date, there were no unexpected outcomes or results from this project that were an effect of implementing this project.

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Additional Information

Table 1. Surviving Trees Planted at the Six Participating Evaluation Sites.

	NDSU (Absaraka)	Bismarck	Dickinson	Williston (City)	Williston (NDSU ARES)	Minot
2016						
Espresso Kentucky Coffee Tree	4	2	0	4	1	0
Marilee Crabapple	4	4	4	4	4	4
Urban Pinnacle Bur Oak	4	1	0	4	0	4
Crimson Spire Hybrid Oak	4	3	0	4	0	2
His Majesty Cork Tree	4	4	0	1	0	0
Prairie Sentinel Hackberry	4	4	4	4	3	4
Commemoration Sugar Maple	4	2	2	4	0	0
Unity Sugar Maple	4	3	2	4	0	0
Fall Fiesta Sugar Maple	4	4	0	4	0	2
Regal Prince Hybrid Oak	4	3	3	4	1	0
Homestead Buckeye	4	4	4	2	0	4
Ironwood (Ostrya)	4	2	0	2	0	0
Gladiator Crabapple	4	4	4	4	4	4
Harvest Gold Mongolian Linden	4	2	0	4	1	0
Prairie Expedition Elm	4	4	4	4	4	4
Amur Maackia	4	3	0	3	0	2
Northern Acclaim Honeylocust	4	4	0	4	3	4
Autumn Gold Ginkgo	4	4	0	1	0	0
Mountain Frost Pear	4	4	4	4	0	0
2017						
Silver Queen silver maple	4	3	3	2	4	4
Northwood Red Maple	4	1	3	3	2	2
Hot Wings Tatarian Maple	4	4	4	4	4	4
Firefall Freeman Maple	4	4	4	4	4	0
Red Baron Crabapple	4	4	4	4	4	4
Swamp White Oak	4	4	2	1	4	4
Boulevard American Linden	4	4	4	4	0	4
Red November Amur Maple	4	4	3	4	3	0
Prairie Stature Hybrid Oak	4	1	1	2	0	0
Prairie Gold Aspen	4	4	0	1	4	0
Prairie Dream Paper Birch	4	3	0	1	3	0
Heartland Catalpa	4	4	3	4	0	0
Chinkapin Oak	4	0	0	2	0	0
Royal Red Norway Maple	4	3	4	0	4	0
Sutherland Caragana	4	4	3	3	4	4
Street Keeper Honeylocust	4	4	4	4	3	4
Pink Spires Crabapple	4	4	4	4	4	4
Purple Robe Black Locust	4	4	0	2	0	0
Ivory Pillar Japanese Tree Lilac	4	4	3	4	4	4
Princeton American Elm	4	4	0	3	4	0
New Horizon Hybrid Elm	4	4	1	4	4	4
Valley Forge American Elm	4	3	3	4	4	0
2018						
Patriot Elm	4	4	4	4	4	4
Mountain Sentinel Aspen	4	4	4	4	4	4
MaacNificent Maackia	4	4	4	4	4	4
Princeton Gold Norway Maple	4	4	4	4	4	4
Spring Wonder Sargent Cherry	4	4	4	4	4	4
Magestic Skies Nothern Pin Oak	4	4	4	4	4	4
Eye Stopper Phellodendron	4	4	4	4	4	4
Ivory Spear Crabapple	4	4	4	4	4	4
Northern Tribute River Birch	4	4	4	4	4	4
Total Surviving Trees	200	171	120	165	120	112
% Survival	100	86	60	83	60	56

State-wide screening of green foxtail for herbicide resistance

Final Report

Project Summary

- The purpose of the project was to screen green foxtail samples from across the state to determine how widespread resistance is and to which herbicides (mostly Group 1 and 2).
- Farmers growing specialty crops will benefit in the short term and long term by understanding herbicide resistance and learning how to properly rotate herbicide chemistries and crops.
- Green foxtail seed was collected from 63 fields across North Dakota. Not all 63 populations had enough seed to be sprayed with all herbicides.
- Resistance to Group 1 herbicides is much more prevalent than with Group 2 herbicides. Over half of the populations were resistant to Puma and Discover.

Project Approach

- Green foxtail seed was collected from 63 fields by NCREC personnel, NDSU Extension agents, and agronomists across North Dakota.
- Many samples were selected because resistance was suspected while some populations were selected from random fields. Thus, this was not a completely random survey.
- Green foxtail seeds were planted in the greenhouse and plants sprayed when about 2-3 inches tall in a spray chamber. Plants were thinned to about 8-10 plants per pot before spraying. A visual control rating was estimated at about 14 days after treatment (DAT). Pictures were taken 14-21 DAT.
- This information was presented at multiple grower meetings in winter meetings in 2016 and 2017, and an International Weed Science meeting in Denver in May 2017.

Goals and Outcomes Achieved

- Original goal was to screen at least 50 green foxtail populations from across the state. We exceeded that by screening 63 populations.
- We determined which herbicide groups the green foxtail populations are resistant to.
- Green foxtail was sprayed with Axial XL, Puma, Discover, Select, Assure II, Everest, Varro, GoldSky, Raptor, Roundup, and Assure II+Select.
- Discover and Puma provided similar control (control or resistance) in 49 of 51 populations (96%). More than 50% of the populations were resistant to Puma and Discover. Discover was more resistant in two populations.

- In 49 of 58 populations (84%), Axial provided similar control to Puma. Seven populations showed significantly more resistance to Puma than Axial. Two were more resistant to Axial than Puma. About 40% of the populations were resistant to Axial.
- Assure II and Puma provided similar control in 49 of 55 populations (89%). 6 of the 55 were more resistant to Puma than Assure II.
- In 32 of 53 populations (60%), Assure II and Select provided similar good control. 21 of 53 populations were resistant to Assure II compared to none for Select. About 38% of the populations were resistant to Assure II. 5 populations showed slight resistance to Select.
- Select + Assure II always provided as good or better control than either product alone. None of the populations (0 of 45) survived Select + Assure II.
- None of the populations (0 of 53) showed any resistance to Raptor.
- None of the populations (0 of 49) showed any resistance to Roundup.
- 2 of 51 were resistant to Everest, Varro, and GoldSky
- 3 of 51 were resistant to Varro and GoldSky, but not Everest.
- Research results were presented at two winter meetings in Williston and Dickinson (Feb and March). Research results were also presented at the International Weed Resistance Conference held in Denver, CO.

Table. Percent of green foxtail populations resistant to common herbicides.

Herbicide	Group	% Resistant
Puma	1-fop	51
Axial	1-den	40
Discover	1-fop	55
Assure II	1-fop	38
Select	1-dim	0
Raptor	2-imi	0
Everest	2-SACT	4
Varro	2-SACT	12
GoldSky	2-TPS	14
Roundup	9	0
Select + Assure II	1-dim + 1-fop	0

Beneficiaries

- Farmers will benefit by better understanding herbicide groups and modes of action and will better understand how to rotate chemistries to delay resistance in the future. Approximately 400 farmers will benefit.
- Two winter grower meetings (about 200 in attendance). Many were crop consultants that will work with many more growers.

- International Weed Science meeting (372 in attendance). This included scientists and industry personnel that will work with many more growers.

Lessons Learned

This information shows that green foxtail resistance varies by population. However, there are some trends that can be useful information to growers:

- None of the populations were resistant to Raptor. Thus, Clearfield crops (wheat, lentil, sunflower, canola) can be used to help manage resistant foxtail.
- Growers should not consider alternating between Puma and Discover as green foxtail responded similarly to both.
- If a grower has foxtail resistant to Assure II, he can likely still control green foxtail with Select.
- Growers should alternate using Group 1 and Group 2 herbicides for foxtail control in wheat.
- Growers should alternate between Assure II and Select for broadleaf crops. Alternatively, some growers are tank mixing the two.

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Project Title: Identification of Pathogen, Soil and Plant Factors Important to Root Rot Development in Field Pea

NOGA#: 15-324

Final Report

Partner Organization
North Dakota State University

Project Summary

North Dakota currently ranks second in pea production in the US, representing approximately 35% of the total, down from a peak of 66% in 2006 (USDA-NASS). Root rot of field peas has been cited by many growers as a main reason for decreased pea acres. This is demonstrated most effectively by grower concerns voiced during formal and informal discussions. These producers have been very vocal in expressing the impact that root rot has had on the decrease in field pea acres over the past decade. In 2007, the economic impact of field pea production in North Dakota reached a high of $141 million, in 2014 that number was reduced to less than $89 million. Montana planted twice the acres to field peas as North Dakota in 2014, and for the first time in 15 years, US pea acreage increased while acres decreased in North Dakota. Field pea production has increased since that time; however, grower concerns over root rot remain. Protecting yield from devastating root rot pathogens can be difficult because there are few effective management practices available to growers.

Multiple root pathogens infect field pea in North Dakota. Generally, root rot symptoms include necrotic lesions, plant stunting and yellowing, delayed development and reduced yield (Lawson and Topham 1985; Xi et al. 1995). In North Dakota, total crop loss has been attributed to Fusarium root rot caused by up to seven *Fusarium* species. *Aphanomyces euteiches*, a globally ubiquitous soilborne pathogen of peas (Papavizas 1974), is also present in North Dakota and infested fields can suffer complete loss (Hagedorn 1984). *Pythium* spp. and *Rhizoctonia solani* are considered less important in North Dakota, but cause pre- and post-emergence damping-off, seed rot, and root rot under favorable conditions (Carling, 2001). Traditional methods to distinguish root rot pathogens require repeated plating over a 2 or 3 week period, but can be effective for low-throughput situations. Multiplex quantitative PCR (qPCR) is more efficient, but only three organisms can be detected in one reaction. The presence of potentially 10 organisms in root tissue of field pea requires the use of four assays, greatly increasing the resources needed for this technique; however, this method remains very effective for medium-throughput situations. Surveys of field pea root rot in North Dakota involved the collection of 3,000 plants, colonized by up to 10 root rotting organisms. This intensive survey greatly benefited from the development of a high-throughput PCR-GBS assay that simultaneously quantified multiple organisms from hundreds of samples. The number of species identified was limited only by the number with available verified sequence data.

The current project was built on results obtained from 2014 SCBG NOGA 14-213 titled "Contribution of Nematode Populations and Soil Properties in Root Rot of Peas". During that project, six groups of plant-parasitic nematodes were detected in soil from 20 North Dakota pea fields surveyed in 2014 including root-knot nematode (*Meloidogyne chitwoodi*), root-lesion nematode (*Pratylenchus* spp.), stunt nematode (*Tylenchorhynchus* spp.), spiral nematode

(*Helicotylenchus* spp.), pin nematode (*Paratylenchus* spp.) and dagger nematode (*Xiphinema* spp.). Densities of pin nematodes were found exceedingly high in some infested fields; up to 15,048 per kg of soil.

The pin nematode (*P. hamatus*) was reported to reduce plant height an average of 50% compared to controls of dryland peas (cv. Columbian) in a greenhouse study conducted in Washington (Riga et al. 2008). However, the effect of pin nematodes on plant growth of field pea cultivars in North Dakota is unknown. Additionally, several *Fusarium* species were commonly isolated from pea roots and *R. solani, A. euteiches*, and *Pythium* species were present at low levels in North Dakota field peas. Preliminary evaluations examined soil characteristics associated with root rot severity. Prior to this, the effect of nematode populations and soil characteristics had not been evaluated for field peas in North Dakota and the effect of pin nematode on plant growth of field pea cultivars grown in North Dakota was unknown.

This project is unique in that it combines evaluations of root diseases, nematodes and soil characteristics and utilizes information and materials collected as part of a parallel project funded by the Northern Pulse Growers Association to improve pea production in North Dakota. This holistic approach will bring together information from many sources to help provide growers with management options for root rot in field peas.

New objectives were developed based on previously obtained results and objectives were continued from the previously funded award to confirm results.

New objectives:

1. Develop an amplicon-GBS high-throughput assay to quantify root rot pathogens of field peas.
2. Determine the importance of seed quality and plant nutrient levels in pea root rot severity.
3. Determine the species and density of root-lesion nematodes from diseased root tissue and their association with soil properties and root rot severity.
4. Isolate pin nematodes from North Dakota pea field soil and establish pure cultures.
5. Determine the effect of pin nematodes on plant growth of five field pea cultivars commonly grown in North Dakota.

Continuing objectives:

6. Examine the association of chemical and physical soil properties with pea root rot severity.
7. Determine the species, density and distribution of plant-parasitic nematodes from soil and their association with soil properties and root rot severity.

Project Approach

PCR-GBS high-throughput assay to quantify root rot pathogens of field peas.

Target organisms most commonly were detected at a higher frequency using the amplicon-GBS technology than traditional plating (culturing) (Table 1). In a few instances, this new technology resulted in slightly lower frequencies; however, we believe this is due to misclassifications using morphological methods. Some of the organisms, particularly *F. avenacuem* and *F. acuminatum*, are very difficult to distinguish morphologically. The assay also detected *Fusarium* spp. in addition to the seven *Fusarium* spp. targeted by the qPCR assay. *R. solani* was not detected from any field samples collected in 2015 or any evaluated using traditional plating assays. Field samples collected in 2015 did not exhibit *R. solani* symptoms. The amplicon-GBS assay did detect *R. solani* from the greenhouse inoculated plants and from pure fungal cultures (Table 2). The amplicon-GBS assay also detected *A. euteiches* and *Pythium* spp. from greenhouse inoculated plants and from pure fungal cultures (Table 2).

Table 1. Frequency of positive identification by field and county of target organisms by traditional plating and the amplicon PCR-GBS methods.

	Traditional plating		Amplicon-GBS	
Target organism	% Fields	% Counties	% Fields	% Counties
F. acuminatum	94	100	88	100
F. avenaceum	98	100	98	100
F. redolens	60	55	70	55
F. solani	30	64	54	82
F. sporotrichioides	44	64	44	64
F. graminearum	52	82	50	82
F. culmorum	38	64	34	45
F. oxysporum	62	100	90	100

Table 2. *Fusarium* species used to inoculate field pea plants under greenhouse conditions, frequency at which the plating method and PCR-GBS assays detected the inoculated species.

Inoculum	Root rot rating (0 to 5)	Traditional plating (% detection)[a]	Amplicon-GBS (% detection)[b]
F. graminearum	1.8	83	100
F. sporotrichioides	1.6	50	100
F. avenaceum	3.2	100	100
F. culmorum	1.4	50	100
F. solani	1.5	83	90
F. redolens	1.3	83	100
F. acuminatum	2.5	100	100
F. graminearum +	2.6	67	92
F. sporotrichioides		0	92
F. avenaceum +	2.5	92	92
F. culmorum		0	92
F. solani +	3.5	25	92
F. redolens +		0	75
F. acuminatum		75	100
Aphanomyces euteiches	4	92	92
Pythium spp.	3.5	83	92
Rhizoctonia solani	4	83	100
Non-inoculated control	0	0	0

[a]Percentage of field pea plants (n = 12) from which the inoculated *Fusarium* species was recovered using a traditional plating method.

[b]Percentage of field pea plants (n = 12) from which the inoculated *Fusarium* species was detected using the amplicon-GBS assays.

Species and density of root-lesion nematodes from diseased root tissue.

Root-lesion nematodes were not detected in diseased root samples obtained from pea fields. However, we frequently detected root-lesion nematodes from pea field soil after harvest. The species of root-lesion nematode was identified as *Pratylenchus neglectus*. Hosting abilities of field pea cultivars to root-lesion nematodes was determined in the greenhouse. Two field pea cultivars, Columbian and Cooper, and two wheat cultivars, Alpowa and Joppa, were selected for the greenhouse trials. Wheat cultivars were selected in this study because lesion nematodes were reported to be parasitic to wheat and farmers in our region commonly rotate field peas with wheats. The first experiment was conducted during the winter with naturally infested soil. The initial population of root-lesion nematodes was 960 per pot per plant, equivalent to 1,600 nematodes/kg of soil. After 11 weeks, the trial was harvested and nematodes were extracted separately from soil and roots. Multiplication rate (MR) was calculated by dividing the final nematode population by the initial population to determine the hosting ability of each cultivar. MR that is equal to 1 indicates that the cultivar only maintains the population level, MR greater than 1 means it increases the nematode population, and MR less than 1 means it decreases the nematode population. MR of the root-lesion nematodes for both field pea cultivars were approximately one; Columbian (MR = 1.0) and Cooper (MR = 0.8). MR of the root-lesion nematodes for both wheat cultivars were greater than five; Alpowa (MR = 6.8) and Joppa (MR = 5.2) (Table 3). The experiment was repeated during summer with a lower initial population of root-lesion nematodes, 210 per pot per plant, equivalent to 350 nematodes/kg of soil. The second experiment was harvested after 13 weeks and nematodes were extracted separately from soil and roots. In this experiment, MR for the root-lesion nematodes in Columbian (3.4) and Cooper (2.6) were greater than two. MR of the root-lesion nematodes for both wheat cultivars were greater than four; Alpowa (12.7) and Joppa (4.6) (Table 3). Results from these greenhouse experiments suggest that root-lesion nematode populations in North Dakota pea fields can infect and reproduce in the field pea cultivars evaluated under greenhouse conditions.

Table 3. Multiplication rate (MR) of lesion nematode in field pea and wheat cultivars under greenhouse conditions.

(Experiment 1 in winter)					
Crop	Cultivar	Initial nematode density per pot per plant (600 g soil)	Final nematode densities in roots and soil		Multiplication rate
			Roots	Soil	
Field Pea	Columbian	960	163	784	1.0
	Cooper	960	152	594	0.8
Wheat	Joppa	960	1,574	3,430	5.2
	Alpowa	960	2,479	4,016	6.8
(Experiment 2 in summer)					
Field Pea	Columbian	210	300	418	3.4
	Cooper	210	90	461	2.6
Wheat	Joppa	210	250	706	4.6
	Alpowa	210	319	2,357	12.7

<u>Isolate pin nematodes from North Dakota pea field soil and establish pure cultures.</u>

The development of a culture for ectoparasitic nematodes, like the pin nematode, has proven difficult under lab conditions; however, we have developed an efficient protocol to increase pin nematodes using susceptible pea plants in the greenhouse. Pure cultures of endoparasitic nematodes, like the root-lesion nematode, have been successfully cultured under lab conditions by inoculating sterilized carrot disks and storing them under controlled temperature and humidity. Field soil samples with pure populations of pin nematodes from 2015 nematode survey were used to increase the nematode populations to set up the experiments for evaluating the effect of pin nematode on plant growth.

Effect of pin nematodes on plant growth of five field pea cultivars commonly grown in ND.

Pin nematodes were reared using three field pea cultivars (Columbian, Aragorn and Cooper) under greenhouse conditions. There was an average ten-fold nematode MR in Columbian followed by Cooper (5) and Aragorn (3) at the initial population density of 300 pin nematodes/200 g of soil. However, the average MR were lower (1 to 3) for these three cultivars at higher initial population densities of 800, 1,100 and 1,400 pin nematodes per 200 g of soil.

Before evaluating the effect of pin nematode on pea plant growth, we performed reproduction study of this nematode in six field pea cultivars at low nematode density (1,500 pin nematodes/ kg of soil) and high nematode density (4,500 pin nematodes/ kg of soil) using naturally infested field soil (Figure 1). At the low nematode density, MR was significantly higher in Columbian (12.5), followed by Arcadia (5.8), Cruiser (3.8), Cooper (3.3), Bridger (2.8), Aragorn (2.0), and Salamanca (1). However, at the high nematode density, nematode MR were lower (1 to 3) compared to the low nematode density.

Figure 1. Reproduction experiments of pin nematodes across several field pea cultivars under greenhouse conditions.

The effect of pin nematodes were evaluated on the plant growth of six field pea cultivars (Columbian, Arcadia, Cruiser, Cooper, Bridger, Aragorn, and Salamanca) at three inoculation (no nematode, low nematode [(1,500 pin nematodes/ kg of soil) and high nematode (4,500 pin nematodes/ kg of soil)] levels. Plant growth parameters including height, yield, dry shoot and root weight were reduced for most of the tested cultivars on average in response to pin nematodes, when compared to the non-inoculated control of each corresponding cultivar, at both low and high nematode inoculation levels (Table 4; Figure 2). However, we only observed a significant interaction between the cultivars and nematode levels for plant height (P = 0.0089) and root weight (P = 0.0336). This suggests that the nematode inoculation levels caused significant impact on the tested cultivars for the changes in plant heights and root weights only. Cvs. Columbian, Salamanca, Bridger, Arcadia and Cruiser displayed significantly reduced plant height when inoculated with pin nematode at the high level compared to control of each cultivar. Cv. Aragorn there did not display a plant height difference when inoculated. Cv. Columbian displayed significant differences in plant height at low and high nematode levels. Only cv. Bridger displayed significantly reduced root weights at both nematode levels. These results indicate that more cultivars from diverse genetic pools should be evaluated to determine the response of field pea cultivars to pin nematodes.

Table 4: Yield, dry stem weight and dry root weight reduction due to pin nematodes compared with the non-inoculated controls

Cultivar	Height reduction (%)		Yield reduction (%)		Dry stem wt. reduction (%)		Dry root wt. reduction (%)	
	Low density	High density	Low density	High density	Low density	High density	Low density	High density
Arcadia	22	37	11	33	38	53	None	32
Columbian	18	36	34	40	28	43	4	31
Bridger	29	29	44	34	37	39	25	55
Cruiser	14	22	16	28	2	26	0	31
Salamanca	25	20	12	7	33	22	18	35
Aragorn	19	19	31	34	24	31	0	43

Low density = 1,500 pin nematodes/kg soil
High density = 4,500 pin nematodes/kg soil

Figure 2. Plant height experiment including cv. Arcadia at three nematode levels 40 days after inoculation (from left to right: non-inoculated control, low and high pin nematode inoculation).

Associations among chemical and physical soil properties, fungi, nematodes and pea root rot severity.

For the purpose of this study, communities were defined by the geographical distribution based on the presence/absence of targeted organisms associated with the pea root rot complex. The community was subsequently characterized by the organism solely present within the community. Five communities were identified and were characterized by *F. acuminatum* (#1), *F. solani* (#2), *A. euteiches* (#3), *F. sporotrichioides* (#4), and *F. redolens* (#5). The communities characterized by *F. solani* and *F. redolens* occurred in too few locations for logistic regression to be performed. Logistic regression analysis was performed on the communities characterized by *F. acuminatum, A. euteiches* and *F. sporotrichioides* to determine the associations between soil properties and disease incidence. The likelihood of community one (characterized by *F. acuminatum*) being present increased as clay content increased (Figure 3). The likelihood of community three (characterized by *A. euteiches*) being present increased as pH and Fe^{2+} levels increase. However, despite the increase in probability due to increasing levels of pH and Fe^{2+}, when potassium levels increased, the overall probability of community 3 being present decreased (Figure 4). The model is set at pH 7 as Fe^{2+} and K^{+} levels increase to better display the logistic regression model. The likelihood of community four (characterized by *F. sporotrichioides*) being present increased as Fe^{2+} and pH increased, the probability of community four being present within a soil decreased, but the overall probability of community four being present within a soil increased as clay content increased (Figure 5). The model is set at pH 7 for this study as Fe^{2+} and clay levels increase to better display the logistic regression model. Results indicated that the likelihood of having disease within a field heavily relied on potassium levels in the soil (Figure 6). In addition, the presence of both, community one (*F. acuminatum*) and community three (*A. euteiches*) increased the likelihood of having diseased plants within a field. The associations between potassium and the presence of communities one and three were significant (p-value < 0.05). A model was generated to predict the probability of diseased plants within a field using potassium, and the presence/absence of communities one and three.

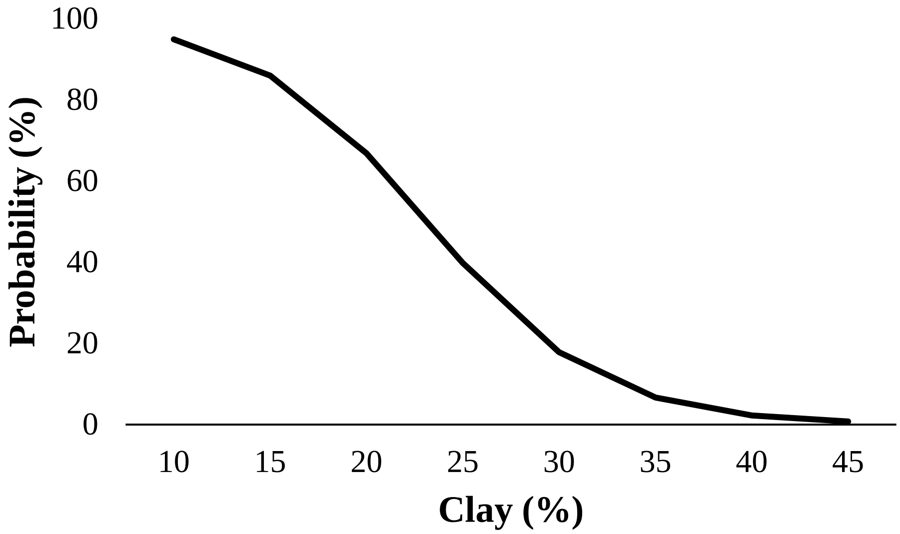

Figure 3. Probability (%) of community one, characterized by *Fusarium acuminatum*, being present in a soil depending on clay percentage increases. Determined by the logistic regression model y = 2.24 + -0.0968 (clay).

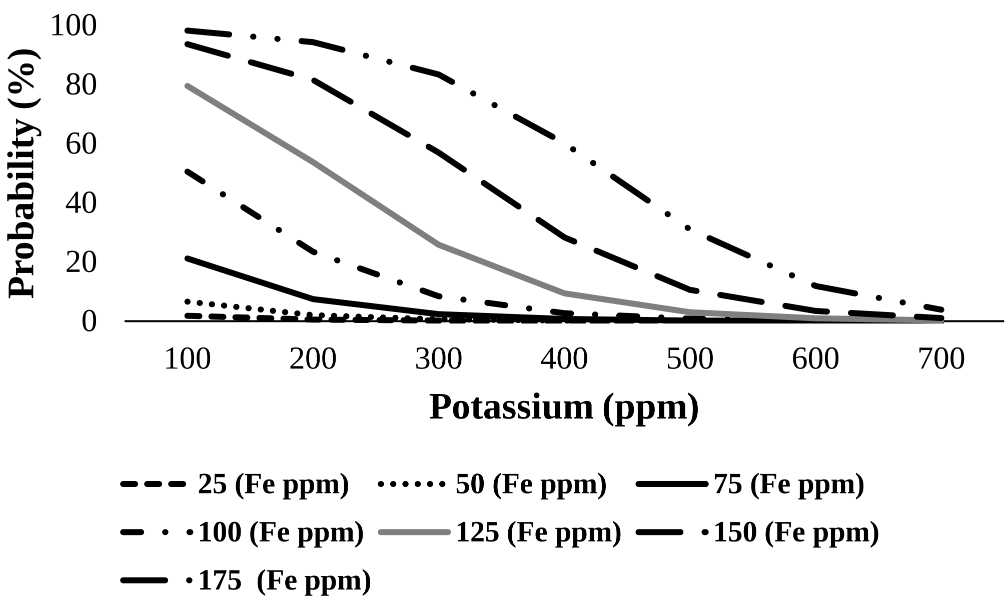

Figure 4. Probability (%) of community three, characterized by *Aphanomyces euteiches*, being present in a soil depending on Fe^{2+}, pH, and K^+ levels. As Fe^{2+} and pH increase the probability of community three being present increases. The overall probability of community three being present declines with the increase in K^+. Determined by the logistic regression model y = -8.9913 + -0.00524 (K^+) + 1.0294 (pH) + 0.0232 (Fe^{2+}). To simplify the representation of the model, pH was set at a constant of 7.

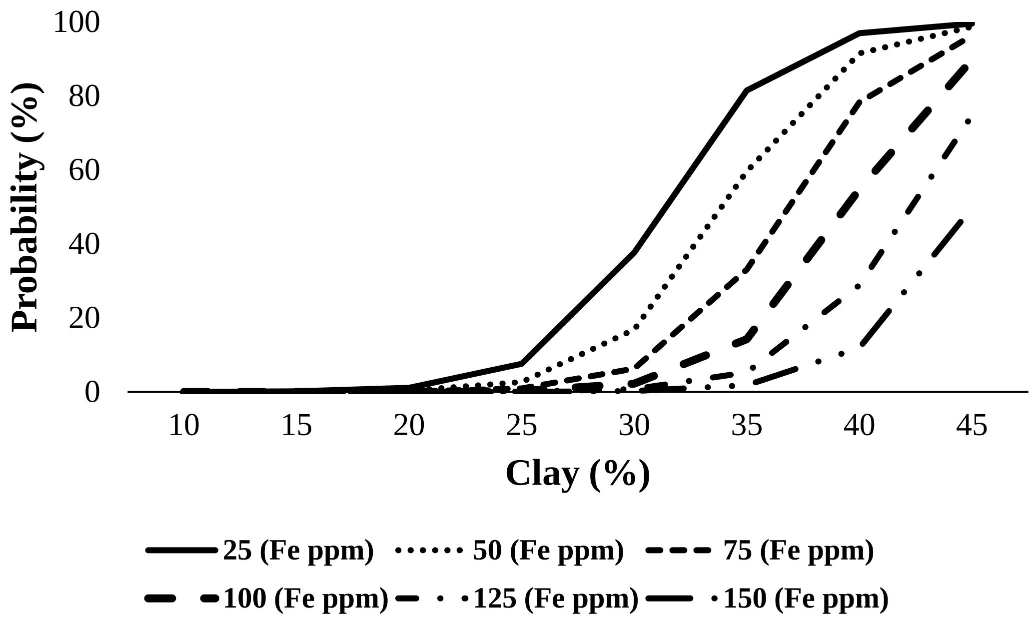

Figure 5. Probability (%) of community four, characterized by *Fusarium sporotrichioides* being present in a soil depending on Fe^{2+}, pH, and clay percentage. As Fe^{2+} and pH increase the probability of community four being present in a soil decreases. The overall probability of community four being present increases with the increase in clay percentage. Determined by the logistic regression model y = 3.3683 + -1.1847 (pH) + -0.019 (Fe) + 0.1727 (clay). To simplify the representation of the model, pH was set at a constant of 7.

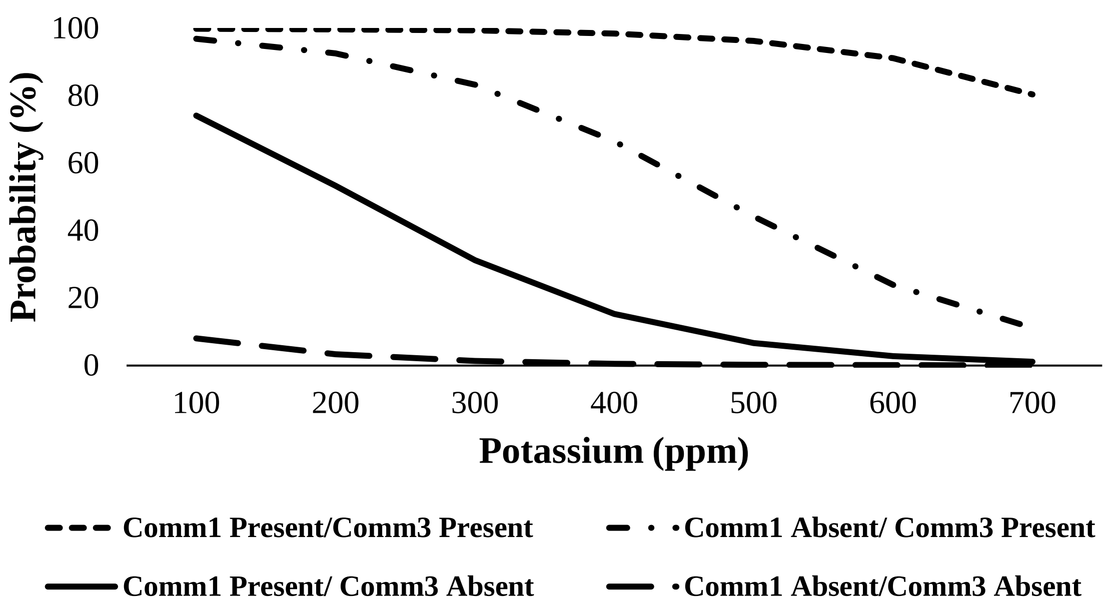

Figure 6. Probability (%) of diseased plants being present in a soil depending on the K^+ and the presence of communities 1 and 3. The probability of diseased plants being present increases in the presence of either community together or alone. Overall probability of diseased plants being present decreases as K^+ increases. Determined by the logistic regression model y = -0.657 + -0.004 (K^+) +1.5145(+/- community 1) + 2.556 (+/- community 3).

Properties of 115 soil samples were analyzed to determine the association of nematode densities with soil factors (texture, organic matter, soil nutrient). There was no significant association between densities of most nematodes and soil properties. However, weak but statistically significant correlations were detected for only the densities of stunt and pin nematodes with soil properties (% of sand, silt, clay and organic matter; soil nitrogen and potash; pH) (Table 5). There was a weak negative correlation between stunt densities and % silt, % clay, % organic matter, soil nitrogen and potash. Similarly, pin nematodes were negatively correlated with % sand and pH. Stunt and pin nematodes had weak positive correlations with % sand and % silt (Table 5).

Table 5. Correlation coefficients between population densities of nematode genera and soil properties in pea field samples in North Dakota

Nematode	Number of samples	Soil properties							
		% Sand	% Silt	% Clay	% Organic matter	pH	Nitrate-N (ppm)	P-Olsen (ppm)	K (ppm)
Paratylenchus	107	-0.22*	0.25**	0.06	0.07	-0.24*	0.06	0.12	0.09
Tylenchorhynchus	76	0.36**	-0.29**	-0.29*	-0.33**	0.16	-0.24*	-0.09	-0.23*
Xiphinema	23	-0.01	0.28	-0.20	0.06	0.07	-0.16	-0.13	0.15
Helicotylenchus	29	0.03	0.18	-0.25	-0.01	0.09	-0.19	-0.19	-0.10
Pratylenchus	21	0.38	-0.29	-0.38	-0.06	0.27	-0.16	0.02	-0.36
Total plant-parasitic nematodes	115	-0.06	0.10	-0.035	-0.04	-0.18	0.06	0.01	-0.02
* and ** indicate the level of significance at P < 0.05 and 0.01, respectively.									

<u>Plant-parasitic nematode species, density and distribution from soil.</u>

Two-hundred forty-three soil samples collected in pea fields from 2014 to 2017 from 16 North Dakota counties were assayed to determine the prevalence, distribution and abundance of plant-parasitic nematodes. The counties included Burke, Divide, Williams, McKenzie, Mountrail, Renville, Ward, McLean, Bottineau, McHenry, Sheridan, Wells, Foster, Griggs, Ramsey, Cavalier, and Pembina. Eight groups of plant-parasitic nematodes were detected during the surveys, including pin, stunt, lesion, dagger, spiral, lance, stubby root and root-knot nematodes (Table 6). Pin and stunt nematodes were most prevalent among the detected plant-parasitic nematodes (Figures 7 and 8), followed by spiral, dagger and lesion nematodes. Average densities of pin nematodes were the highest among the parasitic nematodes in all years (Table 6). Lance, root-knot and stubby root nematodes only occurred in a specific year at very low densities (Table 6; Figure 7).

Table 6. Population densities (in 200 g soil) of plant-parasitic nematodes in soil collected from pea fields in North Dakota from 2014 to 2017.

Nematode (common name)	2014 (N=58)		2015 (N=91)		2016 (N=44)		2017 (N=50)	
	Highest density	Average density	Highest density	Average density	Highest density	Average density	Highest density	Average density
Pin	6,840	909	4,294	470	7,114	1,558	3,666	835
Stunt	1,980	261	207	61	685	149	780	114
Lesion	100	43	88	54	559	105	1,980	224
Dagger	60	26	52	23	70	25	130	41
Spiral	120	64	173	36	739	144	1,100	206
Lance	-	-	-	-	106	41	-	-
Stubby root	-	-	-	-	22	22	-	-
Root-knot	20	20	-	-	-	-	-	-

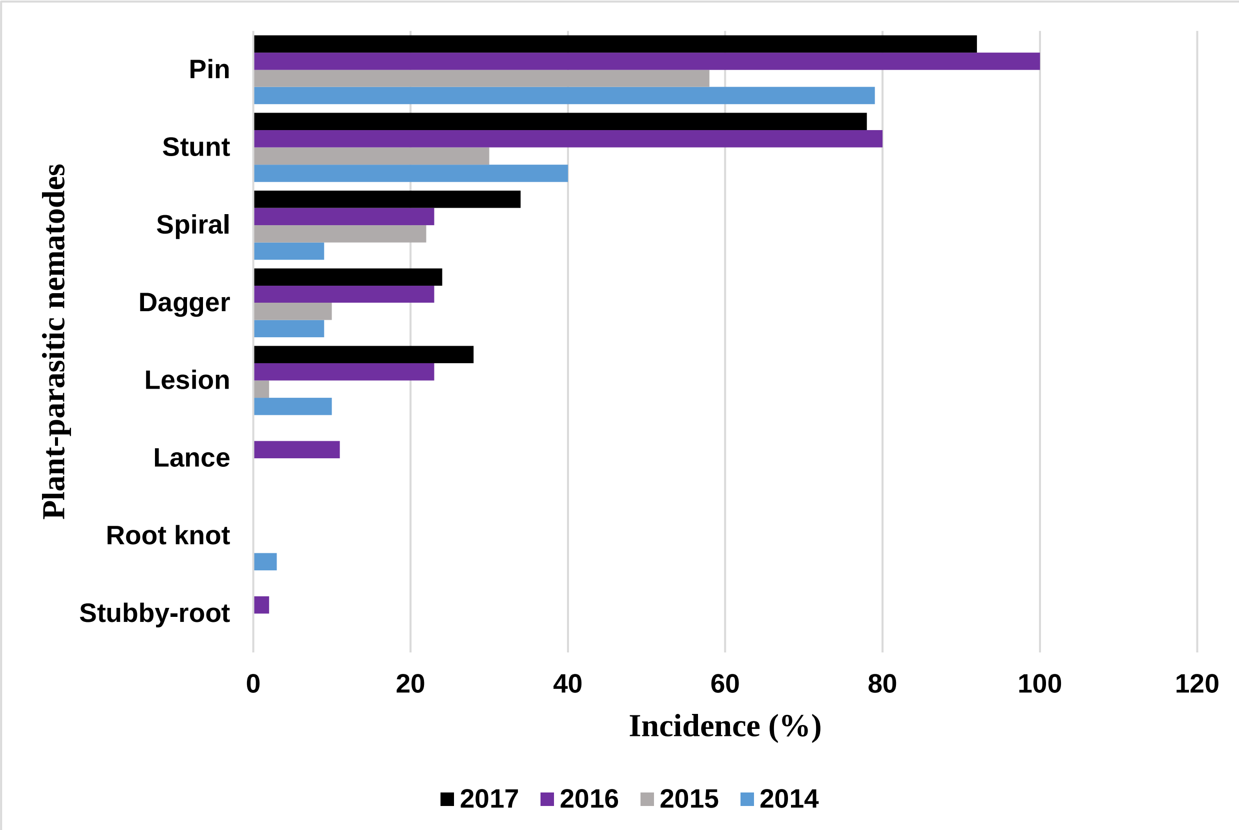

Figure 7. Incidence of plant-parasitic nematodes from 2014 to 2017 in soil from North Dakota pea fields.

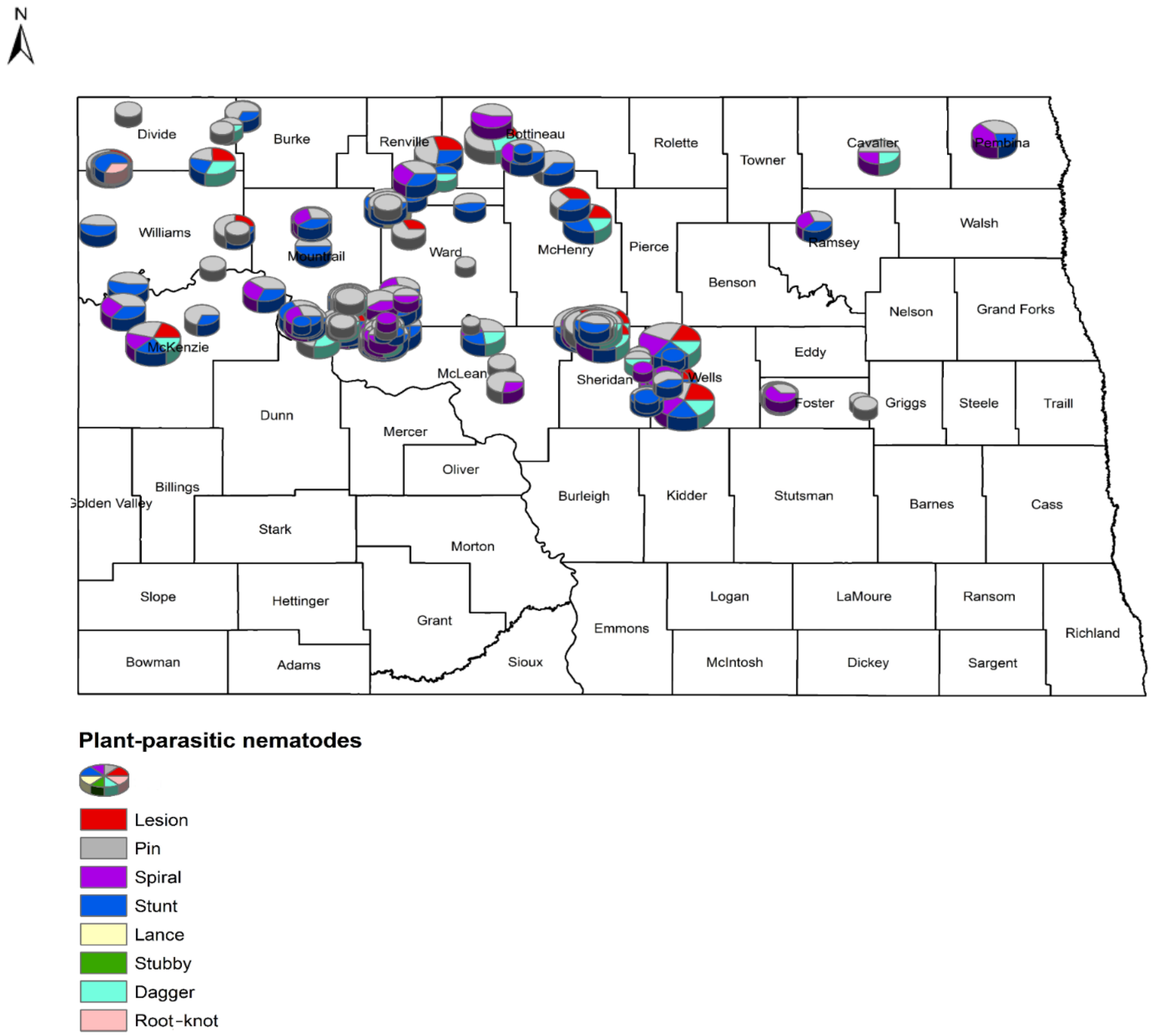

Figure 8. Distribution and abundance of plant-parasitic nematode genera from 2014 to 2017. Nematode numbers were log (1+X) transformed to normalize the abundance data and the distribution map was created using Arcgis version 10.5. Different sized pie-charts represent the proportionate total number of nematodes in sampling sites. A larger pie-chart indicates the higher total nematode number in a particular site compared to a smaller pie-chart which represents lower total nematode number.

Plant-parasitic nematodes including pin (*Paratylenchus nanus*), lesion (*Pratylenchus neglectus*), lance (*Hoplolaimus stephanus*) and stubby root (*Paratrichodorus allius*) nematodes were conclusively identified to species based on molecular evaluations. The species identity of stunt, dagger and spiral nematodes could not be confirmed due to limited sequence information for these species in GenBank. Based on the current sequences available in the database, stunt nematodes from three pea fields had the highest identity with *Tylenchorhynchus annulatus*, *T. thermophilus*, or *Tylenchorhynchus claytoni* (Table 7). Dagger nematodes from two pea fields had high sequence similarity with *Xiphinema thornei*, *X. rivesi*, or *X. americanum*, and spiral nematodes from two fields had the highest identity with *Helicotylenchus digonicus* or *H. minzi* (Table 7). The species identity will be confirmed as more sequences are available in GenBank.

Table 7. Species similarity with known nematode species in NCBI database for two genomic regions, D2-D3 and internal transcribed spacer (ITS).

Field identity	Nematode type	Similar species	Genomic regions	
MB-1	Stunt	*Tylenchorhynchus annulatus*	D2-D3	ITS (1+2)
			98% (689/704)	99% (883/890)
Pea-10 South	Stunt	*Tylenchorhynchus thermophilus*	D2-D3	ITS (1+2)
			97% (689/707)	93% (689/742)
Russ-mid	Stunt		D2-D3	ITS (1+2)
		Tylenchorhynchus thermophilus	97% (689/707)	92% (804/878)
		Tylenchorhynchus claytoni	97% (640/661)	92% (469/512)
MS-2 and MB-3	Dagger		D2-D3	ITS 1
		Xiphinema thornei	100% (769/769)	98% (760/778)
		Xiphinema rivesi	99% (799/800)	99% (766/776)
		Xiphinema americanum	99% (766/771)	99% (769/776)
MB-3 and Russ-mid	Spiral		D2-D3	-
		Helicotylenchus digonicus	99% (701/707)	-
		Helicotylenchus minzi	99% (546/548)	-

<u>Goals and Outcomes Achieved</u>

PERFORMANCE MEASURES:

- Currently, no high throughput assay is available for pea root rotting pathogens (**benchmark = 0**) and this research will result in a PCR-GBS assay for high throughput quantification of nine root rotting pathogens from pea roots (**target = 9**).
  - PCR-GBS assays developed detected 11 root rotting pathogens from pea roots. *F. avenaceum*, *F. culmorum*, *F. graminearum*, *F.sporotrichioides*, *F. redolens*, *F. solani*, *F.acuminatum*, *F. oxysporum*, *Rhizoctonia solani*, *Aphanomyces euteiches*, *Pythium* spp.
- While it is thought that seed quality and plant nutrients play a role in plant establishment, no data is available on the association of seed pathogens, germination rate and seedling nutrient levels with root rot (**benchmark = 0**). This research will generate this data for 40 pea fields (**target = 40**).
  - This target was not reached.
- Currently, no information is known about root-inhabiting nematodes from pea roots (**benchmark = 0**). Lesion nematode is most commonly associated with roots and high levels of this nematode will be likely found in field peas in ND (**target =1**).
  - High levels of lesion nematode were found and this target was reached.
- The nematology program does not have a pure culture of pin nematodes generated from pea fields (**benchmark = 0**). A pure culture to be used in subsequent inoculation will be generated (**target = 1**).
  - Pure cultures of pin nematodes were established from field soil and maintained on a susceptible field pea cultivar.

- No data is available for the effect of pin nematodes on the growth of field pea (**benchmark = 0**). Data will be generated for the effect on five cultivars grown in ND (**target = 5**).
    - Pin nematodes were inoculated onto five pea cultivars and the effect on plant growth was measured. This target was reached.
- In 2014, nematode populations and the effect of soil properties on root rot severity was evaluated in 20 fields in 2014 (**benchmark = 20**). In 2015, these evaluations will be evaluated in 40 pea fields (**target = 60**).
    - Nematode populations were evaluated on more than 40 pea fields. This target was surpassed.

Beneficiaries

- There are over 1500 field pea producers in North Dakota that are members of the Northern Pulse Growers Association that could benefit from this project.

Lessons Learned

- Seed was not collected from growers to evaluate seed quality due to the difficulties in contacting growers during planting. Additionally, growers do not always save or track extra seed individually by field.
- The development of a culture for ectoparasitic nematodes, like the pin nematode, has proven difficult under lab conditions; however, we have developed an efficient protocol to increase pin nematodes using susceptible pea plants in the greenhouse.

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Additional Information

Presentations and publications from these results

- Upadhaya, A., Yan, G. P**.**, Plaisance, A., and Pasche, J. 2017. Plant-parasitic nematodes on field pea and their association with soil edaphic factors in North Dakota. American Phytopathological Society North Central Division Meeting, Champaign, Illinois, June 14-16.
- Upadhaya, A., Yan, G. P., Plaisance, A., Pasche, J., and McPhee, K. 2017. Pin nematode: a potential threat to pea production in North Dakota. Proceedings of the North Dakota Academy of Science 71:45.
- Upadhaya, A., Yan, G. P., Plaisance, A., Pasche, J. S**.**, and McPhee, K. 2016. Identification and reproduction of pin nematode on field pea (*Pisum sativum*) in North Dakota. Society of Nematologists Annual Meeting. Montreal Quebec, Canada. July 17-21, 2016.
- Zitnick-Anderson, K., Simons, K., and Pasche, J. 2017. Multiplex qPCR Assays to Quantify Fusarium species in Root Tissue. North American Pulse Improvement Association Bi-annual Meeting. East Lansing, MI. Nov. 2-3, 2017.

Northern Plains Vegetable Variety Testing II

Final Report

Project Summary

The Northern Plains Sustainable Agriculture Society's Farm Breeding Club (FBC), North Dakota State University (NDSU), and North Dakota Farmers Market & Growers Association continued their collaboration to increase access to superior varieties of selected market classes of vegetable varieties (i.e. carrot, green bean, onion, lettuce, and cucumber) suited to local direct-to-consumer markets with this final year of variety evaluations and outreach. Although there has been ongoing growth in farmers markets and other specialty crop production in North Dakota, there have not been any variety trials for many years. To address this, we employed replicated variety trials at NDSU in Fargo, ND as well as single-replicate, daughter sites at vegetable farms and Tribal College gardens throughout North Dakota with ten varieties for each of the five species of specialty crops, building upon three previous years of specialty crop research. Results from the data we collected at these variety trials have been reported through field day tours, workshops at collaborator events, and through social media. We are pleased to have now finished the four years of work and can now provide reasonable comparisons of good varieties to the growers in North Dakota.

Project Approach

- Communication: Project updates were shared via the NPSAS Farm Breeding Club Facebook page and the NPSAS Germinator newsletter, as well as via eSprout email notes to announce and remind members about upcoming project events.
- Ongoing variety evaluation training: This project has provided training in basic, on-farm experimental design, as well as tools to efficiently and effectively manage on-farm trials and utilize trial results. In 2016 this involved an hour of training and discussion during the late winter Farm Breeding Club meeting held in Medina. Three growers even took part via a web connection. The basics of how the project works have also been presented along with our results at other meetings with other growers as well.
- Source seed for screening: We used the same list of varieties for green beans, nantes-type carrots, slicing cucumbers, romaine lettuce, and yellow, storage onions as we did the last year. NPSAS then distributed the seeds (along with stakes for each variety) to the North Dakota growers in February and March 2016. Onions were grown by Dr. Lee's team at NDSU and by Marvin Baker at Carpio, ND. The project team then distributed onion plants to growers in early May (please see the photo below).
- Project Participants: North Dakota project collaborators included the NPSAS Farm Breeding Club (FBC), Dr. Chiwon Lee (NDSU Plant Science Department), Steve Zwinger (NDSU Carrington Research Extension Center), North Dakota Farmers Market & Growers Association (NDFMGA), Meghan Estvold (Dakota College at Bottineau

Entrepreneurial Center for Horticulture or ECH), Linda Hugelen (Land Grant Extension at United Tribes Technical College or UTTC), and Jeremy Lewis (Nueta, Hidatsa, Sahnish College or NHSC). Farmer cooperators included Marvin & Ilene Baker (North Star Farms, Carpio, ND), Annette & John Carlson (Morning Joy Farm, Cleveland, ND), Glen Philbrick (Hiddendale Farm, Turtle Lake, ND), Eden McLeod (McClusky, ND), Marte Stensli and Bob Nelson (Tangle Tree Ranch, Doyon, ND), and Edd Goerger (Wyndmere, ND).

- Variety evaluations on farm and at NDSU: NDSU and interested growers started onion seeds for ten varieties to grow transplants in February and March of 2015. Other seeds were direct planted at the appropriate time in May or June as weather allowed. NDSU research farm in Fargo (mother site) conducted replicated variety trials (4 replicates) of 5 priority market crops (carrot, green bean, cucumber, onion, and lettuce). Single replicates (one plot of each variety) were evaluated on at least three ND market farms (daughter sites) for each crop. Each grower was trained and received seeds, stakes, an experimental protocol, and data sheets. Crop assignments in 2016 were as follows: Green Beans (Annie Carlson, Steve Zwinger, Marte Stensli, and Eden McLeod), Carrots (Steve Zwinger, Meghan Estvold, Jeremy Lewis, and Linda Hugelen), Cucumbers (Marte Stensli, Linda Hugelen, and Edd Goerger), Lettuce (Marte Stensli, Glen Philbrick, Marvin Baker, and Annie Carlson), and Onions (Glen Philbrick, Steve Zwinger, Eden McLeod, and Marvin Baker). Most crops were grown out in 10 foot rows spaced about three feet apart, surrounded by a few plants of an alternative variety to provide a protective border for the main plots. We used colorful varieties for borders when possible to make separation of the plots easier.
- Field tours and tasting events: We decided that with very light participation at most of our field events in the last three years, we would instead focus on the big field day at NDSU in Fargo and shoot a video of discussions with our growers. Unfortunately, after promoting the event in NPSAS communications, Fargo received a hailstorm in late August and the field tour and all later data collection for crops besides onions and carrots were cancelled. We did shoot the video (see below).
- Compile variety trial results: Variety trial results came in from growers to NPSAS in November and December, 2016. Suman Parajuli, graduate student at NDSU advised by Dr. Lee, worked up the data collected by project participants from this year and over the last four years for presentation to audiences in 2017.
- Dissemination and outreach: The project disseminated critical, replicated trial data on traits of interest to help farmers minimize risk and identify and develop new markets for varieties with traits of interest to consumers. Following basic scientific methods in conducting on-farm variety trials brings a level of assurance to varietal selection, whereas, simply trying a new variety without a good experimental design can give misleading and even invalid information.

Information about this project was posted in the NPSAS Germinator Newsletter that is sent out to members. There are 466 NPSAS members who receive the Germinator and 171 of these are in North Dakota.

We also shot and posted project related videos on YouTube. Our specialty crop videos over the last four years are as follows:

The Farm Breeding Club Plant Variety Screen: Potatoes (https://www.youtube.com/watch?v=siUcpFy6V4w). Using a potato variety screen carried out by a few members a number of years ago, we explain the concept and reasons for doing screens to whittle down the number of varieties to be considered for growing or for further evaluation. This video was posted seven months ago and has had 77 views.

North Dakota Gardeners Talk About On-Farm Specialty Crop Variety Trials (https://www.youtube.com/watch?v=IOzED8jTNTE). This video was shot with some of our collaborating growers in August, 2016. Growers comment on their thoughts about doing variety trials and their utility for serious production. It was uploaded nine months ago and has had 101 views.

Hiddendale Farm Tour: Quinoa, Amaranth, and More (https://www.youtube.com/watch?v=3IDEgqLFuV0). Glen Philbrick, one of our project collaborators, hosted an NPSAS farm tour that included a visit to his specialty crop plots and other projects. This video was uploaded two years ago and has had 475 views.

Farm Breeding Club Tomato Variety Field Day (https://www.youtube.com/watch?v=Edb5OAk1MiI). Marvin Baker was a collaborator on the project throughout, and hosted a field day for his tomato and pepper trials. This video covers a tour of the tomatoes, it was uploaded two years ago, and it has received 138 views.

NPSAS/NDSU Pepper Variety Trial (https://www.youtube.com/watch?v=8YqHbF9jZYY). This is the pepper portion of Marvin Baker's garden tour. The video was produced and uploaded two years ago and has received 112 views.

Garden Variety Trial Field Day at Tangle Tree Ranch (https://www.youtube.com/watch?v=P4181Yzut3o). This video includes highlights from a field day near Doyon, ND led by Marte Stensli and Bob Nelson. The video was uploaded two years ago and it has received 86 views.

NPSAS Vegetable Variety Trials at Prairie Road Organic Seed (https://www.youtube.com/watch?v=N5cI4pi_2vM). This video was shot during a very well attended field day at the Podoll Family Farm near Fullerton, ND. Our project is introduced and the purposes and processes of variety trials explained. The video was uploaded two years ago and it has received 190 views.

All of our Farm Breeding Club videos can be found at our YouTube channel (https://www.youtube.com/channel/UC5rdEz17pAaMt-d8MWsPppA/videos?view=0&shelf_id=0&sort=dd).

Our FBC Facebook page (https://www.facebook.com/pages/Farm-Breeding-Club/165221056860513) has had many postings about our specialty crops project and has been used to share results and to promote upcoming events. We currently have 926 likes for the page.

In addition, the methods and results of the project have been presented during workshops at the North Dakota Local Foods Conferences in Mandan in February, 2017 and at the Bismarck Garden Expo in April, 2017. If we can find additional resources, we would like to put together an Extension Bulletin about specialty crops with Dr. Lee that would feature our four years of efforts.

- Grower surveys: after several years, it has become clear that our gardeners do not like to fill out surveys. Responses have never been overwhelming given the size of our audiences and some times returned surveys have been very few. This year we planned to simply ask a few simple questions at events and gauge the general responses. Of thirty eight attendees of the Garden Expo presentation given by Chiwon Lee, Suman Parajuli, and Frank Kutka, twelve expressed interest in getting copies of our results. This suggests that about one third were very interested in considering variety choices and making possible changes based on the results of our multiyear trials. The North Dakota Farmers Market and Growers Association has also requested a full report.

- The background information and guidance we shared on variety trials could be used for any type of crop, but we only presented this information in discussions of specialty crops with examples and photos of specialty crops to audiences interested in specialty crops.

Goals and Outcomes Achieved

» **Measurable Outcome #1**: *Increase yields and quality of North Dakota-grown vegetables through adoption of better-adapted varieties.*

Performance measure	Number of farmers who adopt top performing varieties
Benchmark	Performance of currently grown vegetable varieties
Target	> 1/2 of farmers attending project events or downloading variety trial data and reports state they are adopting better performing varieties.
Data Collection	Post-event surveys; feedback via social media networks and website.

Responses to questions about our work posted on social media have been very few, much like most of our paper surveys after workshops. This has been a great disappointment. While we did not see half of farmers reporting the use of the varieties we highlighted, at least a third at the last Garden Expo in Bismarck expressed great interest in getting paper copies of the results, and the NDFMGA seeks to further disseminate this information. Surprisingly, some of our growers also expressed strong interest in the border varieties that we used even though we did not collect data on these. Apparently the red onions and red lettuce were very popular in some farmer's markets.

» **Measurable Outcome #2** *Farmer-Scientist-Student-Consumer networks for sharing and collaborating on vegetable variety improvement with unique salable qualities*

Performance measure	Attendance at FBC field events and access of information posted online and through social media platforms
Benchmark	Connection of current project participants, project downloads and social media followers

Target	> ½ of the project attendees "opt in" and interact via social media networks; core project participants contribute posts to our Facebook page and newsletters.
Data Collection	Number of Facebook participants and contributors

Over the years the numbers of attendees at major field events and workshops numbered from 20-60. However, we did not attract nearly as many to the field events at the homes of individual growers in late summer. Some times we had no visitors and sometimes 2-4 would join us, so we cancelled this approach in 2016 to focus on the planned event at NDSU. The best attendance was when a field day incorporated additional activities and was held as part of a larger NPSAS Summer Field Event, as when more than 20 visited the Hiddendale Farm near Turtle Lake in 2014 or when over 60 attended the Carrington Research Extension Center Field Day Organic Tours where our project was sometimes featured (2013, 2015). We did not try to assemble a list serve as even our email group was not of great interest to our growers. Facebook participation has also been very light, although we have gotten a very good number of likes and shares. Personal connections and big events with multiple speakers appear the most efficient ways to reach specialty crop growers.

» **Measurable Outcome #3:** *Increased knowledge of varietal differences, desirable quality traits, potential gains in varietal suitability, and the value of participatory variety evaluation.*

Performance measure	Event attendance, distribution of project publications, & stated learning
Benchmark	2012, 2013, 2014, 2015 NDFMGA surveys
Target	>3/4 of participants indicate increase in self-assessed knowledge level
Data Collection	Post-surveys; attendance; number of publication downloads, print copies distributed, and video views; feedback via social media.

Surveys have not been popular with our audiences and responses have been relatively few. It is much easier to get folks to hold their hands up in response to a question or two at an event and much more informative it would seem. Recent audiences have been very interested in what we have done and are eager to get copies of the results to take home and look over themselves. Generally about 1/3 to 2/3 have seen utility in the work and interest in getting the full data analysis. Social media has been good for broadcasting our information, but has not been as useful for getting people to share their opinions about specialty crops. That said, two years ago we did get good input on a web-based survey about specialty crops that helped us pick out these five crops to evaluate after the link was shared on our Facebook page and via NPSAS communications. Apparently, if you can get one or two dozen growers to respond to a survey you are doing well. Please see above for video views and Facebook page likes.

Beneficiaries

Primary beneficiaries of this project were commercial vegetable growers in North Dakota and of course surrounding states and provinces. Businesses supported by or purchasing from these producers (i.e. seed growers, farm supply retailers, restaurants featuring local food) could also see increased revenues.

According to the North Dakota Department of Agriculture's 2016-17 Local Foods Directory there are over 90 commercial vegetable growers in North Dakota. There are also 50 farmer's markets, 25 CSA operations, and many others dealing with local foods in North Dakota. There are likely many more small home-based farm businesses and home gardeners that would benefit from this project and the production of well-adapted varieties. Those who adopt new varieties based on information from this project should expect greater yields of higher quality vegetables when superior varieties are identified. Our results are a bit mixed, in that clear differences were not always obvious, but the growers who saw the results were in general interested to try something new and now have a clearer idea of what to grow next.

This project continued to bring plant scientists, seed producers, farmers, and their customers together in this collaborative participatory research approach. This feedback loop could increase agronomic suitability and marketability of the produce and the seed supply, enhancing overall efficiency. Variety trials and participatory evaluation enhances efficiency by eliminating guesswork, trial-and-error expense, and risk from the variety selection process. This is especially valuable to beginning and socially disadvantaged farmers, as they gain knowledge of varietal differences, growing characteristics, marketable traits, and how experienced farmers select their varieties. We have certainly reached out to every audience that we could in order to assist all the growers in North Dakota.

Direct grower and consumer input regarding their needs is also the key to guiding future variety development and improvement efforts. This project has provided direct observations and grower and consumer feedback for the plant breeding process to both scientists and graduate students. If ongoing funding can be found for specialty crop varietal improvement at NDSU, we expect that our project will very much help our growers, consumers, and scientists come together to better meet our needs for high quality specialty crops into the future.

Lessons Learned

- Surveys are not popular, and participation is light. After workshops most participants are eager to leave for the next workshop and our complex surveys often were ignored. It is not clear that any paper survey will get many participants. Doing a show of hands worked very well in 2016 and 2017, and the online survey ahead of this present work did get a reasonable number of participants. More intensive survey work is unlikely to receive many willing participants.
- We had weather destruction of several trials throughout this project, and this can only be dealt with by spreading trials out across the region for several years. This is what we did.
- Flexibility and ongoing communication was useful in getting and maintaining our grower participation. Personal communication is especially critical.
- Short, concise descriptions of the methods have been well received during workshops where significant project results or discussions of breeding were presented.
- Small summer field days were not a big priority for the public. We have had no problem getting attendance of workshops at winter conferences or when we combined our specialty crop activities with other topics at summer events. Partner for outreach success!
- Distance communications have not been a big draw, and likely need not be a large priority for future work with our established growers. We have had good public outreach communications via Facebook, but email discussions among our growers did not really go anywhere. We did have a couple of growers take part in our trainings via a weblink, but although we had room for more than a dozen members of the gardening public, no one else took part even after concerted advertising of the opportunity. Trainings and discussions at conferences and field events had much better participation, which was unanticipated. So, train in person and disseminate links, results, and video via the web.
- We now have a very good idea of which varieties of five major crops to recommend!

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Additional Information

What follows are photo documentation of our trials and data tables from the first attempt at analysis of all the data across all four years of the trial. Columns are filled with averages for each variety for a set of parameters that we measured. When those columns have different LSD (Least Significant Difference) letters they are considered to be statistically different from other varieties that have different letters. If they have the same letter, any differences in mean performance are not considered to be statistically significant and may not be real.

Defining Glyphosate and Dicamba Drift Injury Thresholds in Dry Beans, Field Peas, and Potatoes

Final Report

Project Summary

Soybeans continue to be one of the top commodities produced in many areas of the United States, and has nearly matched wheat as the top crop in North Dakota by acres. This project coincides with the release of dicamba-resistant soybean varieties, which has resulted in the increased use of dicamba. Soybean acres often exist adjacent to other broadleaf crops, such as dry beans, field peas, and potatoes, which can be very sensitive to dicamba vapor, drift, or spray-tank contamination. Even with Best Management Practices, incidents of drift have increased since the commercialization of dicamba-tolerant soybeans. Crops such has potatoes may not only be affected by reduced yields, but can also suffer reduced germination in harvested seed tubers.

New analysis techniques and equipment can detect trace amounts of herbicide in a sample plant sample, but the effect of those trace amounts on plant health remains unclear in many cases, and the reliability of the tests in field conditions remains largely unknown. Some products such as atrazine, chlorimuron, clomazone and others have known safe concentrations (ppm or ppb), where plants are not affected by concentrations less than the safe value. This information does not appear to exist for dicamba or glyphosate, even though analytical labs have noted an increased workload of screening for dicamba and glyphosate residues.

Due to increased focus on food safety, it is also important to study potential effects of seed germination and herbicide residue after the plant has been exposed drift events. If herbicide residues can be detected in harvested samples, it jeopardizes the marketability of the crops.

This project is a continuation of a previous Specialty Crop Block Grant awarded in 2014. This continued project builds upon the first study by 1) adding more data to define safe herbicide concentrations, and 2) including harvested samples as part of the analysis to ensure end-use food safety.

Project Approach

This study was conducted at 6 sites in 2015. Two sites each for dry beans, field peas, and potatoes. These crops were grown and sprayed with sub-lethal doses of dicamba and glyphosate. Doses were chosen from previous work which identified a rate range where injury symptoms started to appear in these crops. The ratio of glyphosate and dicamba are the same as what would be used commercially. Three rates of dicamba and glyphosate were used alone and together, plus a non-treated check plot (10 total treatments). These treatments were applied at once each species started to flower, which coincides with when dicamba and glyphosate would typically be applied, and would also be when the plants are most sensitive to herbicide injury. Injury evaluations were taken 10 and 20 days after application. Plant tissue samples were also taken 10 and 20 days after application. Rigorous work was performed to ensure that there was no contamination of the plots during application and during sample collection. Samples were shipped to an analytical lab (South Dakota Agricultural Laboratory) to determine if/how much herbicide remained in the tissue and to compare it to how much visual injury was observed. Plots were harvested for yield and quality data. Subsamples were also sent to the same lab for herbicide residue testing. Another subsample was used to test the germination, as another measure of seed quality. Data were compiled and analyzed for each species. The following is a summary for each crop:

Field Peas:

Field peas were treated with 2.5-25% of typical dicamba use rates in small grains, however they are only 0.6-6% of the dicamba formulation used in soybeans. It is also 0.45-4.5% of glyphosate use-rates. These rates were enough to cause visual injury to the field peas. Field peas received as much as 26% injury 10 days after treatment (DAT), but finished with 17% (Table 1). This only occurred with the highest rate of the combination of the two products. Dicamba caused more injury than glyphosate at the rates used. Glyphosate applied alone did not cause significant injury in the trials.

Leaf tissue analysis revealed that glyphosate was detected in some treatments but that none of the treatments differed statistically from the check. Dicamba was detected at more meaningful levels both 10 and 20 days after treatment. When combined across site-years, the 10 and 20 DAT data do not differ much. Dicamba was present in tissue at the middle and high rates that were used.

Yield was affected by the combination of glyphosate and dicamba at the two highest rates. Yield reductions of 8 and 17% were observed with the middle and high rate of the combined products. No yield reduction was realized with either product alone. Protein was not affected by any treatment. In grain samples, seed germination was not affected by any treatment combination, nor was any significant herbicide residue detected.

It appears that ~10 ppb may be the dicamba leaf concentration that will cause injury to field peas. This could be caused by a 6% application of dicamba mixed with glyphosate. Concentrations less than 15 ppb glyphosate appear to be safe, but were not able to determine an injury threshold for glyphosate. With no residue detected in the grain, field peas that have been affected by low levels of drift should remain safe and marketable.

Table 1. The effect of glyphosate and dicamba drift on field peas.

		Phytotoxicity		Residue Level 10 DAT		Residue Level 20 DAT				Grain Residue		
Treatment	Rate	10 DAT	20 DAT	Dicamba	Glyphosate	Dicamba	Glyphosate	Yield	Protein	Dicamba	Glyphosate	Germination
	fl oz/a	%	%	ppb	ppb	ppb	ppb	%	%	ppb	ppb	%
Check		0	0	0	0	0	0	0.90	29.74	0	0	95.8
Dicamba	0.05	1	0.3	3.1	0	2.4	5.9	0.89	29.62	0	0	95.3
Dicamba	0.25	5.5	3.4	8.7	0	8.9	0	0.88	29.81	0	0	94.8
Dicamba	0.5	12	6.9	11.4	0	17.9	0	0.86	29.84	0	4.1	95.8
Glyphosate	0.1	0.6	0.6	0.4	0	0	0	0.89	29.89	0	0	93.5
Glyphosate	0.5	0.8	1.3	0.3	3.3	0	0	0.91	30.13	0	0	95
Glyphosate	1	3.4	2.8	0.8	0	0	5.2	0.88	29.92	0	0	96.5
Glyphosate + dicamba	0.1 + 0.05	1.4	0.9	2.7	4.7	3.1	0	0.92	29.85	0	4.6	95
Glyphosate + dicamba	0.5 + 0.25	13	8.1	10.5	0	11.7	0	0.82	29.81	0	0	95
Glyphosate + dicamba	1 + 0.5	26.6	17.1	18.4	14.7	17.0	4.5	0.73	29.90	0	0.1	94.5
LSD (0.05)		5.2	3.0	5.2	NS	6.3	NS	0.06	NS	0	NS	NS

Dry Bean:

Glyphosate rates in this study ranged from 0.045-4.5%, while dicamba ranged from 0.25-25% (cereal rate) or 0.06-6% (soybean rate) of the commercial rates. Dry beans were affected heavily by the rates used in this study. Dry bean injury ranged from 5-40% when combined across site-years (Table 2). Both glyphosate and dicamba were damaging to dry beans. Only the lowest rates used did not significantly injure plants.

Dicamba and glyphosate leaf residue data was very difficult to interpret due to the variability. Glyphosate concentrations received from the lab were sometimes several times higher than they should be even after subtracting the check plots from other treatments. At times, the check plots would read several 1000 ppm even though no visible injury occurred. Dicamba was less erratic but suffered a similar result. With either product, there was no consistent meaningful correlation between visual injury and ppb.

Visual injury was more related to final yield data. Yield reductions of up to 80% occurred with the highest rates of both products. Even each product individually caused unacceptably high levels of injury at the highest rates. Only the lowest rates of each product alone and together produced yields similar to the check.

Germination was reduced in the highest rate of dicamba alone and dicamba plus glyphosate. Unfortunately, dicamba was also detected in the grain of the highest rate of dicamba plus glyphosate. However, the yield of that treatment was reduced to the point that it is unlikely a farmer would harvest the crop (14% of the maximum). No threshold was able to be determined for dry beans. This may be an instance where dry beans are not one that be tested via tissue analysis.

Table 2. The effects of glyphosate and dicamba on dry beans.

		Phytotoxicity		Residue Level 10 DAT		Residue Level 20 DAT			Grain Residue		
Treatment	Rate	10 DAT	20 DAT	Dicamba	Glyphosate	Dicamba	Glyphosate	Yield	Dicamba	Glyphosate	Germination
	fl oz/a	%	%	ppb	ppb	ppb	ppb	%	ppb	ppb	%
Check		0	0	0	0	0	0	0.91	0	0	96.7
Dicamba	0.05	4.6	5.5	7	0	62	0	0.83	0	0	94.7
Dicamba	0.25	19.5	21	22	6	1204	1	0.72	1.1	0	95
Dicamba	0.5	22.8	26.4	259	253	625	30	0.33	4.7	0	46.3
Glyphosate	0.1	6.2	5.4	15	11	305	5	0.74	0.5	0	95
Glyphosate	0.5	20.6	16.1	65	1037	414	143	0.54	2	0	95
Glyphosate	1	24.6	25.3	121	510	145	75	0.40	0	0	95.7
Glyphosate + dicamba	0.1 + 0.05	11.4	11.4	8	258	49	108	0.78	0	0	98
Glyphosate + dicamba	0.5 + 0.25	29.3	23.8	85	541	628	38	0.48	2.8	0	94
Glyphosate + dicamba	1 + 0.5	33.4	39.3	262	676	509	185	0.14	8.7	0	33.7
LSD (0.05)		9.1	11.6	192	930	586	100	0.26	5.6	0	18.8

Potatoes:

Potatoes were treated with 0.9-22% of commercial glyphosate rates and 5-100% cereal dicamba rates or 1.2-35% of soybean dicamba rates. The rates used in this study affected potatoes. In both years, glyphosate alone only caused injury at the highest rate. Dicamba caused injury at all rates alone or combined with glyphosate. In contrast to the other crops. Visual injury was often not different when dicamba was applied alone versus together with glyphosate.

Both dicamba and glyphosate were detected in the tissue samples (Table 3). The sampling correlated fairly well to yield and injury data. The highest rates of dicamba and glyphosate caused detectable levels of residues. Lower rates of dicamba were trending upward but were not statistically different than the check. With potatoes the timing of the sampling could influence the ability to properly detect herbicide residues. At 10 DAT the levels of glyphosate and dicamba were generally much higher than at 20 DAT. AT 10 DAT, 500 ppm of dicamba appears to be damaging, but at 20 DAT, 300 ppm appears to be damaging. Glyphosate appears to be difficult to sample for once again with results being fairly erratic (see below for more). Daughter tubers had meaningful levels of herbicide residue as well. These residue levels (even glyphosate) translated better into reduced germination,

Yield was affected by the highest rates of each product and the middle rates of dicamba alone and with glyphosate. Marketable yield showed much the same response, except that the differences were much more severe.

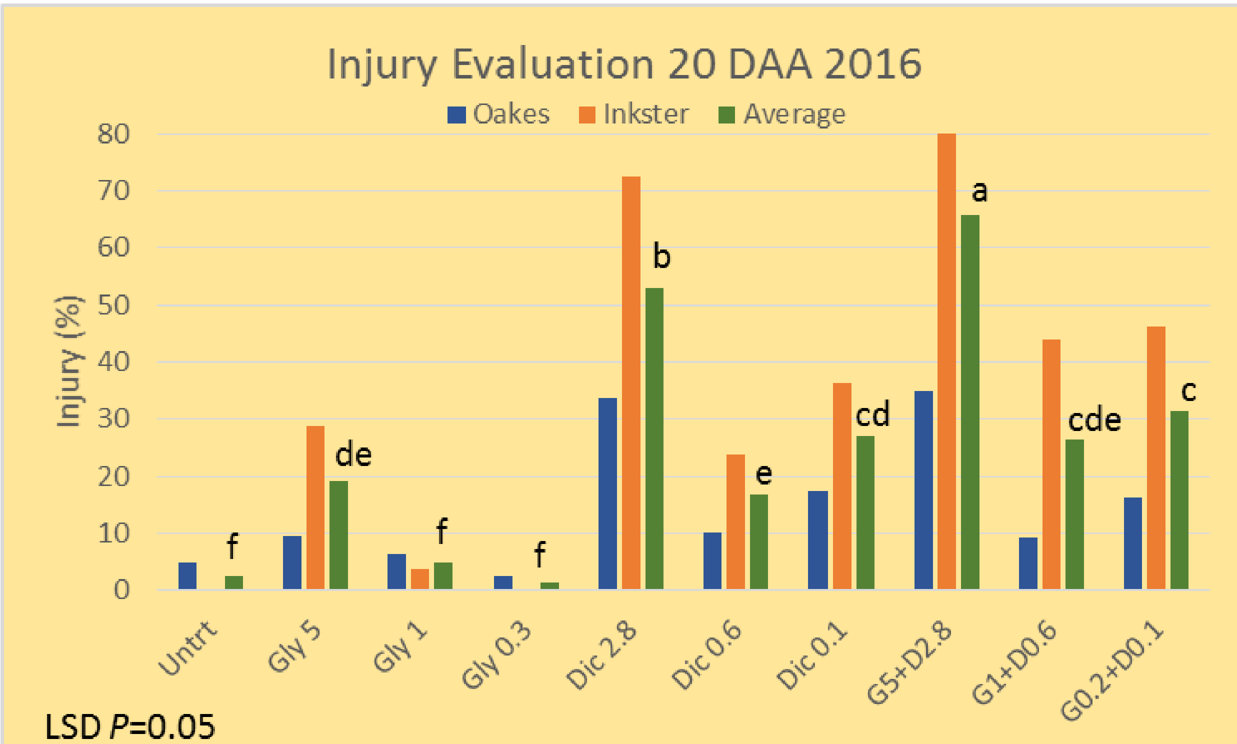

Figure 1. Injury of potatoes taken 20 days after herbicide applications.

Table 3. Glyphosate and dicamba effect on potatoes.

						Daughter Tuber Residues			
		Residue Level 10 DAT		Residue Level 20 DAT		Dicamba		Glyphosate	
Treatment	Rate	Dicamba	Glyphosate	Dicamba	Glyphosate	Inkster	Oakes	Inkster	Oakes
	fl oz/a	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb
						0	0	0	0
Check		0	0	0	0	0	0	0	0
Dicamba	0.05	237	0	73	6	0	0	0	0
Dicamba	0.25	614	0	299	147	0	0	0	0
Dicamba	0.5	2089	0	431	0	0	0	0	0
Glyphosate	0.1	6	13	4	17	0	0	0	0
Glyphosate	0.5	31	76	30	12	0	0	0	0
Glyphosate	1	54	616	0	144	0	0	0	0
Glyphosate + dicamba	0.1 + 0.05	254	0	29	1	0	0	0	0
Glyphosate + dicamba	0.5 + 0.25	475	35	239	16	75	0	21	0
Glyphosate + dicamba	1 + 0.5	3246	656	893	364	115	0	50	19
LSD (0.05)		1246	816	586	100				

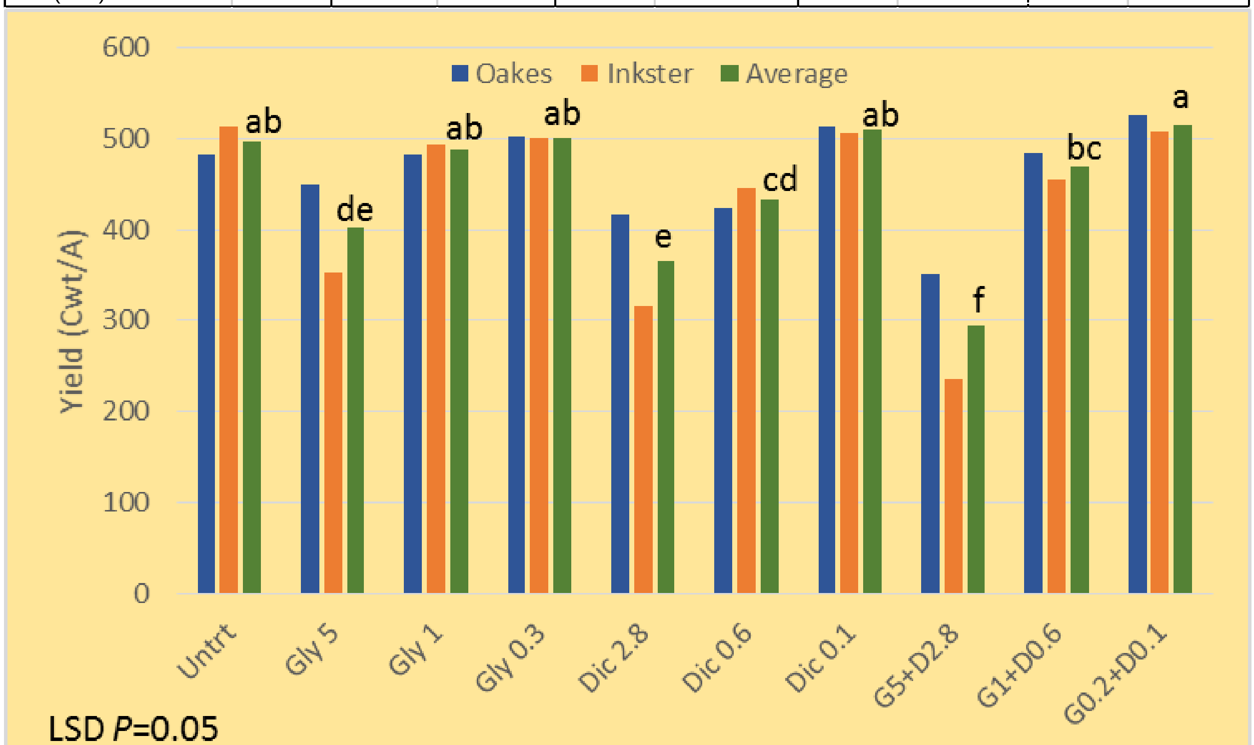

Figure 2. Total yield of potatoes following glyphosate and dicamba applications.

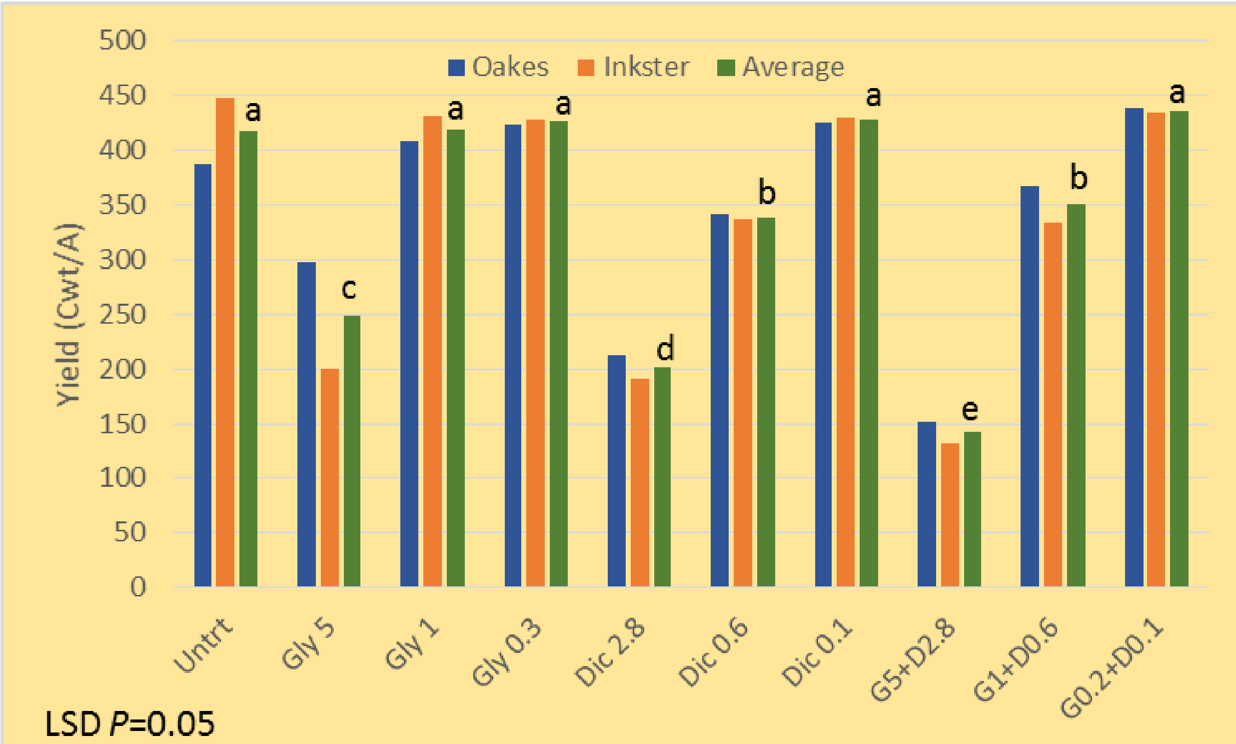

Figure 3. Marketable yield following glyphosate and dicamba applications.

Summary:

The levels of herbicide concentration with this study should be sufficient to measure causality, however, the highest detected concentrations many times do not correspond to the highest application rates. Once again, the relationships between ppb and yield are only moderate at best across herbicide dose and species. Overall dicamba was more predictive of response than glyphosate, but only when averaged across sites. Sometimes there was little or no correlation between dicamba and yield at an individual site. When averaged across sites and species, glyphosate did not cause a consistent concentration in leaf tissues. Overall, without the visual injury and yield information, the herbicide residue data would not mean much in this study, representing a challenging situation to producers who may have been affected by drift. Instead of relying on tissue testing, it will be necessary to use that data as supporting evidence only, in determining if a drift situation has occurred. See the 'lessons learned' section for a description of how to collect tissue samples, based on our experiences with this project.

How to collect samples for pesticide residue analysis

Here are a few comments that should be made regarding how to collect leaf samples for lab submission. In our trial the top 4" were collected from many plants to get a total of 40g per product that was tested (glyphosate + dicamba = 80g total needed). This represents that part of the plant that is most vigorously growing and would be the most affected by herbicides. In order to ensure an accurate test, samples should be collected from an area that has not been affected by an herbicide. Samples should then be collected from an area with only minor damage, followed by areas with more damage. Between each sampling group, be sure to change gloves to make sure there is no contamination between groups. Send the samples to the lab as quickly as possible to prevent the leaves from molding or deteriorating. Samples should be taken soon after injury is evident.

You also have to know which product to test for. Labs have a unique test that they use for each herbicide and so it will cost twice as much to check for two herbicides and three times as much to test for three herbicides; so you have to have an idea about which product caused damage. Once you get the results back it is important to know that a single test result does not mean anything. You have to compare test results from healthy parts of the field to results from the affected areas. This is because many lab procedures may show that a product is present when it may actually be something called "background noise" from the equipment which means that even plants never exposed to an herbicide may appear to have a herbicide concentration present. Tests will come back as either ppm (parts per million) or ppb (parts per billion). What does this mean? Again, that number on its own does not explain much as there is no standard concentration that will or will not cause plant injury, which is why you can only compare an affected plant to a healthy one.

In our studies, we have found that visual injury is a more conclusive method to determine herbicide injury than testing plants for herbicide residue. Herbicide residue information would be better suited as information used to substantiate visual evidence of injury rather than as a stand-alone method for showing injury. As seen in the results injury can occur with very little residue detected, but at the same time no injury may occur even though high residue levels are seen. Overall, there was a relationship between the amount of product applied and the amount detected in the leaves, but it was not strong enough to prove causality.

Goals and Outcomes Achieved

Besides the results shared above (see that section for important outcomes and data), this information has been shared at professional meetings and Extension events over the past year (outlined below). The final compiled information was just generated recently and will be shared at future outreach events. This information is also being used to create a threshold database located in the North Dakota Weed Control Guide. The information from this study will appear in the 2018 version of that publication.

Ultimately, we were able to complete all the tasks we set out to do. We have a good set of data with which to predict yield loss based on injury symptoms observed in the field. Unfortunately the tissue tests we conducted were less clear than we had hoped, even with upmost care in sampling and applications. We were able to determine some thresholds for dicamba residues in plants, but glyphosate was more difficult. This lesson in tissue collection and data analysis was still quite valuable. Many of the weed science experts at NDSU were involved with this project and have now become more familiar with what a tissue test can and can't explain. The key is that tissue tests alone can't resolve drift claims on their own. We had one goal for this project, which was to determine at what level of dicamba and glyphosate can you expect yield or quality loss. We accomplished this with all crops via visual injury and in some cases, supported by tissue tests.

Beneficiaries

Parts of this information were shared several times over the last year. The largest event is the Wide World of Weed Workshop held for farmer, crop consultants, and other ag professionals. The session for this topic had nearly 300 people in attendance. Last year, this focused primarily on the potato and dry bean portion. Another Extension even last year included a presentation at the Northern Pulse Growers Association annual meeting that focused on the field pea portion of the work. This even was attended by about 90 people. The potato data was also presented at a potato growers meeting (not sure on attendance). Preliminary data was also shared at the North Central Research Extension Center and Carrington Research Extension Center field days last year. The Minot tour had roughly 150 attendees while the Carrington ag tours had roughly 250 attendees.

The dry bean and potato data was also shared at 3 professional meetings this past winter. Each meeting had breakout sessions with roughly 30 research peers at each session.

The meetings where this information has been shared is intended to be a 'train the trainer' setup. The information gleaned from the meetings is intended to be used by others, increasing the impact of the information. 2017 is the first year that dicamba has been labeled for use in soybeans. The timeline for this project unfortunately means that we won't know the full impact the data will have, but we know it will be crucial for our area. In Arkansas (where applications are made earlier in the year), dicamba has been banned due to the amount of drift complaints. Our study was conducted at the perfect time to ensure that we have the proper information for North Dakota in order to protect the specialty crops that we are working with in this study. The impact of this study is just beginning.

A few other notes about impact. Page 106 in the 2017 North Dakota Weed Control Guide has some preliminary information related to this study. That publication is distributes over 70,000 copies each year. Previous results from this study have also been published on the North Dakota Weed Research website. The data was also published in the 2016 Carrington Research Extension Center annual report.

Lessons Learned

Based on our mixed experiences with tissue testing, below is a set of instructions that we are utilizing to maximize the chances for meaningful results in a tissue test:

How to collect samples for pesticide residue analysis

Here are a few comments that should be made regarding how to collect leaf samples for lab submission. In our trial the top 4" were collected from many plants to get a total of 40g per product that was tested (glyphosate + dicamba = 80g total needed). This represents that part of the plant that is most vigorously growing and would be the most affected by herbicides. In order to ensure an accurate test, samples should be collected from an area that has not been affected by an herbicide. Samples should then be collected from an area with only minor damage, followed by areas with more damage. Between each sampling group, be sure to change gloves to make sure there is no contamination between groups. Send the samples to the lab as quickly as possible to prevent the leaves from molding or deteriorating. Samples should be taken soon after injury is evident.

You also have to know which product to test for. Labs have a unique test that they use for each herbicide and so it will cost twice as much to check for two herbicides and three times as much to test for three herbicides; so you have to have an idea about which product caused damage. Once you get the results back it is important to know that a single test result does not mean anything. You have to compare test results from healthy parts of the field to results from the affected areas. This is because many lab procedures may show that a product is present when it may actually be something called “background noise” from the equipment which means that even plants never exposed to an herbicide may appear to have a herbicide concentration present. Tests will come back as either ppm (parts per million) or ppb (parts per billion). What does this mean? Again, that number on its own does not explain much as there is no standard concentration that will or will not cause plant injury, which is why you can only compare an affected plant to a healthy one.

In our studies, we have found that visual injury is a more conclusive method to determine herbicide injury than testing plants for herbicide residue. Herbicide residue information would be better suited as information used to substantiate visual evidence of injury rather than as a stand-alone method for showing injury. As seen in the results injury can occur with very little residue detected, but at the same time no injury may occur even though high residue levels are seen. Overall, there was a relationship between the amount of product applied and the amount detected in the leaves, but it was not strong enough to prove causality.

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Improving Management of Fusarium Root Rot of Field Peas by Quantifying Impacts of Common Herbicides

Final Report

Project Summary

Fusarium root rot of field peas is one of the most important production constraints for field peas in North Dakota and other regions of North America. The disease causes significant yield loss and is difficult to manage; fungicide seed treatments have limited efficacy, tillage practices have no impact, resistant varieties are not available, and, due to the broad host range and persistence in the soil of the causal pathogens, crop rotation is of limited effectiveness. Severe outbreaks of Fusarium root rot occurred on field peas in North Dakota in 2011, 2013 and 2014, and the disease – together with another root disease, Aphanomyces root rot – is threatening the continued production of field peas in parts of North Dakota. Fusarium root rot is most severe on plants under physiological stress, and most herbicides registered for use on field peas and some long-residual herbicides used on small grains prior to peas are known to cause physiological stress on field peas. Producers have long suspected that many herbicides may exacerbate Fusarium root rot in peas, but the impact of herbicides on Fusarium root rot has not been rigorously investigated and remains poorly understood. This project expanded research initiated in a Specialty Crop Block Grant awarded in 2014 by significantly expanding assessments of pre-emergence herbicide applications to encompass all commonly used products and by adding a second study location in Minot, ND (with weed scientist Brian Jenks) to confirm that results are consistent across environments differing in soil characteristics.

Project Approach

Project execution (tasks completed): Field trials were established in Carrington and Minot, ND in accordance to the work plan. Field trials were direct-seeded into wheat stubble with laboratory-produced pathogen inoculum applied in-furrow with the seed; herbicide treatments were imposed in accordance with label instructions; and root rot severity was assessed at full bloom and early pod-fill, the growth stage at which the disease generally becomes significant. A severe hail storm on early July in Carrington caused severe damage to the field peas, precluding additional data collection at that study location. In Minot, the trials were brought to completion; the incidence of wilt symptoms associated with severe root rot were assessed at mid- and late pod-fill, and seed yield and quality were assessed after harvest. Major findings were disseminated at outreach events during the winter meeting season and at summer field tours.

Major findings:

1. The combined use of common pre- and post-emergence herbicides resulted in a statistically significant increase in root rot severity in one of the four field trials, with root rot severity increasing from 33% (in the non-treated control) to 47% in three commonly used herbicide programs.
2. A modest increase in the wilt symptoms associated with severe root rot was observed with a commonly employed pre- and post-emergence herbicide program in one of two field trials in Minot. The herbicide applications were associated with an increase in the incidence of wilt symptoms from 3 to 6% despite no observed differences in root rot severity. These results parallel results from Carrington in 2015 and suggest that the stress of processing herbicides may sometimes reduce the tolerance of field peas to root disease.

3. No post-emergence herbicides applied to wheat the previous season increased Fusarium root rot in field peas.
4. The results suggest that commonly used herbicides may sometimes exacerbate root rot in field peas. The factors that caused increased root rot severity or increased root rot-associated wilt symptoms in some field trials, but not other field trials, were not immediately apparent, precluding the development of guidelines for when herbicides are at greatest risk of exacerbated root rot.

Role of project partners: Co-investigator Brian Jenks and his staff in Minot executed the research trials conducted in Minot, executing all tasks in those studies except production of pathogen inoculum, assessment of root rot severity, and completion of final reports.

Ensuring that the project funds only benefitted specialty crops: Results from this study will be directly applicable to field peas and fresh-market peas and partially applicable to snap beans and dry edible beans, all of which are designated specialty crops by the USDA. Findings from this study will be largely irrelevant for peas and beans sold at farmers markets or locally through CSAs, as products sold through such outlets are often exclusively or nearly exclusively organic.

Goals and Outcomes Achieved

1. Level of disease pressure and consistency of disease pressure across plots within field trials:
   - In all of the field trials, the targets for disease pressure (minimum 20% root severity) were met. Average root rot severity in the non-treated controls ranged from 33 to 65% across the field trials conducted as a part of this project, values that are well within the range of root rot severity observed in problematic commercial production fields.
   - In all of the field trials, the target for consistency of disease pressure across treatments (maximum CV of 40) was met. The coefficients of variation for root rot severity data ranged from 10.4 to 22.2 across the field trials, indicating that the impact of the herbicide treatments was relatively consistent across replicates within each study.
   - In the two field trials in which yield could be assessed, the target for consistency of field pea yields (maximum CV of 15) was met in one field trial (CV=14.7) but not the other field trial (CV=22.2). The factors causing the relatively high variability in yields in one of the trials were not immediately apparent.
2. Successful outcomes:
   - The study demonstrated that common herbicide programs sometimes, but not always, increase root rot severity or root rot-associated wilt symptoms in field peas, suggesting that the observations of producers that herbicides can impact root rot may be correct.
   - The study suggests that herbicide applications may only moderately increase the risk of root rot in field peas; common herbicide programs increased root rot severity in field peas in only one of four field trials and contributed to a modest increase in wilt symptoms in one of two field trials. These results suggest that herbicide usage is unlikely to be a significant contributor to root rot-associated crop loss in most situations.
3. Dissemination of research findings to stakeholders:
   - Major findings from this study were communicated directly to approximately 220 stakeholders (primarily producers, crop advisors and industry agronomists, but also extension personnel and researchers) through talks given at research and outreach events in 2017 and will be communicated to an estimated additional 250 to 300 stakeholders at scheduled talks at major extension events in South Dakota, Montana, and North Dakota in early 2018. This exceeds the goal of directly reaching 300 stakeholders through talks.

- Because of unresolved questions associated with the inconsistent root rot and root rot-associated wilt symptom responses to herbicide applications observed in this study, publishing results from this project will be delayed until follow-up research is completed. However, the total outreach goal of reaching 400 stakeholders (300 through talks and 100 through results published online) will be met through direct contact with stakeholders through outreach talks.

4. Adoption of research findings by producers.
   - The goal of at last 100 stakeholders modifying their production practices on the basis of the findings from this study is likely to be met once follow-up research is completed and rigorous conclusions can be reached relative to when herbicides can be expected to exacerbate root rot in field peas.

Beneficiaries

This project primarily benefited producers of field peas but is also expected to benefit fresh pea producers. Fusarium root rot is an important disease in all regions of the United States where peas are grown, and the project is expected to benefit most of the approx. 2,400 producers growing field peas on approx. 665,000 acres annually in the United States and many of the approx. 6,000 producers growing fresh-market peas on approx. 200,000 acres annually in the United States.

Lessons Learned

The field trials conducted as a part of this project demonstrated that common herbicide programs utilized in field peas can sometimes, but not always, exacerbate Fusarium root rot in field peas. The factors which determine when herbicides applications are most likely to increase the risk of Fusarium root rot in peas remain unclear.

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Advancing Value Added North Dakota Export of Specialty Dry Bean

Final Report

Project Summary

Value added exports of specialty dry beans can be advanced by metabolic innovations for enriching their bioactive health benefits. Globally value added development of diet-based and safe anti-hyperglycemic bioactives from plant-based foods is a major emerging focus of consumer diet demands to manage type 2 diabetes and other chronic diseases. Dry bean (Black, Kidney, Pinto, and Navy) based foods enriched with health relevant bioactives offer excellent strategy to incorporate healthy outcomes into regular dietary strategies for prevention and management of type 2 diabetes and associated cardiovascular complications. Edible dry bean with improved health relevant bioactive profiles provide safe dietary antidote targeting against type 2 diabetes and associated cardiovascular complications. To incorporate dry bean in dietary management strategies for improving glucose metabolism it is important to screen the bioactive profiles in the right target commercial cultivars and determine their potential role for diets to potentially mitigate type 2 diabetes through rapid *in vitro* assays. Therefore, the major goal of this project was to screen and improve health relevant bioactive profiles of commercial dry bean cultivars with natural bioprocessed elicitors for enhancing value added health relevant bioactives for advancing global exports from North Dakota. To achieve the goals and objectives of this project, greenhouse (**Activity 1**), field (**Activity 2**), and laboratory based (sprout models) (**Activity 3**) experiments were conducted to improve human health relevant bioactive profiles in four different market classes of edible dry beans (black, pinto, navy, and red kidney beans). Overall, improvement of phenolic bioactives and associated antioxidant and anti-hyperglycemic properties of edible dry beans was observed with natural bioprocessed elicitors (soluble chitosan oligosaccharide-COS and Gro-Pro; amino acid enriched marine peptide @1%) in the greenhouse and in the field. Furthermore, black and red kidney bean sprout models was used to improve value added health targeted phenolic bioactives and associated antioxidant and anti-hyperglycemic functionalities relevant for dietary interventions targeting against type 2 diabetes and associated complications. The research fund was solely dedicated to improve specialty dry beans for bioactive enrichment and value added export opportunities. The research outcomes of this specialty crops project will advance both domestic and global export market opportunities and will help ensure higher economic returns to the dry bean growers of North Dakota.

Project Approach

Activity 1:Greenhouse Experiment:

Stage 1 Experiment: Initially phenolic antioxidants and anti-hyperglycemic properties of all four dry bean market classes (Table 1) were evaluated using *in vitro* assay models to obtain baseline data (Table 2).

Stage 2 Experiment: Greenhouse experiments (from 2016-2017 period) were conducted with four cultivars (Table 1) of each edible dry bean market class for bioactive enrichment using foliar treatments with natural elicitors (bioprocessed soluble chitosan oligosaccharide-COS mimic fungal cell wall response, and Gro-Pro amino acids enriched marine peptide). Initially different concentrations of natural elicitors were evaluated to optimize the doses (@1%,2%,5%,

& 10%) for foliar applications in edible dry beans. Elicitors @1% concentration resulted higher levels of phenolic bioactives and associated antioxidant activity in all four dry bean market classes and therefore selected for this study. The elicitors were applied as foliar treatments during pod filling stage of dry beans at AES Greenhouse (NDSU, Fargo, ND). All dry bean plants were grown in potting mix and standard fertilizer and irrigation application were maintained. Beneficial nematode was applied in weekly basis to control Thrips in the greenhouse. Following harvest dry bean seeds were dried, milled, and extracted (aqueous extract to simulate food grade extraction) in the laboratory for biochemical analysis. Entire experiment was repeated two times in the greenhouse for all four edible dry beans (Figure 1)

Activity 2 Field experiment:
We conducted field experiments (2015-2017) with black bean in Eastern North Dakota (NDSU Research plots), pinto, navy, and red kidney bean cultivars using COS (1%) foliar treatment during pod filling stage. A concentration of 1g/L was sprayed at 50 mL m^{-2} using two passes during the pod filling stage with a backpack sprayer. We have completed all biochemical analysis for soluble phenolic content, antioxidant activity, anti-hyperglycemic properties (α-amylase and α-glucosidase inhibitory activities using *in vitro* assays) of all four edible dry bean market classes from field experiment.

Activity 3. Sprout Experiment:
Dry bean sprout study: To understand seed elicitation responses (COS & Gro-Pro) in dry bean cultivars, black bean and red kidney bean sprout experiment and phenolic antioxidant-linked functionalities of black bean and red kidney bean sprouts were determined. For black bean cv. Eclipse and for red kidney bean cv. Pink Panther was used. Black bean and red kidney bean seeds were treated with COS (1, 2, 5, & 10 gm/L) and Gro-Pro (1, 2, 5, & 10 ml/L) and incubated in dark for sprouting under controlled environment (Figure 3). Entire experiment was repeated three times. From this experiment we found that 1 gm/L (COS) and 1 mL/L (Gro-Pro) doses are optimum for seed elicitation for dry beans and results of the optimum dose were included in the report. A seed treatment incubation strategy was used, with a four-hour incubation period at 25°C prior to dark germination of the seeds. Black and kidney bean seeds were treated with COS (1 g/L) and Gro-Pro (1 mL/L) solution. The control was treated with distilled water. Seed (50 g) were added to 450 mL of solution for each experimental group. Treated and untreated solution was drained after 4 hours and seeds were kept in a dark incubator set to 25°C for the duration of the study in 500 mL beakers, with each treatment being rinsed and drained periodically to maintain germination.

Dissemination of Results:
We will disseminate the results to dry bean growers through North Harvest Bean Growers Association and through NDSU Extension and GIFSIA website (https://www.ag.ndsu.edu/GIFSIA). The findings will also be presented in the national and international plant/food/health science conferences. Information will also be shared with dry bean exporters, especially in Asia where diabetes epidemic is growing rapidly and has significant demand for legumes including dry beans. We will also be writing manuscripts from this study for publication in peer reviewed international journals and these publications will be made available when published.

Goals and Outcomes Achieved

Overall Key Findings

- All four dry bean market classes have shown high phenolic bioactive-linked antioxidant activity from greenhouse and field experiment and can be advanced as value added specialty crop targeting non-communicable chronic diseases.
- Moderate to high α-amylase and low to moderate α-glucosidase inhibitory activity was also observed in all four edible dry bean market classes and this has relevance for use in value added dietary solutions against chronic hyperglycemia commonly associated with type 2 diabetes.
- Improvement of phenolic bioactive linked- antioxidant and anti-hyperglycemic functionalities was observed with bioprocessed elicitors as foliar treatments (@1%) during pre-harvest pod filling stages.
- Sprouting coupled with seed elicitor treatment is an effective strategy to improve human health relevant phenolic bioactives and associated functionalities in edible dry beans.

Specific Findings Based of Three Different Activities

- **Activity 1:** The original goal of the proposed specialty crop research grant was to conduct the greenhouse study and to optimize the natural elicitor treatments for field applications during this period. Among edible dry bean market classes high phenolic –linked antioxidant activity was observed in kidney and pinto bean followed by black and navy beans (Table 1). High phenolic antioxidant activity of edible dry beans was also positively correlated with its anti-hyperglycemic properties and was confirmed by moderate α-amylase and low to moderate α-glucosidase enzyme inhibitory activities which has significant relevance for its value added dietary use against hyperglycemia commonly associated with type 2 diabetes (Table 1). Significant improvement in phenolic content and total antioxidant activity was also observed in all four edible bean market classes after foliar application with COS (1% solution) and Gro-Pro (1% solution), however the improvement was more evident in black and pinto bean cultivars (Table 3 & 5). Among cultivars Eclipse and Zorro (Table 2) of black bean and Monterey and Stampede (Table 4) of pinto bean had highest increase in phenolic-linked antioxidant activity (10-20%) after natural elicitor foliar treatments during pod filling stage. The increase in phenolic linked antioxidant activity with bioprocessed elicitors has significant relevance for enrichment of human health relevant bioactive profiles in edible dry beans that can advance it as a value added specialty crop in improved diets targeting non-communicable chronic diseases such as type 2 diabetes and associated complications globally. The anti-hyperglycemic properties (alpha-amylase and alpha-glucosidase inhibitory activity of dry beans also improved, however the improvement is more cultivar specific (Table 3, 5, 7, 9). We have achieved our goal and completed all activities for bioactive enrichment with black, pinto, and navy bean cultivars on time and later with red kidney beans. We have communicated the initial findings with North Harvest Bean Growers association and will present key findings of this study to dry bean growers of the Northern Plains. In collaboration with ND growers we want further advance the value added health benefits of ND Specialty crops such as the dry bean market classes targeted in this study.

- **Activity 2:** The enhancement of phenolic content and antioxidant activity in black pinto, and navy bean cultivars were found both in the field and in the greenhouse. This result fulfills our first objective to enhance phenolic-antioxidant profile for value added health benefits in edible dry beans with natural bioprocessed elicitors (Table 10,11,12,13,14,15,16,17). The improvement of phenolic content and antioxidant activity in edible dry bean with COS foliar treatment ranged between (2-10%) however the response to natural elicitor foliar treatments were specific to the cultivar and edible dry bean market class. Edible dry bean cultivars with superior phenolic-linked antioxidant and anti-hyperglycemic properties relevant to support dietary management of type 2 diabetes and associated complications have been identified. Health relevant phenolic bioactive enrichment in edible dry beans with natural elicitors would potentially expand the global export of specialty dry beans of North Dakota, where type 2 diabetes rates are rapidly increasing. The key findings of the proposed specialty crop grant were communicated to the edible dry bean growers and commodity group at North Harvest Bean Growers annual grant meeting (March 2017), Fargo, ND and will continue to disseminate the results to the dry bean growers and dry bean industry to integrate this value addition into their marketing and export strategies.
- **Activity 3:** Improvement of phenolic-linked antioxidant capacity in black bean and red kidney bean sprouts was observed (Figure 4,5,6,7,8,9,10,11). The entire experiment was carried out for three times and all results exhibited significant improvement of total soluble phenolic content and antioxidant activity in black beans with seed elicitation strategy during sprouting. Results from black bean and red kidney bean sprout study provide additional value added information on potential use of natural elicitors to improve phenolic-linked human health benefits in edible dry beans for advancing specialty crop exports. We have also investigated the potential carbon flux shift during sprouting for biosynthesis of health relevant bioactives and antioxidant enzymes through stimulation of redox-linked proline associated pentose phosphate pathway (Data are not included in the report).

Beneficiaries

Beneficiaries of this project include specialty dry bean growers, dry bean commodity groups, dry bean exporters, other specialty crop research and consumers from North Dakota, Great Plains region and other parts of the United States. There are at least 200 plus growers in the NortHarvest commodity growers region who will benefit directly from this project. We have already communicated our previous findings to the North Harvest Dry Bean Growers Meeting (March, 2017) and will continue to communicate the findings to the specialty dry bean growers and other stakeholders for advancing value added exports. There are nearly 50,000 pre-diabetic consumers in ND, 30 million nationally, and 450 million globally that will benefit from a cost effective dietary antidotes with high bioactive and higher fiber bean diets for incorporating into more effective prevention and management of type 2 diabetes related chronic diseases.

Lessons Learned

We have completed all required experiments and achieved the goal of the proposal. The key findings are based on repeated greenhouse experiments and one-year field experiment. One of the major challenge is the effect of seasonal variations (environmental and growing condition) from different years and effect of different locations on bioactive profiles of specialty dry beans. Therefore, multi-years field trials in the future with similar objectives will provide more reliable data to prove the concept. Another challenge is the optimization of timing of foliar application of bioprocessed elicitors during flowering and pod filling stages as maturity days of dry bean varies among cultivars and dry bean market classes.

Further another positive indication and lesson is that the cold stressed environment and related stressed ecologies of Northern Great Plains offer metabolic opportunities for targeting improvement of health bioactives from stimulated food crops compound associated with stress resilience. Building this strategy more widely for other North Dakota specialty crops can provide value added advantages for enhanced market opportunities as well as benefit livelihoods and our health care needs.

Contact Information

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Additional Information (Data Tables and Figures from the Study)

Table 1. Edible dry bean cultivars selected for bioactive enrichment in greenhouse & field trials.

Edible Dry Bean Market Class	Black	Pinto	Navy	Kidney
Cultivars	Eclipse Zorro Loretto Zenith	Windbreaker Lariat Stampede Monterrey	Medalist Ensign Vista Avalanche	Montcalm Redhawk Pink Panther Roise

Table 2. Baseline value of total soluble phenolic content, total antioxidant activity (DPPH based radical scavenging activity, alpha-glucosidase inhibitory activity (%), alpha-amylase inhibitory activity (%) of four different cultivars of black, pinto, navy, and red kidney beans.

Dry Bean Market Class	Cultivars	Total Phenolic Content (mg/g D.W.)	Total Antioxidant Activity (DPPH based Inhibition %)	Alpha-Amylase Inhibitory Activity (%)	Alpha-Glucosidase Inhibitory Activity (%)

Black Bean	Eclipse	1.56	70	55	28
	Zorro	1.34	62	52	34
	Loretto	1.52	68	51	31
	Zenith	1.45	60	52	30
Pinto Bean	Windbreaker	1.67	74	54	45
	Lariat	1.88	72	60	42
	Stampede	1.82	68	58	48
	Monterrey	1.8	70	56	39
Navy Bean	Medalist	1.24	48	48	22
	Ensign	1.26	54	34	20
	Vista	1.42	52	36	28
	Avalanche	1.45	50	38	26
Red Kidney Bean	Montcalm	3.9	74	52	38
	Redhawk	3.82	69	56	34
	Pink Panther	3.98	71	50	37
	Roise	2.78	72	49	35

Table 3. Total soluble phenolic content and total antioxidant activity of black bean cultivars from greenhouse experiment without (control) and with (soluble chitosan oligosaccharide-COS & Gro-Pro @1%) foliar treatments.

Dry bean Market class	Cultivars	Total Soluble Phenolic Content (mg/g D.W.)			Total Antioxidant Activity (DPPH based Inhibition %)		
		Control	COS	Gro-Pro	Control	COS	Gro-Pro
Black Bean	Eclipse	1.5	1.58	1.52	70	74	72
	Zorro	1.32	1.48	1.46	65	68	64
	Loretto	1.51	1.54	1.56	64	70	71
	Zenith	1.45	1.49	1.50	62	72	74

Table 4. Alpha-amylase and alpha-glucosidase inhibitory activity of black bean cultivars from greenhouse experiment without (control) and with bioprocessed elicitor (soluble chitosan oligosaccharide-COS & Gro-Pro @1%) foliar treatments.

Dry bean Market class	Cultivars	Alpha-Amylase Inhibitory Activity (%)			Alpha-Glucosidase Inhibitory Activity (%)		
		Control	COS	Gro-Pro	Control	COS	Gro-Pro
Black Bean	Eclipse	51	52	55	25	28	29
	Zorro	52	50	54	32	34	30
	Loretto	50	46	52	31	35	36
	Zenith	48	49	56	30	32	35

Table 5. Total soluble phenolic content and total antioxidant activity of pinto bean cultivars from greenhouse experiment without (control) and with (soluble chitosan oligosaccharide-COS & Gro-Pro @1%) foliar treatments.

Dry Bean Market class	Cultivars	Total Soluble Phenolic Content (mg/g D.W.)			Total Antioxidant Activity (DPPH based Inhibition %)		
		Control	COS	Gro-Pro	Control	COS	Gro-pro
Pinto Bean	Windbreaker	1.62	1.8	1.74	52	60	62
	Lariat	1.84	1.82	1.79	56	52	55
	Stampede	1.8	1.88	1.84	50	57	59
	Monterrey	1.78	1.8	1.86	48	52	53

Table 6. Alpha-amylase and alpha-glucosidase inhibitory activity of pinto bean cultivars from greenhouse experiment without (control) and with bioprocessed elicitor (soluble chitosan oligosaccharide-COS & Gro-Pro @1%) foliar treatments.

Dry Bean Market class	Cultivars	Alpha-Amylase Inhibitory Activity (%)			Alpha-Glucosidase Inhibitory Activity (%)		
		Control	COS	Gro-Pro	Control	COS	Gro-Pro
Pinto Bean	Windbreaker	50	55	56	44	42	42
	Lariat	58	50	51	40	45	44
	Stampede	55	60	62	48	50	51
	Monterrey	50	60	60	40	54	53

Table 7. Total soluble phenolic content and total antioxidant activity of navy bean cultivars from greenhouse experiment without (control) and with (soluble chitosan oligosaccharide-COS & Gro-Pro @1%) foliar treatments.

Dry Bean Market class	Cultivars	Total Soluble Phenolic Content (mg/ g D.W.)			Total Antioxidant Activity (DPPH based Inhibition %)		
		Control	COS	Gro-Pro	Control	COS	Gro-Pro
Navy Bean	Medalist	1.2	1.25	1.21	50	54	55
	Ensign	1.2	1.28	1.25	51	60	56
	Vista	1.36	1.34	1.36	49	52	50
	Avalanche	1.4	1.42	1.44	47	50	52

Table 8. Alpha-amylase and alpha-glucosidase inhibitory activity of navy bean cultivars from greenhouse experiment without (control) and with bioprocessed elicitor (soluble chitosan oligosaccharide-COS & Gro-Pro @1%) foliar treatments.

	Cultivars	Alpha-Amylase Inhibitory Activity (%)	Alpha-Glucosidase Inhibitory Activity (%)

Dry Bean Market class		Control	COS	Gro-Pro	Control	COS	Gro-Pro
Navy Bean	Medalist	52	54	56	20	28	27
	Ensign	38	42	53	24	26	22
	Vista	40	48	52	30	34	28
	Avalanche	45	50	51	28	32	34

Table 9. Total soluble phenolic content and total antioxidant activity of red kidney bean cultivars from greenhouse experiment without (control) and with (soluble chitosan oligosaccharide-COS & Gro-Pro @1%) foliar treatments.

Dry Bean Market class	Cultivars	Total Soluble Phenolic Content (mg/ g D.W.)			Total Antioxidant Activity (DPPH based Inhibition %)		
		Control	COS	Gro-Pro	Control	COS	Gro-Pro
Red Kidney Bean	Montcalm	1.84	1.9	1.79	74	76	78
	Redhawk	1.8	1.86	1.84	69	74	70
	Pink Panther	1.82	1.8	1.82	71	73	74
	Roise	1.81	1.84	1.85	72	72	75

Table 10. Alpha-amylase and alpha-glucosidase inhibitory activity of red kidney bean cultivars from greenhouse experiment without (control) and with bioprocessed elicitor (soluble chitosan oligosaccharide-COS & Gro-Pro @1%) foliar treatments.

Dry Bean Market class	Cultivars	Alpha-Amylase Inhibitory Activity (%)			Alpha-Glucosidase Inhibitory Activity (%)		
		Control	COS	Gro-Pro	Control	COS	Gro-Pro
Red Kidney Bean	Montcalm	46	46	48	38	40	42
	Redhawk	44	48	49	34	32	38
	Pink Panther	46	50	51	37	44	42
	Roise	48	52	47	35	40	44

Table 10. Total soluble phenolic content and total antioxidant activity of black bean cultivars from field experiment without (control) and with (soluble chitosan oligosaccharide @1%) foliar treatments.

Dry bean Market class	Cultivars	Total Soluble Phenolic Content (mg/g D.W.)		Total Antioxidant Activity (DPPH based Inhibition %)	
		Control	COS	Control	COS
Black Bean	Eclipse	1.62	1.70	74	76
	Zorro	1.56	1.78	76	80
	Loretto	1.67	1.64	75	74
	Zenith	1.78	1.75	78	80

Table 11. Alpha-amylase and alpha-glucosidase inhibitory activity of black bean cultivars from field experiment without (control) and with bioprocessed elicitor (soluble chitosan oligosaccharide @1%) foliar treatments.

Dry bean Market class	Cultivars	Alpha-Amylase Inhibitory Activity (%)		Alpha-Glucosidase Inhibitory Activity (%)	
		Control	COS	Control	COS
Black Bean	Eclipse	46	42	22	25
	Zorro	47	54	24	32
	Loretto	44	49	26	30
	Zenith	40	57	20	26

Table 12. Total soluble phenolic content and total antioxidant activity of pinto bean cultivars from field experiment without (control) and with (soluble chitosan oligosaccharide @1%) foliar treatments.

Dry Bean Market class	Cultivars	Total Soluble Phenolic Content (mg/ g D.W.)		Total Antioxidant Activity (DPPH based Inhibition %)	
		Control	COS	Control	COS
Pinto Bean	Windbreaker	1.94	1.99	55	64
	Lariat	1.75	1.85	60	70
	Stampede	1.78	1.82	62	71
	Monterrey	1.84	1.88	67	73

Table 13. Alpha-amylase and alpha-glucosidase inhibitory activity of pinto bean cultivars from field experiment without (control) and with bioprocessed elicitor (soluble chitosan oligosaccharide @1%) foliar treatments.

Dry Bean Market class	Cultivars	Alpha-Amylase Inhibitory Activity (%)		Alpha-Glucosidase Inhibitory Activity (%)	
		Control	COS	Control	COS
Pinto Bean	Windbreaker	43	60	34	49
	Lariat	55	64	43	47
	Stampede	56	58	40	52
	Monterrey	52	67	46	58

Table 14. Total soluble phenolic content and total antioxidant activity of navy bean cultivars from field experiment without (control) and with (soluble chitosan oligosaccharide @1%) foliar treatments.

Dry Bean Market class	Cultivars	Total Soluble Phenolic Content (mg/ g D.W.)		Total Antioxidant Activity (DPPH based Inhibition %)	
		Control	COS	Control	COS
Navy Bean	Medalist	1.31	1.36	60	65
	Ensign	1.36	1.40	61	68
	Vista	1.39	1.42	54	67
	Avalanche	1.45	1.48	59	65

Table 15. Alpha-amylase and alpha-glucosidase inhibitory activity of navy bean cultivars from field experiment without (control) and with bioprocessed elicitor (soluble chitosan oligosaccharide @1%) foliar treatments.

Dry Bean Market class	Cultivars	Alpha-Amylase Inhibitory Activity (%)		Alpha-Glucosidase Inhibitory Activity (%)	
		Control	COS	Control	COS
Navy Bean	Medalist	55	56	25	24
	Ensign	48	58	22	28
	Vista	49	54	34	36
	Avalanche	50	60	30	32

Table 16. Total soluble phenolic content and total antioxidant activity of red kidney bean cultivars from field experiment without (control) and with (soluble chitosan oligosaccharide @1%) foliar treatments.

Dry Bean Market class	Cultivars	Total Soluble Phenolic Content (mg/ g D.W.)		Total Antioxidant Activity (DPPH based Inhibition %)	
		Control	COS	Control	COS
Red Kidney Bean	Montcalm	1.89	1.92	72	74
	Redhawk	1.85	1.94	70	76
	Pink Panther	1.9	1.96	75	80
	Roise	1.84	1.9	76	80

Table 17. Alpha-amylase and alpha-glucosidase inhibitory activity of red kidney bean cultivars from field experiment without (control) and with bioprocessed elicitor (soluble chitosan oligosaccharide @1%) foliar treatments.

Dry Bean Market class	Cultivars	Alpha-Amylase Inhibitory Activity (%)		Alpha-Glucosidase Inhibitory Activity (%)	
		Control	COS	Control	COS
Red Kidney Bean	Montcalm	50	58	42	45
	Redhawk	52	60	44	42
	Pink Panther	48	61	47	50
	Roise	56	62	49	50

Figures

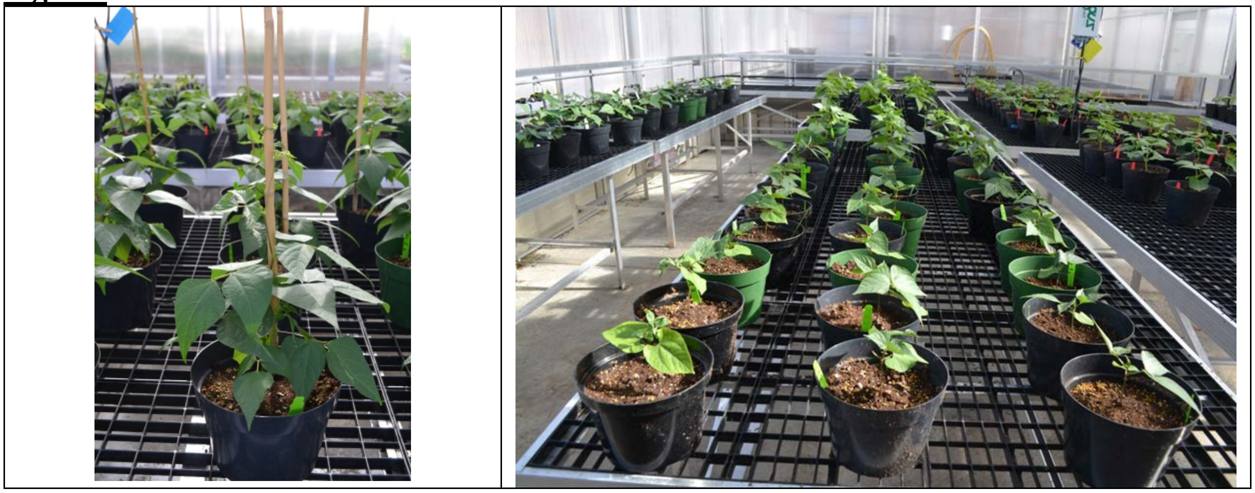


Figure 1. Greenhouse Experiments with Edible Dry Beans

Figure 2. Foliar application of Chitosan Oligosaccharide (COS @1%) in dry beans during pod filling stages

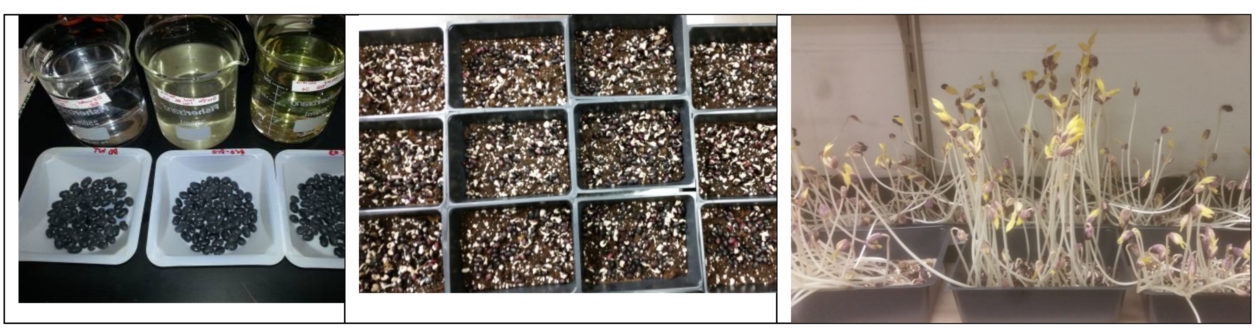


Figure 3. Black Bean Sprout Experiment with Natural Elicitors (COS & Gro-Pro)

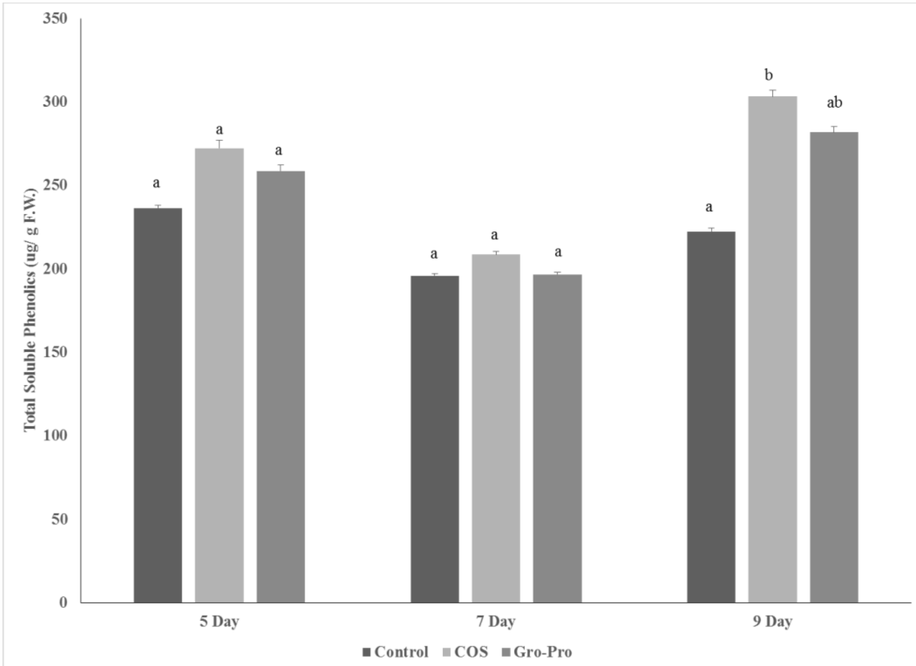


Figure 4. Total soluble phenolic content (µg/ g F.W.) of black bean sprouts at 5, 7, and 9 days post seed priming treatments with soluble chitosan oligosaccharide (COS) and marine hydrolysate (Gro-Pro). Different letters indicate significant differences between seed elicitation treatments within each time point at the $p<0.05$ level.

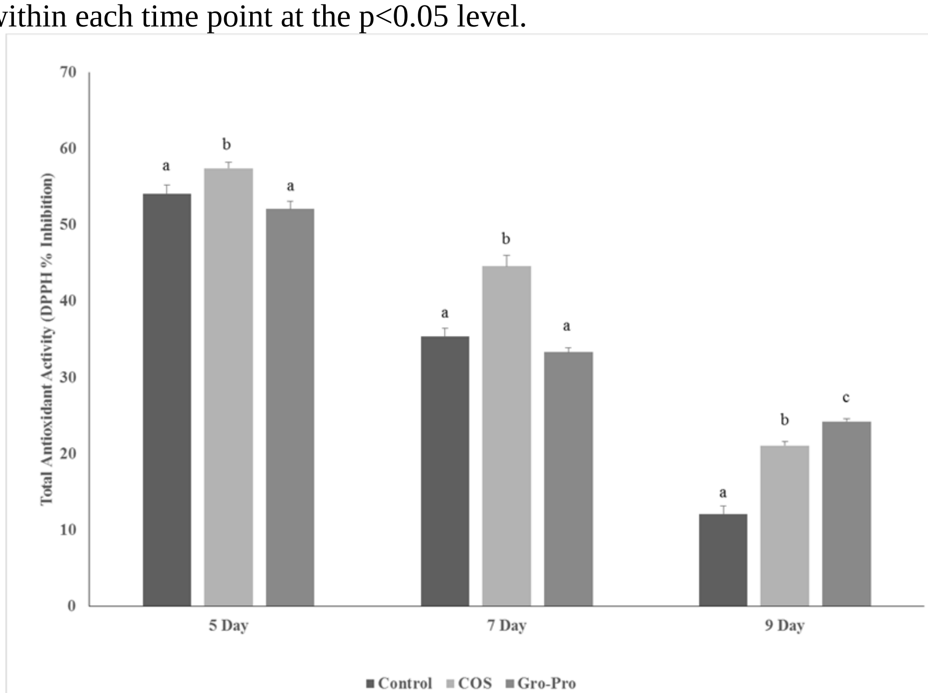


Figure 5. Total antioxidant activity (DPPH % inhibition.) of black bean sprouts (bioactive extraction) at 5, 7, and 9 days post seed priming treatments with soluble chitosan oligosaccharide (COS) and marine hydrolysate (Gro-Pro). Different letters indicate significant differences between seed elicitation treatments within each time point at the $p<0.05$ level.

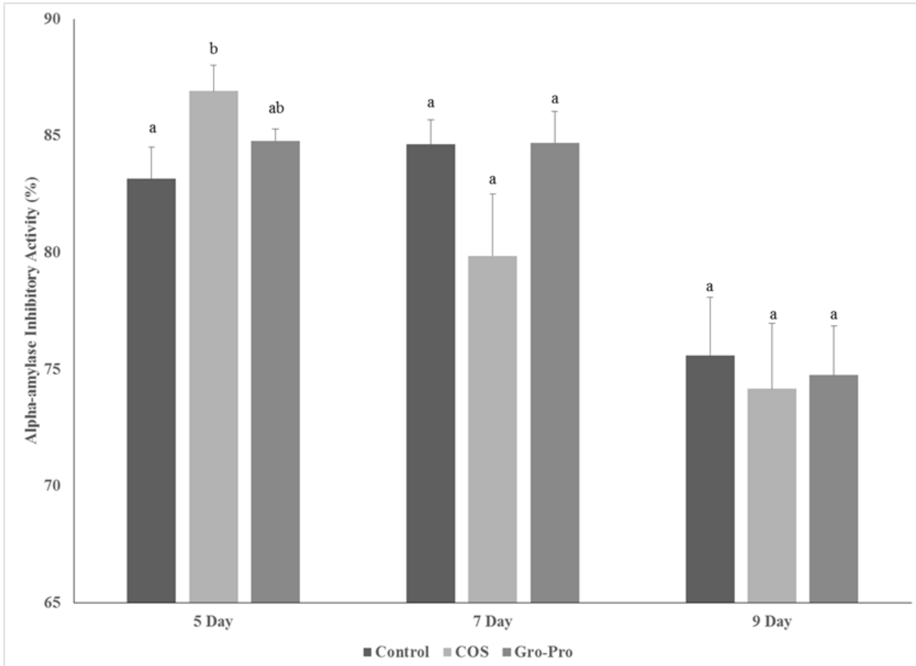


Figure 6. Alpha-amylase inhibitory activity (%) of undiluted black bean sprouts (bioactive extraction) at 5, 7, and 9 days post seed priming treatments with soluble chitosan oligosaccharide (COS) and marine hydrolysate (Gro-Pro). Different letters indicate significant differences between seed elicitation treatments within each time point at the p<0.05 level.

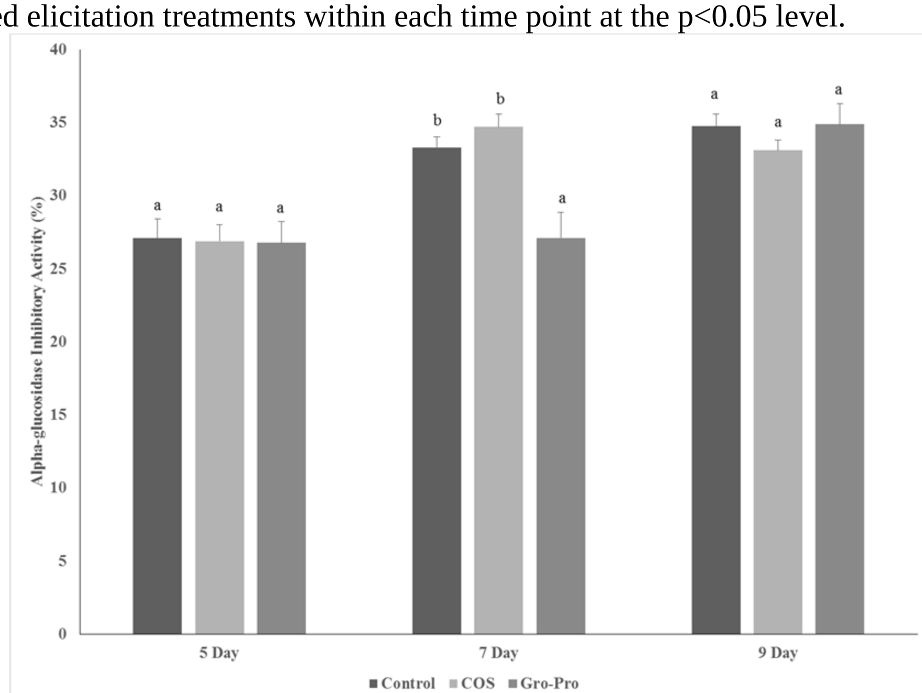


Figure 7. Alpha-glucosidase inhibitory activity (%) of undiluted black bean sprouts (bioactive extraction) at 5, 7, and 9 days post seed priming treatments with soluble chitosan oligosaccharide (COS) and marine hydrolysate (Gro-Pro). Different letters indicate significant differences between seed elicitation treatments within each time point at the p<0.05 level.

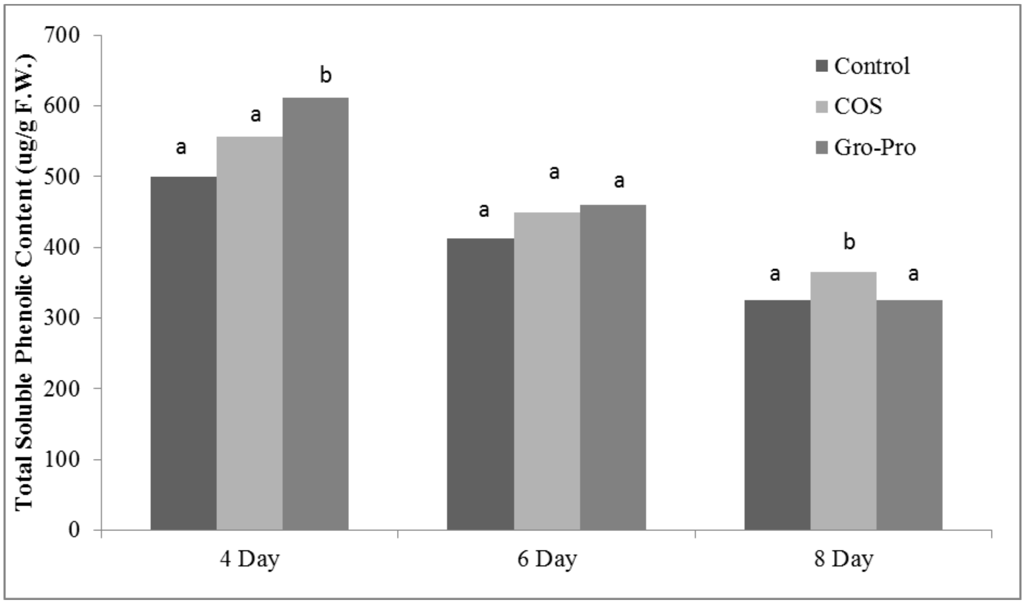


Figure 8. Total soluble phenolic content (µg/ g F.W.) of kidney bean sprouts at 4, 6, and 8 days post seed priming treatments with soluble chitosan oligosaccharide (COS) and marine hydrolysate (Gro-Pro). Different letters indicate significant differences between seed elicitation treatments within each time point at the p<0.05 level.

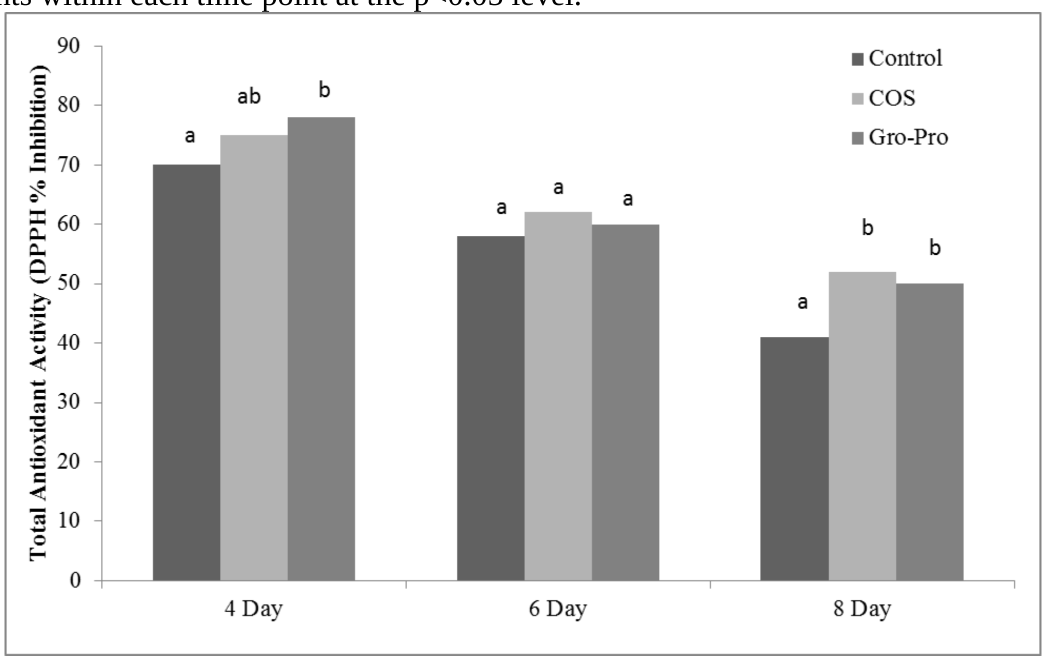


Figure 9. Total antioxidant activity (DPPH % inhibition.) of kidney bean sprouts (bioactive extraction) at 4, 6, and 8 days post seed priming treatments with soluble chitosan oligosaccharide (COS) and marine hydrolysate (Gro-Pro). Different letters indicate significant differences between seed elicitation treatments within each time point at the p<0.05 level.

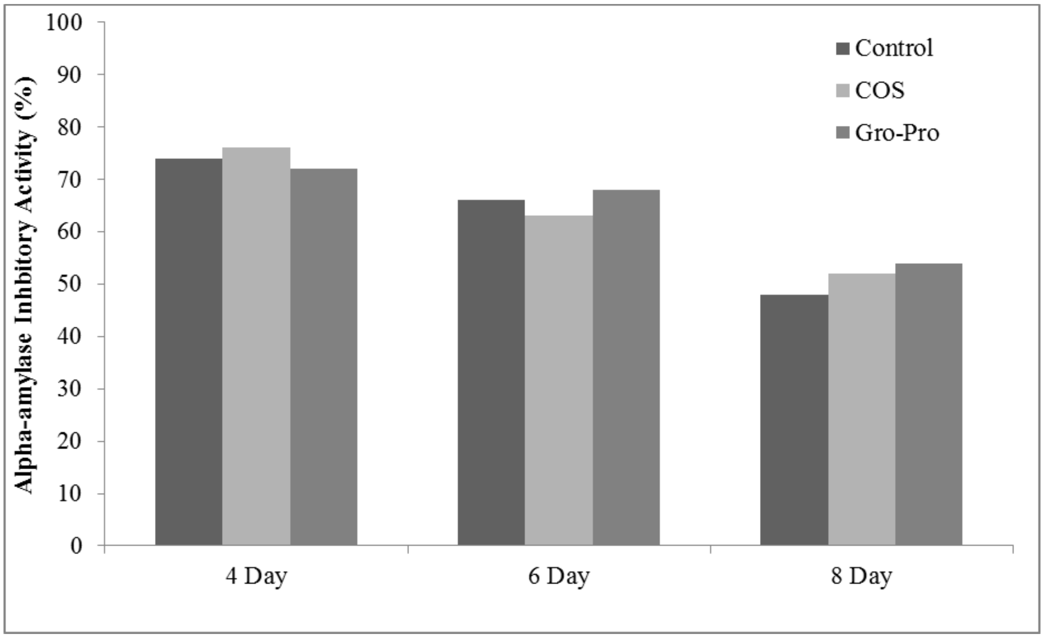


Figure 10. Alpha-amylase inhibitory activity (%) of undiluted kidney bean sprouts (bioactive extraction) at 4, 6, and 8 days post seed priming treatments with soluble chitosan oligosaccharide (COS) and marine hydrolysate (Gro-Pro). No statistically significant differences in α-amylase inhibitory activity was observed at $p<0.05$ confidence level

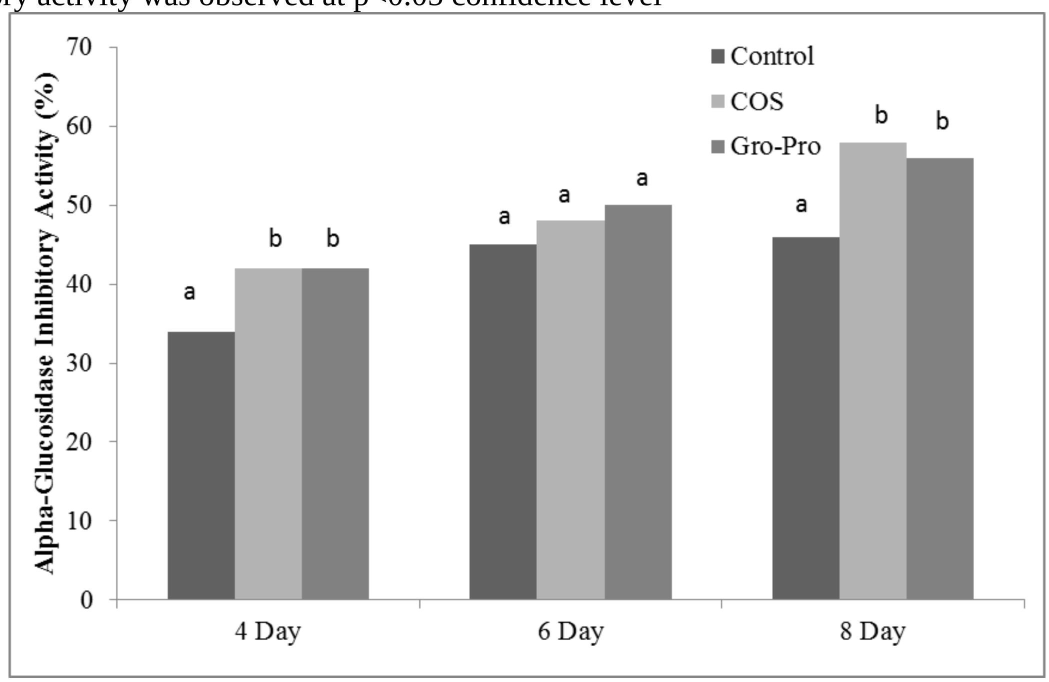


Figure 11. Alpha-glucosidase inhibitory activity (%) of undiluted kidney bean sprouts (bioactive extraction) at 4, 6, and 8 days post seed priming treatments with soluble chitosan oligosaccharide (COS) and marine hydrolysate (Gro-Pro). Different letters indicate significant differences between seed elicitation treatments within each time point at the $p<0.05$ level.

Project Title
Assessing the Potential for Remote Sensing of Potato Virus Y in Potato Seed Fields

NOGA#
15-329

Final Report

Partner Organization
North Dakota State University

Project Summary

- Did the grantee provide a background for the initial purpose of the project, which includes the specific issue, problem, or need that was addressed by this project?
- Did the grantee establish the motivation for this project by presenting the importance and timeliness of the project?
- If the project built on a previously funded project with the SCBGP or SCBGP-FB, did the grantee describe how this project complimented and enhanced previously completed work?

NOGA15-329 was a request for a second year of funding for the project titled 'Assessing the Potential for Remote Sensing of Potato Virus Y in Potato Seed Fields', and started as NOGA14-208. A third submission building upon this work was proposed and accepted for FY16 'Assessing the Potential for Remote Sensing of Potato Virus Y in Potato Seed Fields – Nutrient Deficiency' as NOGA 16-235. Results to date for the FY15-329 are summarized here.

Seed potato production in North Dakota (ND), Minnesota, and across North America (NA) has been impacted significantly by Potato Virus Y (PVY), particularly since the early 2000s when new strains began emerging in NA certified seed potato fields. North Dakota ranks second for certified seed potato production (Potato Association of America 2018) after Idaho. PVY is an aphid vectored, non-persistent virus, that may cause major yield losses for commercial potato producers (Gray et al 2010). Several PVY strains impact NA potato production including PVY^{O}, PVY^{N}, PVY^{NTN}, and $PVY^{N:O}$, the latter being the most prevalent in ND (Gudmestad personal communication). Traditionally, aphids such as the green peach aphid and potato aphid have been the most efficient and important vectors; however, new vectors, such as the soybean aphid, have been identified as very effective vectors (Davis et al 2005, Gray et al 2010). For certified seed potato producers particularly, asymptomatic cultivars, those cultivars that when infected lack visual symptoms, or those fields with PVY^{N}, PVY^{NTN}, and the recombinant $PVY^{N:O}$ strains, generally express mild or no foliar symptoms. Visual assessment techniques and management tools have often been unreliable, making a rapid, accurate assessment method for PVY infection in certified potato seed fields an important advancement, simplifying management, certification, and resistance breeding efforts. PVY infection generally decreases chlorophyll content, causing a decline in the amount of reflected light energy, particularly in the Near Infrared (NIR) wavelengths. Advances in the collection and analysis of reflected light energy provide an opportunity to develop remote sensing techniques for field or individual seed lot diagnosis. Four research objectives were established: 1) To determine the wavelengths of reflected light that are associated with PVY of seed potato plants. 2) To determine if these wavelengths are discernible in the greenhouse and field for new NDSU cultivar releases, advanced selections, and commonly grown cultivars by ND certified seed potato producers. 3) To determine if PVY strains are discernible from one another in the greenhouse and field using spectral data, and 4) To determine if PVY infection can be differentiated from nutrient deficiency (specifically nitrogen) in the greenhouse and field, using spectral data. Reflectance associated with PVY infection in new cultivar releases from the NDSU potato breeding program, advancing potato selections, and common commercially grown cultivars in ND was explored in field and greenhouse trials.

Objective 3 was modified to include only PVYN:O since that is the strain found in ND and thus available for our work. It was determined that due to the large data sets involved and the difficulty in keeping materials clean in the field and greenhouse that a separate experiment was needed to address Objective 4. A second student was added and NOGA 16-235 was approved to provide a second year of work specific to this objective. Thus work on Objective 4 is not presented here, but will be summarized in the Final Report for NOGA 16-235.

Project Approach

- Were the activities and tasks performed during the entire grant period briefly summarized? This section should discuss the tasks provided in the Work Plan or the approved project proposal. This includes significant results, accomplishments, conclusions and recommendations, as well as favorable or unusual developments.
- If the overall scope of the project benefitted commodities other than specialty crops, did the grantee indicate how project staff ensured that funds were used to solely enhance the competitiveness of specialty crops?
- Did the grantee detail the significant contributions and role of project partners in the project?

Potato Genotype and PVY Presence: Effect on Reflectance
(Objectives 1-3)
Trials were conducted at the North Dakota State University (NDSU) Agriculture Experiment Station (AES) Greenhouse in Fargo, ND. Two trials were conducted consecutively, in the same greenhouse unit, from 2015 to 2016. The trial area was 9.73 m x 7.29 m. An Argus Control Systems unit (Argus Control Systems, Ltd., Surrey, BC) maintained conditions in the greenhouse unit at 22 °C during the day and 15 °C at night, with de-humidification to initiate at 80 %. Supplementary lighting consisted of 600-watt high pressure sodium lamps (P. L. Light Systems, Inc., Beamsville, ON) with a photoperiod of 16:8 hours. The plots were watered with an on-site source. A randomized complete block designs (RCBD) with a split plot in time (split block) arrangement and three replicates was used. There were 16 treatments consisting of eight cultivars, both virus free and $PVY^{N:O}$ infected. Plots (experimental units) consisted of four plants in a rectangular tub (17.78 x 38.1 x 50.8 cm; Winco®, DWL International Trading Inc., Lodi, NJ). Plant material consisted of minitubers of the commercially available cultivars Dakota Pearl, Dakota Ruby, Red LaSoda, Red Norland, Russet Burbank, and Satina, and NDSU potato breeding program selections ND7132-1R and ND8305-1. Trial 1 was planted June 11-12, 2015,

and Trial 2 on November 17, 2015. To determine viral presence and strain, the terminal leaflet was taken from the first mature leaf on a stem from each of the plants in each plot for enzyme-linked immunosorbent assay (ELISA) testing. Leaflets from the first trial were collected August 3, 2015, and January 4, 2016.

Spectral data was recorded three times at three-week intervals using a Flame Miniature spectroradiometer (Ocean Optics, Dunedin, FL), utilizing the software OceanView v1.5 (Ocean Optics, Dunedin, FL), with a SpectroClip Probe clamping sensor (Ocean Optics, Dunedin, FL) beginning four weeks after emergence, July 13, August 7 and August 28, 2015. A primary leaflet from a mature leaf was collected from a chosen stem from each of the four plants in each plot, and reflectance was measured. A handheld SPAD 502 Plus Chlorophyll Meter (Spectrum Technologies, Inc., Plainfield, IL) was implemented to estimate the chlorophyll content, measuring the difference in optical density at 650 nm and 940 nm, by measuring five primary leaflets on a mature leaf from a stem for each plant in each plot. Methodology differed slightly for Trial 2. Using the same techniques and equipment utilized in the initial greenhouse trial, analyses began four weeks after emergence, December 22, January 6, and January 22, 2016. To ensure the same stem was used throughout the trial, stems used for spectral measurements and virus and strain determination were marked using a cable-tie. As opposed to the first greenhouse trial, in which data was collected three times at intervals of three weeks, data was collected three times at intervals of two weeks. The normalized differential vegetative index (NDVI) was determined for each plant within plots utilizing spectral data obtained by way of the spectroradiometer. The visible (VIS) light range and the near-infrared (NIR) light range were analyzed for NDVI. To determine the reflectance value for the VIS red light range, the average was taken from the sum of the reflectance proportions at wavelengths from 600 to 680 nm. Using this same method, the reflectance value for the NIR light range was determined based on wavelengths from 760 to 900 nm. The NDVI was calculated by means of the following equation and the value for each plant was used as a representative value in the analysis:

$$NDVI = \frac{Avg.\ NIR - Avg.\ Red}{Avg.\ NIR + Avg.\ Red}$$

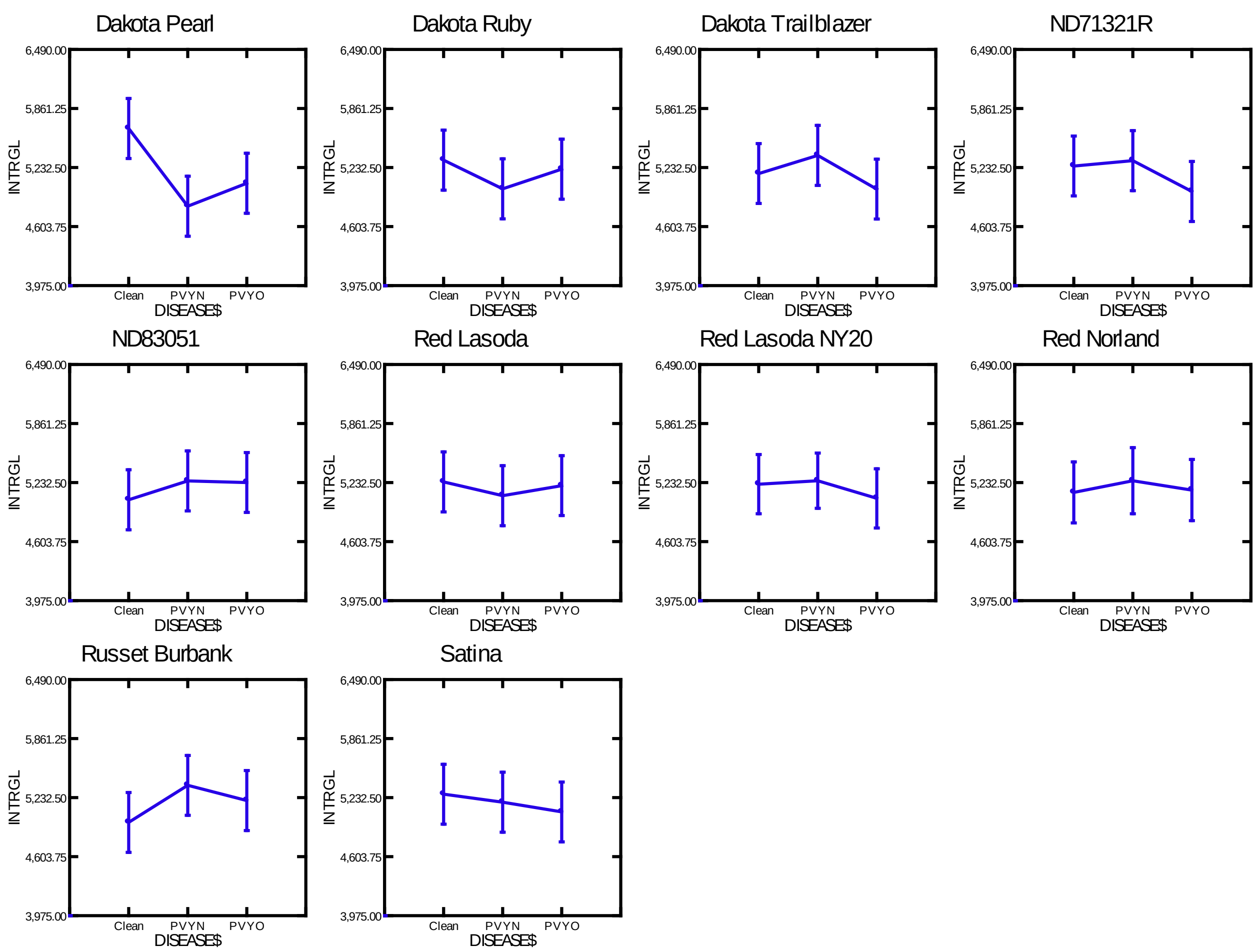
The SPAD for each plant within plots was determined by averaging the total sum of the five leaflets' measurements from each plant. The resulting value was used as a representative value in the analysis. Statistical analysis was conducted using a PROC MIXED model with the restricted maximum likelihood estimation method in SAS 9.4 (SAS Institute Inc., Cary, NC). Clone, viral status, and day of measurement were treated as fixed effects, while replicate was considered a random effect. Differences were evaluated at $p \leq 0.05$.

Based on ELISA results, it was determined that PVY did not spread to virus free treatments in the greenhouse trials. Analysis of variance (ANOVA) using effects of week, clone, clone x week, virus, virus x week, clone x virus and clone x virus x week interactions indicated significant differences across the three dates of measurement, clone, clone x week, clone x virus, and clone x virus x week for NDVI and Trial 1. Similarly, ANOVA results indicated significant differences for the three dates of measurement and clone x week; however, in Trial 2, the interactions of clone x virus and clone x virus x week were not significantly different. SPAD meter results indicated significance for clone and for clone x week, but no significant differences were found for week, virus, virus by week, clone by virus, and the three-way interaction clone by virus by week.

Analysis of individual weeks indicated no significance for NDVI for virus infection, but significant differences for clone and clone x virus for July 13, 2015 readings. Similarly, results for August 8 and August 28 NDVI indicate no significance for virus infection, significantly different NDVI reflectance for clone, but marginal differences for the clone x virus interaction.

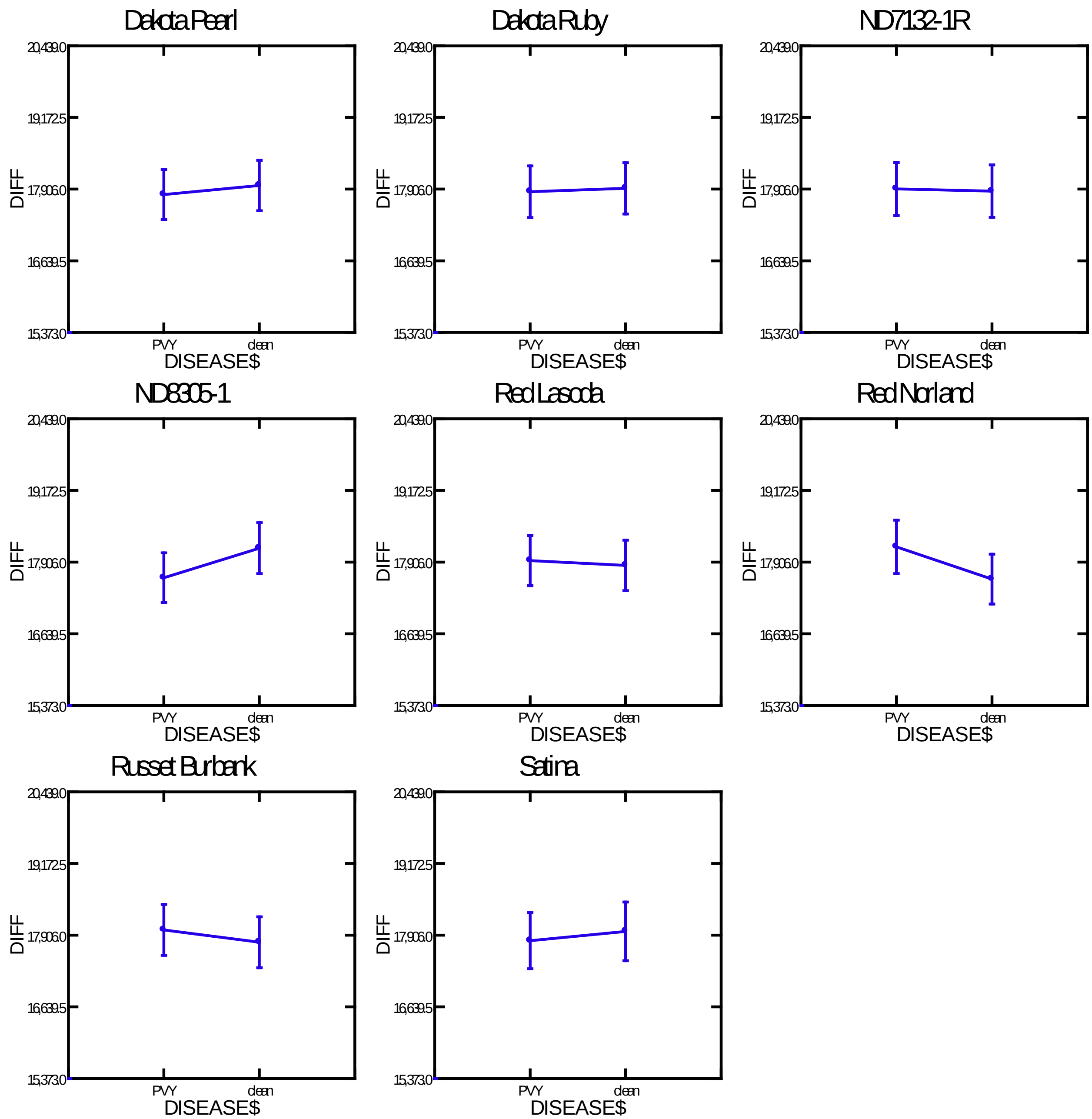
Interpretation of the 2015 field trial spectral data demonstrate genotype (varietal) and virus strain interactions (figures below). Ideally, for this technology to be useful for adoption we will be able to see differences between PVY infected plants and disease free plants using reflectance monitoring via remote sensing, including PVY infection of those genotypes that are asymptomatic carriers of PVY. Chlorophyll content was lower in both the field and greenhouse experiments for PVY infected clones.

Least Squares Means



Leaf clip date (reflectance) from the summer 2015 greenhouse study is similar. Indicating a difference in reflectance for most clones between PVY infected and disease free plants.

Least Squares Means



In another greenhouse study using three advancing potato selections (ND092217ABC-85, ND071401CB-1 and ND081621B-48Russ, PVY infection was not a significant effect using NDVI, but was using Soil Adjusted Vegetative Index (SAVI), which compensates for bare soil reflection from the tubs.

In summary, the combination of these data presented from the many greenhouse and field trials indicate clone and clone x infection (interaction) differences for reflectance, but generally not for infection alone. Further analysis and interpretation of these data is on-going.

Influence of Mineral Oil on Spectral Reflectance of Potato Foliage

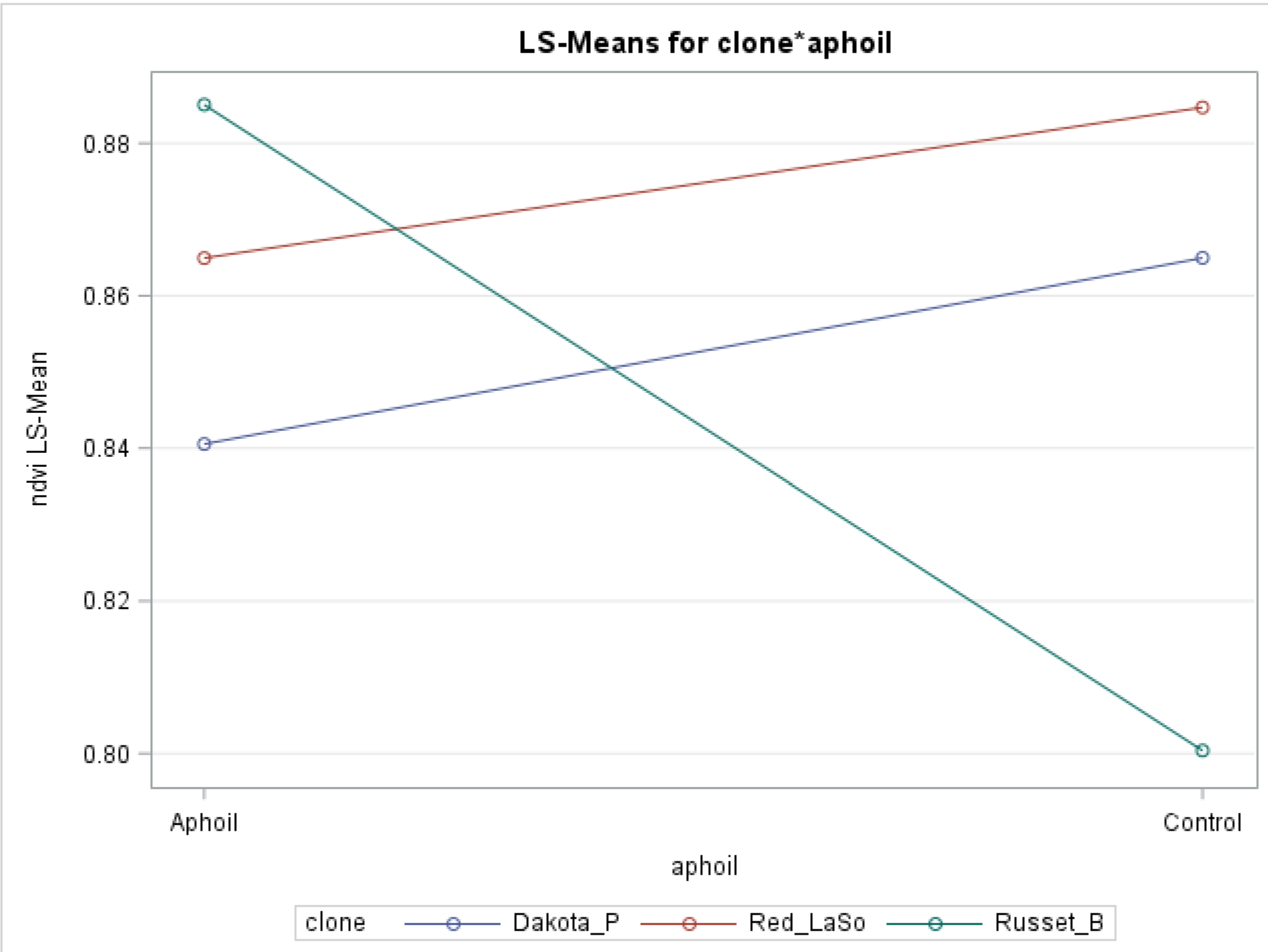
The use of mineral oils in potato production is a significant tool in managing PVY. Due to PVY spread in field trials addressing objectives 1-3 above in 2015, utilization of Aphoil, a mineral oil used by certified seed potato producers to deter aphid activity, was used in the field in 2016. For effective use of spectral analysis as a tool for recognizing viral presence in potato production, it is necessary to determine if mineral oil application alters reflectance of potato foliage. The goal of the experiment was to test the hypothesis that reflectance would not be significantly different between potato plants treated and not treated with mineral oil within the same cultivar.

The trial was conducted at the North Dakota State University (NDSU) Agriculture Experiment Station (AES) Greenhouse in Fargo, ND in 2016. The trial area was 9.73 m x 7.29 m. An Argus Control Systems unit (Argus Control Systems, Ltd., Surrey, BC) maintained conditions in the greenhouse unit at 22 °C during the day and 15 °C at night, with de-humidification to initiate at 80 %. Supplementary lighting consisted of 600-watt high pressure sodium lamps (P. L. Light Systems, Inc., Beamsville, ON) with a photoperiod of 16:8 hours. The plots were watered with an on-site source; once roots were established watering mats were utilized and set to turn on for 15 minutes twice a day. A randomized complete block design (RCBD) with a split plot arrangement (six treatments consisting of three cultivars and pant material sprayed with Aphoil® (Loveland Products, Inc.®, Greeley, CO) and the unsprayed control), and four replicates was used. Plots consisting of three plants in a rectangular tub (17.78 x 38.1 x 50.8 cm; Winco®, DWL International Trading Inc., Lodi, NJ) were the experimental units. Plant material consisted of minitubers of the commercially available cultivars Dakota Pearl, Red LaSoda NY-20, and Russet Burbank. The trial was planted July 16, 2016. Aphoil® was sprayed September 29, 2016 at a concentration of 4 % in water at a rate of 87 mL/m^2 with a 110 ° fan nozzle (TeeJet #11001, Spraying Systems Co., Wheaton, IL) over treated units. Spectral data was taken three times, at one, four, and eight days post-spray (September 30, October 3 and October 7), using a Flame Miniature spectroradiometer (Ocean Optics, Dunedin, FL), utilizing the software OceanView v1.5 software (Ocean Optics, Dunedin, FL), with a SpectroClip Probe clamping sensor (Ocean Optics, Dunedin, FL). A distal primary leaflet on a mature leaf of a chosen stem from one plant was marked with a cable-tie for each plot, and reflectance was measured. The same leaflet was used for the whole trial. The NDVI was determined for each plot utilizing spectral data obtained by way of the spectroradiometer. The visible (VIS) light range and the near-infrared (NIR) light range were analyzed for NDVI. To determine the reflectance value for the VIS red light range, an average was taken from the sum of the reflectance proportions at wavelengths from 600 to 680 nm. Using this same method, the reflectance value for the NIR light range was determined based on wavelengths from 760 to 900 nm. The resulting NDVI value for each plant was used as a representative value in the analysis. The NDVI was calculated by means of the following equation:

$$NDVI = \frac{Avg.\ NIR - Avg.\ Red}{Avg.\ NIR + Avg.\ Red}$$

Statistical analysis was conducted using a PROC MIXED model with the restricted maximum likelihood estimation method in SAS 9.4 (SAS Institute Inc., Cary, NC). Clone and Aphoil® treatment were treated as fixed effects, while replicate was considered a random effect. Differences were evaluated at $p \leq 0.05$.

Initially, differences in reflectance were observed between treated and untreated plants for Dakota Pearl and Russet Burbank, but not for Red LaSoda. Analysis of variance for the October 7 readings indicated no significant differences across clone or aphoil treatment; however, significant differences were found for clone x aphoil treatment. The Russet Burbank control had a slightly lower NDVI than the other treatments.



A further understanding of the interaction is warranted. As with the main project summarized above, data analysis and interpretation is ongoing. However, based on these results, the utilization of mineral oils and the resulting effects on reflectance, indicate that the use of mineral oils should not result in misinterpretation of reflectance results.

<u>Goals and Outcomes Achieved</u>

- Did the grantee supply the activities that were completed in order to achieve the performance goals and measurable outcomes identified in the approved project proposal or subsequent amendments?
- If outcome measures were long term, was a summary of the progress made towards this achievement provided?
- Did the grantee provide a comparison of actual accomplishments with the goals established for the reporting period?
- Did the grantee clearly convey completion of achieving outcomes by illustrating baseline data that has been gathered to date and showing the progress toward achieving set targets?
- Did the grantee highlight the major successful outcomes of the project in quantifiable terms?

Per the project proposal, four research objectives were established: 1) To determine the wavelengths of reflected light that are associated with PVY of seed potato plants. 2) To determine if these wavelengths are discernible in the greenhouse and field for new NDSU cultivar releases, advanced selections, and commonly grown cultivars by ND certified seed potato producers. 3) To determine if PVY strains are discernible from one another in the greenhouse and field using spectral data, and 4) To determine if PVY infection can be differentiated from nutrient deficiency (specifically nitrogen) in the greenhouse and field, using spectral data. Reflectance associated with PVY infection in new cultivar releases from the NDSU potato breeding program, advancing potato selections, and common commercially grown cultivars in ND was explored in field and greenhouse trials. Several problems were encountered over the course of the experimentation, including those listed as lessons learned. Additionally, objective 4 is being addressed in a subsequent project.

While analysis and interpretation continue, our results indicated reflectance varies for genotype (clone or cultivar) and for the clone x virus infection interaction, but generally not for PVY infection alone. As a result of a lesson learned during field experimentation, a further outcome was obtained from this research, that addressing the utilization of mineral oil as a means to minimize aphid activity in certified seed potato fields. These findings indicate that the use of mineral oils should not result in misinterpretation of reflectance results. Thus, producers, crop consultants, certification personnel and others should be able to use reflectance data as a means for identifying PVY infection and/or different potato cultivars in fields. Further interpretation will provide guidelines for appropriate wavelengths and more stringent guidelines for stakeholder adoption.

Results to date have been briefly shared with potato producers and industry personnel during the annual Northern Plains Potato Growers Association (NPPGA) annual field days in 2016, 2017 and 2018; similarly at the NPPGA Research Reporting Conference and MN Area II Potato Research meetings during the fall/winter. The work was also mentioned at the NCCC215 (potato breeding and genetics north central regional coordinating committee) meetings in 2017 and 2018 in my research summary. Additionally, one graduate student (SA Falde) presented some of his results at two different Potato Association of America (PAA) meetings (2016 and 2017). To date, he has not finished writing his thesis and thus Dr. MacRae and I cannot put together a popular article (or articles...one on remote sensing PVY and one on our results using the Aphoil), or put together refereed journal articles (planning the American Journal of Potato Research or other journal(s) as appropriate, of which we are planning two papers...one based on the results indicating reflectance varies for genotype (clone or cultivar) and for the clone x virus infection interaction, but generally not for PVY infection alone, and the second journal article would focused on the utilization of mineral oil as a means to minimize aphid activity in certified seed potato fields; our results indicate that its use does not confound or result in misinterpretation of reflectance results based on clone/pvy incidence. The information will be published once the student has completed his thesis and defended his work (hopefully this spring 2019 semester). This SCBG will be acknowledged in our documents.

<u>Beneficiaries</u>

- Did the grantee provide a description of the groups and other operations that benefited from the completion of this project's accomplishments?
- Did the grantee clearly state the number of beneficiaries affected by the project's accomplishments and/or the potential economic impact of the project?

The primary beneficiaries of this project are the certified seed potato producers, approximately 60, in ND and MN. ND is the second largest certified seed potato producing state. Commercial producers in this region rely on these producers for high quality certified seed. Thus, protecting the ND/MN seed industries would have a positive impact on the more than 250 producers in ND and MN (Northern Plains Potato Growers Association and Minnesota Area II producers) by safeguarding the +$199M in sales generated by the ND potato industry in 2016. The value of the ND 2017 production exceeded $279.2M. About 14,000 acres of certified seed were produced in 2017 in ND, making up just under 20% of ND potato acreage.

Additionally, this technology application may have global potential, as potato ranks fourth for food crop production worldwide.

<u>Lessons Learned</u>

- Did the grantee offer insight into the lessons learned by the project staff as a result of completing this project?
- Did the grantee provide any unexpected outcomes or results that were an effect of implementing this project?
- If goals or outcome measures were not achieved, did the grantee identify and share the lessons learned to help others expedite problem-solving?

Many lessons were learned along the way, as several difficulties and setbacks resulted. These included:
The initial experiment using two strains (PVY^{O} and PVY^{N}) for inoculation, resulted in only one, strain being found as the strains recombined to form $PVY^{N:O}$. Thus, separation of the strains in different locations (ie. greenhouse rooms or growth chambers) must occur in order to guarantee that they remain independent. Since $PVY^{N:O}$ is nearly exclusive in ND, we chose to move on using it.

PVY moves rapidly in the field. Initial field experiments were quickly completely infected (based on ELISA testing), rather than having the clean controls clean and the PVY infected treatments infected. This led us to use Aphoil, as growers often do, to limit virus spread. Because of this use of Aphoil, we determined we would need to know if the product impacts reflectance. Thus this phase of the project was initiated. We also abandoned field trials trying to evaluate clean versus PVY infected clones due to movement by aphid vectors and possibly by Colorado Potato Beetle (CPB), in favor of greenhouse experiments. A collaborating scientist (Dr. Ian MacRae, UMN-Crookston) is now currently working on movement by CPB during defoliation.

In addition to these larger lessons, several small 'learnings' resulted from our efforts, primarily aimed at details regarding sampling and data collection (identification of plant part for example). These included using the same leaf for the readings (reflectance and SPAD), identifying it using a small plastic zip tie (loose enough to not damage the leaf but easily found), doing readings on the 'clean' materials first so as to not infect with $PVY^{N:O}$, and sterilizing the SPAD meter and clip between samples to minimize mechanical spread of PVY.

Contact Information

- Name the Contact Person for the Project
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  - Email Address

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Additional Information

- Did the grantee provide any additional information available (i.e. publications, websites, photographs) that is not applicable to any of the prior sections?

The masters student has not completed his thesis yet. When completed, defended, and accepted for printing it will be available at the NDSU library. If any publications result (particularly for the Aphoil experiments) this Specialty Crop Block Grant funding will be acknowledged.

The graduate student presented his project at the Potato Association of America Annual Meeting at Grand Rapids, MI, July 31-August 4, 2016. Falde, SA, AP Robinson, GA Secor, I MacRae, and AL Thompson. 2017. Remote sensing of Potato Virus Y. Am J Potato Res 94:221.

The graduate student presented his project at the Potato Association of America Annual Meeting in Fargo, ND, July 23-27, 2017. Falde, SA, AL Thompson, and IV MacRae. 2018. Influence of mineral oil on the spectral reflectance of potato leaves. Am J Potato Res 95:214.

Project Title
Placing Value on Seasonal Fruits and Vegetables

NOGA#

Final Report

Partner Organization
North Dakota Department of Agriculture (NDDA)

Project Summary

This project had two activities:

Activity 1

This activity was designed to explore the seasonal pricing of fruits and vegetables being sold across North Dakota. The purpose of the work was to first collect current price information for fruits and vegetables in the state. Background information. Five years ago, this information was collected by another organization, but with cost of living and inflation over the years the validity of that data was being questioned. This organization did not have the resources to conduct a similar pricing study as they had done years ago. It is important for beginning and long-time growers to have up to date information on pricing to make more informed business decisions and to help increase the competitiveness of the specialty crops they sell. In addition, through the 2014 farm bill, USDA AMS - Livestock, Poultry and Grain Market News Division had been tasked with gathering current local market prices for fruits and vegetables. They contacted NDDA for our assistance in gathering pricing information from producers, this was the motivation for this project. The second reason was to try to understand why and how producers set the prices for the produce they grow and sell. This activity was a new project for the NDDA and not previously funded with specialty crop block grant funds.

Activity 2

Background information, North Dakota at the time the grant was written, was one of eight states that did not have a WIC Farmers Market Nutrition Program (WFMNP). And was also one of 11 states with limited or no access to the Senior Farmers Market Nutrition Program (SFMNP) for its residents over the age of 65. This activity was to develop a feasibility study to identify resources needed for the state of North Dakota to administer a Senior Farmers Market Nutrition Program (SFMNP) and a Women, Infant and Child Farmers Market Nutrition Program (WICFMNP). This work was needed because there are many questions that need to be answered and resources that needed to be identified before a program can be implemented. A feasibility study is the first step. NDDA was motivated to do this project because producers and groups had approached NDDA asking the question, why North Dakota does not offer these programs when so many other states do. This activity was a new project for the NDDA and not previously funded with specialty crop block grant funds.

Project Approach

Activity 1

As outlined in the state accepted plan of work, the NDDA marketing specialist reviewed the old pricing study, developed how the current survey would be administered. In February 2016, at the state local foods conference the process for data collection for the upcoming growing/selling season was announced. At the conference, a representative from the United States Department of Agriculture - Agriculture Marketing Service (USDA-AMS) came and talked about collection of data and how our state project would tie into a national effort at AMS to gather more market-specific data for fruits and vegetables. After the conference, a follow up email went out to all farmers market managers. Fifteen market managers responded that they wanted to participate in the project.

All participating specialty crop growers in the state recorded prices during the same week in the months of July, August and September. Prior to the data collection weeks, each market manager was sent a packet with a letter explaining the purpose and worksheets for growers to fill out. The worksheet asked for the city where produce was being sold, price of produce, and unit of measure for that price. Growers were only to put prices of produce they had for sale that day. The worksheets did not ask for the grower's name. This was intentional because the NDDA did not want to disclose individual producer prices. The worksheets were then given back to the market manager and mailed back to the NDDA. The NDDA then took all that information and reported only the high and low price for specialty crops for the city and collection week. This informant was shared on the NDDA website. This information was also shared with USDA-AMS, Dakota College at Bottineau and producers via social media and direct emails. The data collected in 2016 was also shared at the 2017 state local foods conference in February as outlined in the accepted plan of work. At the 2017 conference, growers showed enough interest in the information that was collected, that the NDDA made the decision to go beyond the initial scope of the grant and collect pricing for the 2017 growing season for the same three months.

Activity 2

As outlined in the state plan of work, the NDDA marketing specialist and consultant met to discuss the details of this activity and how to collect the necessary information to compose the feasibility study. Throughout this project, the NDDA marketing specialist consulted with the state department of human services to further their knowledge of the two programs as that state agency is responsible for both the SFMNP and the WIC FMNP. After the first meeting the consultant was going to work on developing the survey and brainstorming ideas to send the survey out to the target audiences. In March of 2016, the consultant's contract expired, and the decision was made to not renew. The NDDA had a meeting to discuss how to proceed forward with this work. It was determined that the NDDA marketing specialist did not have the resources to complete the work the consultant was going to do. Upon advice from others in the department, the NDDA marketing specialist contacted the University of North Dakota (UND). UND had a department that specialized in survey collection, after contacting UND the NDDA marketing specialist discovered that due to state budget cuts that department had been downsized and could not do this project. The NDDA marketing specialist was then passed on to another UND department that was interested in doing the work. This department wanted to do a pre-study first before doing the work outlined in the grant. The NDDA marketing specialist visited with the specialty crop block grant administrator and was told that this was not going to fulfill the grant obligations. At that point, discussions with UND ended. The marketing specialist then explored other options with North Dakota State University (NDSU) and extension. In the spring of 2017, NDSU agreed to do the work. A Memorandum of Understanding (MOU) needed to be written between NDDA and NDSU before any work could start. For reasons out of the control of the NDDA marketing specialist there were delays in drafting that document. In the summer of 2017 NDSU expressed concerns if there was enough time left in the grant to produce a quality product. A MOU was never signed with NDSU. All work including any surveys needed as stated in the initial grant were going to be completed by the consultant or contractor. The NDDA marketing specialist decided to survey specialty crop growers at the 2018 local foods conference. The survey was used to fulfill the obligations written into the grant to identify growers level of interest in participating in the SFMNP and the WIC FMNP as well as identify any barriers that would prevent them from participating.

Goals and Outcomes Achieved

Activity 1

The goal of creating a database of current market prices for specialty crops was achieved. The database can be viewed by going to: https://www.nd.gov/ndda/nd-farmers-market-pricing. To date, this page has received over 1,000 pageviews according to website analytics. An online survey was developed and the link to the survey was sent out to 93 producers and posted on social media to expand the reach. There were 20 respondents to the survey with the following results: 50 percent of those responding said they had seen the data and 46.15 percent of the respondents said they will use the data to help determine future pricing for their specialty crops. This grant looked at the seasonal pricing of fruits and vegetables. According to the survey, 60 percent of respondents do not adjust their prices during the selling season and 40 percent adjust their prices one to two times a season. The number one reason a specialty crop producer raised their prices was because they were the only one at the market who had that specific specialty crop for sale. The number one reason a producer lowered the price was due to oversupply. These survey results were verified by the pricing data collected. For example, crops such as tomatoes and cucumbers were higher priced early in the season, but the prices fell the months of August and September as supplies increased and more growers reported their prices. However, it is worth noting that when the NDDA marketing specialist went to the farmers market and visited with specialty crop producers, the decision to lower price was very subjective. The marketing specialist observed that the drop in price was driven by the producers and not the consumers. One of the specialty crop growers noted on their pricing sheet that they sold peas at a price that did not cover their cost of production, but in their market the price they sold them for is all the market would bear.

Activity 2

The final goal of creating a feasibility study was not achieved for the reasons outlined in the project approach. The performance measure was partially achieved by the survey that was done at the 2018 state local foods conference. Eight surveys were completed. Of those that responded, 38 percent said they were not comfortable accepting vouchers as a form of payment and 63 percent said they did not have access to a credit card machine at the market where they sell. Based on the surveys received, this did help to answer some of the initial questions about resources needed to properly administer the programs at the market level in the grant application. The NDDA marketing specialist, in conversations with state agencies, learned that in a few years there would be a change to accept payment for these programs using EBT, like how they accept SNAP benefits now. With 63 percent of specialty crop growers who responded to the survey not having access to EBT machines at the market, this will prove to be a future challenge that will need to be addressed. The survey also identified there was a strong interest to learn more about these programs from a specialty crop growers' standpoint, with 87.5 percent responding that way. The benchmark for this number was zero as stated in the initial accepted grant application.

Beneficiaries

The groups that benefited from this project were the estimated 125+ specialty crop growers in the state and the 1,000+ individuals that came to the website to see the prices of specialty crops sold in the state. Other organizations that benefited from this work were USDA-AMS, NDSU, Dakota College at Bottineau and the North Dakota Farmers Market and Growers Association (NDFMGA). Because the organizations listed above are outside of the NDDA's control, an accurate number of individuals who benefited from us sharing that data is hard to calculate.

Lessons Learned

Activity 1

Lessons learned during this activity focused on the actual collection of the prices. The first lesson learned was that there was no standardization for selling certain specialty crops at farmers markets. This posed a challenge as the NDDA tried to compare prices. To try to overcome this challenge in the second year of collecting prices on certain specialty crops, the NDDA specified how the prices were reported. Some growers chose to ignore this and reported pricing in their own unit of measure anyway. The second lesson learned was during the second year the prices were recorded, over 50 percent of the markets that reported the first year did not report the second year. The NDDA marketing specialist's level of engagement with the farmers markets was the same both years. When the NDDA marketing specialist contacted markets who did not participate, many reasons were given. Some insight as to why this occurred could be that prices do not change that much from year to year and specialty crop growers did not see the value in reporting prices two years in a row. The information provided was all voluntary and after the first year there may not have been enough perceived value to do it again a second consecutive year. The third lesson learned was the underestimation of the resources needed to input the data received. The data got reported but it was often a month or two old.

Activity 2

There were three lessons learned with this project. First, from the time when this grant was written to the time when it was approved and implemented, there were questions if this work could be accomplished by one person. After meeting with NDDA senior staff it was determined to not hire a single contractor to do this work, but to contract with an organization that has better experience in gathering the data that was needed. The decision was to use an in-state college that had the staff, knowledge and people resources to gather the information in a timely and creditable manor. Changing direction to go with a university with all these attributes lead to the second lesson learned. Time loss, since using a university was not part of the initial plan of work time was spent identifying and discussing the project with a university that could do the work that was outlined in the grant. After several months of calls and emails a department at UND agreed to do the work but that lead to the third lesson learned. Budget vs. scope, for the budget in the grant, UND could do pre-analysis on how best to do a feasibility study to accomplish the goals written into the grant. To do a feasibility as written into the grant UND indicated that the cost would be at least twice what was initially written into the grant. When this was proposed to the SCBG administrator their proposal to do a pre-analysis only was turned down because it would not address the goals of the grant. The lessons learned from this activity will be good to remember if the NDDA or any other organization tries to do a similar project like this in the future.

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Additional Information

Management of Potato Mop Top Tuber Necrosis Using Cultivars That Do Not Express the Disease

Final Report

Project Summary

The goal of this research is to determine the susceptibility of potato cultivars important to the North Dakota potato industry to Potato Mop Top Virus (PMTV)-induced tuber necrosis. Potato cultivars used in the table, chip, and French fry market sector grown in the state of North Dakota were included in the trial. The measureable outcome will be the quantifiable level of susceptibility that commercially acceptable potato cultivars grown in the region has to PMTV-induced tuber necrosis. The cultivars were screened for susceptibility in a field trial conducted in 2016 in a field known to be infested with PMTV. In total, 60 cultivars were screened. Fifty six of these cultivars expressed symptoms of PMTV-induced tuber necrosis. Tubers from the remaining seven cultivars that did not show PMTV symptoms were randomly assayed for the presence of the virus by RT-PCR. Four cultivars showed no visual symptoms but tested positive for PMTV and are rated as insensitive to the virus and but not resistant to infection. This was the second year of cultivar testing for sensitivity to PMTV-induced tuber necrosis and builds on a previous funded project from FY2014 SCBGP funding.

Project Approach

Screen red, white, yellow, and russet-skinned potato cultivars for their susceptibility to tuber-induced necrosis caused by PMTV.

The ND potato industry is relatively small with approximately 90,000 acres grown each year and approximately 188 potato growers. There are two French fry factories in the state, one in Grand Forks, the other near Jamestown. In addition to the French fry industry that is dominated by russet-skinned cultivars, white-skinned chip potatoes are grown primarily under non-irrigated conditions and red- and yellow-skinned cultivars are grown for the tablestock market. All of these market sectors are affected by powdery scab and by PMTV-induced tuber necrosis. The seed industry is also affected by these diseases since both can be disseminated either on or in the seed tuber. PMTV was first reported in 2003 in Maine and in North Dakota in 2010. Since its first detection, PMTV has been found in eight of the top ten potato-producing states. Based on the known spread of PMTV in the USA over the past 13 years since it was first detected, it is very likely that this disease will become increasingly more important over the next decade. The approach of this project was to proactively provide research-based disease management tactics to the potato industry before the disease reaches cataclysmic economic levels as it has for one grower.

Goals and Outcomes Achieved

- 60 potato varieties were evaluated for their sensitivity to PMTV-induced tuber necrosis in 2016. This was the second and final year of field screening.
- The second grading was performed from January 18 to February 16th, 2017, respectively.
- Potato cultivars were graded for PMTV incidence and severity. Significant differences were found among clones in PMTV-induced tuber necrosis incidence ($P < 0.0001$) and severity ($P < 0.0001$).
- PMTV tuber necrosis incidence ranged from 0 to 65% across cultivars. PMTV tuber necrosis severity ranged from zero to 0.38.

- A total of 56 clones have shown PMTV-induced tuber symptoms at varying levels of sensitivity from highly sensitive to moderately insensitive. Four cultivars did not show any symptoms to PMTV-induced tuber necrosis and were rated as insensitive to the virus.
- All potato cultivars have been classified into one of four sensitivity categories based on two years of field evaluation data; insensitive to PMTV-induced tuber necrosis; moderately insensitive, moderately sensitive; and sensitive.
- A draft manuscript to publish these data in a refereed journal article is being prepared. This article will be the basis of an updated extension bulletin (A1777) that will be distributed to the potato growers in the region by the fall of 2017. The data will be posted and immediately available on the NDSU website in June, 2017; http://www.ndsu.edu/potato_pathology/potato_pathology/potato_diseases/
- Combined results from 2015 and 2016 field studies on PMTV sensitivity among cultivars was presented at a national potato meeting in San Francisco on January 4-6, 2017.
- Results of this research were also presented at potato research meetings in Grand Forks, ND on February 21, 2016 to 103 regional potato industry representatives.
- PMTV-induced tuber necrosis sensitivity among potato cultivars was also presented upon request at a national virus meeting in San Diego, CA on March 9, 2017 to 54 potato scientists and other national potato industry representatives.
- Tuber necrosis in red-skinned cultivars was significantly higher than for any other market class of potato. Tuber necrosis in white-skinned cultivars was highest in the internal susceptible control cultivar Nicolet and was significantly higher than any other cultivar for this market class. In yellow- and russet-skinned cultivars PMTV-induced tuber necrosis was very low and significantly lower than red- or white-skinned cultivars.
- Overall, expression of PMTV-induced tuber necrosis was much as expected and very similar to preliminary studies. PMTV symptom expression was much higher in 2016 than 2015.
- Based on the collective results from two years of studies, we are able to make definitive recommendations to the potato industry regarding the susceptibility of potato cultivars to PMTV-induced tuber necrosis in each market class.

Beneficiaries

- There are approximately 188 potato growers in the state, two French fry processing plants and three major tablestock packing facilities. All of these growers and potato associated industries will benefit from this research. The economic impact and benefit to the industry cannot be calculated at this time as it will accumulate over the course of the next decade as the disease becomes more important and prevalent. However, it is reasonable to assume that the economic impact will be very significant given the rapid spread of this disease.
- Data and the results of the disease susceptibilities for all potato cultivars will be disseminated at potato grower meetings such as the Northern Plains Potato Growers Association Research Conference, the International Crop Expo, and the MN Area II Potato Growers Annual Meeting. Additionally, Dr. Gudmestad is a regular presenter at grower meetings for the two French fry processors in the state, Simplot, Inc. and Cavendish Farms.
- Disease susceptibility ratings will also be disseminated via extension pamphlets that will be prepared in collaboration with Dr. Andy Robinson, ND/MN Potato Extension

Specialist. Additionally, the data will be made available on Dr. Neil C Gudmestad's NDSU home page where research results are made available to the public. There are no means by which to monitor the number of growers that view the results of this research project on this website.

- http://www.ndsu.edu/potato_pathology/
- http://www.ndsu.edu/potato_pathology/potato_pathology/potato_diseases/
- Preliminary data potato cultivar susceptibility to PMTV from the 2015 trial were presented to the Cavendish Farms staff (12 in attendance) on December 9, 2015. It was agreed that a more complete presentation on the data would be provided in a research meeting with them in November/December, 2016.
- Preliminary data on the susceptibility of potato cultivars to PMTV were presented at a research meeting of the Northern Plains Potato Growers Association on February 16, 2016 in conjunction with the 2016 International Crop Expo. There were 103 participants at the meeting.
- Combined results from 2015 and 2016 field studies on PMTV sensitivity among cultivars was presented at a national potato meeting in San Francisco on January 4-6, 2017 to 86 potato industry representatives.
- PMTV-induced tuber necrosis sensitivity among potato cultivars was also presented upon request at a national virus meeting in San Diego, CA on March 9, 2017 to 54 potato scientists and other national potato industry representatives.
- All participants at the meetings in which preliminary data was presented agreed that this research will be very beneficial to the entire potato industry in North Dakota.

Lessons Learned

- There were no problems or delays experienced in the research conducted during the growing season or during post-harvest evaluations.
- Expression of PMTV-induced tuber necrosis was lower than expected during the first evaluation after harvest. However, during the second evaluation, approximately 75% of the cultivars showed symptoms of PMTV-induced tuber necrosis.
- Regardless of the increase in PMTV tuber symptoms in storage, we need to insure that we do everything we can to facilitate infection. PMTV is vectored by the powdery scab pathogen which requires high soil moisture for successful infection. We have discussed this problem with the cooperator, and the field trial will receive a "double irrigation" during each irrigation event to insure that soil conditions conducive to infection are established.
- We learned that we must be pro-active in insuring that our field plot is adequate irrigated for our purpose. We have a lot of other field trials in the vicinity of the potato mop top trial, so staff members are stopping at this field site multiple times during each week to verify that the field moisture is at a level sufficiently high level of disease pressure.

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Additional Information

- PMTV-induced tuber necrosis in Red Norland, the most common red-skinned cultivar grown in North Dakota.

Project Title

Host Preference and Phenology of Spotted Wing Drosophila

NOGA#

15-331

Partner Organization

North Dakota State University

Project Summary: Purpose and Motivation

In 2013, spotted wing drosophila (*Drosophila suzukii* Matsumura) was first discovered in North Dakota. Spotted wing drosophila (SWD) is an invasive insect pest with a wide host range that includes specialty fruit crops such as strawberries, raspberries, cherries, grapes, and other thin-skinned fruit. This pest is particularly devastating because the female fly uses her saw-like ovipositor to lay eggs in firm, ripening fruit as opposed to other vinegar flies that attack overripe, rotting fruit. Eggs hatch before the fruit ripens and larvae render the fruit unmarketable. This invasive pest threatens the livelihood of ND small fruit producers (specialty crop producers).

This project studied the biology and timing of SWD infestations in North Dakota fruits and attempted to identify specialty crop species and cultivars that are less susceptible to infestation. The project had three objectives: (1) evaluate SWD's host preference for common and newer ND fruits; (2) determine the phenology (timing) of SWD appearance in ND fruits through trapping; and (3) test for a correlation between skin thickness and SWD damage using a penetrometer in grapes and juneberries. All field activities were conducted by Caitlin Krueger, a graduate student, and hourly workers.

This project did not build upon a previously funded specialty crop block grant. Furthermore, the project did not benefit commodities other than specialty crops.

Project Approach and Activities Performed

Objective One: Evaluate SWD's host preference for common and novel North Dakota fruits.

- Exclusion netting was placed over common North Dakota cultivars of aronia, currant, grape, haskap, Juneberry, and sour cherry. All tested fruit was sourced from mixed orchard research sites at the NDSU Absaraka Horticultural Research Farm (46°59'29.38"N 97°21'15.64"W,) and the NDSU Carrington Research and Extension Center (47°30'35.54"N 99° 7'14.39"W).
- When fruit ripened, bagged branches from each available cultivar were collected and ripe berries were gently trimmed from their stems.
- Berries were sorted by cultivar and used for no-choice infestation testing in a randomized complete block design. SWD artificial diet was used as a control. Berries were placed three to a cup to be infested with two adult male and two female flies for forty-eight hours.
- After adults were removed the infested cups were allowed to sit for a minimum of two weeks, with daily checks to record any emerging larvae and adults. After new flies stopped emerging the fruit was dissected. The number of larvae, pupae, and adults were recorded.
- Data was collected from spring 2017 through June 30, 2018.

Objective One Results: This study determined that SWD attempted to infest all tested fruit crops with the exception of aronia, where no larvae were observed across all trials and replications outside of the diet control (Figure 1). Aronia does not appear to be a preferred host for SWD in ND.

Trials in other fruits showed trends for some cultivars that could influence future recommendations to specialty crop stakeholders and orchards looking for resistant or less favorable cultivars for SWD. However, more data is necessary before definitive recommendations can be made. Research and analysis will continue beyond the term of this grant. The data presented in the rest of this section is preliminary data and has not been analyzed for statistical significance.

Currants were readily infested by SWD in the no-choice trials (Figures 2-3). However, the cultivars Blackcomb, Nechaka, Tahsis, and Tiben showed relatively low infestation rates of larvae, pupae, and adults compared to other currant cultivars.

Overall SWD infestation of grape cultivars common to ND was quite low (Figure 4), with no successful infestation seen on the M1, or Marechal Foch cultivars and relatively low numbers on everything else but the control. Juneberry testing indicated that Honeywood was the least preferred variety (Figure 5) though overall numbers were high in other cultivars tested. Sour cherry (Figure 6) showed similar infestation rates between the two tested varieties.

Conclusions Objective One: The resulting data suggests that common aronia and grape cultivars are at low risk for SWD damage and require minimal or no control measures to manage SWD damage. This is important information for specialty crop producers. Other crops such as currant, Juneberry, and sour cherry are more readily accepted as hosts for SWD and will need to be carefully managed to prevent crop losses in the field. This data also suggests potential cultivars that are less vulnerable, or less favorable to SWD and may be recommended for future planting by specialty crop producers.

Objective Two: Determine the phenology (timing) of SWD appearance in ND fruits through trapping in selected fruit crops

- Traps were placed at two North Dakota mixed orchard research sites during the 2016, 2017 and 2018 production seasons: NDSU Absaraka Horticultural Research Farm (46°59'29.38"N 97°21'15.64"W,) and the NDSU Carrington Research and Extension Center (47°30'35.54"N 99° 7'14.39"W)
- In 2016, Phercon® liquid traps (Figure 7) with *D. suzukii* pheromone lures (Trécé, Inc., Adair, OK) were placed throughout both sites to monitor for the seasonal occurrence and densities of SWD. Traps were placed in transect lines spaced at 5-m intervals for all available small fruits at each site. Trapping began the first week of July and continued through the middle of August.
- In 2017, AlphaScents™ sticky traps (Figure 8) with *D. suzukii* pheromone lures (Alpha Scents Inc., West Linn, OR) were used to monitor for the seasonal occurrence and densities of flies at each fruit production site. Traps were spaced at a distance of approximately 5 m apart, with larger spacing for locations with buffer crops or areas of no plantings. Trapping began in May and continued through the start of grape harvest in September, 2017.
- Trapping continued in early 2018, beginning in May and using the same model of AlphaScents™ sticky traps and continued through June 30, 2018.
- For all years, traps were changed weekly and evaluated for the presence of SWD and recorded the number of male and female flies present per trap.

- Weekly captures averages were calculated for each fruit transect and location and graphed to identify potential patterns in SWD yearly phenology in ND.

Objective Two Results: Results in graphic form, broken down by year, location, and crop are presented in figures 9-28. Results for the 2018 season have not been graphed as no SWD were detected prior to the ending of this grant (June 30, 2018).

Objective Two Conclusions: The data collected from 2016, 2017, and 2018 (Figures 9-28) strongly suggests that SWD does not overwinter in ND and instead migrates annually to reinfest portions of the state. First yearly detection usually occurs during the first week in July, following a pattern first observed by Extension personnel. This suggests that early-season fruits such as Haskaps, and early ripening Juneberries and strawberries may avoid SWD damage entirely.

Populations of SWD for the 2016 and 2017 season rapidly increased after initial detection, suggesting they quickly utilized potential hosts. Populations of SWD at both sites continued to build through mid-August before rapidly decreasing into low but detectible numbers through September. This may be due to a reduction in available hosts at both orchard sites, where after mid-August very few ripe berries can be found on any plants until the grape crops mature in mid-September. For specialty crop production growers that produce ripe fruit into early fall, management efforts will likely need to be sustained until hard frosts set in.

Objective Three: Test for a correlation between skin thickness and SWD oviposition damage in grapes and juneberries

- In 2016-2017 an evaluation of the puncture force needed to penetrate the fruit skin of multiple cultivars of Juneberry (2017) and grape (2016-2017) was collected at the point where the fruit was determined to be ripe both visually and by flavor.

- Berry samples were processed by gently clipping the berries from the stems within 48 hours of harvest and randomly selecting 20 undamaged berries per cultivar for testing. Each individual berry was weighed, punctured with a penetrometer and had its polar and equatorial diameter taken.
- Average penetrometer values were calculated from the 20 samples and graphed (Figures 29-30).

Objective 3 Results: Results from the 2016-2017 penetrometer testing of grape cultivars (Figure 29) show there are significant differences between cultivar skin toughness both between cultivars and across years. This range may be attributed to the year to year differences in field conditions (a hot dry 2017 vs a cooler wetter 2016) or the date of harvest, which was earlier in 2017. Juneberry cultivars tested (figure 30) were remarkably similar for the 2017 season.

Objective 3 Conclusions: From comparing 2017 results with host preference testing data, there appears to be little to no connection between penetrometer values and reproductive success on tested cultivars. The grape cultivar Alpenglow had one of the highest penetrometer values, suggesting resistance to infestation. However, Alpenglow grapes also had one of the highest rates of infestation in the host preference trials. Juneberry results were so similar that there was little to no influence in the reproductive success of SWD.

While not definitive, initial results suggest that skin toughness among small ND fruits is not the deciding factor in host preference or resistance. A further exploration of other factors, such as acidity and sugar content needs to be incorporated into future studies.

<u>Outreach and Outcomes</u>

The work plan called for attending two conferences to conduct a benchmark survey on spotted wing drosophila (SWD) infestations. Dr. Esther McGinnis (NDSU Extension

Horticulturist) and Dr. Harlene Hatterman-Valenti (NDSU High Value Crops Professor) attended the 2016 ND Farmers Market and Growers Association Conference and the 2016 ND Grape and Wine Association Conference respectively to conduct the initial survey of fruit producers. The survey was used to introduce fruit producers to SWD, assess their level of knowledge regarding SWD management practices and to identify which fruits were infested. Overall, most fruit producers had a low level of knowledge regarding SWD. Only 3 out of 33 fruit producers reported trapping and monitoring for SWD. The following percentage of fruit producers reported infestations in the following fruit: raspberries (27%), strawberries (9%), tart cherry (6%), black currant (3%), apricot (3%), haskap (3%), Juneberry (3%), plum (3%), and chokecherries (3%). Seven producers claimed economically significant yield losses due to SWD.

NDSU Plant Diagnostician, Jesse Ostrander, presented a poster entitled *Emerging Insect Pest—Spotted Wing Drosophila in North Dakota* at the National Meeting of the National Plant Diagnostic Network in Washington D.C., March 8-12, 2016.

Graduate student Caitlin Krueger prepared a poster entitled *Drosophila suzukii* in North Dakota: Distribution and Future Research. This poster was presented to the International Congress of Entomology meeting in Orlando, FL in September 2016.

Caitlin Krueger presented information on SWD identification, management techniques, and preliminary research data to specialty crop stakeholders at the North Dakota Grape and Wine Association in early February 2017. In addition, she gave a presentation at the 2017 NDSU Extension High Tunnel Conference in which she taught specialty crop stakeholders to identify SWD and how to build monitoring traps. Many high tunnel producers grow fruits in the protected environment of high tunnels.

In a post-conference survey from the 2017 NDSU Extension High Tunnel Workshop, 18 specialty crop producers reported an increase of knowledge regarding SWD identification and trapping.

In November 2017, Caitlin Krueger traveled to Denver, CO for the Entomological Society of America annual conference and gave an oral talk entitled, Seasonal activity of spotted wing drosophila, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), in small fruit production sites in North Dakota.

In May 2018, Dr. Esther McGinnis gave a presentation on spotted wing drosophila to the NDSU Extension High Tunnel Community of Practice that summarized identification, life cycle, monitoring, best management practices, and research results. Over 100 people participated in the live webinar or watched the YouTube video:
https://www.youtube.com/watch?v=DW94JLDuEFU

In addition, Drs. Esther McGinnis and Janet Knodel published a newly revised Extension publication on Spotted Wing Drosophila in June of 2018:
https://www.ag.ndsu.edu/publications/crops/integrated-pest-management-of-spotted-wing-drosophila-in-north-dakota

We have not published two peer-reviewed journal articles. We will continue to collect data until the end of the 2018 season. At that time, we will finish analyzing the data and attempt to publish articles in 2019. We will also continue to engage with specialty crop stakeholders and continue to share our research results.

Lessons Learned:

The 2016 season saw a number of issues, including difficulties in sustaining a healthy SWD colony. Despite being an invasive insect, SWD colonies are difficult to maintain in captivity. Colony diet was the first problem that was encountered. Initially, the colonies were fed organic raspberries. However, we learned that organic raspberries are heavily infested with other *Drosophila* spp. that contaminated our SWD colony. We decided to order and use artificial *Drosophila* diet to prevent colony contamination. While expensive, artificial diet prevented contamination of the SWD colony wither other species.

Colony environment was also an issue. The SWD colonies were very sensitive to wind desiccation and high temperatures. Growth chambers, a climate-controlled greenhouse, and incubation chambers have all proven too hot or windy and led to high mortality and impacted our 2017 host preference data. These issues led to the loss of 2017 and 2018 trials for haskap cultivars. We then learned to keep the SWD colonies in the lab.

Frequent hail events in 2016 destroyed traps at both locations, prompting the switch to sticky traps which are more tolerant of impact damage. Hot dry conditions in the field in 2017 led to far earlier maturation of some crops and forced a reduction in the number of cultivars tested when the SWD colony numbers had not built sufficiently for larger tests.

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Figures:

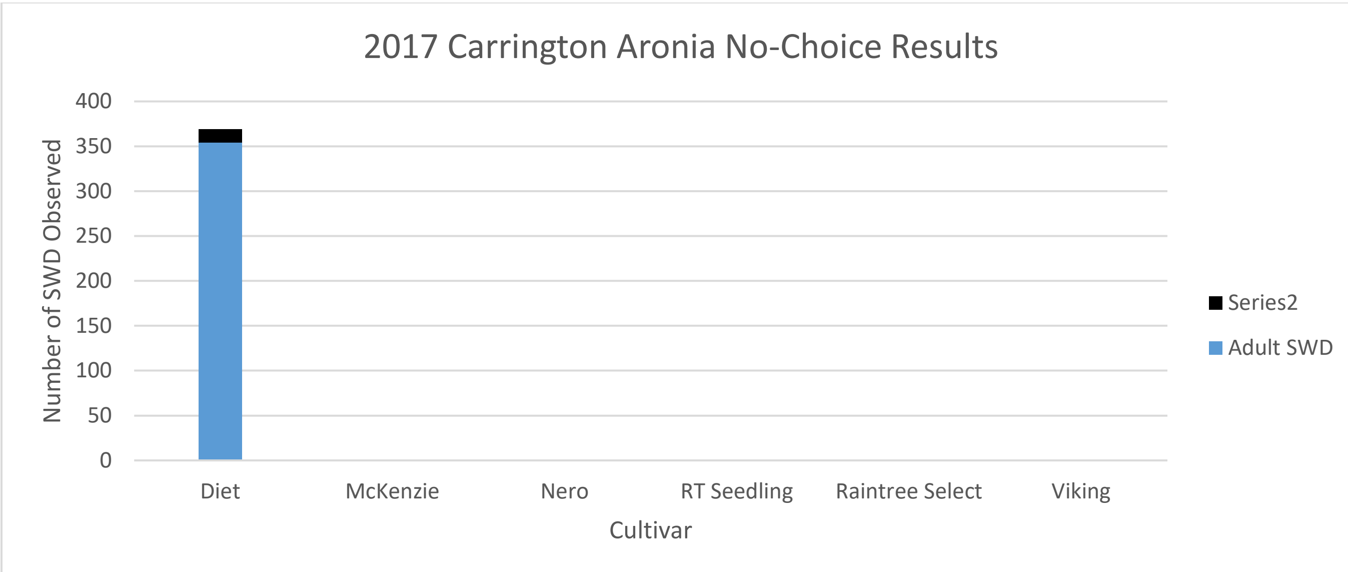

Figure 1: A summary of the total number of observed larvae and un-emerged pupae, and adult SWD on Carrington grown aronia fruit cultivars of in a no-choice experiment conducted in 2017.

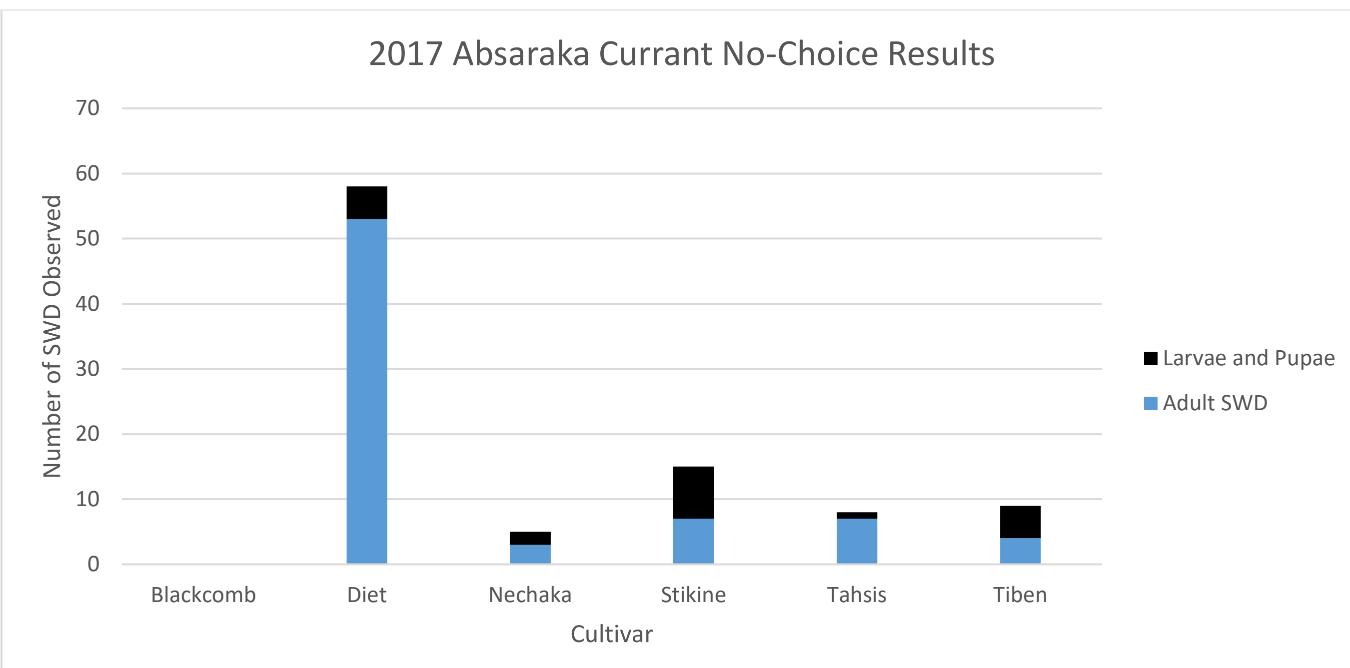

Figure 2: A summary of the total number of observed larvae and un-emerged pupae, and adult SWD on Absaraka grown currant fruit cultivars of in a no-choice experiment conducted in 2017.

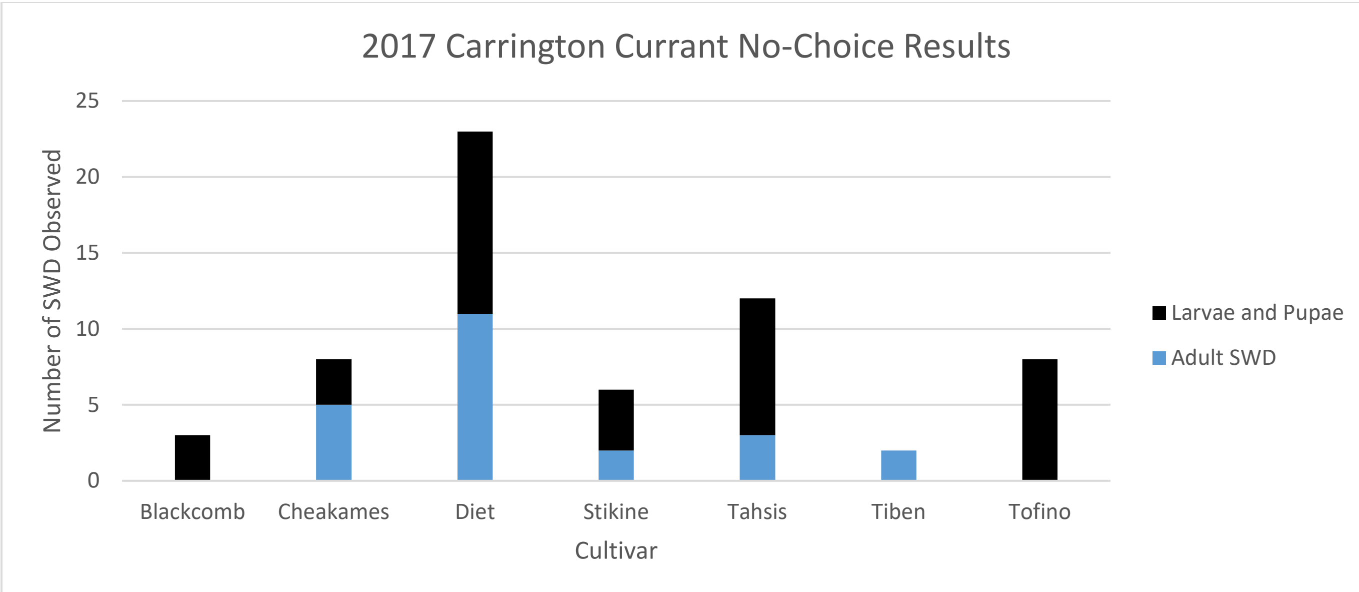

Figure 3: A summary of the total number of observed larvae and un-emerged pupae, and adult SWD on Carrington grown currant fruit cultivars of in a no-choice experiment conducted in 2017.

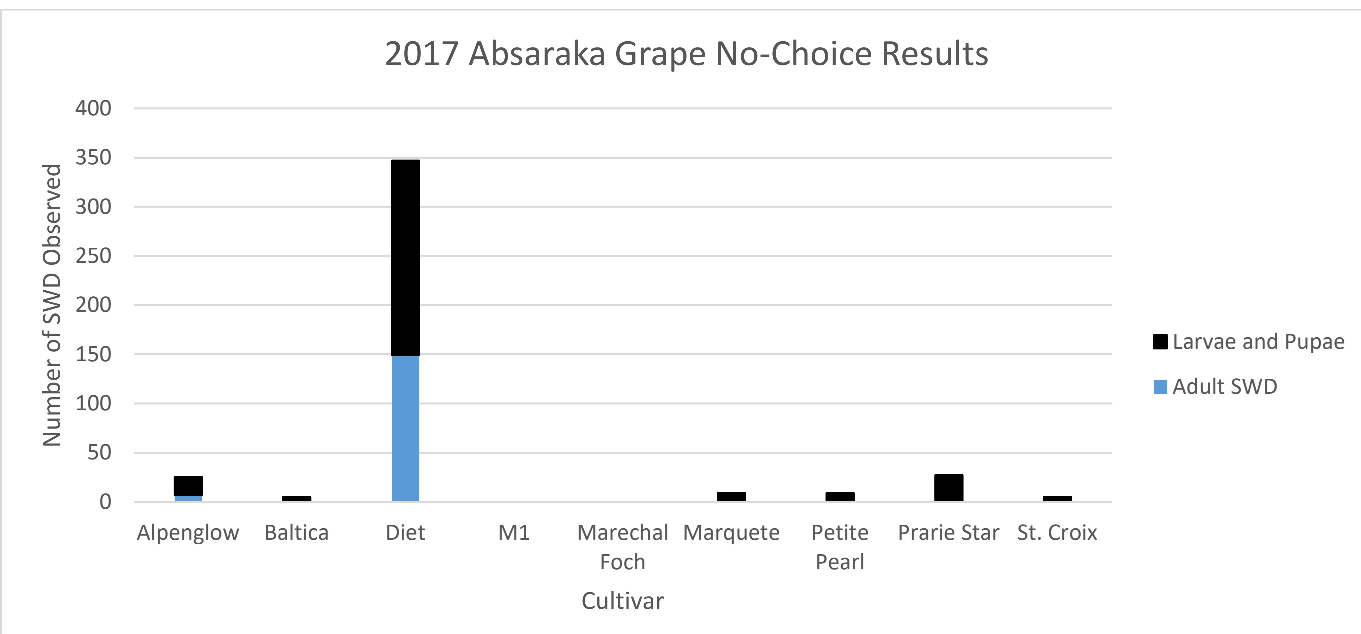

Figure 4: A summary of the total number of observed larvae and un-emerged pupae, and adult SWD on Absaraka grown grape fruit cultivars of in a no-choice experiment conducted in 2017.

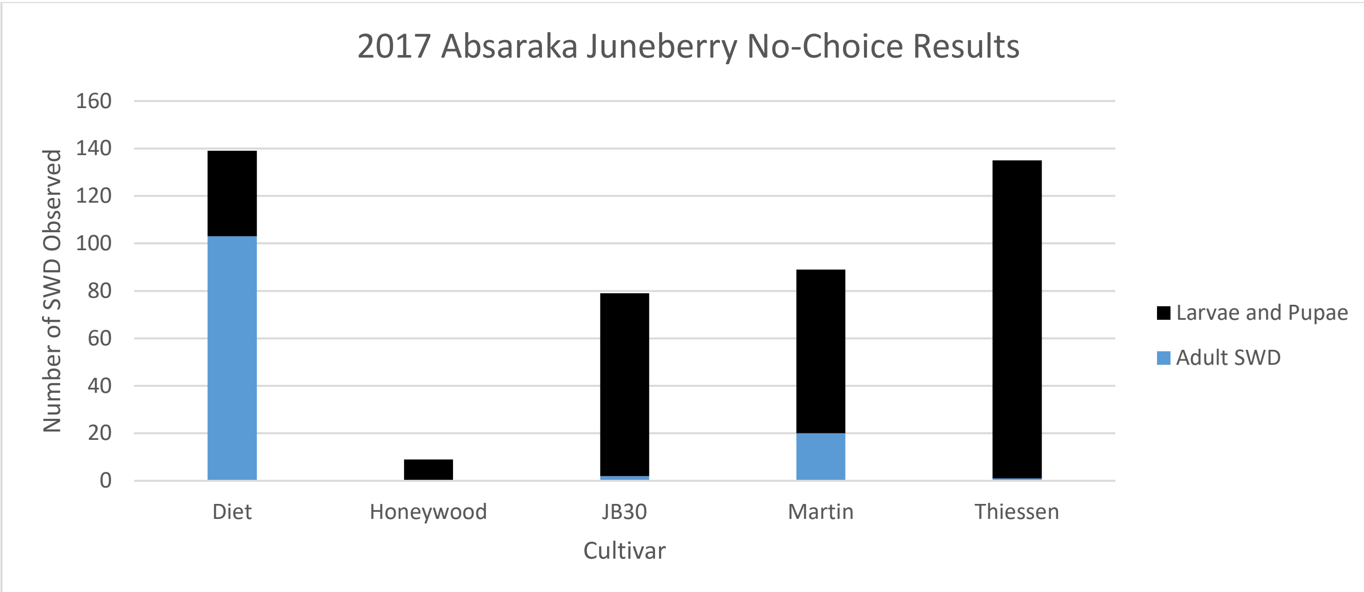

Figure 5: A summary of the total number of observed larvae and un-emerged pupae, and adult SWD on Absaraka grown Juneberry fruit cultivars of in a no-choice experiment conducted in 2017.

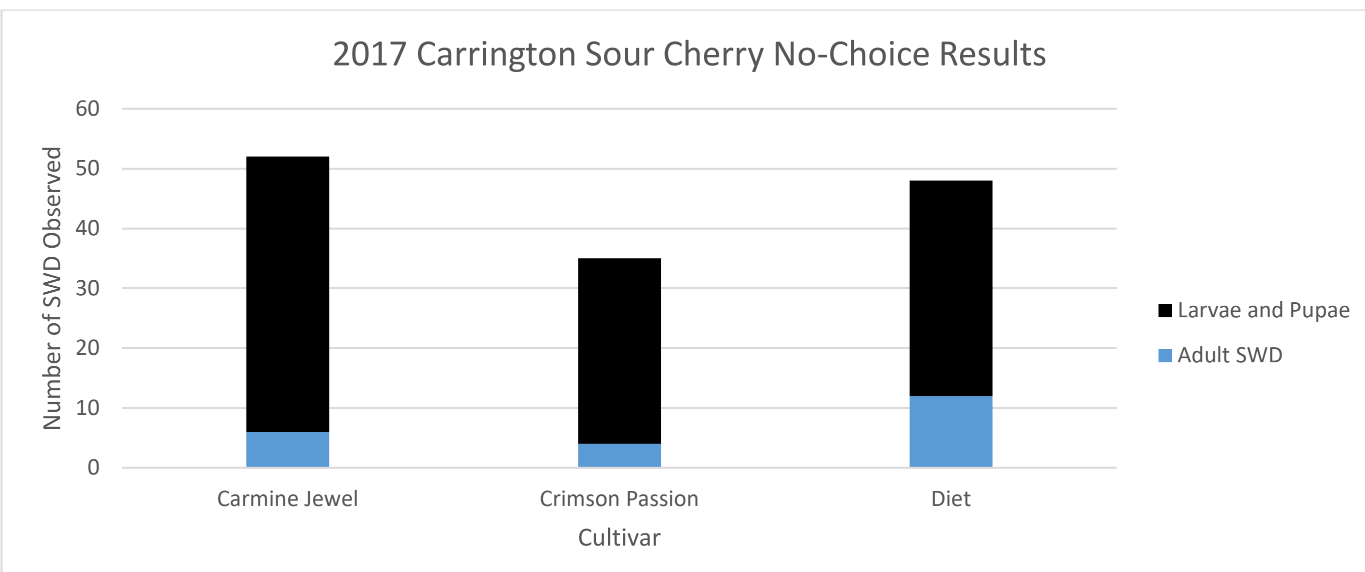

Figure 6: A summary of the total number of observed larvae and un-emerged pupae, and adult SWD on Carrington grown Sour Cherry fruit cultivars of in a no-choice experiment conducted in 2017.

Figure 7: Pherocon® liquid trap with SWD lure in grape plantings at Carrington in 2016.

Figure 8: Alphascents™ yellow sticky trap with SWD lure in a wooded area at Carrington in 2017.

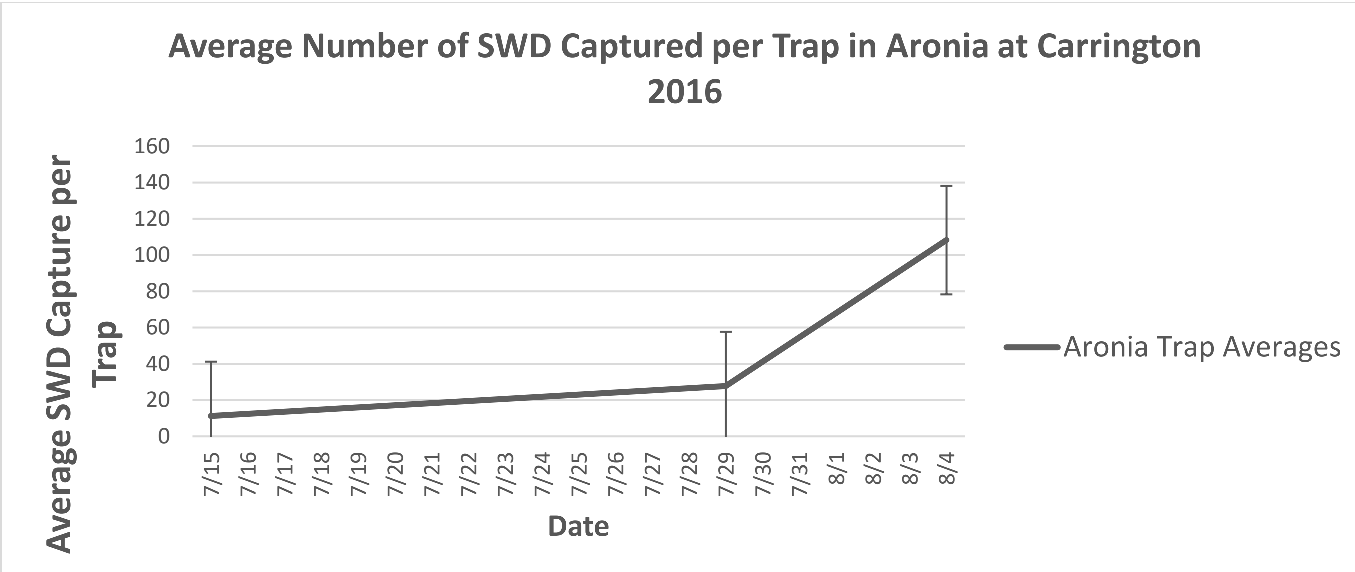

Figure 9: A summary of Pherocon® trap capture averages in an aronia transect at Carrington 2016.

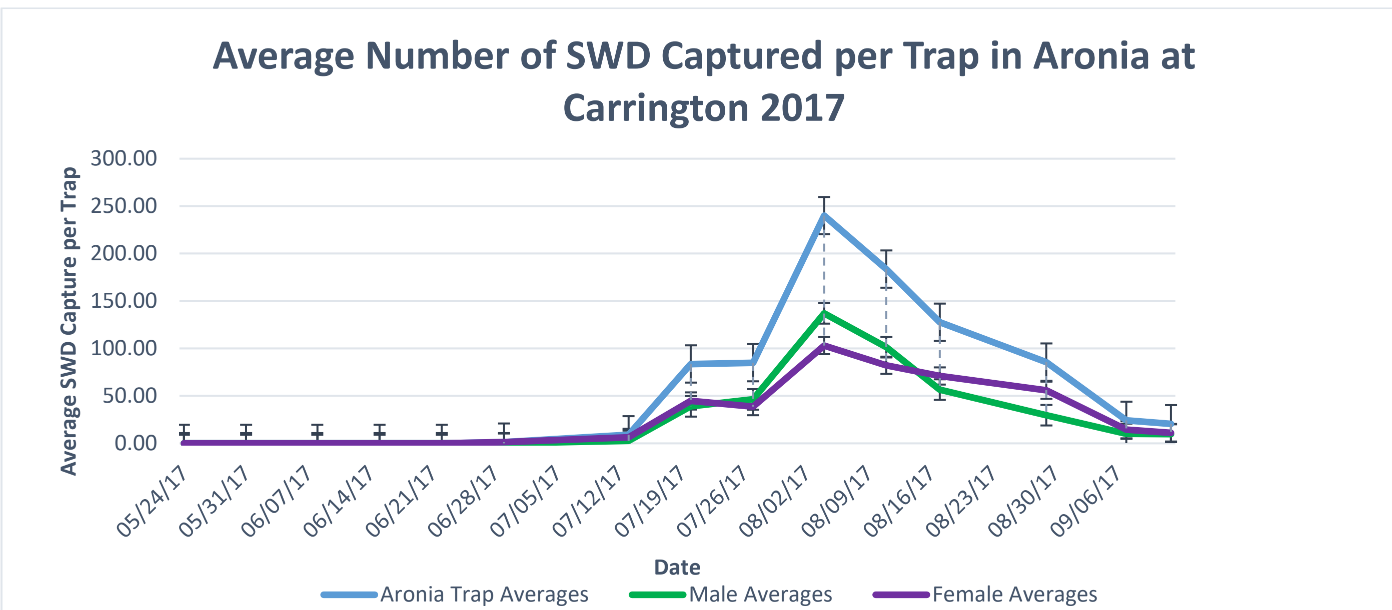

Figure 10: A summary of Alphascents™ trap capture averages in an aronia transect at Carrington 2017, with a comparison of total trap capture averages to male and female averages.

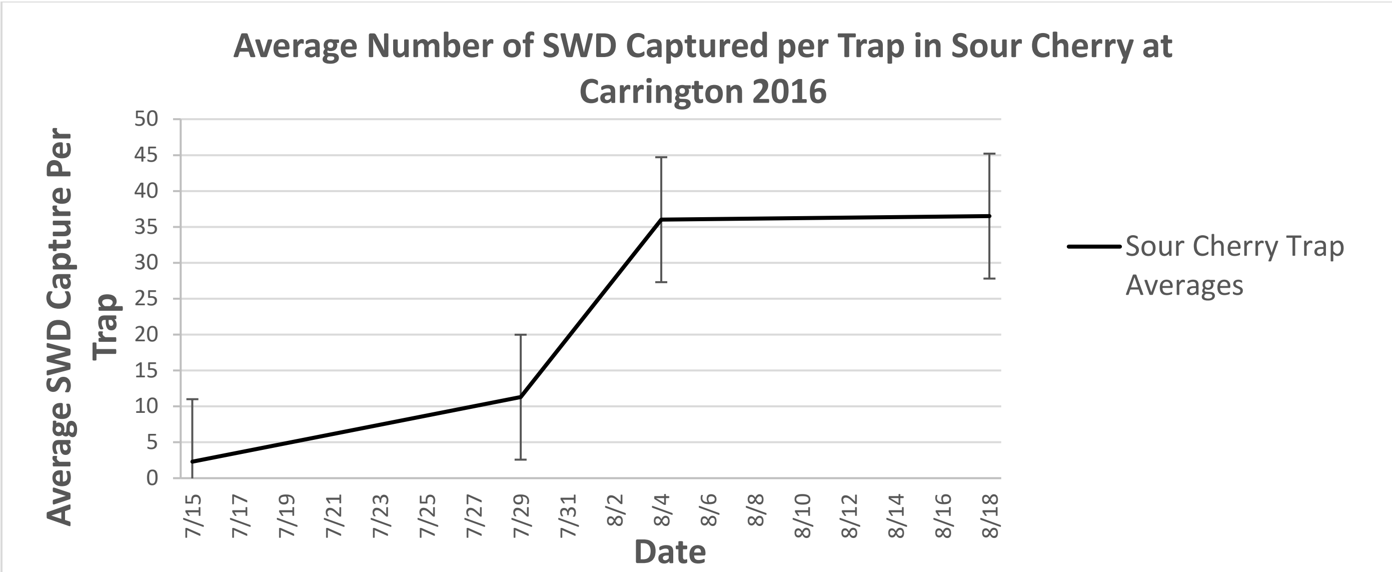

Figure 11: Figure 3: A summary of Pherocon® trap capture averages in a sour cherry transect at Carrington 2016.

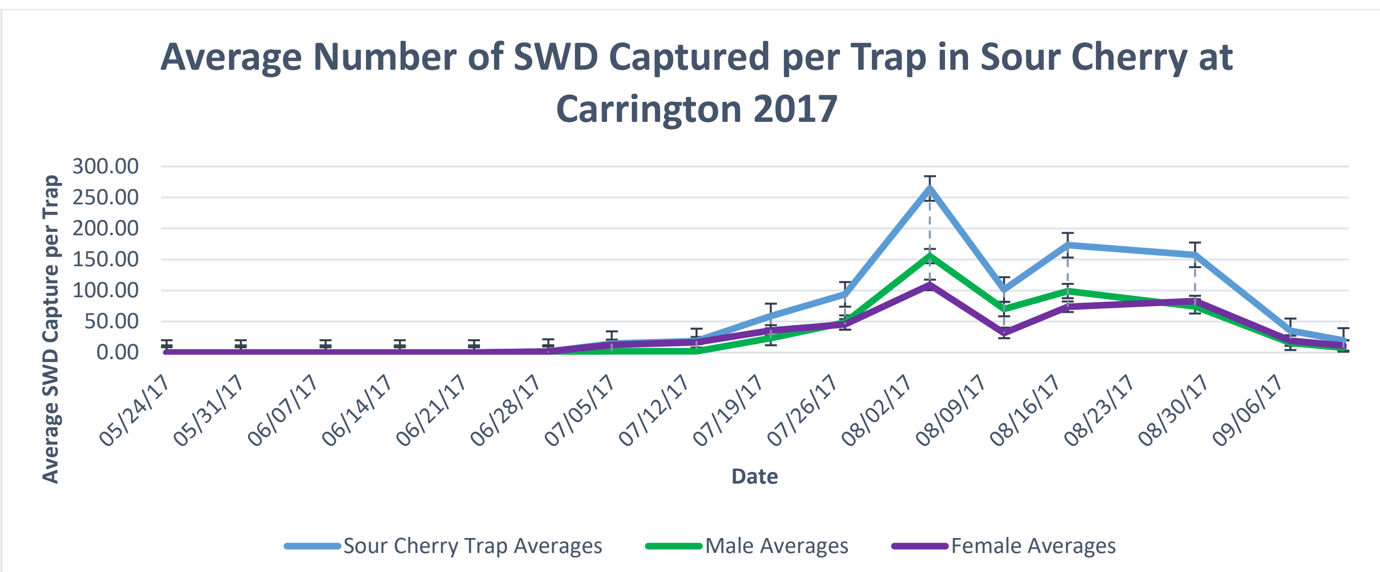

Figure 12: A summary of Alphascents™ trap capture averages in a sour cherry transect at Carrington 2017, with a comparison of total trap capture averages to male and female averages.

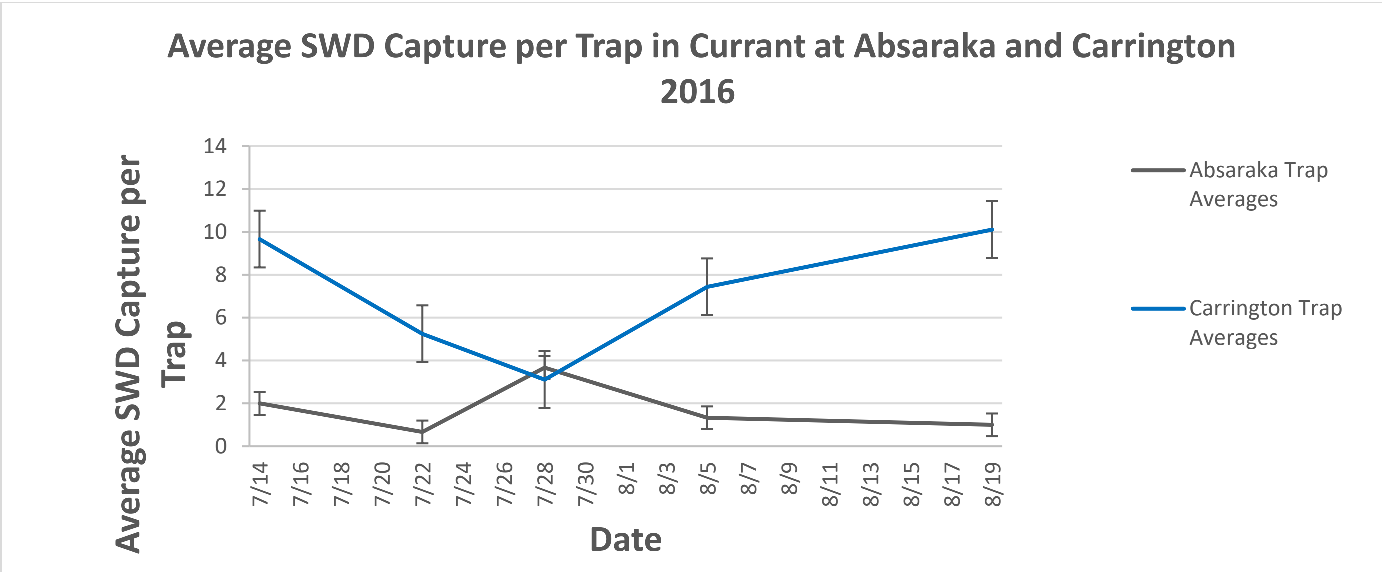

Figure 13: A summary of Pherocon® trap capture averages in currant transects at Absaraka and Carrington 2016.

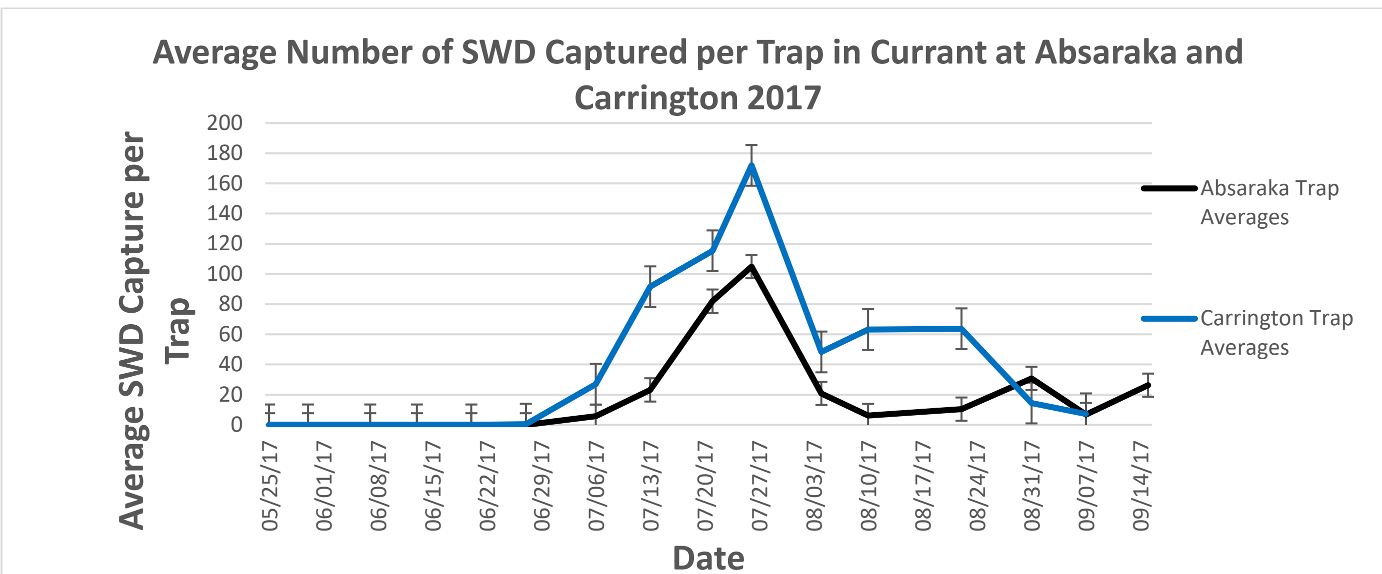

Figure 14: A summary of Alphascents™ trap capture averages in currant transects at Absaraka and Carrington 2017.

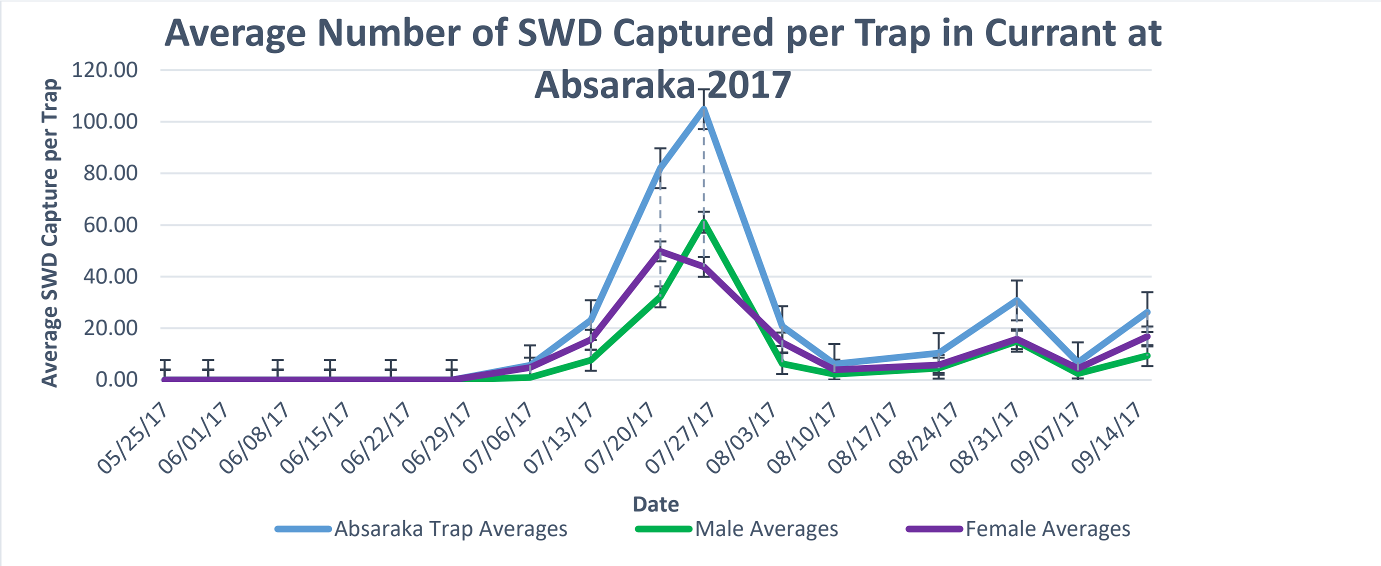

Figure 15: A summary of Alphascents™ trap capture averages in a currant transect at Absaraka 2017, with a comparison of total trap capture averages to male and female averages.

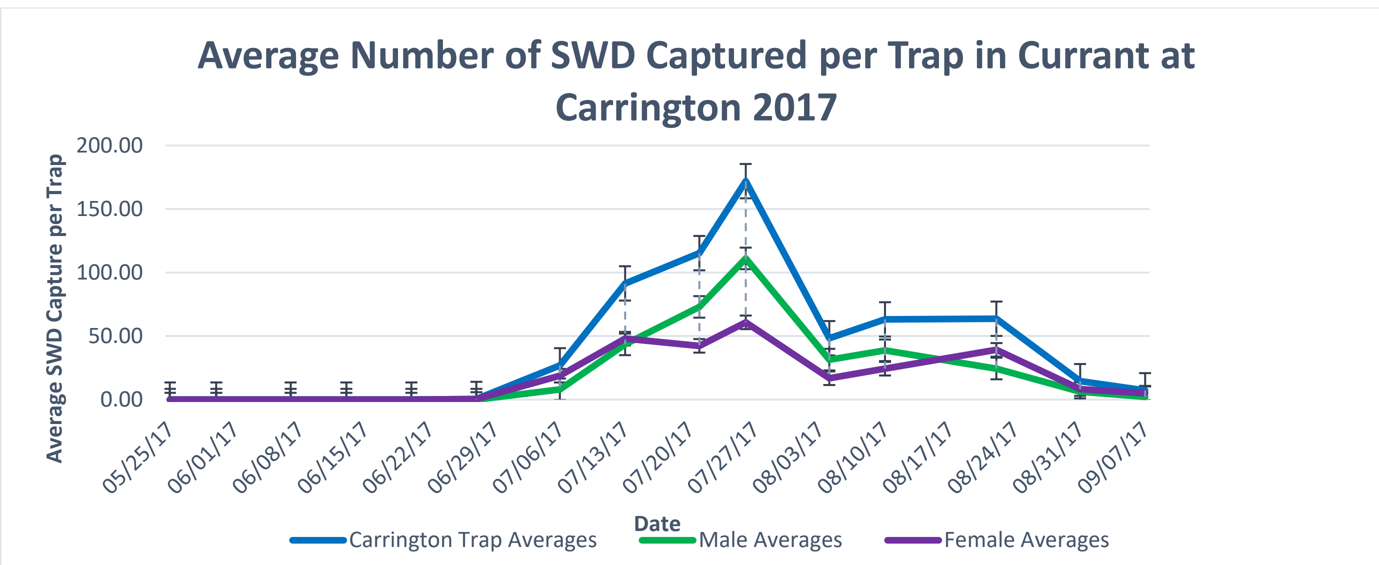

Figure 16: A summary of Alphascents™ trap capture averages in a currant transect at Carrington 2017, with a comparison of total trap capture averages to male and female averages.

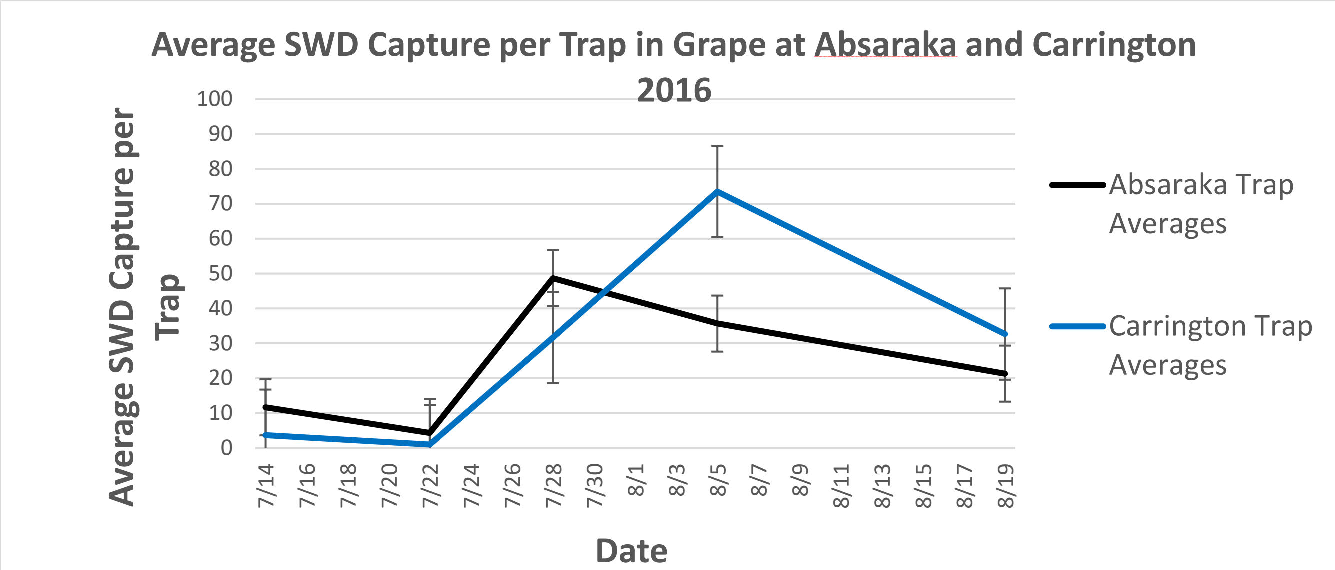

Figure 17: A summary of Pherocon® trap capture averages in grape transects at Absaraka and Carrington 2016.

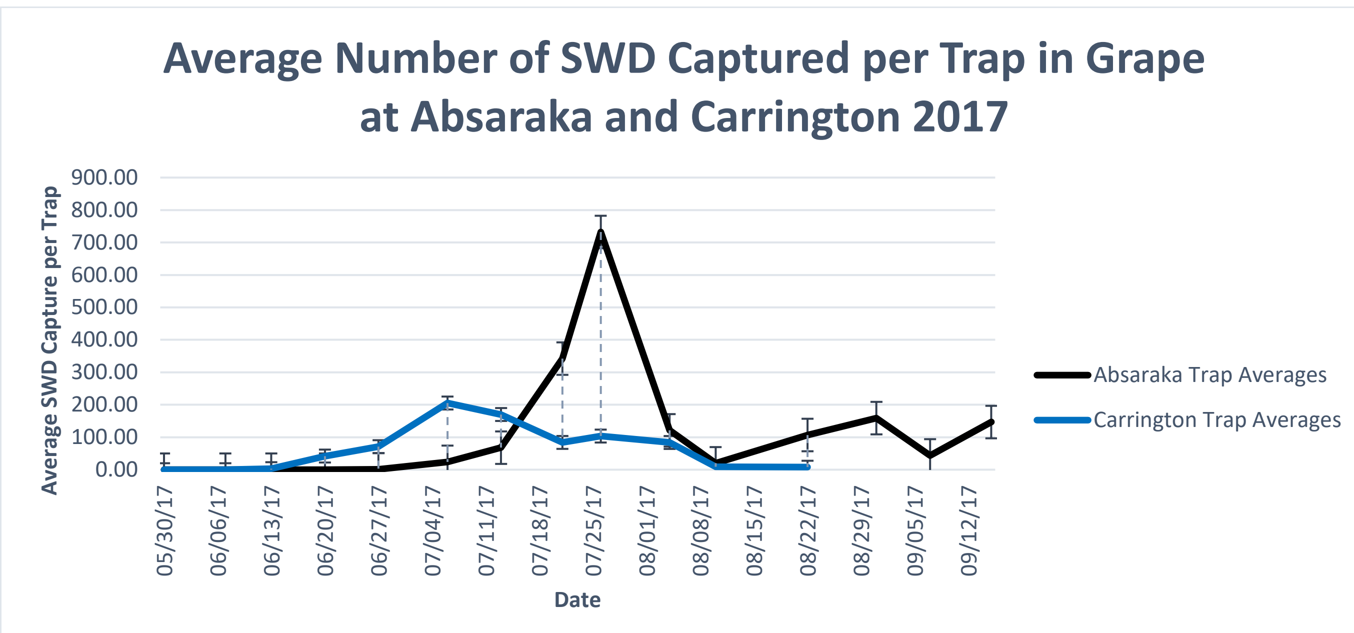

Figure 18: A summary of Alphascents™ trap capture averages in grape transects at Absaraka and Carrington 2017.

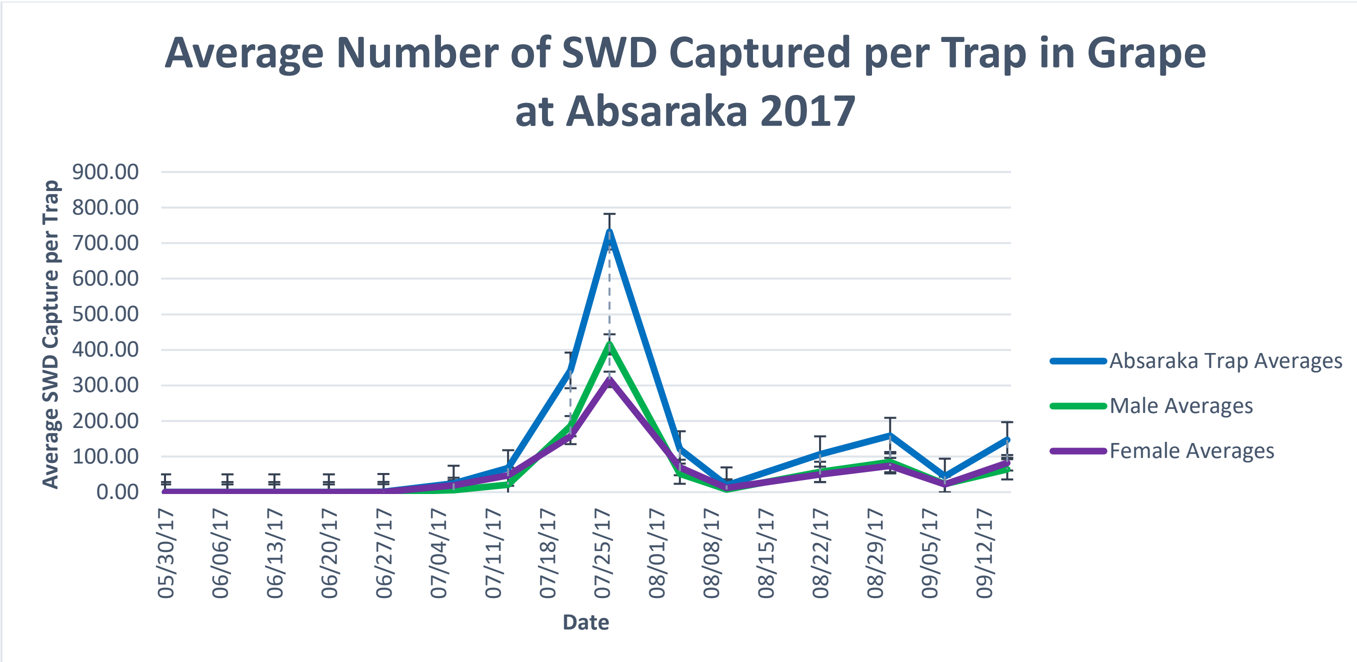

Figure 19: A summary of Alphascents™ trap capture averages in a grape transect at Absaraka 2017, with a comparison of total trap capture averages to male and female averages.

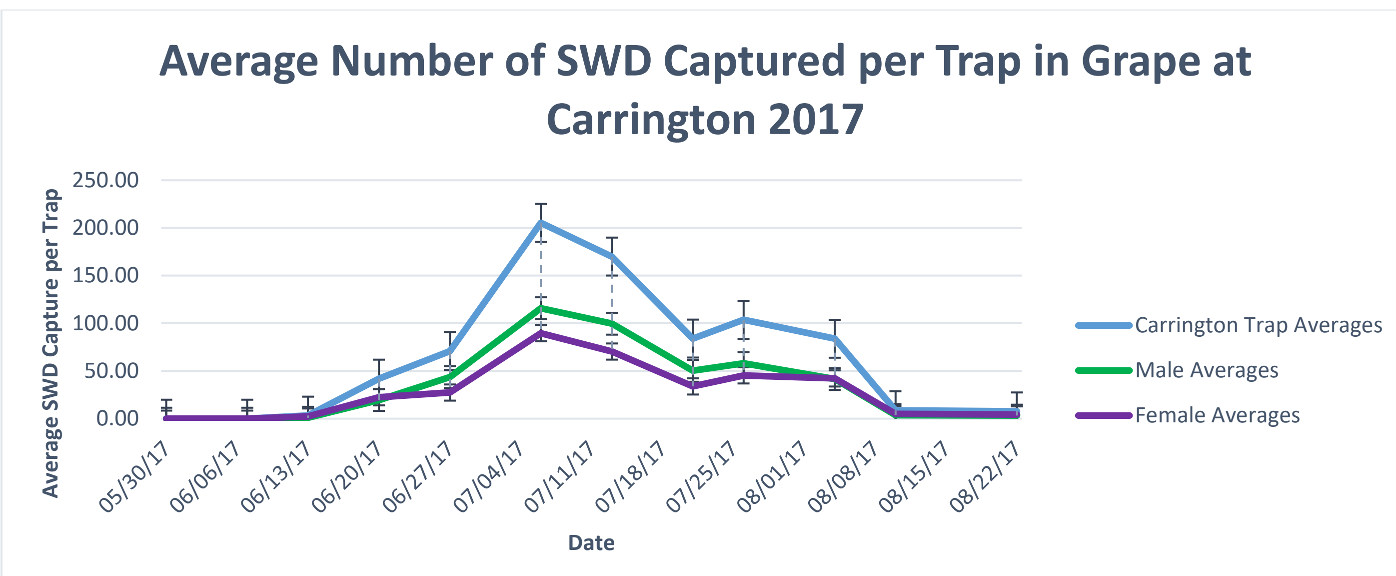

Figure 20: A summary of Alphascents™ trap capture averages in a grape transect at Carrington 2017, with a comparison of total trap capture averages to male and female averages.

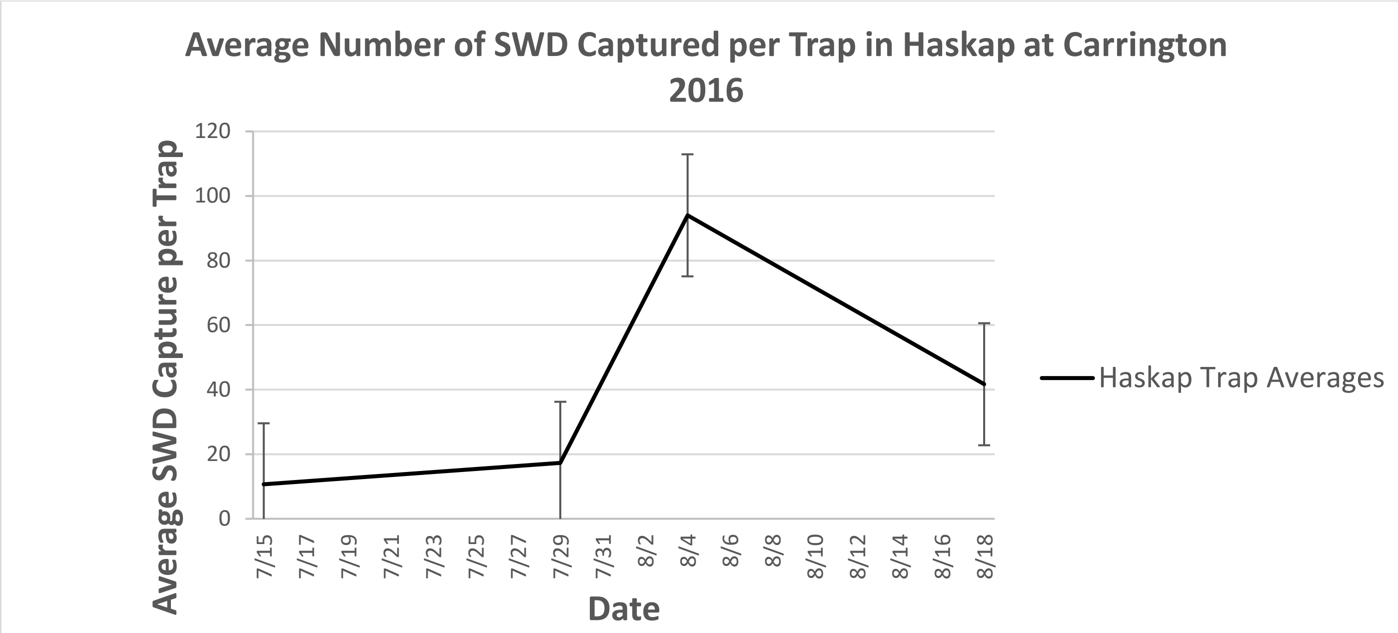

Figure 21: A summary of Pherocon® trap capture averages in haskap transects at Carrington 2016.

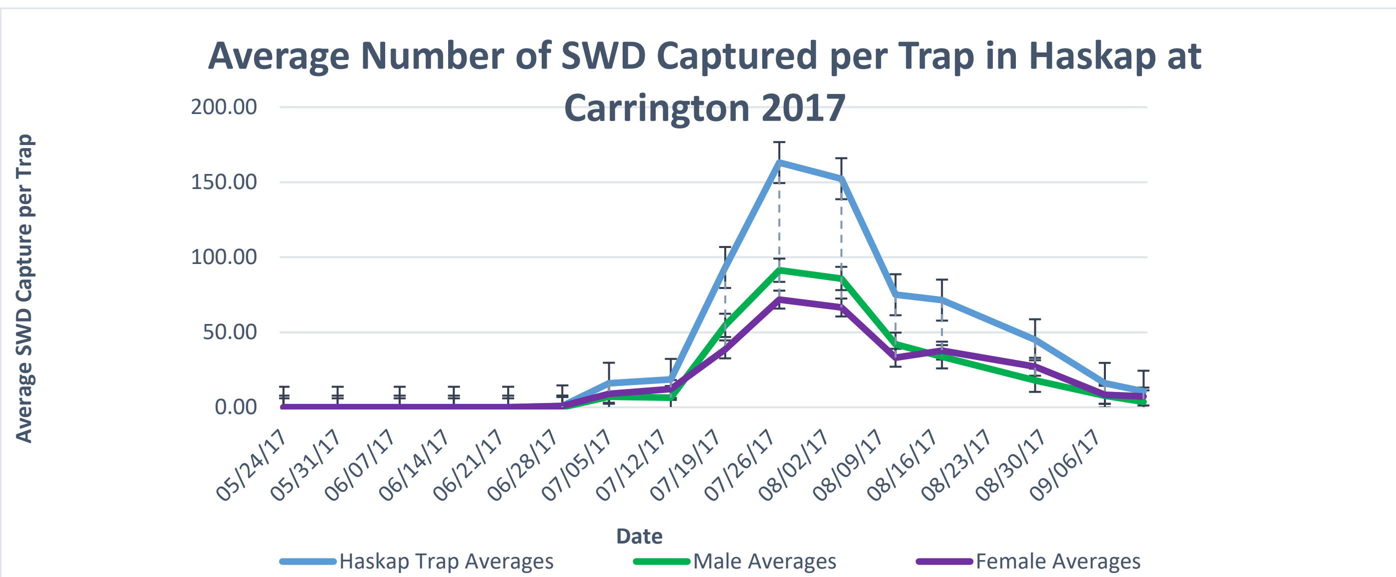

Figure 22: A summary of Alphascents™ trap capture averages in a haskap transect at Carrington 2017, with a comparison of total trap capture averages to male and female averages.

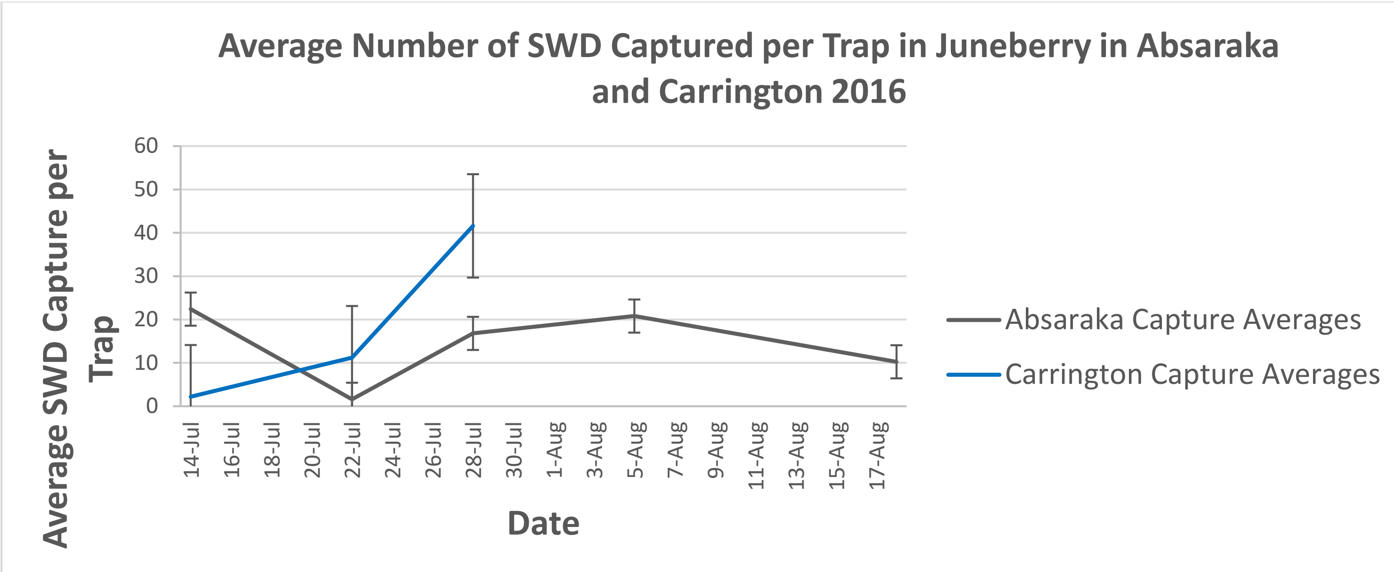

Figure 23: A summary of Pherocon® trap capture averages in Juneberry transects at Absaraka and Carrington 2016.

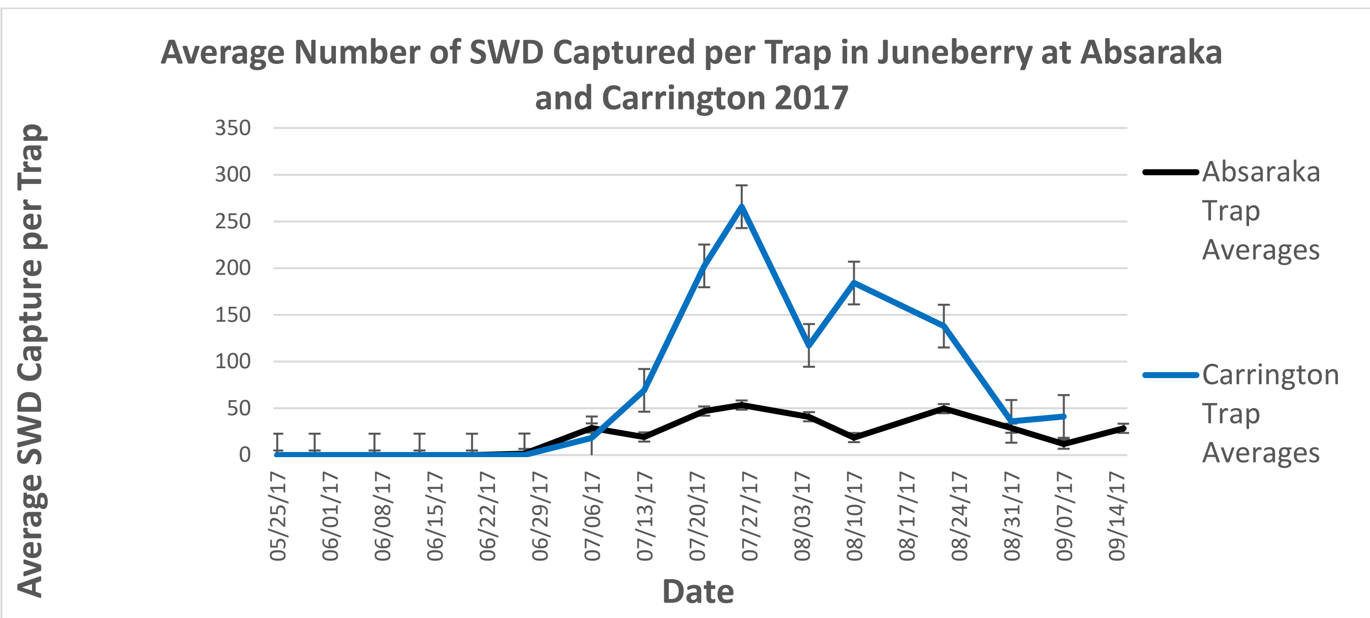

Figure 24: A summary of Alphascents™ trap capture averages in Juneberry transects at Absaraka and Carrington 2017.

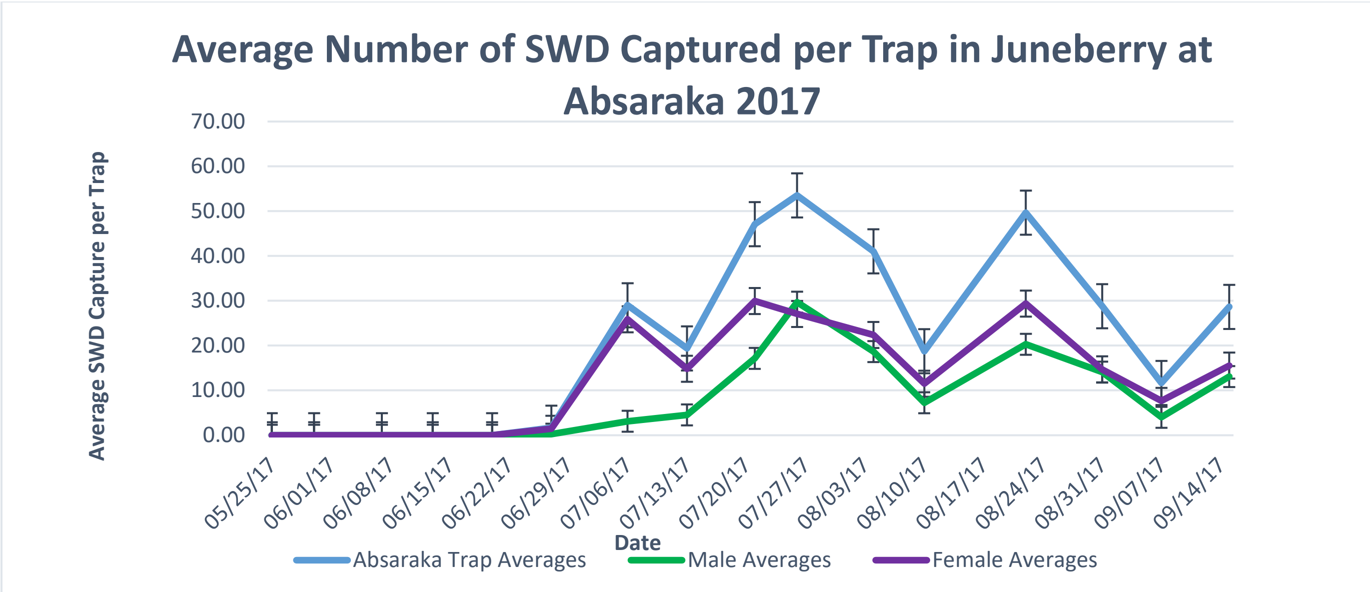

Figure 25: A summary of Alphascents™ trap capture averages in a Juneberry transect at Absaraka 2017, with a comparison of total trap capture averages to male and female averages.

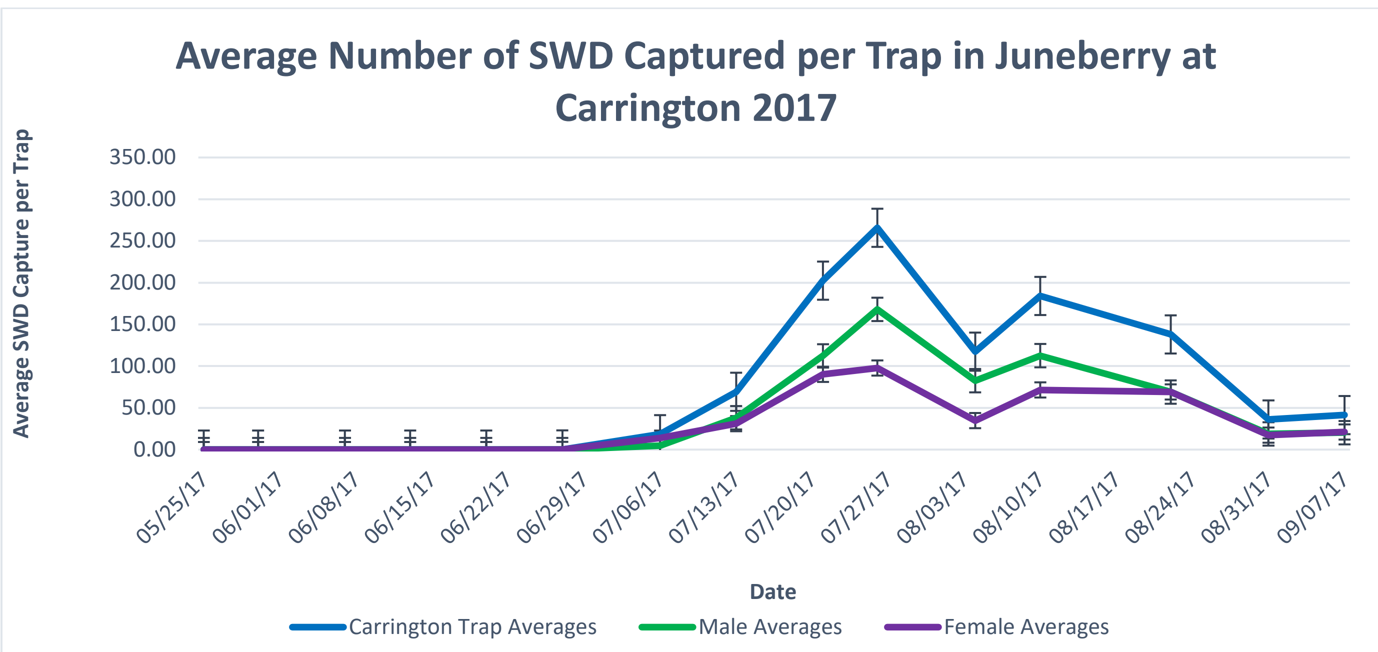

Figure 26: A summary of Alphascents™ trap capture averages in a Juneberry transect at Carrington 2017, with a comparison of total trap capture averages to male and female averages.

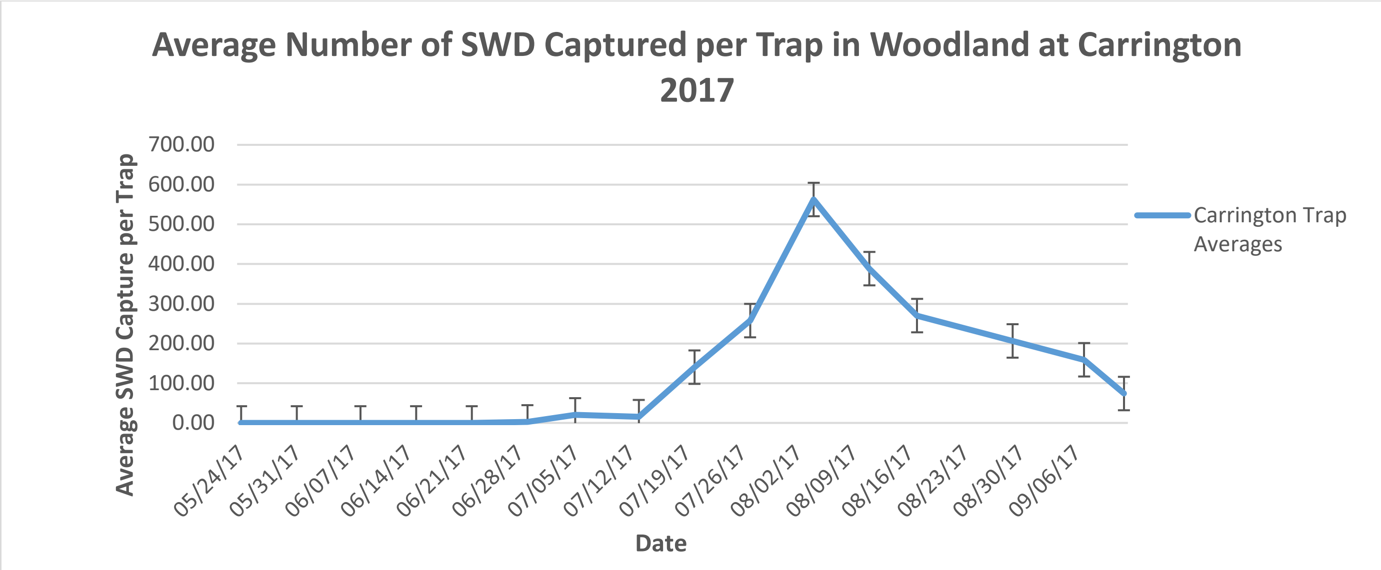

Figure 27: A summary of Alphascents™ trap capture averages in a woodland transect at Carrington 2017.

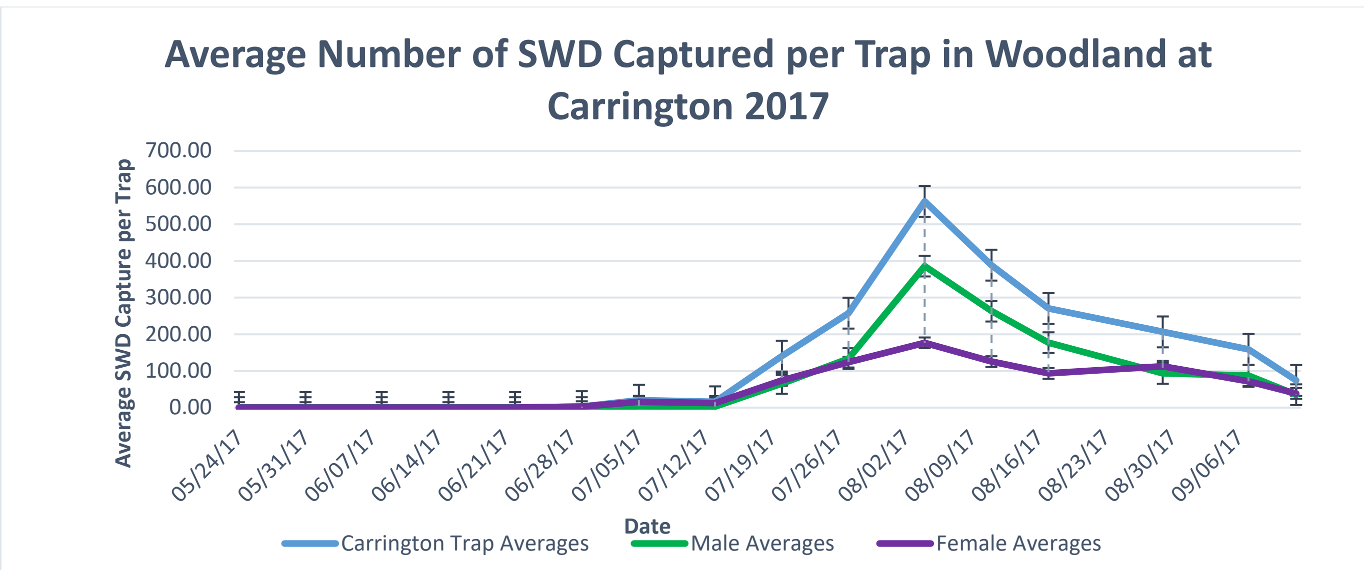

Figure 28: A summary of Alphascents™ trap capture averages in a woodland transect at Carrington 2017, with a comparison of total trap capture averages to male and female averages.

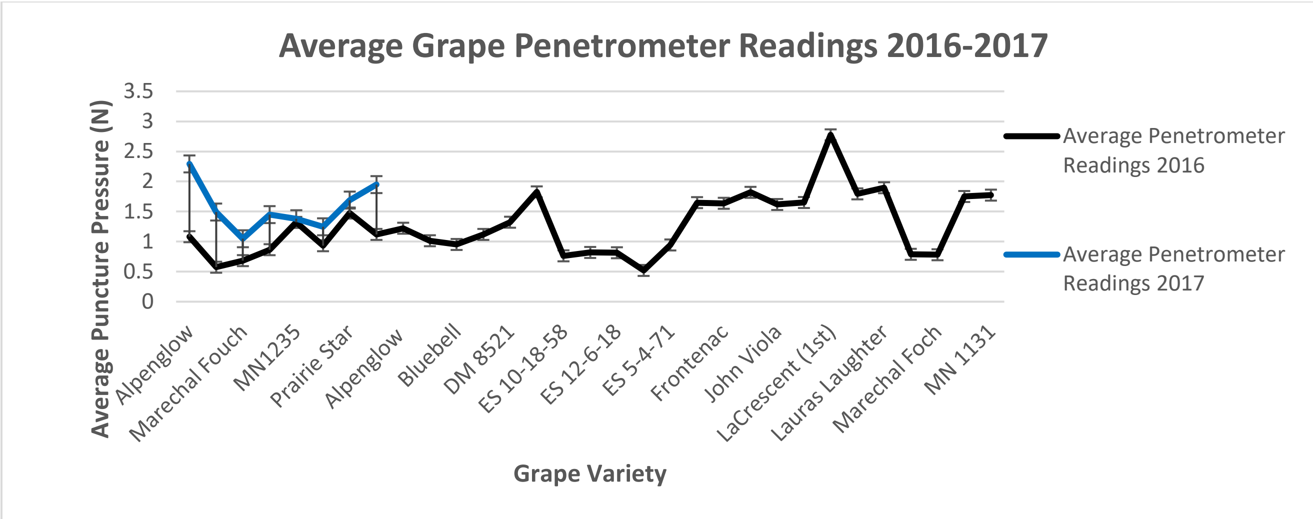

Figure 29: A summary of average penetrometer values for Absaraka grape cultivars in 2016 and 2017.

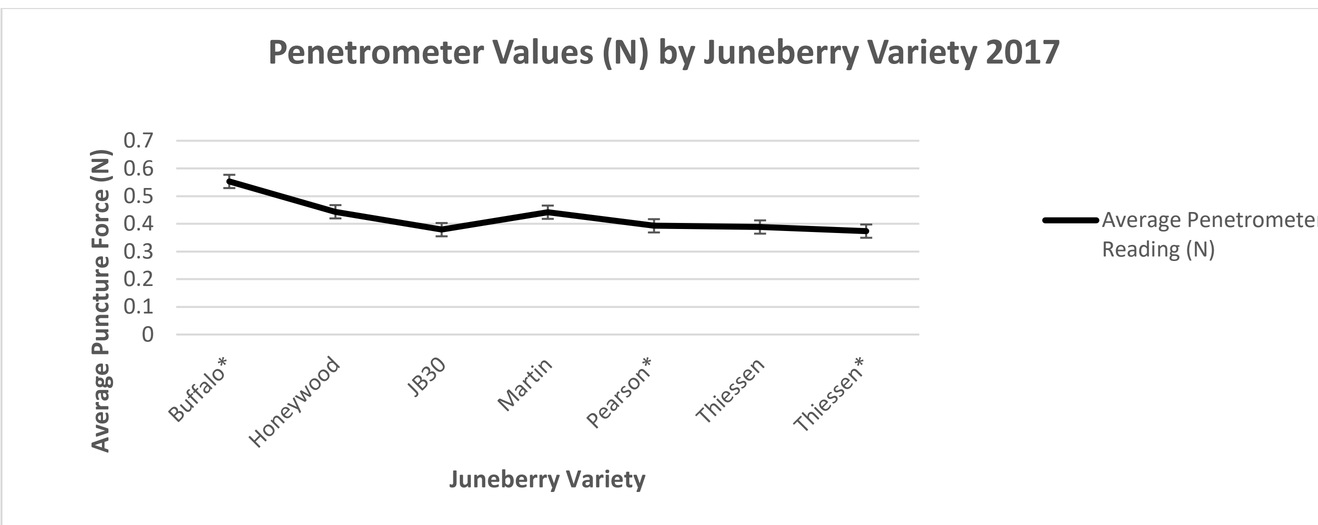

Figure 30: A summary of average penetrometer values for Absaraka grape cultivars in 2016 and 2017.

Development of Super Confection Sunflower Effectively Resistant to Downy Mildew and Rust

Final Report

Project Summary

Downy mildew (DM), incited by *Plasmopara halstedii*, is an important cause of yield loss in sunflower. The disease can cause heavy losses of up to 50-95% in cool wet years and adversely affects other aspects of seed quality. The best way to control this disease is to use sunflower hybrids with resistance to the disease. Unfortunately, no DM resistant germplasm and commercial hybrid are available in confection sunflower. The objectives of this proposed project are 1) incorporation of DM resistance identified in oil-type sunflower into confection sunflower, 2) molecular mapping of DM resistance genes, and 3) combining DM and rust resistance genes in a single genetic background. The confection germplasms with DM resistance combined with rust resistance will be provided to the private seed industry for incorporation into finished hybrids. Resistance to both DM and rust, two severe diseases in sunflower, is an important agronomic factor in keeping U.S. confection sunflower competitive. This is a three-year project to transfer DM resistance genes from oil-type sunflower into confection sunflower because it needs eight generations of cross and backcrosses to complete this process. In the year-three, we transferred a novel DM R-gene, Pl_{20}, discovered from the wild sunflower species *Helianthus argophyllus* into cultivated sunflower, and molecularly mapped the gene to the linkage group 8 of the sunflower genome. The molecular markers for the novel gene was identified that can be used in sunflower breeding programs. The discovery of new sources of resistance to DM will provide a more efficient, durable, and environmentally friendly host plant resistance to sustain sunflower as economically viable crop. We have released the three finished lines, HA-DM2, HA-DM3, and HA-DM4 with the gene combinations of Pl_{Arg} (DM R-gene) plus R_{12} (rust R-gene), Pl_{17} plus R_{13a}, and Pl_{18} plus R_{13a} to the public in 2017. HA-DM2, HA-DM3, and HA-DM4 represent the first confection germplasm with combined resistance to both downy mildew and rust.

Project Approach

I. The selection of homozygous double-resistant plants from BC_4F_2 populations with the gene combinations of Pl_{Arg} plus R_{12}, Pl_{17} plus R_{13a}, and Pl_{18} plus R_{13a} was conducted by marker-assisted selection, and the selected double R plants were advanced to BC_4F_3 generation.

II. The selected BC_4F_3 families were further tested for resistance to downy mildew and rust to confirm the results of marker-selection. The BC_4F_3 families with good seed set were selected and planted in Glyndon field for seed increase and agronomic trait evaluation in 2016 growing season. Days to 50% flowering and plant height were recorded in the field plots. The heads were bagged for self-pollination, and harvested in October 2016.

III. Genotyping-by-sequencing (GBS) was used to target a novel downy mildew resistance gene Pl_{20} from wild *Helianthus argophyllus* for sunflower. For DM tests of the BC_1F_3 mapping population, 40 seeds from each of the 114 $BC_1F_{2:3}$ families were germinated at 22-24 °C in a germinator, and at least 30 seedlings were inoculated with isolate of *P. halstedii* race 734. The single nucleotide polymorphism (SNP) markers resulting from GBS analysis were, respectively, classified into 17 LGs, and markers belonging to each of the 17 LGs were separately analyzed using DM phenotypic data to detect linkage.

IV. The 2017 NSA Research Forum was held on January 11 - 12, 2017 in Fargo, ND. The postdoctoral researcher, Dr. Guojia Ma, of this project described the significant progress that has been made in developing confection sunflower DM resistance.

V. The Focus Group annual meeting was held on February 21, 2017 and the lead researcher, Dr. Lili Qi, provided an update on the results and progress of this research project.

VI. *The Sunflower* magazine, a publication owned by the National Sunflower Association, had an article summarizing this project in the March/April 2017 issue.

Goals and Outcomes Achieved

I. Create BC_4F_2 generation in winter greenhouse, select homozygous plants by DNA markers and advance to BC_4F_3 generation

1. The BC_4F_2 generations from the cross of HA-R6/HA-DM1, HA-R6/HA 458, and CONFSCLR5/RHA 464 were harvested in September 2015. A total of 188 BC_4F_2 plants with the gene combination of Pl_{17} plus R_{13a}, were tested using DNA markers linked to the genes Pl_{17} and R_{13a}, respectively. Twelve homozygous BC_4F_2 plants were selected and advanced to BC_4F_3 generation.
2. A total of 376 BC_4F_2 plants with the gene combination of Pl_{18} plus R_{13a}, were tested using DNA markers linked to the genes Pl_{18} and R_{13a}, respectively. Thirty-two homozygous BC_4F_2 plants were selected and advanced to BC_4F_3 generation.
3. A total of 214 BC_4F_2 plants with the gene combination of Pl_{Arg} plus R_{12}, were tested using DNA markers linked to the genes Pl_{Arg} and R_{12}, respectively. Six homozygous BC_4F_2 plants were selected and advanced to BC_4F_3 generation.

II. Greenhouse test BC_4F_3 for resistance and field evaluation BC_4F_3 for agronomic characteristics in growing season

1. A total of 12 homozygous BC_4F_3 families with gene combination of Pl_{17} plus R_{13a} were selected from 188 BC_4F_2 plants using DNA markers linked to Pl_{17} and R_{13a}, and harvested in the early 2016. The selected BC_4F_3 families were further tested for resistance to downy mildew and rust to confirm the results of marker-selection in April 2016. The four BC_4F_3 families with good seed set were selected and planted in 16 rows of Glyndon field for seed increase and agronomic trait evaluation in 2016 growing season. Days to 50% flowering and plant height were recorded in the field plots. A total of 240 heads were bagged for self-pollination, and harvested in October 2016.

2. A total of 32 homozygous BC_4F_3 families with gene combination of *Pl_{18}* plus *R_{13a}* were selected from 376 BC_4F_2 plants using DNA markers linked to *Pl_{18}* and *R_{13a}*, and harvested in the early 2016. The selected BC_4F_3 families were further tested for resistance to downy mildew and rust to confirm the results of marker-selection in April 2016. The five BC_4F_3 families were selected and planted in the 18 rows of Glyndon field for seed increase and agronomic trait evaluation in 2016 growing season. Days to 50% flowering and plant height were recorded in the field plots. A total of 41 heads were bagged for self-pollination, and harvested in October 2016.
3. A total of six homozygous BC_4F_3 families with gene combination of *Pl_{Arg}* plus *R_{12}* were selected from 214 BC_4F_2 plants using DNA markers linked to *Pl_{Arg}* and *R_{12}*, and harvested in the early 2016. The selected BC_4F_3 families were further tested for resistance to downy mildew and rust to confirm the results of marker-selection in April 2016. The four BC_4F_3 families with good seed set were selected and planted in the 16 rows of Glyndon field for seed increase and agronomic trait evaluation in 2016 growing season. Days to 50% flowering and plant height were recorded in the field plots. A total of 240 heads were bagged for self-pollination, and harvested in October 2016.

III. Release three confection sunflower germplasms resistant to downy mildew and rust

The three finished lines of BC_4F_4, HA-DM2 (*Pl_{Arg}* plus *R_{12}*), HA-DM3 (*Pl_{17}* plus *R_{13a}*), and HA-DM4 (*Pl_{18}* plus *R_{13a}*), have been released to the public in 2017.

IV. Complete molecular mapping of a new DM resistance gene *Pl_{20}* derived from *H. argophyllus*

Molecular mapping of a novel DM *R*-gene introduced from a wild species *H. argophyllus* PI 494578 is finished and the paper has been published in Theoretical and Applied Genetics. The BC_1F_2 and BC_1F_3 population were developed from the cross of HA 89/PI 494578. Phenotyping of the 114 BC_1F_2-derived BC_1F_3 families (30 seedlings from each family, total 3,420 seedlings) was completed, and genotyping of the 114 BC_1F_2 individuals, together with the parents, was conducted using genotyping-by-sequencing approach. Single dominant gene named *Pl_{20}* conferring the DM resistance in PI 494578 population was mapped to LG8 of the sunflower genome, and has been introgressed from wild sunflower species into cultivated sunflower. Diagnostic DNA markers have been developed for marker-assisted selection in breeding programs.

For germplasm release, a total of 62 plants were identified as homozygous resistant from 300 BC_2F_2 individuals of HA 89/PI 494578 by DM testing in the greenhouse and DNA markers linked to *Pl_{20}*, and 32 of them were advanced to BC_2F_3 generation. Four of the resulting homozygous DM-R BC_2F_3 families with good seed set were further confirmed by both downy mildew resistance evaluation in the greenhouse and by markers linked to *Pl_{20}* gene in lab in March 2017. These four homozygous DM-R BC_2F_3 families will be grown in 4 rows each in Glyndon field in 2017 summer for seed increase and agronomic performance evaluation.

Beneficiaries

The beneficiaries of this research are broad and varied. It is estimated that 825 farmers nationwide (half of whom are in ND) will benefit directly by this project. There are five confection sunflower hybrid seed companies producing confection hybrids that would utilize the DM resistant gene(s) placed in a confection germplasm background. All of them have ND research locations. There are nine major confection sunflower processing plants located in ND, TX, MN and KS. There are numerous local receiving stations where seed is gathered and stored. The seed is later delivered to one of the nine plants for final processing. In order to satisfy customer needs, these processors must start with a quality product that is of the proper color, size and test weight. These plants are complete with the latest equipment such as electric eye sorters to insure that poor quality seed will be eliminated. Some of these plants take the seed to a consumer ready packaging. That includes roasting, salting, adding a variety of spices and distributing the products directly to sales outlets. According to Agricultural Economics Report No. 327-S “Economic Contribution of the United States Sunflower Industry”, the direct impact of the confection sunflower industry is $275.7 million annually. That study was based on economic surveys conducted in 1991-93. It is very safe to assume that the 2016 number would be considerably greater.

In the last several years confection inshell sunflower has become a nationally distributed product. It can be found in retail shops across the country. There are several national brands including David & Sons and Frito Lay brands, plus numerous regional brands. These companies depend on a consistent supply of product. Inshell confection sunflower has become a substitute for chewing tobacco among professional baseball players. Today, inshell sunflower is a popular snack for young athletes emulating their professional heroes.

The 2017 NSA Research Forum was held on January 11 - 12, 2017 in Fargo, ND. The postdoctoral researcher, Dr. Guojia Ma, of this project described the significant progress that has been made in developing confection sunflower DM resistance. This meeting was attended by 150 individuals consisting of growers, industry and public researchers. It is these individuals who will take the project to the final step of incorporating the identified resistant genes into their elite sunflower lines and then cross into finished hybrids, which would be ultimately planted by farmers in field.

The Focus Group annual meeting was held on February 21, 2017 and the lead researcher, Dr. Lili Qi, provided an update on the results and progress of this research project. The Focus Group consists of 30-40 industry and grower leaders along with researchers who help formulate long range sunflower research strategy.

The Sunflower magazine, a publication owned by the National Sunflower Association, had an article summarizing this project in the March/April 2017 issue. This publication is mailed to all sunflower producers and reach approximately 25,500 growers, researchers and industry individuals. The article is of special interest to the estimated 825 confection sunflower growers and another 500 crop consultants, and is archived on the NSA website for future reference for growers, consultants and breeders.

*Crop consultants are a very important group to reach since they advise their farmer clients on all aspects of crop production from seed selection to harvesting.

Lessons Learned

In the Glyndon field of summer, 2016, BC_4F_3 families with gene combination of *Pl₁₈* plus *R₁₃ₐ* showed bad rooting and later development, resulting in very few or none seeds when harvesting. This delays the preparation of seeds for registration, investigation of agronomic traits in field, and release process for one season. We re-selected new homozygotes from BC_4F_2 generations.

In August, 2016, a total of 564 BC_4F_2 plants derived from different BC_4F_1 with gene combination of Pl_{18} plus R_{13a} were tested using DNA markers linked to the genes Pl_{18} and R_{13a}, respectively. One-hundred eight homozygous BC_4F_2 plants with both *R* genes were identified and 56 were advanced to BC_4F_3 generation in the greenhouse. The BC_4F_3 families were tested for both downy mildew and rust resistance and advanced to BC_4F_4 generation for seed increase in the greenhouse. A total of 200 heads of BC4F4 plants were harvested in the early June of 2017.

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Additional Information

Publications (2015-2017)

1. Ma GJ, Markell SG, Song QJ, Qi LL (2017) Genotyping-by-sequencing targeting of a novel downy mildew resistance gene Pl_{20} from wild *Helianthus argophyllus* in sunflower (*Helianthus annuus* L.). Theor. Appl. Genet. DOI 10.1007/s00122-017-2906-4.

2. Qi LL, Talukder ZM, Hulke BS, Foley ME (2017) Development and dissection of diagnostic SNP markers for the downy mildew resistance genes Pl_{Arg} and Pl_8 and maker-assisted gene pyramiding in sunflower (*Helianthus annuus* L.). Molecular Genetics and Genomics 292:551-563.

3. Qi LL, Foley ME, Cai XW, Gulya TJ (2016) Genetics and mapping of a novel downy mildew resistance gene, Pl_{18}, introgressed from wild *Helianthus argophyllus* into cultivated sunflower (*Helianthus annuus* L.). Theor Appl Genet 129: 741-752

4. Qi LL, Seiler GJ (2016) Registration of an oilseed sunflower germplasm HA-DM1 resistant to sunflower downy mildew. Journal of Plant Registrations. 10:195-199. Log No. 324221

5. Ma GJ, Seiler GJ, Markell SG, Gulya TJ, Qi LL (2016) Registration of two double rust resistant germplasms, HA-R12 and HA-R13 for confection sunflower. Plant Regist 10: 69-74

6. Qi LL, Long YM, Jan CC, Ma GJ, Gulya TJ (2015) Pl_{17} is a novel gene independent of known downy mildew resistance genes in the cultivated sunflower (*Helianthus annuus* L.). Theor Appl Genet 128:757–767

7. Qi LL, Ma GJ, Long YM, Hulke BS, Markell SG (2015) Relocation of a rust resistance gene R_2 and its marker-assisted gene pyramiding in confection sunflower (*Helianthus annuus* L.). Theor Appl Genet 128:477–488

8. Qi LL, Long YM, Ma GJ, Markell SG (2015) Map saturation and SNP marker development for the rust resistance genes (R_4, R_5, R_{13a}, and R_{13b}) in sunflower (*Helianthus annuus* L.). Mol. Breed 35: 196

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Ma GJ, Markell SG, Qi LL (2017) 2016 progress for development of super confection sunflower effectively resistant to downy mildew and rust. Presentation at the National Sunflower Association Research Forum, January 11-12, 2017, Fargo, ND. Available: http://www.sunflowernsa.com/uploads/63/ma_2016.progress.super.confection_2017.pdf

Qi LL, Talukder Z, Hulke BS, Foley ME (2016) Development of SNP markers linked to the downy mildew resistance gene Pl_8 in sunflower. Presentation at the National Sunflower Association Research Forum, January 12-13, 2015, Fargo, ND. Available: http://www.sunflowernsa.com/uploads/resources/881/snp.markers_qi.2016.pdf

Ma GJ, Markell SG, Qi LL (2016) 2015 progress for development of super confection sunflower effectively resistant to downy mildew and rust. Presentation at the National Sunflower Association Research Forum, January 12-13, 2016, Fargo, ND. Available: http://www.sunflowernsa.com/uploads/resources/880/super.confection_ma.2016.pdf

Ma GJ, Markell SG, Qi LL (2015) Development of super confection sunflower effectively resistant to downy mildew and rust. Presentation at the National Sunflower Association Research Forum, January 7-8, 2015, Fargo, ND. Available: http://www.sunflowernsa.com/uploads/resources/798/super.confection_ma_2015.pdf

Qi LL, Jan CC, Foley ME, Cai XW (2015) 2014 progress for molecular mapping of the downy mildew resistance genes in sunflower. Presentation at the National Sunflower Association Research Forum, January 7-8, 2015, Fargo, ND. Available: http://www.sunflowernsa.com/uploads/resources/797/molecular.mapping_qi_2015.pdf

Sunflower Magazine Mach/April 2015

Update: Downy mildew confection resistance project. Page 24.

Sunflower Magazine Mach/April 2016

Update: Downy mildew confection resistance, USDA lab's progress points toward releases in 2017. Page 26-27.

Sunflower Magazine Mach/April 2017

Downy Mildew-Resistant Confection Germplasm, Multi-year Project at USDA Fargo Lab Resulting in Releases to Public in 2017. Page 22.

Project Title
Development and evaluation of anti-spore treatments for improved management of American Foulbrood Disease in honeybees
NOGA#
15-333
Final Report

Partner Organization
North Dakota State University

Project Summary
North Dakota produces more honey than any other state in the nation. In 2014 there were 522,940 colonies (hives) maintained by 223 registered beekeepers. In 2013 North Dakota produced 33 million pounds of honey with an economic value of over $67 Million. American Foulbrood disease (AFB) is caused by the bacterium *Paenibacillus larvae* and is considered the most dangerous infectious disease of honeybees. There are currently only two methods approved for control of AFB in North Dakota:

1. Burning contaminated hives and all associated equipment.
2. Antibiotic treatment with approved antibiotics.

Once AFB is detected in a hive, antibiotic treatment must continue indefinitely due to the continual presence of *P larvae* spores, which are resistant to all antibiotics. However, honey production practices require that antibiotics be removed at least 6 weeks prior to the harvest of honey from a treated hive. Because of the residual bacterial spores still present in the hive, active AFB disease often recurs within this time period. This puts both the original hive and neighboring hives at risk again. For this reason, burning is the usual method of mitigating AFB despite its heavy economic cost: loss of the hive, all equipment produce, and all current and future honey production.

The purpose of this project is to reduce losses to North Dakota honey production caused by AFB. By preventing relapse of AFB when antibiotic treatment is discontinued, apiarists will be able to recoup much of the losses they would encounter if they were to destroy all infected hives and associated equipment. North Dakota experienced a spike in AFB cases in 2013 and the prevalence of this disease is on the rise globally, making this project extremely timely. In addition, the prevalence and duration of antibiotic use in agriculture is under increasing scrutiny and any new advances that can lower these figures will be favorable.

The overarching objective of this project is to identify means by which one hundred percent of all spores within a hive can be rendered sensitive to antibiotics, thus preventing relapse of AFB when treatment is discontinued.

Project Approach
Two specific objectives were identified for this project:

1. Determine which chemical agents lead to the greatest amount of germination of *P. larvae* spores, either alone or in combination.
2. Determine the extent to which artificial induction of *P larvae* spore germination enhances antibiotic killing and chemical disinfection of tainted equipment.

1. **Project Activity**	**2. Who will do the work?**	**3. When will the activity be accomplished?**	**4. Current Status**
Identify highly qualified GS and UGs to participate in the project	NAF (GS) KG (UG)	completed	
Begin project and obtain research supplies including chemicals known to cause germination in other spore forming species. PI and research team meet to discuss objectives and deliverables	All Participants	completed	
Objective 1: Submit protocols to NDSU and SR Institutional Biosafety Committee and Safety Office	NAF and KG	completed	
Objective 1: Train GS and UGs in spore production and germination assays	NAF, KG, GS, UGs	completed	
Objective 1: Produce *P larvae* endospores for subsequent analyses	GS, UGs	completed	
Objective 1: Clone *P larvae* germinant receptors into *B subtilis* for ectopic expression studies	NAF, GS	Not approved	This aim was not approved for further work. We are focusing the remainder of the grant efforts on testing chlorine dioxide as a sterilant of *P. larvae*.
Objective 1: Test *P larvae* spores and recombinant *B subtilis* spores for germination triggered by diverse chemical signals	GS	completed	
Objective 2: Determine kinetic profiles of germinants to determine those most active at low concentrations	GS	complete	Task complete for uric acid germination.
Objective 3: Evaluate germinant-antibiotic and germinant-disinfectant	GS, UGs	complete	Task complete. Tests were conducted to determine the most

combinations for killing of *P larvae* spores in aqueous solution			effective concentration and exposure time of chlorine dioxide.
Objective 4: Evaluate germinant-antibiotic and germinant-disinfectant combinations for killing of P larvae spores on solid surfaces associated with hives and apiary equipment	GS, UGs	Feb. 2017 – June 2018	Objective 3: Evaluate germinant-antibiotic and germinant-disinfectant combinations for killing of P larvae spores on solid surfaces associated with hives and apiary equipment
Data analysis and manuscript preparation	KG, GS	In prep	Data analysis and manuscript preparation
Disseminate results at American Federation of Beekeeping Annual Meeting and North Dakota association meetings	KG	In prep	These results will be ready for presentation after the grant ends. If other funds are available, the PI will attend and present in 2020.

The overall scope of the project did not benefit commodities other than specialty crops.

NDSU was the only project partner listed on the grant. NDSU faculty (Greenlee) supervised the students and the research and will be a co-author on the resulting manuscript.

<u>Goals and Outcomes Achieved</u>

Both objectives were met. First, we developed methods for generating endospores for two type strains and 5 North Dakota isolates of *Paenibacillus larvae*. We identified the ND isolates as part of the ERIC I family using phenotypic and molecular methods.

We identified uric acid as the sole germinant for three of the isolates. This is a significant finding in that it differs from a previous literature report that claims uric acid is not a sole germinant for *P. larvae* spores. We tested all common amino acids, sugars, salts, and nucleic acid derivatives for germination with no positive results.

We tested the effect of several disinfectants on *P. larvae* as spores and after triggering germination with 1mM uric acid. Disinfectants include Citrus II Hospital Germicidal Deodorizing Cleaner, 70% Isopropyl Alcohol, Benzarid hospital grade cleaner, 10%Bleach, Dettol Liquid, 37% Formaldehyde, Rescue RTU Disinfectant and Deodorizer, and chlorine dioxide gas. Ten percent bleach and gaseous chlorine dioxide led to the highest inactivation of spores with 100% killing rate for vegetative bacterial cells after germination. This is significant, because gaseous chlorine dioxide can be scaled up to disinfect buildings and equipment.

To determine the optimal concentration of chlorine dioxide, we are in the process of determining the LD50 (lethal dose) and the LT50 (lethal time). Thus far, in aqueous solutions, chlorine dioxide gas is effective at very low concentrations and very short time periods (< 30 min). We are also testing the efficacy of chlorine dioxide gas to kill spores and vegetative cells on wood, wax, and steel, all common surfaces in bee-keeping equipment. At 1 hour of exposure, varying chlorine dioxide concentrations are able to kill 100% of *P. larvae* spores.

One of the objectives, to clone *P. larvae* germinant receptors into *Bacillus subtilis* for ectopic expression studies, was not able to be completed. Unfortunately, we were unable to obtain approval for this objective. The Institutional Biosafety Committee and Safety Office was not comfortable with genetically modifiying bacteria in this way, given the importance of honeybees to ND.

We are currently in the process of finishing up this project. We are running one more replicate and then will conduct statistical analyses. Once these are complete, the manucscript will be submitted for publication and the results will be presented at an upcoming meeting.

Beneficiaries

Any apiarist that produces honey will benefit from this project. Smaller apiarists (with fewer hives) will benefit considerably since any loss of production hurts these producers disproportionately. Consumers of honey will also benefit. In 2014 there were 522,940 colonies (hives) at 12,521 apiaries maintained by 223 registered beekeepers. These local producers and other regional producers are expected to benefit from future foulbrood treatment regimes that are developed as a result of the research conducted in this project.

In 2013 North Dakota produced 33 million pounds of honey with an economic value of over $67 Million. In the same year, 13 apiaries (out of 11,050 in the state) suffered losses from AFB. This is admittedly a low number, but we must keep in mind that AFB is under passive surveillance, so this is likely an underestimate of the current prevalence of AFB in the state. In 2014, researchers in the project director’s laboratory sampled honey from 12 North Dakota and Minnesota producers and found that *P. larvae* spores were present in half of the samples. Importantly, these came from presumably AFB-free colonies and apiaries, indicating that the state is not yet rid of AFB and that major outbreaks could occur in the future if apiary management practices change (such as if preventative antibiotic use decreases as organic farming trends increase).

Lessons Learned

This project presented many challenges. Some of those were unforeseen accidents that could not have been predicted or prevented, such as the original PI leaving NDSU, the roof collapse at Southern Research Institute, and two relocations of the graduate student on this project. One challenge that may have been preventable was the IBC not approving the one protocol. However, if the original PI had remained at NDSU, that would likely not have been a problem because his lab was certified for that level of biosafety required for that research.

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Additional Information

- https://currentprotocols.onlinelibrary.wiley.com/doi/abs/10.1002/cpmc.45
- https://currentprotocols.onlinelibrary.wiley.com/doi/10.1002/cpmc.46

<u>Project Title:</u> Rust-proofing dry edible beans for the Northern Great Plains via guided pre-breeding and breeding efforts

<u>Project NOGA Number:</u> 15-334

Final Report

Partner Organization
North Dakota State University

<u>Project Summary:</u>

North Dakota is the primary dry bean producing state in the US, accounting for approximately 32% of the total acreage. In 2016, North Dakota dry beans were valued at nearly $250 million USD (NASS, 2018). Several market classes of dry beans of meso-american descent, including pinto, black and navy, are produced primarily in the northeast corner of the state. In 1994, yield losses of 16% were attributed to bean rust, caused by *Uromyces appendiculatus*, representing nearly $14 million in economic losses to North Dakota growers (Gross and Venette, 2001). Additionally, fungal teliospores and urediniospores have been reported to survive North Dakota winters (Gross and Venette, 2001; Venette et al., 1978). This suggest the possibility of fungal sexual reproduction, even though aecia are not commonly observed in the field. The incorporation of the *Ur-3* resistance gene into cultivars effectively managed the disease until 2008 when the pathogen overcame this resistance (Markell et al., 2009). This new race, designated 20-3, is virulent on resistant genes *Ur-3, Ur-6, Ur-7*, and uncharacterized resistance from Montcalm. In the last 7 years, bean rust has grown in importance in North Dakota, reported by dry bean growers as one of the three most destructive diseases (Knodel et al., 2011-2018). In 2017, bean rust was identified in 37% of fields surveyed. While the application of fungicides can reduce the damage caused by dry bean rust, the use of resistant cultivars is the most economical method of disease management. However, with no effective resistance genes incorporated into available cultivars, the need for understanding pathogen races present in the state is crucial in identifying new sources of host resistance to this very damaging pathogen.

This project identified 15 virulence phenotypes of *U. appendiculatus* among 118 single pustule isolates collected from dry bean fields displaying symptoms of rust in 2015 and 2016. Previously identified race 20-3 was by far the most prevalent, 77% and 58% of isolates in 2015 and 2016, respectively. Isolates of the other 14 races were observed substantially less frequently, between 1% and 17%. This drastically limits the host resistance genes available to breeders to *Ur-11* and the yet to be characterized gene in PI260418.

RAD-GBS was performed on 67 single pustule *U. appendiculatus* isolates using the Ion-Torrent S5 sequencing platform. The relatedness measure suggested the presence of diversity within and among the isolates belonging to the same race, providing further evidence that the *U. appendiculatus* population in North Dakota is undergoing sexual reproduction and is more diverse than virulence phenotypes suggest. Results from this research increase our understanding of population dynamics and diversity in *U. appendiculatus* and will assist common bean breeding for rust resistance.

A total of 163 lines from the North Dakota Experimental Agricultural Station breeding program, 29 commonly grown cultivars and 103 accessions from the Mesoamerican Diversity Panel (MDP) from 7 dry bean market classes were evaluated for reaction to *U. appendiculatus* races 20-3 and 29-3. Additionally, 188 and 49 accessions from the Durango and Andean Diversity Panels, respectively, were screened with U. appendiculatus races 20-3 and 27-7. This total of 532 exceeds the target for germplasm screening (450 accessions). Sources of resistance were found in each market class. This identified germplasm is being utilized in crosses by the NDAES breeding program for incorporation into future cultivars. The identification of resistant germplasm in each market class among the advanced germplasm in the NDAES program is of utmost importance in the race to incorporate resistance into adapted cultivars. These germplasm have been previously selected based on agronomic performance, yield, and other characteristics important to North Dakota dry bean growers.

Our current recommendation to growers is to scout fields for rust and apply effective fungicides for disease management under conducive environmental conditions. While some lines in the breeding material and diversity panel evaluated were resistant to the two most commonly identified races, races of *U. appendiculatus* have been identified in the state that overcome all but two currently identified host resistance genes. While it remains pertinent to work towards the incorporation of currently effective host resistance genes into new cultivars, the incorporation of these genes into cultivars may potentially select for pathogen isolates that overcome currently effective genes, rendering them useless, as recently occurred with race 20-3. Therefore, future research should also focus on the identification and incorporation of quantitative rust resistance in dry beans.

Project Approach

The research outlined below was performed during this granting period as outlined in the proposal 'Workplan'.

Rust surveys were conducted in 2015 and 2016 across North Dakota dry bean growing counties (Fig. 1). GPS coordinates, the prevalence and severity of rust was recorded at five locations within each field surveyed in a 'W' pattern. Three to five leaves displaying rust pustules were collected from each of 1 to 5 locations in each field included in the survey, depending on the incidence, severity and pattern of disease in the field. Leaves were subsequently dried and stored for the development of single pustule *U. appendiculatus* isolates. Urediniospores were collected from pustules on field samples using a specialized vacuum. These bulk spore samples were stored in a vial at 4C until single pustule isolates could be developed from each sample. Bulk urediniospores were inoculated onto susceptible pinto bean line PI14 (UI 114) with a cotton swab using 5% tween 20 as a surfactant. Inoculated plants were incubated in humidity chambers overnight with a distilled water mist was engaged for 15 seconds every 4 minutes. Plants were moved to a greenhouse room for disease development in fiberglass boxes topped with net fabric to limit spore movement between plants. When pustules developed but were still closed, typically seven to ten days post-inoculation, spores from 3 to 5 discrete pustules were collected from plants inoculated with each bulk spore sample. Urediniospores from these single pustules were inoculated onto PI14 and plants were incubated as described above. Inoculations were repeated up to three more times to generate an amply volume of spores of each single pustule isolate to conduct virulence phenotyping and next generation sequencing. Each sample was identified by field, sample number within the field and single pustule number for clear record-keeping (Tables 1 and 2).

Urediniospores from each single pustule isolate were inoculated onto the common bean standard differential set consisting of 12 cultivars/lines, to identify the virulence phenotype as described above (Table 1). The *U. appendiculatus* inoculum was a solution of 5mg of urediniospores/10mL of soltrol applied using a hand sprayer on both primary leaflets. The host:pathogen interaction was scored 14 days post-inoculation by measuring ten pustules chosen arbitrarily on each of the primary leaflets using a 6× Pocket Comparator (Edmund Optics Inc., Cat. #30-585). Uredinial sizes ranging from <.03mm to 12.0 mm; correspond to a reaction type from 1 to 6. Where a disease reaction of 3,4 and 4,3 or greater was considered a virulent reaction. A race identification was generated for each single pustule isolate by averaging pustule diameters within each differential line using the bean rust scale (Steadman et al., 2002). The phenotypic data were used to construct a dendrogram using average clustering method of hierarchical clustering.

Mean Disease Score (MDS), calculated from phenotypic ratings on the 12 standard common bean rust differential lines, was used to compare differences in isolate virulence. The average virulence phenotype [rating scale from 1 (avirulent) to 6 (large pustules)] was determined across all 12 lines in the rust differential set to give a MDS for each single pustule *U. appendiculatus* isolate. Statistical analyses were performed using SAS software to determine if significant differences were observed in MDS between/among fields and counties from which isolates originated. A heat map was created from the MDS values to visualize which regions of North Dakota currently have the most diverse *U. appendiculatus* population.

A Restriction site Associated DNA-Genotyping by Sequencing (RAD-GBS) was performed on 67 *U. appendiculatus* isolates and sequenced using the Ion Torrent S5 platform. The sequencing data were trimmed for quality and read length in CLC genomics workbench v8.0. A single isolate race typed as 20-3 was used to perform a denovo assembly using a publicly available tool SPAdes v3.11.1 with default parameter suggested for a Ion-torrent sequencing and obtained a reference files containing 140 sequence tags/contigs. The reference contigs were used to map the sequencing data from 67 isolates and obtain variants (SNPs/Indels) for downstream analysis. The variant calling files were used to select the 47 *U. appendiculatus* isolates with <90% missing data. A total of 976 variants with minor allele frequency (MAF) <0.05 and 60% missing data were obtained for final assay. The variants were used to construct neighbor-joining tree in Tassel software and measure relatedness measure using identify by state (IBS) matrix in JMP Genomics and principle component analysis (PCA) was performed.

Commonly grown cultivars, advanced breeding germplasm from North Dakota Experimental Agricultural Station and the MDP were screened with *U. appendiculatus* isolates belonging to races 20-3 and 29-3. Race 20-3 was chosen because it was by far the most frequently detected. Only 5 isolates were determined to be race 29-3 during this survey; however, this was the most virulent race identified, with the exception of race 29-7. One isolate of race 29-7 was identified in 2016, after the screening had been initiated with race 29-3. Race 29-7 is virulent on *Ur-5,* in addition to those resistance genes overcome by race 29-3. For each *U. appendiculatus* race, 10 plants per line were inoculated with a urediniospore solution, incubated and scored as described above. All lines were evaluated in the first trial, the reaction of those lines displaying resistance was confirmed in a second trial.

This work did not directly benefit commodities other than specialty crops.

Goals and Outcomes Achieved

Goal 1: D*etermine the virulence and genetic diversity of* Uromyces appendiculatus *in ND*

Field surveys conducted in 2015 and 2016 were targeted across the major dry bean growing counties of North Dakota (Fig. 1A). In 2015, 62 dry bean samples were collected from 26 fields across 8 counties (Fig. 1B). In 2016, 56 samples were collected from 35 surveyed fields across 8 counties. Grand Forks, Grafton and Pembina are the primary dry bean producing counties where samples were collected across both years.

A total of 62 and 53 single pustule *U. appendiculatus* isolates were generated from samples collected in North Dakota in 2015 and 2016, respectively. Approximately 77% of isolates from 2015 were identified as race 20-3 (Table 1; Fig. 2 and 3). This race is virulent to the *Ur-3, Ur-6, Ur-7* genes and the uncharacterized resistance in Montcalm. Race 28-3 was identified in approximately 8% of the isolates and is virulent on differential line PC50, which contains *Ur-9* and *Ur-12*, in addition to those genes overcome by race 20-3. Isolates belonging to race 20-2 represented 3.2% of isolates collected in 2015. These isolates are similar to race 20-3, but do not overcome *Ur-7*. Isolates of other races each represented less than 2% of total isolates. In addition to resistance genes overcome by previously described races, the race 28-7 isolate is virulent on *Ur-5* and the 29-3 isolate is virulent on *Ur-4*. In 2016; nearly 59% of *U. appendiculatus* isolates were identified as race 20-3 (Table 2; Fig. 2 and 3). Rust race 21-3 was identified in 17% of the isolates, a substantial increase from the less than 2% of isolates of this race in 2015. Race 21-3 is virulent on *Ur-4* in addition to those genes overcome by isolates of race 20-3. Race 29-3 was identified in 8.5% of the isolates. Race 29-3 is virulent on *Ur-4*, *Ur-9*, *Ur-12*, in addition to those overcome by isolates of race 20-3. Several races of *U. appendiculatus* described here have not been previously identified in North Dakota.

Mean Disease Severity (MDS) was calculated by averaging virulence score across the 12 standard dry bean differential lines. MDS of all isolates evaluated ranged from 1.9 to 4.6. A geographical heat map was produced to visually represent the data across both years. *U. appendiculatus* isolates collected from dry bean fields near Grafton, Pembina and Devils Lake had the highest MDS (Fig. 4). No significant differences in MDS were observed across counties. However, significant differences in MDS across fields were observed within Benson and Walsh counties. Additionally, significant differences in MDS were observed when comparing all fields. These indications that the most virulent *U. appendiculatus* isolates are located in pockets where dry beans are most intensely farmed is not surprising, but this confirmation will allow us to track the movement of these isolates across years and provides a better understanding of the overall situation in the state.

While not originally proposed as part of this project, notable observations were made across the 79 isolates determined to be race 20-3. The 20-3 virulence phenotype expressed on PC-50 (*Ur-9, Ur-12*), Early Gallatin (*Ur-4*) and Mexico 235 (*Ur-3+*) ranged from hypersensitive to small-intermediate pustule sizes (0.2-0.4mm) (Fig. 5). This variation suggest more pathogen diversity is present than is discernable via traditional race classifications. Hierarchical clustering (Average Method) (UPGMA) was conducted to analyze genetic relation using MDS. All races clustered together in distinct clades, with the exception of isolates belonging to race 20-3 (Fig. 6). Race 20-3 was recurrently present in multiple cluster, suggesting that the race is more diverse than what can be characterized with virulence phenotypes alone. A second hierarchical clustering analysis was conducted including isolates of race 20-3 only (Fig. 7). The pattern of clustering observed reflected trends observed in virulence phenotyping. Isolates displaying a type 3, 2,3 or

3,4 virulence on PC-50 differential line clustered together. Similar observations were carried through on other differentials/clusters.

RAD-GBS was performed on 67 single pustule *U. appendiculatus* isolates collected in North Dakota to study the genomic diversity of the pathogen population using the Ion-Torrent S5 sequencing platform. The sequencing data were trimmed for quality and read length in CLC genomics workbench v8.0. A de novo assembly was performed on a single isolate of race 20-3 to generate reference sequence tags for variant calling. The reference contigs were used to map the sequencing data from 67 isolates and obtain variants (SNPs/Indels) for downstream analysis. The variant calling files was used to select the 47 samples/isolates with missing data < 90%. A total of 976 variants (target = at least 3,000) with minor allele frequency (MAF) <0.05 and missing data 60% were obtained for final assay. The variants were used to construct neighbor-joining tree in Tassel software and measure relatedness measure using identify by state (IBS) matrix in JMP Genomics. The phenotypic data were used to construct a dendrogram using average clustering method of hierarchical clustering. The lack of a reference U. appendiculatus genome, and the immense size of the genome resulted in fewer variants than originally predicted. A reference genome is currently being constructed. Comparing the genetic information generated in this research to that reference genome will allow for further, more powerful analyses.

U. appendiculatus isolates ranged from 0.62 to 1 proportion of identity based on identity by state (IBS) matrix relationship (Fig. 8). The relatedness measure also suggested the presence of diversity within and among the isolates belonging to the same race, providing further evidence that the *U. appendiculatus* population in North Dakota is undergoing sexual reproduction and is more diverse than virulence phenotypes suggest. A dendogram created using Neighbor-joining tree in Tassel indicates some pairing with similar or identical races exists; however, race 20-3 appears in multiple clusters, indicating great diversity in the race and suggest a sexual reproducing population (Fig. 9). Principal component analysis (PCA) based on genetic information indicates clustering and outliers in the data (Fig. 10). This supports the theory that there to be some recombination (possibly sexual) that cannot be observed solely on the virulence phenotype. Further genotypic diversity analyses are ongoing. Results from this research increase our understanding of population dynamics and diversity in *U. appendiculatus* and assist common bean breeding for rust resistance. Previously reports of suggesting sexual reproduction in this pathogen based on the observations of teliospores (sexual spores) surviving North Dakota winters and aecia present in dry beans fields are aligned with the results of this study based on modern methods.

Goal 2: *Identify which known genes and genomic locations should be incorporated in the next generation of rust resistant bean varieties.*

A detailed explanation of the pathogen races, and resistance genes overcome is included in the previous section. To identify germplasm with resistance to *U. appendiculatus*, 163 lines from the NDAES breeding program, 29 commonly grown cultivars and 103 accessions from the MDP Panel from 7 dry bean market classes were evaluated for reaction to races 20-3 and 29-3 (Tables 3 and 4). Additionally, 188 and 49 accessions from the Durango and Andean Diversity Panels, respectively, were screened with U. appendiculatus races 20-3 and 27-7 (Tables 5 and 6). This total of 532 exceeds the target for germplasm screening (450 accessions). Sources of resistance were found in all market classes. This identified germplasm is being utilized in crosses by the NDAES breeding program for incorporation into future cultivars. The identification of resistant germplasm in each market class among the advanced germplasm in the NDAES program is of utmost importance in the race to incorporate resistance into adapted cultivars. These germplasm have been previously selected based on agronomic performance, yield, and other characteristics important to North Dakota dry bean growers.

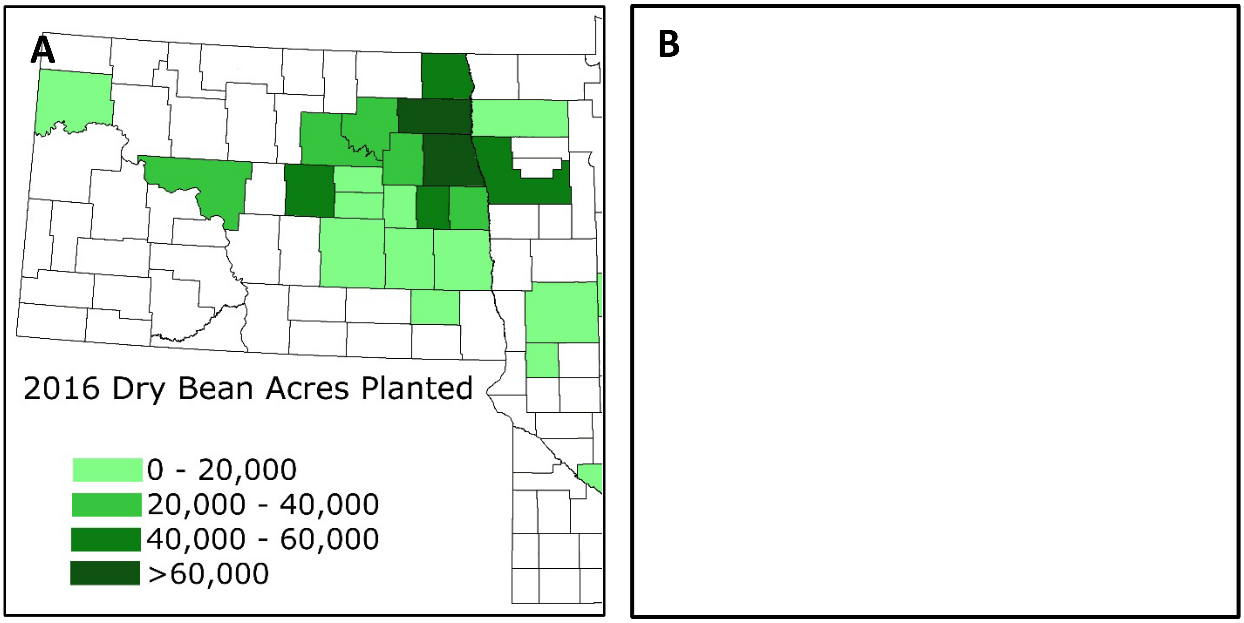

Figure 1. Dry bean acres planted in North Dakota in 2016 (A) and the location of dry bean fields surveyed in 2015 (red) and 2016 (purple) (B).

Table 1. Virulence phenotype of 62 *Uromyces appendiculatus* single pustule isolates collected from dry beans fields in North Dakota in 2015.

Single pustule isolate	Race ID	Differential Line											
		EG	RP	M	PC50	GGW	PI260418	GN1140	Aurora	Mex309	Mex235	CNC	PI181996
UP15-1-2-SP3	17-3	5,6	1	3,4	H	5	1	5,6	6,5,4	1	1	1	1
UP15-1-4-SP	20-3	1	1	4	3	5,4	3	6	4,5,4	1	3,2	1	1
UP15-1-4-SP6	20-3	H	1	5,6,4	1	5,6	1	6	4,3	1	3,2	1	1
UP15-1-4-SP(2/2)	28-11	H	1	4	4,3	5,6	2,3	6	4	2,3,4	4,3,2	3,2	1
UP15-1-5-SP1	20-3	H	1	5,6,4	1	4,5,3	3	6	6,5	1	3,2	1	1
UP15-1-5-SP6	20-3	H	1	6,5	H	5,4	3,2	6	6	1	3,2	1	1
UP15-1-5-SP3	28-3	H	2	3,4	3,4	5	3,4	6,5	6,5	3,2	3,2	2,3	1
UP15-1-5-SP5	20-3	1,3	1	4,5	1	6,5	2	6	3,4	1	1	1	1
UP15-1-6-SP1	20-3	H	1	5,3,4	H	5,4	3,4	6	6	1	3,2	1	1
UP15-1-6-SP3	20-3	1	1	4,3	1	5,4	1	5,4	5,6	1	2	1	1
UP15-1-6-SP6	20-3	H	1	4,5,3	1	5,4	2,3,5	6,5	4,5,3	1	2	1	1
UP15-2-1-SP3	20-11	H	1	5,6	H	5,6	1	5	5,6	1	4,3	1	1
UP15-2-1-SP9	29-3	6,4	2,1	4,3	4,5	6	3,2	6	5,6,4	1	1	1	1
UP15-2-1-SP6	20-3	1	1	3	1	5,4	1	6	4	1	1	1	1
UP15-2-1-SP4	20-3	H	1	5,4,3	2,3	4	1	6	5,4	1	2	1	1
UP15-2-2-SP5	20-3	H	2,3	6	2,1	5,6	3,2	6	6	2,3	3,2	1	1
UP15-2-2-SP6	20-3	H	1	6	H	6,5	1	6,5	6	1	1	1	1
UP15-2-3-SP1	21-3	4,3	2,3	4,5	3	5,6	2,3	6	5,4	2,3	3,2	1	1
UP15-2-3-SP2	20-3	H	1	6,5	H	6,5	1	6	6	1	3,2	1	1
UP15-2-3-SP4	20-3	H	1	6,5	H	6,5	1	6	6	5,6	1	1	1
UP15-2-5-SP2	20-3	H	1	4,3	3,2	5,4	3	6,5	5	2	3,2	2	1
UP15-2-5-SP4	20-3	H	1	5,6	H	6,5	1	6	5,6	1	2,3	3,2	1
UP15-3-3-SP1	20-3	H	1	5,4	H	6	1	6	6	1	1	1	1
UP15-4-2-SP1	20-3	H	1	4,3	H	6	1	6	6	1	1	1	1
UP15-4-2-SP3	20-3	H	1	6,5	H	5,6	1	6,5	6	1	1	1	1
UP15-4-2-SP2	20-2	1	1	4	1	4	1	3,2	4	1	1	1	1
UP15-6-1-SP1	20-3	1	1	6	1	5,6	1	6	4	1	1	1	1
UP15-6-1-SP2	20-3	H	1	5,4	H	6,5	3,4	6	6,5	2,3	3,2	1	1
UP15-14-1-SP7	20-3	1	1	5,4	1	5,6	3,2	6	6	1	1	1	1
UP15-28-SP2	28-3	1	1	5,4,3	4	5	1	6	5,6,4,3	1	2,3	1	1
UP15-28-SP3	20-3	1	1	5,4,3	H	5,4	2	6	6	1	3	3,2	1
UP15-29-SP6	28-7	H	H	5,4	5,4	5,6	-	4,5	4,3	5,4	2,1	1	1
UP15-30-2-SP2	28-3	1	2	4,3,5	3,4,5	4,5,3	3,2	6,5	5,4	1	3,4	1	1
UP15-30-2-SP3-SP3	20-3	H	1	4,3,5	H	6,5	1	6	6,5	1	3,2	3,2	1
UP15-31-2-SP4	28-3	1	1	5	4	5,4	-	6,5	4,5	1	1	1	1
UP15-34-1-SP	20-3	H	1	4,3	3,2,4	6,5	2,3	6	5,4	2,3	3	1	1
UP15-2W-1-SP2	20-3	H	1	6,5,3	H	5,6	1	6	4,5	1	1	1	1
UP15-35-2-SP1	20-3	H	1	6,5	H	6,5	3	6	6,5	2,3	2,3	1	1
UP15-35-2B-SP1	20-3	H	1	4,5	1	5,6	3	6	6,5	1	1	1	1
UP15-2W-2-SP	20-3	H	1	5,6	3,4	5,4,6	3,2	5,4	5,6	1	3,4	1	1
UP15-2W-2-SP6	20-3	1	1	5,4	H	6	3,2	6	5,4,6	1	1	1	1
UP15-2W-4-SP1	20-3	H	1	5,4	H	5,4	2	6,5	5,4,6	1	2,3	1	1
UP15-2W-4-SP2	20-3	H	1	5,4,6	H	6,5	1	6	5,6	1	2,3	1	1
UP15-2W-4-SP3	20-3	H	1	5	H	6,5	1	6	6	1	3,2	1	1

Single pustule isolate	Race ID	Differential Line											
		EG	RP	M	PC50	GGW	PI260418	GN1140	Aurora	Mex309	Mex235	CNC	PI181996
UP15-4w-1-sp5	20-2	H	1	4,3	H	6	1	2	5	1	2	1	1
UP15-4W-1-SP8	20-3	H	1	6,5	H	6	3,2	6,5	6	2	3,2	1	1
UP15-7W-1-SP6	20-3	2	1	4,3	2	5,4,6	-	4,5	1	1	1	1	1
UP15-7W-2-SP4	20-3	H	H	5	2	6	3,2	6	6,5,4	1	1	1	1
UP15-7W-3-SP2	28-3	1	1	4,5	3,4	5,4	2,3	6	6,5	1	3,4	1	1
UP15-7w-4-SP3	20-3	H	1	6	H	5,6	3,4	5,6,4	6	2	2,3	1	1
UP15-7W-4-SP7	20-3	H	3	6.5	H	6	3.2	5.4	4.5	1	1	1	1
UP15-7W-4-SP6	20-3	H	1	4,5	1	6,4	1	6	5,4	1	2,3	1	1
UP15-7W-4-SP8	20-3	1	1	4,3	1	4,3,2	1	6,5	5,4	1	1	3	1
UP15-8W-1-SP2	20-3	H	2	4	3,4	4,3	2,3	6,5	4,3	1	4,3,4	3	1
UP15-8w-3-SP2	20-3	H	1	6,5	H	6	1	6	4,3	1	1	1	1
UP15-8W-3-SP3	20-3	H	1	5,6	H	6	1	6,5	6,5	1	1	1	1
UP15-8W-3-SP5	20-3	H	1	5,4	H	5,4,6	3	6,5	5	1	2	1	1
UP15-9W-3-SP2	20-3	H	1	4,5	H	6,5	3	6	6,5	1	2,3	1	1
UP15-9W-3-SP5	20-3	H	1	5,4,6	H	5	4,5	6,5	5,6	1	1	1	1
UP15-10W-2-SP1	20-3	H	H	5,3	H	5,4	1	6,5	4,5	1	1	1	1
UP15-10W-2-SP4	20-3	H	1	6	H	6	3,	6	4,6	2,3	2,3	1	1
UP15-10W-3-SP3	20-3	1	1	6	1	6,5	1	6	6	5,4	1	1	1

Reaction grades 1= No visible symptoms; H= Necrotic spots without sporulation (Hypersensitive Response); 2,3= Reaction 2 with few type 3; 3,2= reaction type 3 with few type 2; 3= Uredinia <0.3 mm in diameter; 3,4= reaction 3 with few type 4; 4,3= Reaction 4 with few type 3; 4= Uredinia 0.3-0.49 mm in diameter; 4,5= Reaction 4 with few type 5; 5,4= Reaction 5 with few type 6; 5= Uredinia 0.5-0.8 mm in diameter; 5,6= Reaction 5 with few type 6; 6,5= Reaction 6 with few type 5; 6= Uredinia 0.8-1.2 mm in diameter. (-)= missing data.

Table 2. Virulence phenotype of 56 *Uromyces appendiculatus* single pustule isolates collected from dry beans fields in North Dakota in 2016.

Single pustule isolate	Race ID	Differential Line											
		EG	RP	M	PC50	GGW	PI260418	GN1140	Aurora	Mex309	Mex235	CNC	PI181996
UP16-F23-SP1	16-3	H	H	3,4	1	5,4	3,4	6	5,4	1	3,4	1	1
UP16-9-2-SP3	20-1	H	1	5,4	H	5,4	-	4,5	4,3,2	3,2	4,3	3,2	1
UP16-13-3-SP3	20-1	H	1	5,4	H	6,5	-	6	1	1	1	1	1
UP16-1-2-SP1	20-3	4,3	3,2,1	4	3,1	6,5	1	6	6	2,3,1	4,3	4,3	1
UP16-5-3-SP1	20-3	H	1	5,6	H	5,6	-	5,6	4	3,2,1	1	2,3	1
UP16-3-2-SP1	20-3	4,3	1	5,6	3	5,6	-	6,5,4	6	3,2,4	-	4,3	1
UP16-9-2-SP5	20-3	H	1	4,3	3,4	5,4	1	6	6	1	3	3,2	1
UP16-10-3-SP1	20-3	3,4	1	4,3	H	6,4,5	-	6	5,6,4	1	1	1	1
UP16-13-3-SP1/1	20-3	1	1	5,4	1	6,5	-	6,5	6,5,4	1	1	1	1
UP16-16-2-SP1	20-3	H	1	5,4	H	6,5	-	6,5	6,5	1	1	1	1
UP16-16-2-SP3	20-3	H	1	6,4	H	6,5	-	6	4,3	1	1	1	1
UP16-17-1-SP1	20-3	4,3,2	1	5,4	4,3,1	5,6	1	6	6	3,2	4,3	3,2	1
UP16-17-1-SP2	20-3	H	1	4	H	6,5	-	5,6	6,5	1	1	1	1
UP16-17-1-SP2	20-3	2	1	6,5,4	2	5,6	-	6	5	1	1	1	1
UP16-17-2-SP2	20-3	3,4	1	5,4	H	5,6	-	6	5,4,3	1	3	1	1
UP16-17-2-SP3	20-3	H	1	6	H	6	-	6	6	1	1	1	1
UP16-14-SP1	20-3	3,2,1	3,2	6,5	1	5	-	6	5,4,6	1	1	2,3	1
UP16-F5-SP2	20-3	1	1	4,3	1	5,4	-	6,5,4	4,5,3	1	1	1	1
UP16-F12-SP2	20-3	H	1	4,3	H	5,6	-	6	4,3	1	1	1	1
UP16-F12-SP3	20-3	H	1	4,5	H	6,5	-	5,6	4	1	1	1	1
UP16-F14-SP1	20-3	2,3	2,3	6,5	H	5,6	6	4,5	1	1	3,2	1	1
UP16-F14-SP2	20-3	2,1	1	6	1	6	-	6	6,5	1	3	1	1
UP16-F15-SP1	20-3	3,4	1	4,5	3	5	-	5,4	5	1	1	1	1
UP16-F15-SP3	20-3	H	H	6,5	H	6		6	6	1	1	1	1
UP16-F16-SP1	20-3	3,2	1	4,3	1	5,4	3	6,5	4,5	1	3	1	1
UP16-F16-SP3	20-3	H	1	6	H	5,6	-	6,5	6,5	1	1	1	1
UP16-F18-SP1	20-3	3,4	3,2	4,3	1	5,4	-	5,4	5,4	3,2	3,2	3,2	1
UP16-F18-SP2	20-3	H	1	6,5	H	5,4,6	-	6	5,4	1	1	1	1
UP16-F19-SP2	20-3	H	1	6	H	6	3	6	6	2	3,2	2	1
UP16-F20-SP1	20-3	1	1	6,5	H	6	-	6	6	1	1	1	1
UP16-F22-SP7	20-3	4,3	1	5,4	4,3	6,5	4	6	6	3,4	3,4	3,2	1
UP16-F27-SP2	20-3	3,2	1	4,5	H	4	1	5,6	4	1	3	1	1
UP16-F31-SP1	20-3	H	1	5,4	H	6	3	6	6	2	3,4	3,2	1
UP16-F12-SP3	20-3?	H	1	5,4	1			6	6,5,4	1	1	1	1
UP16-16-2-SP2	20-7	1	1	5,4,3	H	5,4	-	-	6,5	4,3,5	3	2,3,1	1
UP16-3-1-SP1	21-3	5	H	4,5	H	4	1	5	4	1	1	1	1
UP16-8-2-SP1	21-3	5,4	3	4,5	3,2	6	2,3	6	6	2	2	3,2	1
UP16-12-1-SP2	21-3	5,4	1	5,6	H	6,5	4,3	6	6	2	3,2	2	1
UP16-12-1-SP3	21-3	4,3	3,2	5,4	H	6	-	6	6	2	3,4	3,4	1
UP16-12-1-SP4	21-3	4,5	3,4	5,4	3,2,4	5,4	-	6	4,3,5	3,4	3,2	3	1
UP16-13-3-SP1	21-3	5,4	1	5,4	1	6	-	6,5	5,6	1	1	1	1
UP16-15-1-SP1	21-3	4,3	2,3	5,6	H	4,5	-	4,5	6	3,2	3,4	2,3	1
UP16-16-1-SP1	21-3	4,3	1	3,4	2,1	5	1	6,5	5	1	2	1	1
UP16-16-3-SP1	21-3	4,3	1	4,3,5	3,4	4,5	-	5	6	2,3	3,2	1	1

Single pustule isolate	Race ID	Differential Line											
		EG	RP	M	PC50	GGW	PI260418	GN1140	Aurora	Mex309	Mex235	CNC	PI181996
UP16-8-1-SP1	24-3	3,2,1	1	3	4,1	5,4	-	4,5	4,5	1	3,2	1	1
UP16-10-1-SP1	27-7	H	1	4,3	4,3	4,3	-	6	6	4	3,2	1	1
UP16-10-2-SP1(SP4	27-7	5,4	4,3	4,3,2	6,3	6	-	6	6	5,6	3	3,2	1
UP16-F16-SP2	28-3	3,4	1,3,4	5,4	5,4	6	3	6	6	1	3	3,2	1
UP16-F19-SP3	28-3	2	2,3	5,6	5,4	6,5	-	6	6	3,2	2,3	2	1
UP16-F22-SP1	28-3	H	2	4,5	4,3	5	-	6	5,4	1	1	1	1
UP16-F27-SP1	28-3	3,4	1	4	4	5,4	-	6	6	3,4	4,3	3,2	1
UP16-12-2-SP1	29-3	5,6	1	6,5	4,3	5,6	-	6	6,5	1	1	1	1
UP16-13-2-SP1	29-3	5,4	1	6,5	4,3	5,4	-	6	6	1	3,2	3,2	1
UP16-17-2-SP1	29-3	6,5	3,2	5,4	5	6	-	6	6	2	3,2	1	1
UP16-F38-SP1	29-3	5,4	1	4,5	4	5,6	-	6	4,3	1	1	1	1
UP16-6-1-SP1	29-7	4,5	1	5	5,4,3	6,5	-	6	6	6	4	4,3	1

Reaction grades 1= No visible symptoms; H= Necrotic spots without sporulation (Hypersensitive Response); 2,3= Reaction 2 with few type 3; 3,2= reaction type 3 with few type 2; 3= Uredinia <0.3 mm in diameter; 3,4= reaction 3 with few type 4; 4,3= Reaction 4 with few type 3; 4= Uredinia 0.3-0.49 mm in diameter; 4,5= Reaction 4 with few type 5; 5,4= Reaction 5 with few type 6; 5= Uredinia 0.5-0.8 mm in diameter; 5,6= Reaction 5 with few type 6; 6,5= Reaction 6 with few

Figure 2. Phenotype of race 20-3. Hypersensitive response on BD1 (Early Gallatin) and BD4 (PC-50); Virulent on BD3 (Montcalm), BD5 (GGW), BD7 (GN1140)) and BD8 (Aurora). Avirulent on BD2 (Redland Pioneer), BD6 (PI260418), BD9 (Mexico 309), BD10 (Mexico 235), BD11 (CNC), and BD12 (PI161994).

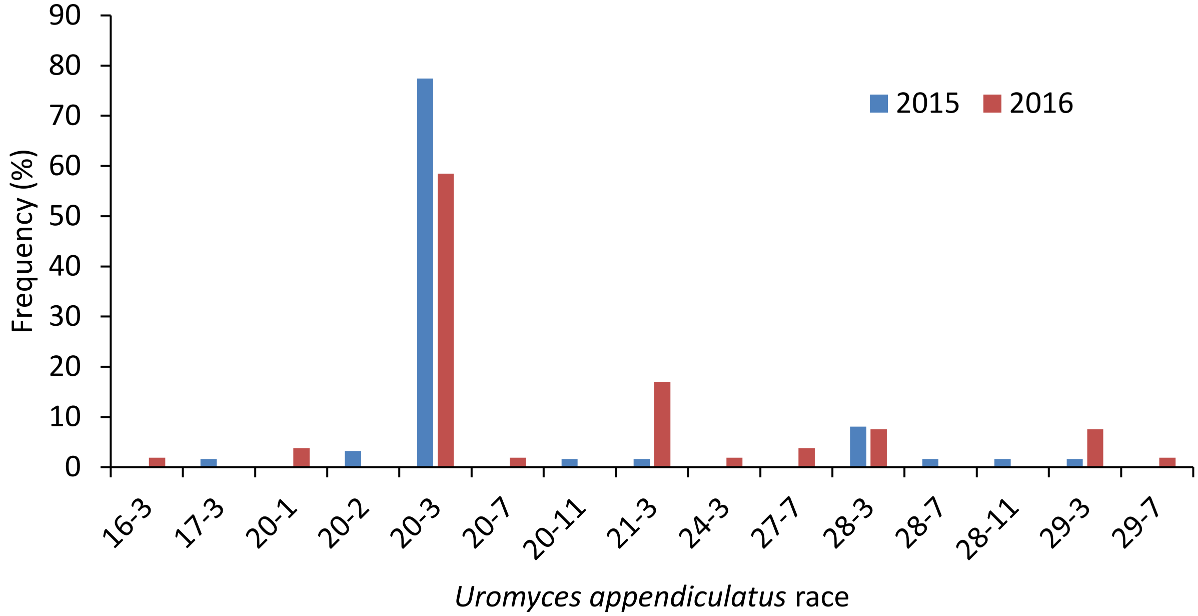

Figure 3. Frequency of races identified among 115 *Uromyces appendiculatus* isolates collected in North Dakota and 2015 and 2016.

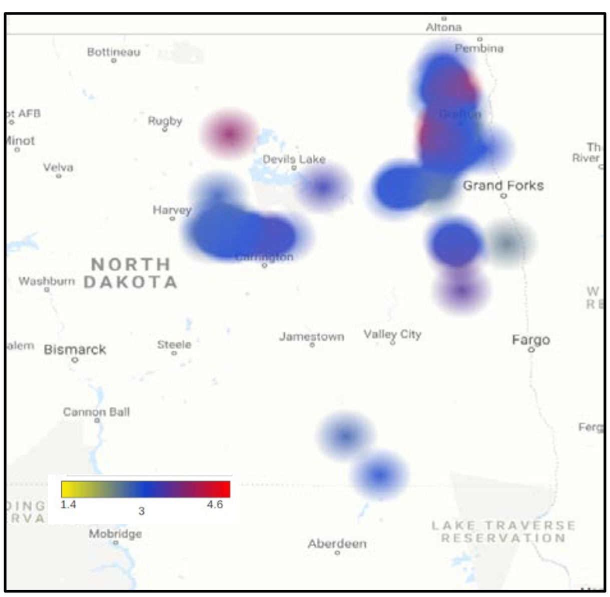

Figure 4. Geographical heat map based on Mean Disease Severity (MDS) of *Uromyces appendiculatus* single pustule isolates collected in 2015 and 2016.

Figure 5. *Uromyces appendiculatus* virulence phenotype reactions; hypersensitive response (A), pustule size 0.5 mm (B) and 1.0 mm (C).

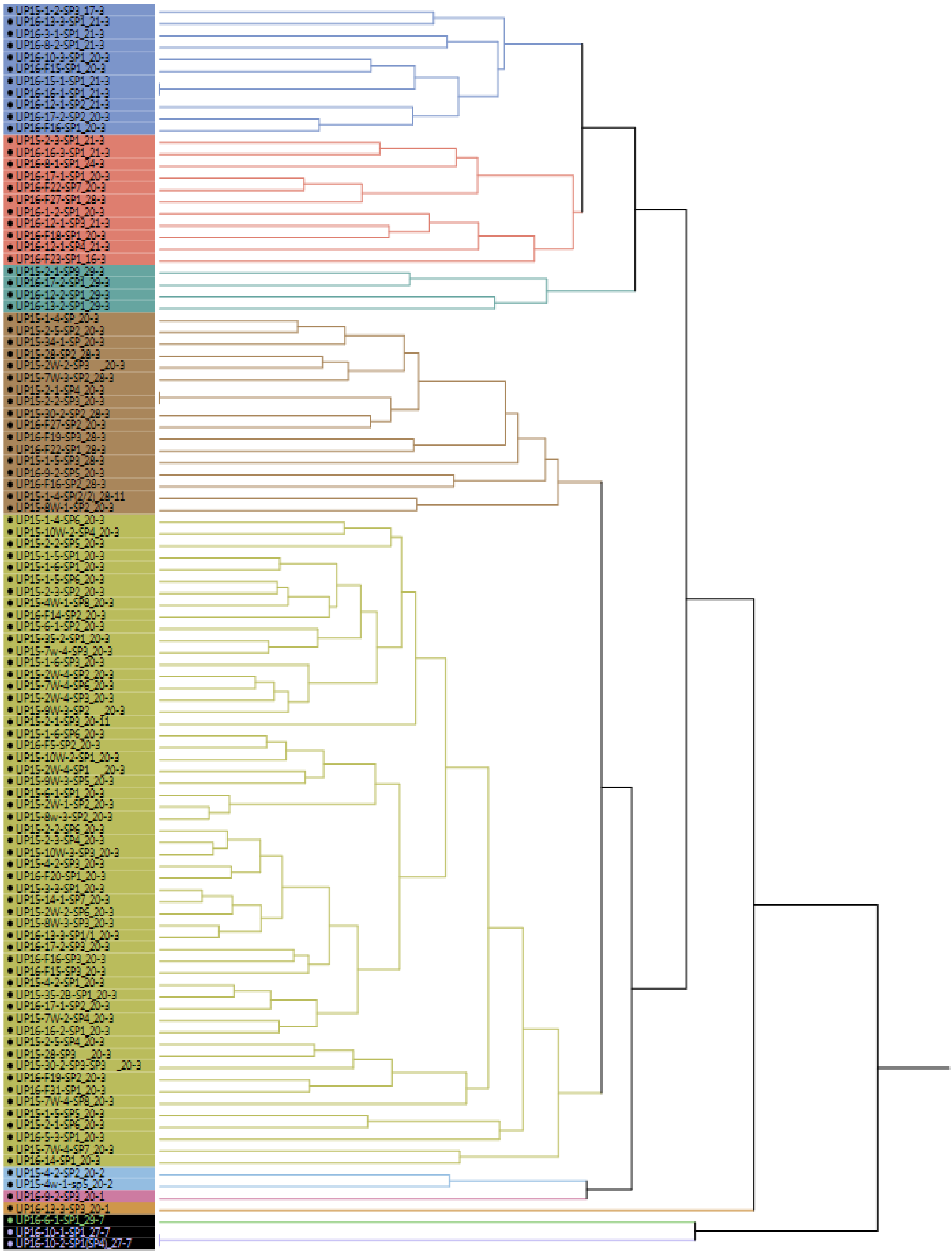

Figure 6. Hierarchical clustering (Average method) of 109 bean rust isolates based on virulence phenotype.

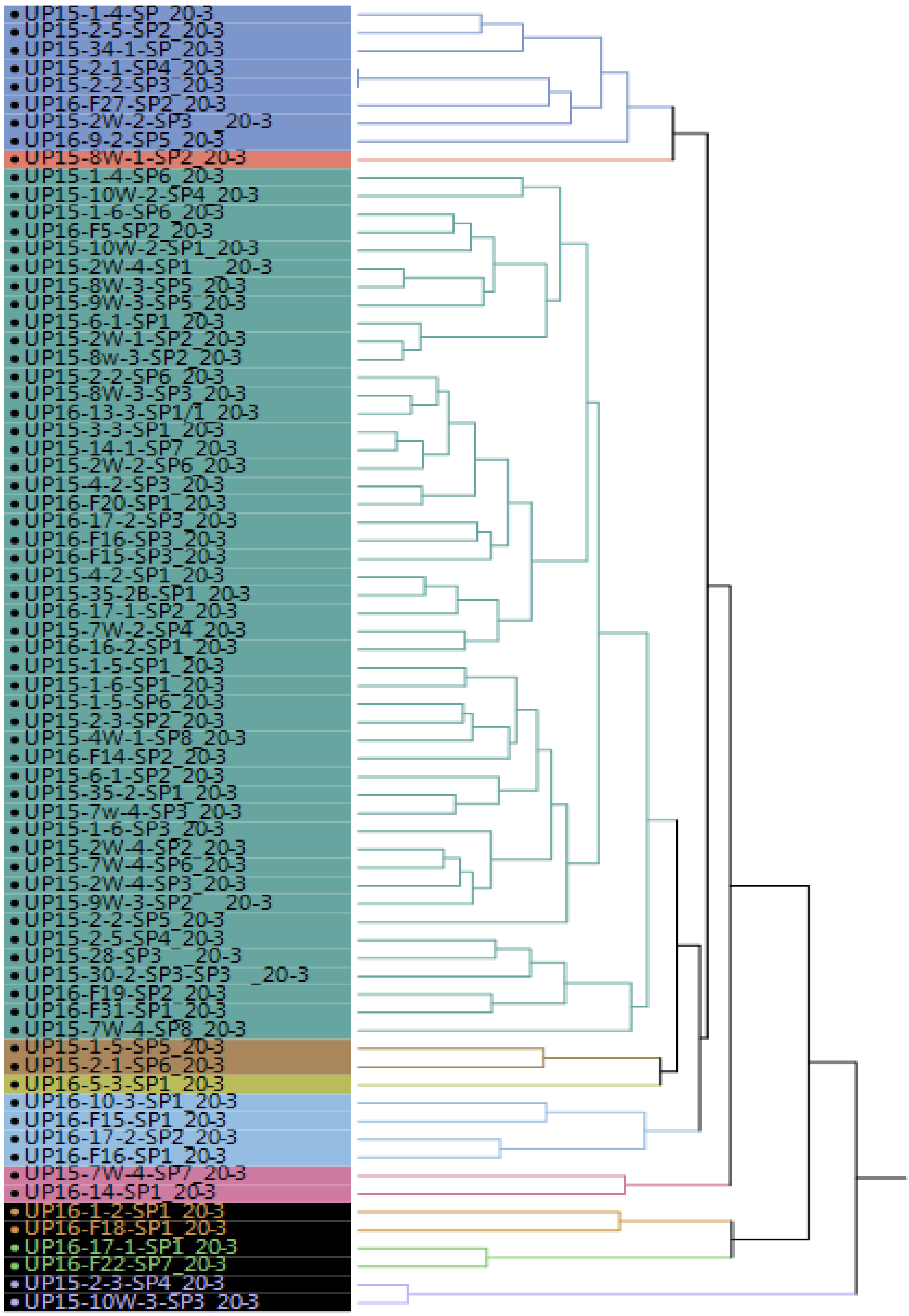

Figure 7. Hierarchical clustering (Average method) of isolates race-typed as 20-3 based on virulence phenotype

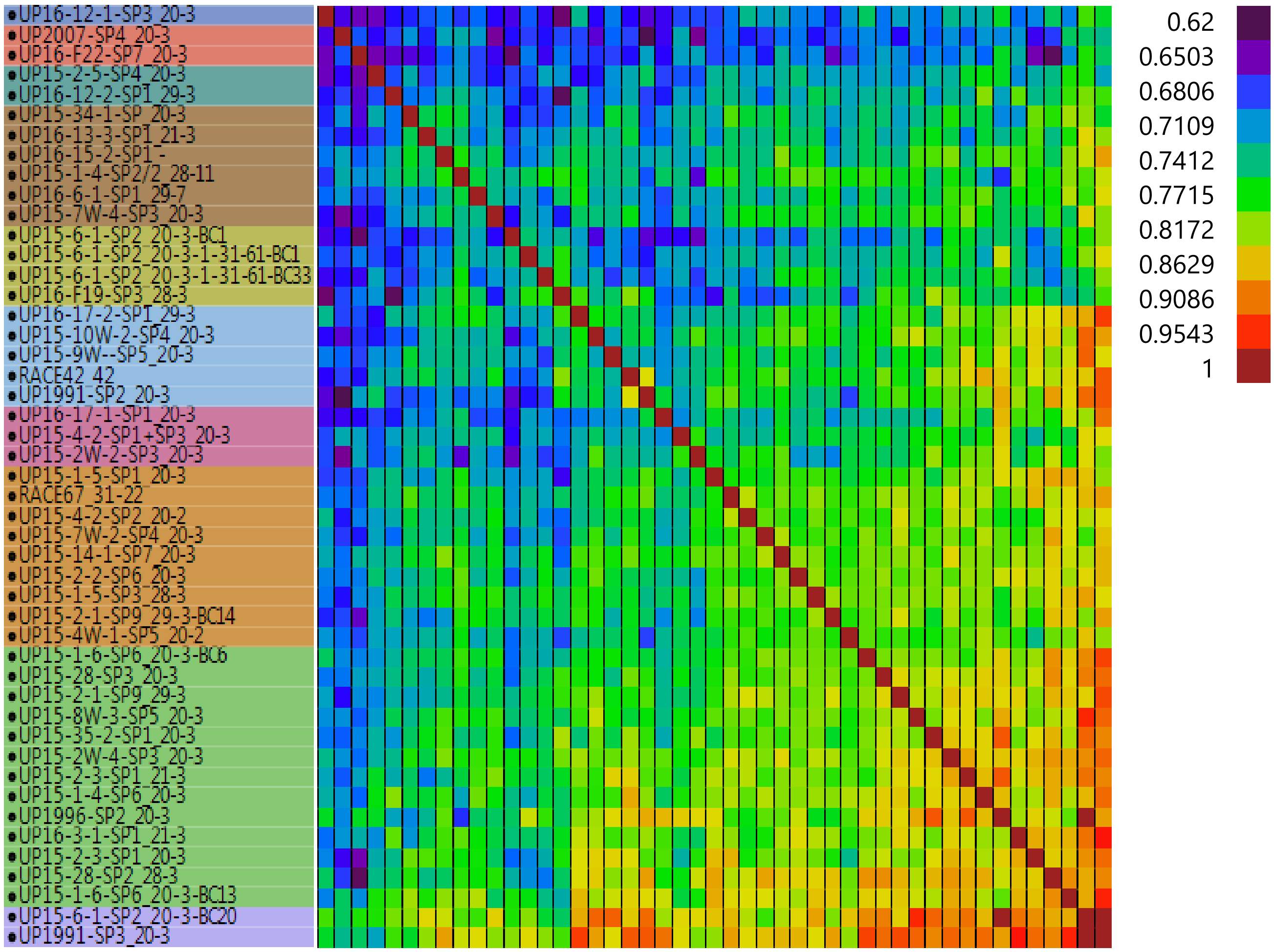

Figure 8. Heat map showing Identity by state (IBS) matrix relationship matrix of 42 *Uromyces appendiculatus* isolates collected in North Dakota.

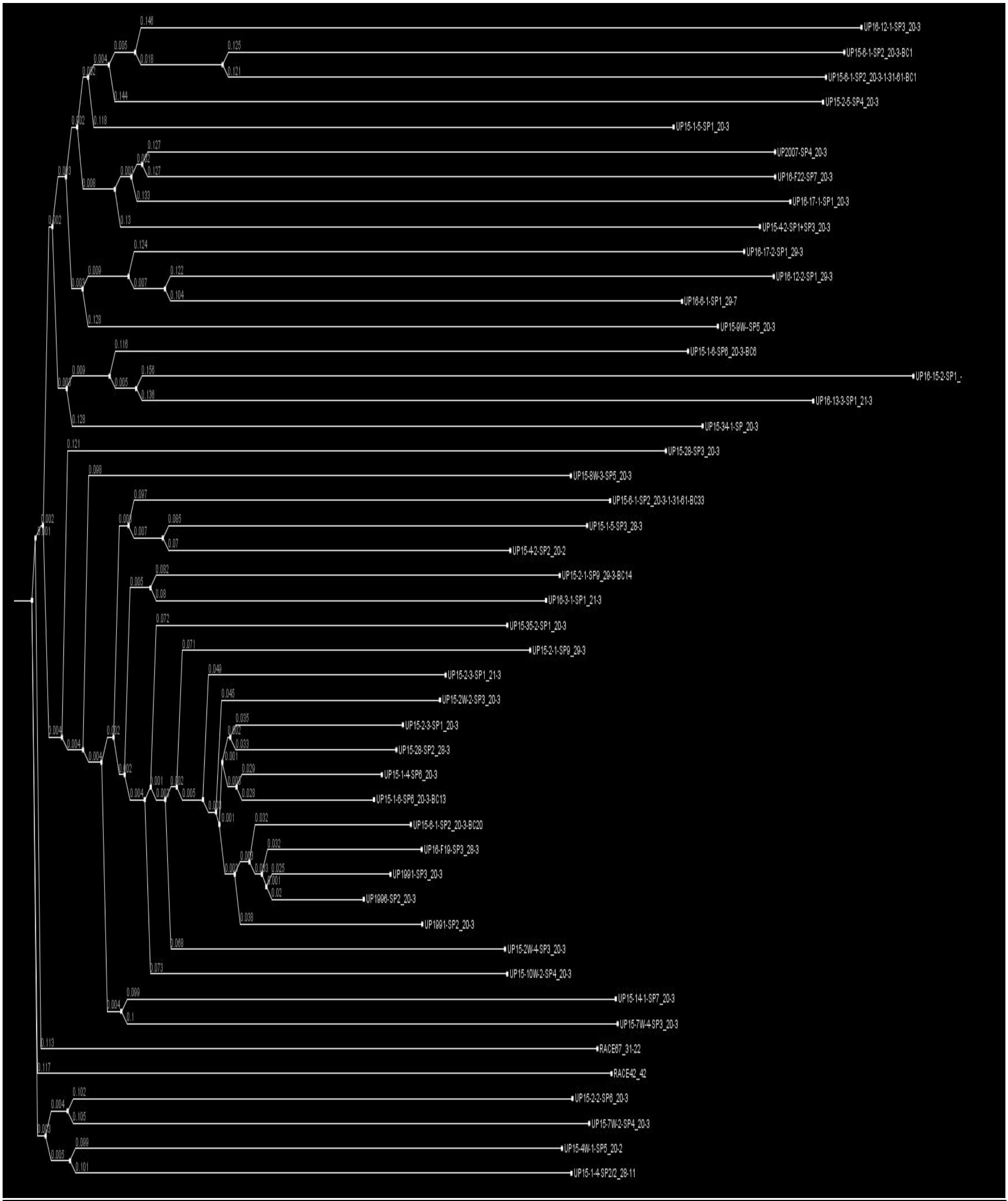

Figure 9. A dendogram of *Uromyces appendiculatus* isolates based on genotypic data created using Neighbor-joining tree method.

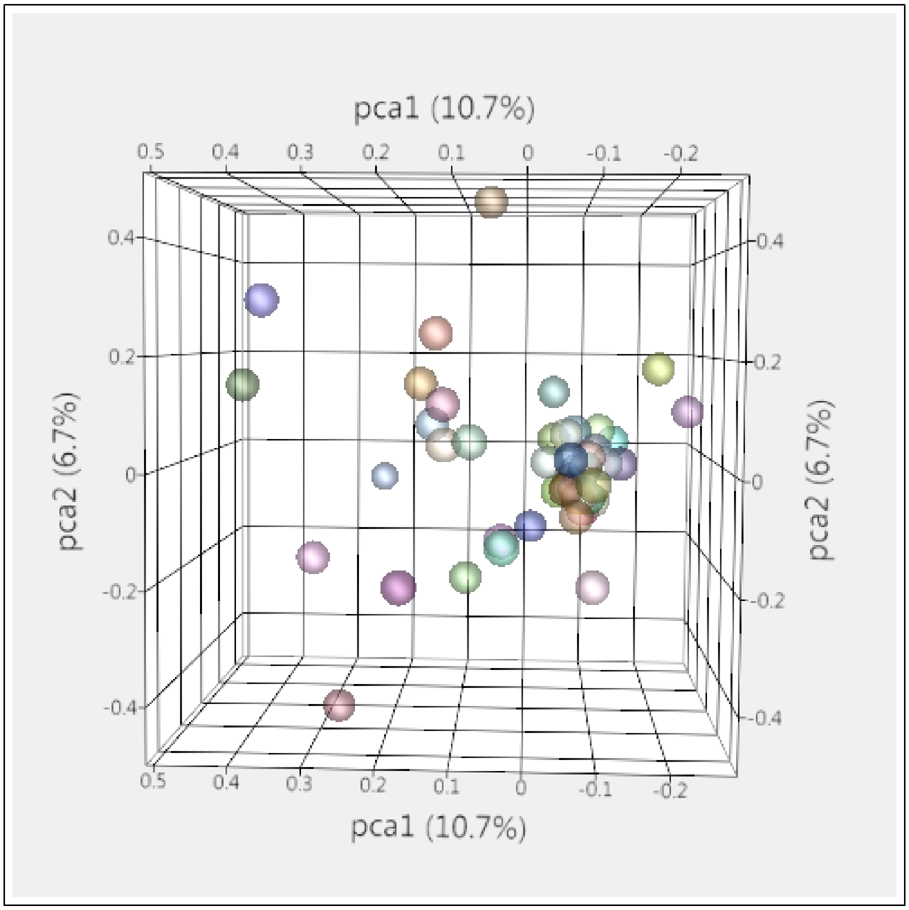

Figure 10. Principal Component Analysis (PCA) illustrates relatedness of *Uromyces appendiculatus* isolates collected in North Dakota based on 3 dimensional clustering.

Table 3. Reaction of advanced pinto, black, light red kidney and great northern bean germplasmfrom the NDEAS breeding program to*Uromyces appendiculatus* (dry bean rust) race 20-3 and 29-3. Ratio of Resistant : Susceptible reaction on 10 plants.

Market class	Genotype	20-3	20-3	29-3	29-3	29-3
Pinto						
1	LaPaz	5R-5S	4R:6S	2R:8S	2R:8S	
2	Lariat	2R-8S	S	S		
3	Monterrey	2R-8S	S	S		
4	Windbreaker	3R-5S	S	S		
5	ND121229	4R-6S	S	S		
6	ND121237	R	6R:4S	4R:6S	5R:2S	S
7	ND121315	3R-7S	5R:5S	S		
8	ND121352	S	S	4R:6S		
9	ND121429	2R-7S	S	S		
10	ND121447	6R-3S	9R:1S	R	R	
11	ND121448	R	R	R	R	
12	ND121449	R	R	R	R	
13	ND121453	R	R	R	R	
14	ND121479	3R-7S	S	6R:4S	S	
15	ND121508	R	2R:8S	R	R	
16	ND121531	4R-6S	S	S		
17	ND121540	5R-5S	S	3R:7S		
18	ND121560	R	8R:2S	R	R	
19	ND131502	2R-7S	8R:2S	4R:6S		
20	ND131543	6R-2S	6R:4S	S	S	
21	ND131551	1R-9S	6R:4S	S		
22	NDF140646	S	S	S		
23	NDF140658	6R-4S	8R:2S	S	S	
24	NDF140660	3R-7S	S	S		
25	NDF140663	4R-3S	S	S		
26	NDF140673	4R-6S	7R:3S	S		
27	NDF140682	2R-7S	S	S		
28	ND131405	8R:2S	S	S		
29	ND131406	1R-11S	S	S		
30	ND131413	S	S	S		
31	NDF140505	R	S	S		
32	NDF141506	1R-11S	S	S		
33	NDF141507	S	S	S		
34	Palomino	9R-1S	S	S		
35	SDIP-1	3R-9S	S	S		
36	Vibrant	S	S	S		
37	WM-2	9R-1S	S	S		
Great northern						
1	Aries	S	S	S		
2	Draco	S	S	S		
3	Matterhorn	6R-7S	S	S		
4	Powderhorn	S	S	S		

Market class	Genotype	20-3	20-3	29-3	29-3	29-3
5	Taurus	S	S	S		
6	ND09726	1R-9S	S	4R:6S		
7	ND112828	5R-8S	S	7MR:3S	S	
8	ND112843	1R-11S	S	S		
9	ND121630	S	S	S		
10	ND121660	4R-6S	S	S		
11	ND121676	S	S	S		
12	ND121686	1R-9S	S	1R:9S		
13	ND121692	5R-5S	S	S		
14	ND121698	2R-8S	S	S		
15	NDF140812	S	S	S		
16	NDF140813	3R-10S	S	S		
17	NDF140820	S	S	S		
18	NDF140831	5R-7S	S	S		
19	NDF140832	3R-9S	S	S		
Small rec						
1	Merlot	S	S	S		
2	Rosetta	S	6R:4S	2R:6S		
3	Ruby	S	S	S		
4	Sedona	S	S	S		
5	ND112929	S	3R:7S	S		
6	ND121885	S	S	S		
7	NDF140712	S	2R:8S	S		
8	NDF140719	S	S	S		
9	NDF140720	S	S	2R:8S		
10	NDF140722	S	4R:6S	2R:8S		
11	NDF140723	S	S	6R:4S	S	
12	NDF140725	S	2R:8S	S		
13	NDF140726	2R:5S	7R:3S	3R:6S		
14	NDF140735	S	4R:6S	S		
15	NDF140736	S	S	S		
16	NDF140741	R	R	R	9R:1S	
17	NDF140743	7R:5S	2R:8S	2R:8S		
18	NDF140750	S	S	1R:9S		
Navy						
1	Ensign	S	S	S		
2	Medalist	1R:9S	S	S		
3	T9905	5R:5S	S	S		
4	ND070612	1R:9S	S	S		
5	ND122056	S	S	3R:7S		
6	ND122080	S	S	S		
7	ND122081	S	S	1R:9S		
8	ND122082	S	S	3R:7S		
9	ND122105	S	S	1R:9S		
10	ND122114	3R:7S	2R:8S	S		
11	ND131705	R	9R:1S	7R:3S	1R:8S	S
12	ND131762	S	S	S		

Market class	Genotype	20-3	20-3	29-3	29-3	29-3
13	ND131807	2R:8S	8R:2S	8R:2S	S	
14	ND131905	S	S	S		
15	ND131990	R	8R:2S	R	R	
16	ND132023	S	S	S		
17	ND132048	S	S	2R:8S		
18	ND132049	S	S	S		
19	ND132126	S	S	S		
20	ND132208	S	S	S		
21	ND132234	S	S	S		
22	ND132254	R	S	7R:3S	5R:5S	
23	ND131900	S	S	S		
24	ND131717	S	S	S		
Black						
1	Blackcat	2R-8S	S	S		
2	Eclipse	7R-3S	S	9R:1S	S	
3	Loreto	9R-1S	S	S		
4	Zenith	1R-9S	S	1R:7S		
5	Zorro	3R-8S	S	S		
6	ND060769	9R-1S	6R-4S	1R:9S	7R:3S	
7	ND071089	R	R	R	R	
8	ND071244	R	R	R	R	
9	ND071249	R	R	R	R	
10	ND071257	R	R	R	R	
11	ND081144	6R-4S	S	S		
12	ND081147	9R-1S	S	S		
13	ND081247	R	S	8R:2S	S	
14	ND081295	S	S	1R:9S		
15	ND081340	9R-1S	S	1R:9S		
16	ND132375	3R-7S	4R-6S	S		
17	ND132381	2R-8S	S	S		
18	ND132392	R	S	3R:7S		
19	ND132571	R	R	R	R	
20	ND132581	R	R	6R:1S	R	
21	ND132590	R	R	9R:1S	R	
22	ND132599	R	R	R	R	
23	ND132617	R	S (BH)	S		
24	ND132698	R	R	R	R	
25	ND132700	9R-1S	5R-5S	R	R	
26	ND132719	R	S(NBP)	R	4R:6S	
27	ND132788	4R-6S	S	S		
28	NDF120002	R	R	S	S	
29	NDF120039	8R-2S	S	S		
30	NDF120055	8R-1S	10R-3S	R	R	
31	NDF120066	R	8R-2S	R	R	
32	NDF120070	8R-2S	8R-2S	7R:3S	R	
33	NDF120117	3R-7S	S	S		
34	NDF120253	8R-2S	2R-8S	S		

Market class	Genotype	20-3	20-3	29-3	29-3	29-3
35	NDF120259	R	R	R	R	
36	NDF120272	R	R(SBP)	R	R	
37	NDF120276	R	8R-2S	R	R	
38	NDF120287	R	S	R	R	
39	NDF120299	6R-4S	S	S		
40	NDF120304	5R-5S	S	S		
41	NDF120329	5R-5S	S	6R:4S	5R:5S	
42	NDF120334	R	S	3R:7S		
43	NDF120346	R	5R-5S	7R:3S	R	
44	NDF120366	2R-7S	S	3R:7S		
Dark red kidney						
1	Montcalm	4R-10S	S	6R:4S	S	
2	Redhawk	10R-5S	S	R	1R:9S	
3	Talon	R	R	9R:1S	4R:6S	2R:8S
4	1885	3R-9S	S	8R:2S	3R:7S	
5	1941	R	R	R	8R:2S	
6	1988	R	R	R	R	
7	1998D	R	R	5R:5S	8R:2S	
8	2000	8R-3S	7R:3S	8R:2S	5S:5R	
9	2001	10R-7S	R	R	9R:1S	
10	2006	R	R	9R:1S	5R:5S	
11	2010	10R-3S	8R:2S	R	9R:1S	
12	2013	R	R	R	4R:6S	
13	2015	R	R	6R:4S	7R:1S	
14	2016	2R-10S	S	R	3R:7S	
15	2026	R	R	R	9R:1S	
16	2036	12R-1S	S	3R:7S	S	
17	2038	8R-5S	3R:7S	R		
18	2044	5R-11S	R	5R:5S		
19	2046	6R-6S	S	R	1R:9S	
20	2073	9R-3S	9R:1S	R	R	
21	2074	R	R	R	R	
22	2077	3R-8S	S	9R:1S	1R:9S	
23	2096	2R-10S	S	8R:2S	3R:7S	
24	2112	5R-7S	S	R	S	
25	2113	3R-9S	S	R	2R:8S	
26	2114	10R-3S	2R:8S	9R:1S	1R:9S	
27	2116	8R-4S	S	R	S	
28	2118	7R-4S	3R:7S	R	3R:7S	
29	2131	4R-8S	3R:7S	R	2R:8S	
30	2133	3R-11S	S	6R:4S	S	
Light red kidney						
1	Foxfire	R	R	8R:2S	8R:2S	
2	Pink Panther	3R-8S	S	9R:1S	1R:9S	
3	Rosie	3R-11S	S	R	1R:9S	
4	1878	7R-6S	S	7R:3S	1R:9S	
5	1882	3R-11S	S	R	1R:9S	

Market class	Genotype	20-3	20-3	29-3	29-3	29-3
6	1914	5R-10S	1R:9S	9R:1S	1R:9S	
7	1922	R	R	5R:5S	9R:1S	
8	1932	7R-7S	1R:9S	7R:3S	3R:7S	
9	1936	9R-5S	S	5R:5S	2R:8S	
10	1995	R	R	9R:1S	R	
11	1996	R	R	R	R	
12	1998L	R	R	8R:2S	R	
13	2030	4R-10S	S	2R:8S		
14	2035L	1R-12S	S	7R:3S	R	
15	2040	4R-9S	S	9R:1S	S	
16	2070	R	R	R	8R:2S	
17	2082L	11R-1S	7R:3S	5R:4S	R	
18	2102	S	S	7R:3S	S	
19	2120	6R-8S	S	3R:7S		
20	2121	3R-10S	S	3R:7S		

Table 4. Preliminary results of Mesoamerican Diversity Panel with rust races 20-3 and 29-3. Results presented in ratio of Resistant (R):Susceptible (S).

Market class		Genotype	Race 20-3	Race 29-3
Pinto				
	1	A285	R	S
	2	A-801	2R:8S	R
	3	BAT477	S	1R:9S
	4	SEA10	4R:6S	1R:9S
Black				
	1	115M Black Rhino	8R:2S	7R:3S
	2	92BG-7	9R:1S	9R:1S
	3	A-55	1R:9S	S
	4	AC-Black Dimond	S	S
	5	AC-Harblack	S	S
	6	Aifi Wuriti	8R:2S	R
	7	B05055	S	S
	8	Bandit	S	S
	9	Black Knight	S	S
	10	Black Magic	9R:1S	S
	11	Black Velvet	6R:4S	R
	12	Blackhawk	1R:9S	1R:9S
	13	Blackjack	R	R
	14	CDC Expresso	S	S
	15	CDC Jet	7R:3S	S
	16	CDC Nighthawk	S	S
	17	Condor	S	S
	18	Cornell 49-242	9R:1S	R
	19	Domino	8R:2S	R
	20	DPC-4	R	9R:1S
	21	Eclipse	9R:1S	R
	22	F04-2801-4-1-2	5R:5S	3R:7S
	23	F04-2801-4-5-1	7R:3S	8R:2S
	24	F04-2801-4-6-6	2R:8S	S
	25	Harrowhawk	S	S
	26	I9365-31	R	9R:1S
	27	ICB-10	1R:9S	R
	28	ICB-3	S	1R:9S

Market class		Genotype	Race 20-3	Race 29-3
Navy				
	1	AC Compass	8R:2S	S
	2	AC Polaris	S	S
	3	Albion	1R:9S	S
	4	Arthur	S	S
	5	Avalanche	S	S
	6	Avanti	4R:6S	S
	7	BelMiNeb-RMR	R	5R:5S
	8	BelMiNeb-RMR	R	9R:1S
	9	BelMiNeb-RMR	9R:1S	R
	10	C-20	S	S
	11	CDC Whitecap	S	S
	12	Crestwood	S	S
	13	Ensign	S	-
	14	Envoy	S	S
	15	F07-004-9-1	S	S
	16	Fleetwood	S	S
	17	Huron	S	S
	18	HY 4181	S	S
	19	Hyden	S	S
	20	Laker	S	S
	21	Lighthing	S	S
	22	Mackinac	S	S
	23	Mayflower	-	-
	24	McHale	S	S
	25	Medalist	S	S
	26	Michelite	1R:9S	9R:1S
	27	Midland	S	S
	28	Morales	9R:1S	S
	29	Morden 003	S	S
	30	N05324	S	R
	31	Nautica	S	S
	32	Navigator	S	S
	33	Neptune	6R:4S	8R:2S

Market class	Genotype	Race	
		20-3	29-3
29	Jaguar	S	S
30	Loreto	S	5
31	Midnight	9R:1S	1R:9S
32	ND021574	S	4R:6S
33	ND021717	9R:1S	6R:4S
34	OAC Gryphon	S	S
35	Orca	5R:5S	S
36	Phantom	R	R
37	PR 0443-151	R	R
38	Puebla 152	S	4R:6S
39	Raven	R	R
40	Shania	R	R
41	Shiny Crow	S	S
42	T-39	9R:1S	7R:3S
43	UCD 96114	S	S
44	UI-906	S	S
45	UI-911	S	S
46	Xan 176	-	S
47	Zorro	S	S

Market class	Genotype	Race	
		20-3	29-3
34	Newport	R	6R:4S
35	Norstar	S	S
36	NW-395	S	S
37	OAC Laser	S	S
38	OAC Rex	S	S
39	OAC Seaforth	S	S
40	Reliant	S	S
41	Sanilac	S	S
42	Schooner	4R:6S	S
43	Seabiskit	S	S
44	Seafarer	S	S
45	Seahawk	S	S
46	Swan Valley	9R:1S	R
47	T9903	S	S
48	T9905	S	S
49	USWA-50	3R:7S	4R:6S
50	Verano	S	S
51	Vista	3R:7S	S
52	Voyager	S	S

Table 5: Virulence phenotype for 194 genotypes in the Durango Diversity Panel (DDP) to the two most commonly identified rust (*Uromyces appendiculatus*) races identified in North Dakota.

Accession No.	Genotype	Race 20-3	Race 27-7	Accession No.	Genotype	Race 20-3	Race 27-7
1	6R-42	6	5,6	41	CDC Pintium	5,4	6,5
2	92US-1006	6,5	5,4	42	CDC Rosalee	4,3	5,6
3	ABCP-15	5,4	5,6	43	CDCWM-2	6	6
4	ABCP-17	6,5	6,5	44	CENTA Pupil	4,3	5,6
5	ABCP-8	6,3	6,5	45	Chase	6,5	6,5
6	ABC-Weihing	3,4	5,6	46	Common Pinto	5,6	6,5
7	AC Earlired	6	5	47	Common Red Mexican	6,5	6
8	AC Early Rose	6	6,5	48	Coulee	5,6	5,6
9	AC Island	6,5	5,6	49	Coyne	4,3	5,6
10	AC Ole	5,6	5,6	50	Croissant	6,5	6,5
11	AC Pintoba	6,5	6,5	51	Dehoro	1	5
12	AC Redbond	1	1	52	Desert Rose	1	3
13	AC Resolute	5,4	6,5	53	DOR 364	6	6,5
14	AC Scarlet	1	4	54	Durango	6	5,6
15	Agassiz	6,5	6	55	Ember	5,6	6,5
16	Amadeus 77	1	1	56	Emerson	6,5	6
17	Apache	1	1	57	F07-014-22-2	6	6,5
18	Arapaho	6,5	5	58	F07-449-9-3	6	6,5
19	Aztec	4,5	6	59	Fargo	6,5	6
20	Baja	6,5	5,6	60	Fiesta	6	5,4
21	BelDakMi-RR-5	1	1	61	Fisher	6,5	6,5
22	BelMiNeb 1	1	1	62	Flint	5,4	5,6
23	BelMiNeb 2	1	1	63	Focus	6	5,6
24	BelMiNeb 5	1	1	64	Frontier	6	5,6
25	BelMiNeb-RMR-3	1	1	65	Gala	6	6,5
26	BelMiNeb-RR-2	1	1	66	Garnet	5,4	6,5
27	BelNeb 2	1	1	67	Gemini	6,5	6
28	BelNeb-RR-1	1	1	68	Gloria	6,5	6,5
29	Beryl	5,4	6,5	69	GN Harris	4,5	6,5
30	Beryl R	1	1	70	GN Star	6,5	6,5
31	Big Bend	6	5,6	71	GN#1Sel27	-	-
32	Bighorn	4,5	4,5	72	GN9-1	6,5	6
33	Bill Z	6,5	5,4	73	GN9-4	6,5	6,5
34	Buckskin	6,5	6,5	74	Grand Mesa	5,6	6
35	Burke	6,5	6,5	75	GTS-900	3,2	6,5
36	Buster	4,5	6,5	76	Harold	6,5	6,5
37	CDC Camino	6,5	6,5	77	Hatton	5,4	6
38	CDC Crocus	5,4	6,5	78	Holberg	6,5	6
39	CDC Nordic	5,6	6,5	79	I06-2575-17	6	6,5
40	CDC Pinnacle	5,4	6,5	80	I9365-25	2,3	3,4

Accession No.	Genotype	Race	
		20-3	27-7
81	I9365-5	2,1	6,5
82	IBC 301-204	-	1
83	ICB-12	2,1	1
84	Indeterminate Jamaica Red	-	5,6
85	INTA Precoz	-	-
86	IP08-2	6	6,5
87	Ivory	2,4	6,5
88	Jackpot	3,4	6,5
89	JM-126	2,3	5,6
90	JM-24	6,5	6,5
91	Kimberly	5,6	6,5
92	Kodiak	5,4	6,5
93	La Paz	6,5	6,5
94	Le Baron	5,4	6,5
95	Mariah	6	6,5
96	Marquis	4,5	6,5
97	Matterhorn	6	6
98	Maverick	6	5,6
99	6,7	5,4	5,6
100	Medicine Hat	6,5	6,5
101	Merlot	4,3	6,5
102	Montrose	5,6	5,6
103	ND040494-4	6,5	6,5
104	ND041062-1	6	1
105	ND060197	4,3	5,4
106	ND-307	5,6	6
107	NDZ06249	4,3	6,5
108	NE1-09-13	6,5	6,5
109	NE1-09-19	6,5	6,5
110	NE1-09-20	6	5,6
111	NE1-09-22	6	5,6
112	NE1-09-9	6,5	5,6
113	NE2-09-1	1	1
114	NE2-09-10	6	6,5
115	NE2-09-3	1	1
116	NE2-09-4	4,3	5,6
117	NE2-09-8	1	1
118	Nodak	6,5	5,6
119	NW-410	5,6	6,5
120	NW-590	6,5	5,6
121	NW-63	5,4	6,5
122	Orion	6,5	6
123	Othello	6	6,5
124	Ouray	6,5	5,6
125	P07863	5,4	5,6
126	Pindak	H	H
127	Pink Floyd	1	1
128	PK915	6	6,5
129	PK9-7	5,6	6,5
130	Poncho	6	6,5
131	PR 0340-3-3-1	1	1
132	PR 0401-259	4,3	5,6
133	PT7-2	5,6	6,5
134	PT9-17	4,5	6
135	Quincy	6	6
136	Red Ryder	6	6
137	ROG 312	6,5	6,5
138	Rojo Chiquito	1	1
139	Roza	6,5	6
140	S08418	6,5	6
141	Santa Fe	6,5	6
142	Sapphire	6	6,5
143	Sawtooth	6	6
144	SDPI-1	6	6
145	Sedona	6	6,5
146	Sequoia	6	6,5
147	Shoshone	4,3	5,6
148	Sierra	6,5	5,6
149	Sonora	6	6,5
150	SR7-3	5,6	6
151	SR9-4	6,5	6
152	Stampede	3,4	4,3
153	Starlight	4,3	5,6
154	TARS09-RR004	1	1
155	TARS09-RR007	6,5	6,5
156	UI-59	5,6	6
157	TARS09-RR023	3,4	5,6
158	TARS09-RR029	3,4	6,5
159	TARS-VCI-4B	3,2	4,5
160	UCD 9623	5,6	5,6
161	UI-111	6,5	6
162	UI-114	4,3	6,5
163	UI-123	5,6	6,5
164	UI-126	6	6,5

Accession No.	Genotype	Race 20-3	Race 27-7	Accession No.	Genotype	Race 20-3	Race 27-7
165	UI-196	6	6	177	USPT-CBB-5	6,5	5,6
166	UI-228	6	6	178	USPT-WM-1	1	3,4
167	UI-239	6	6,5	179	USRM-20	5,4	6,5
168	UI-3	6,5	6,5	180	USWA-12	4,5	5,4
169	UI-37	6	6	181	USWA-13	6	6
170	UI-425	6	6	182	USWA-61	6,5	6,5
171	UI-537	6	6,5	183	Victor	6	6,5
172	URS-117	5,6	6,5	184	Vision	6	5,6
173	US-1140	6	5,6	185	Viva	6,5	6,5
174	USPT-ANT-1	H	4,3	186	Win Mor	6,5	6
175	USPT-CBB-1	1	1	187	Windbreaker	5,4	5,6
176	USPT-CBB-3	6	6,5	188	Yolano	6	6,5

Reaction grades 1= No visible symptoms; H= Necrotic spots without sporulation (Hypersensitive Response); 2,3= Reaction 2 with few type 3; 3,2= reaction type 3 with few type 2; 3= Uredinia <0.3 mm in diameter; 3,4= reaction 3 with few type 4; 4,3= Reaction 4 with few type 3; 4= Uredinia 0.3-0.49 mm in diameter; 4,5= Reaction 4 with few type 5; 5,4= Reaction 5 with few type 6; 5= Uredinia 0.5-0.8 mm in diameter; 5,6= Reaction 5 with few type 6; 6,5= Reaction 6 with few type 5; 6= Uredinia 0.8-1.2 mm in diameter. (-)= missing data.

Table 6: Virulence phenotype for 49 genotypes in the Andean Diversity Panel (ADP) to the two most commonly identified rust (*Uromyces appendiculatus*) races identified in North Dakota.

Accession No.	Genotype	Race		Accession No.	Genotype	Race	
		20-3	27-7			20-3	27-7
1	02-385-14	4,5	4,5	26	Litekid	4,5	4,5
2	Bellagio	6,5	6,5	27	Micran	4,5	5,6
3	Beluga	3,2	3,4	28	Montcalm	6,5	6,5
4	Blush	5,6	4,5	29	Myasi	H,3,2	H
5	Capri	5,6	4,3	30	ND061106	4,3	4,5
6	Cardinal	5,4	6	31	Pink Panther	4,3	4,5,6
7	CDRK	4,5	5,4	32	Pompadour B	1	6
8	CELRK	2,3	6,5,4	33	Red Hawk	4,3	6
9	Charlevoix	2,1	5,6	34	Red Kanner	H	H
10	Chinook 2000	3,2	5,4	35	Red Kloud	H	H
11	Crimson	3,2	6	36	Red Kote	3,4	6
12	Dolly	6,5	6	37	Red Rider	6,5	6,5
13	Drake	3,4	6,5	38	Royal Red	4,3	4,5
14	Etna	6,5	5,4	39	Sacramento	3,4	5,4
15	Fiero	3,4	3,4,5	40	Silver Cloud	4,3	6,5
16	Fox Fire	H	H	41	UC Canario 707	3,4	5,6
17	G-122	3,4	5,6	42	UC Nichols	4,3	5,6
18	Hooter	5,6	6	43	UCD 0801	4,3	5,4,6
19	Isabella	5,4	6	44	USCR-9	5,6	6
20	Jalo EEP558	3,2	3,4	45	USCR-CBB-20	3,2	6,5
21	K-42	4,5	4,3,5	46	USDK-CBB-15	6,5	5,6
22	K-59	5,4	6,5	47	USWK-CBB-17	3,2	5,4
23	Kardinal Kidney	4,5	4,5	48	VA-19	5,4	5,4,6
24	Krimson	5,4	6	49	Wallace 773-V98	3,4	5,6
25	Lassen	5,6	6				

Reaction grades 1= No visible symptoms; H= Necrotic spots without sporulation (Hypersensitive Response); 2,3= Reaction 2 with few type 3; 3,2= reaction type 3 with few type 2; 3= Uredinia <0.3 mm in diameter; 3,4= reaction 3 with few type 4; 4,3= Reaction 4 with few type 3; 4= Uredinia 0.3-0.49 mm in diameter; 4,5= Reaction 4 with few type 5; 5,4= Reaction 5 with few type 6; 5= Uredinia 0.5-0.8 mm in diameter; 5,6= Reaction 5 with few type 6; 6,5= Reaction 6 with few type 5; 6= Uredinia 0.8-1.2 mm in diameter. (-)= missing data.

Beneficiaries:

The direct beneficiaries of the outcomes of this project are North Dakota dry bean producers. Producers in other regions of the US may also benefit from the outcomes of this project, as they could use the information, germplasm or varieties produced as a result of this project. Based on the 2007 census of Agriculture, beans are produced on 1,682 North Dakota farms. This number can be used to represent the number of bean producers in the state.

Dry bean pathologists, breeders and geneticists around the world also will benefit. A better understanding of *U. appendiculatus* virulence diversity and the frequency of virulence to widely utilized resistance genes (particularly *Ur-3*) will allow scientists to assess the risk of future epidemics and determine which gene(s) or genetic location(s) are most likely responsible for resistance to bean rust. The identification of resistance genes effective to the North Dakota bean rust pathogen population, and identification of new sources of resistance that can be utilized in the development of rust resistant varieties will provide a cost effective method to limit yield losses caused by dry bean rust. Additionally, widespread adoption of varieties with effective resistance has the potential to prevent epidemics for long periods of time, as it did in North Dakota for about 10 years starting in the late 1990's.

Lessons Learned

- Infecting bean plants under greenhouse conditions can be challenging, requiring very specific humidity and temperature parameters. As temperature and humidity fluctuated across the seasons, adaptations were required to successfully complete phenotypic evaluations.
- Seed of the set of 12 differential lines needed to complete virulence phenotyping is limited in availability; therefore, seed increases were required for all lines throughout the duration of this project. Producing seed of photosensitive lines PI260418, Mexico235 and PI141996 was particularly challenging, requiring the use of growth chambers set to strict growth conditions. This limited the speed at which the project could progress.
- The collection of infected dry bean samples is restricted to a short time period, from the time rust is first identified in the field until the plants start to senesce. At that point, the teliospore stage is initiated and these spores are not able to re-infect dry beans, resulting in the inability to produce single pustule isolates.
- Genome assembly from RAD-GBS results was challenging due to the lack of a reference genome. *De novo* assembly, as was conducted is this study, allows to conduct very informative genetic analyses; however, availability of a reference genome facilitates more in depth and broad-reaching analyses including tagging regions associated with resistance to dry bean rust to that reference. Unfortunately, the genome of *U. appendiculatus* is extremely large in comparison to other fungal species and a large amount of repetitive DNA sequences exist, making assembly difficult and resource intensive.

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Additional Information

Preliminary research results were presented to the Northarvest Bean Growers, at five professional scientific meetings with abstracts or proceedings published and at two additional venues (see citations below).

Scientific meetings

- Monclova-Santana, C., Markell, S. G. Brueggeman, R. S. Acevedo, M. Sharma Poudel, R. and Pasche, J. S. 2018. Population structure of *Uromyces appendiculatus* in North Dakota. Biology of rust-host interactions and the future of durable disease resistance. A Satalite Meeting of the International Congress of Plant Pathology. July 29, 2018, Boston, MA, USA.

**Ms. Monclova was invited by the scientific committee of the international rust workshop to present this research.*

- Monclova-Santana, C. Markell, S. G., Brueggeman, R. S., Osorno, J. M., Acevedo, M., Sharma-Poudel R., and Pasche, J. S. 2018. *Uromyces appendiculatus* severity in common bean producing counties in North Dakota. (Abstr.) Phytopathology (In Press).
- Monclova-Santana, C., Pasche, J., Markell, S., Acevedo, M., and Osorno, J. 2018. Population structure of the dry bean rust pathogen *Uromyces appendiculatus* in North Dakota. Plant Science Graduate Student Symposium, University of Winnipeg, Manitoba Canada.
- Monclova-Santana, C. Markell, S. G., Acevedo, M., and Pasche, J. S. 2018. *Uromyces appendiculatus* prevalence in dry bean fields in North Dakota. Annu. Rep. Bean Improv. Coop. 61:7-8.

**Ms. Monclova-Santana was awarded the graduate student travel award.*

- Monclova-Santana, C., Pasche, J. S., Markell, S. G., and Acevedo, M. 2017. Dry bean rust (*Uromyces appendiculatus*) survey in North Dakota and evaluation of dry bean advanced breeding lines. (Abstr.) Phytopathology 107:S5.165.

**Ms. Monclova-Santana was awarded second place in the graduate student competition for this oral presentation.*

Additional presentations

- Monclova-Santana, C. and Pasche, J. 2018. Rust: A Dry Bean Disease Story in North Dakota. 3 Minute Thesis Showcase, University of North Dakota, Grand Forks, ND.
- Monclova-Santana, C. and Pasche, J. 2018. Rust: A Dry Bean Disease Story in North Dakota. 3 Minute Thesis Competition, North Dakota State University, Fargo, ND.

**Ms. Monclova-Santana was awarded second place for this oral presentation.*

- Pasche, J. S., and Markell, S. G. Disease and Research Update. Northarvest Bean Growers Bean Day. Jan. 20, 2017; Fargo, ND.

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Identification of Root-Resilience Traits in Dry Bean Genotypes for Increased Productivity

NOGA# 15-335

Final Report

Partner Organization

North Dakota State University

Project Summary

North Dakota is the leading producer of dry bean in the US, accounting for almost 40% of the total production. Unfortunately, seed yields at the farm level fall short of the yield potential observed under optimal conditions. The main cause of this yield gap is abiotic stress (waterlogging, drought, soil fertility, etc.) combined with the presence of numerous diseases affecting different plant parts. The root system is a key component of plant growth and in the case of dry bean, it is generally considered weak or less efficient when compared to other crops grown in the region. Breeding efforts at NDSU devoted to genetically improve the robustness of the root system at early developmental stages should reduce the yield gap currently observed in the region.

Through this project, we were able to identify new bean germplasm that confers resistance/tolerance to the following abiotic/biotic stresses: waterlogging/flooding, Fusarium root rot, and Rhizoctonia root rot. Genetic/genomic analyses also allowed to identify genomic regions associated with these good traits. This genetic material us being currently used in generating new crosses within the breeding program. This project also allowed us to confirm the results from a previously funded SCBGP grant where genotypes were identified as being tolerant to flooding under greenhouse conditions. This new project allowed us to confirm the greenhouse conditions mimic field conditions by producing very highly correlated results. In addition, the results about this project have been shared with the rest of the scientific community as well as farmers and others in the bean industry.

Project Approach

- Genotypes within the Andean Diversity Pool (ADP) and the Middle-American Diversity Pool (MDP) were phenotypically screened for tolerance to root rot pathogens in the greenhouse. The pathogens, *Fusarium* and *Rhizoctonia*, were screened separately. Each experiment consisted of 2 trials with 3 replications and 3 plants per replication in an augmented design. Plants of each line were inoculated with the pathogen and roots were scored 10-14 days post inoculation. Ten MDP, and 5 ADP genotypes demonstrated resistance to *Rhizoctonia*. Eighteen MDP and 15 ADP genotypes were more resistant to *Fusarium* than the industry standard genotype VAX3.

- Waterlogging tolerance was determined for 326 genotypes related to common bean as well as the genotypes within the ADP and MDP. The experiment was a randomized complete block design with a split plot arrangement consisting of 4 replications and 2 plants per replication. All genotypes were evaluated in the greenhouse. Genotypes were germinated. When the primary leaves started to unfold plants were submerged in water to 5 cm above the soil line leaving the leaves above the water. After 10 days of water stress, chlorophyll content (SPAD index) and adventitious rate scores were collected. After drying, whole plant weight, shoot weight, root weight and hypocotyl length were collected. Approximately 15 genotypes were tolerant to flooding, 10 newly identified.

- A subset of 10 flooding tolerant and 10 flooding susceptible genotypes were evaluated in the field using a randomized complete block design with a split plot arrangement and three replications. Flooding was induced in the field using a sprinkler system to ensure soil saturation on the stress plots. Unstressed plots were rainfall fed. Flooding tolerance was evaluated after 10 days of flooding conditions. Results from the greenhouse waterlogging experiments were highly correlated with the results found under field conditions. This confirmed the new greenhouse method for flood tolerance screening adequately represented field conditions and would be a good and quick method to evaluate germplasm before placing the germplasm in the field.

- The published method to screen for plant zinc efficiency was not successful. Various other methods were attempted to screen for plant zinc efficiency including growth in various substrates ranging from sand to hydroponic solutions. No differences were identified within the preliminary set of genotypes screened, suggesting an inadequate screening method. Consultations with various plant physiologist, soil scientists and other experts lead to the discontinuation of this objective.

- A SNP data set was available for both the ADP and MDP genotypes. A Genome-Wide Association Study (GWAS) was undertaken for each trait to identify genomic region affecting root resilience. Rhizoctonia root rot was associated with at least six genomic regions, Fusarium root rot was associated with at least five genomic regions, and flooding tolerance was identified in at least six genomic regions. Results for all three traits are very contrasting between the ADP vs. MDP, suggesting the presence of different mechanisms in these two sets of germplasm due to their genetic divergence. Similar results have been reported for other traits such as disease resistance, abiotic stress, and seed shape and size, among others. One loci on chromosome PV08 was associated with differences in root weight, total weight, shoot weight, hypocotyl length and SPAD index under flooding condition indicating a possible major gene. One candidate gene near this location is a homolog of RAP2.6L which known to improve flooding tolerance in other species by ameliorating hydraulic and oxidative stress. A loci associated with germination under flooding condition was identified on Pv02 and two candidate genes were proposed that are known to affect germination under stress or anaerobic conditions in other species.

Goals and Outcomes Achieved

- The short-term goal was to identify genotypes with resistance/tolerance to root rot pathogens, water logging and low levels of zinc in the soil. The zinc portion of the project was dropped due to previously stated reasons. The goal was accomplished by screening a minimum of 700 genotypes under greenhouse conditions for resistance/tolerance to two pathogens and flooding. The benchmark was 5 genotypes known to have resistance/tolerance to each of the root stresses. Our target was to identify additional genotypes with resistance/tolerance. We successfully identified approximately 58 genotypes with resistance/tolerance to either root rot or flooding. At least two genotypes had resistance/tolerance to both root rot and flooding.

- The second short-term goal was to identify genomic regions associated with resistance/tolerance to root rot and flooding. The benchmark was zero characterized genomic regions for root rot and flooding in dry beans. Our target was one region for each trait. We successfully identified more than one genomic region for each trait. Root rot resistance was associated with numerous regions. Flooding tolerance was associated with numerous regions and includes the identification of 2 candidate genes related to germination rate and 1 gene candidate having a significant impact on whole plant weight, root weight, hypocotyl weight, and SPAD index.

- The long-term goal is the release of a commercial cultivar with multiple root resiliencies. The benchmark is no commercial cultivars exist with effective root resiliencies to multiple biotic and abiotic stresses. Towards this target, a first set of crosses have been made to incorporate the identified resistance/tolerance into germplasm adapted to the region.

Beneficiaries

- Over 2,000 growers in the region produce dry beans. The results from these experiments have been disseminated to these bean growers during the North Harvest Bean Days in 2017 and 2018 as well as during various field days.

- Results have been communicated with over 150 plus scientists working on dry bean through various scientific meetings and publications including meetings of the International Bean Improvement Cooperative and the International Conference on Legume Genetics and Genomics. The long term effect of these results allow the incorporation of these root resilience traits into dry bean breeding programs worldwide.

Lessons Learned

- After experimenting with different methods for screening for Zn-uptake efficiency in the greenhouse and asking some plant physiologists and other experts in the area, we decided not to continue pursuing this objective. For future studies, we will make sure we develop some preliminary data/results to show that the methods to be used are reliable and effective in accomplishing the objective. We were a bit naïve thinking that the available methods were going to work fine with no previous testing before this proposal.

- Field experiments for root resilience characters were difficult to set up for even a small number of genotypes. Greenhouse evaluation is a critical first step to allow selection of the best genotypes for field evaluation, no more than 30 genotypes.

- The large database of genotypic and phenotypic information from this project required additional personnel to process and complete the analysis in a timely manner.

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Additional Information

At least 2 peer reviewed articles have been published (see references below) and results/findings from this project have been shared with ND bean growers at field days and the annual Bean Day. In addition, presentations and posters have been done at several scientific meetings.

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Effect of soybean cyst nematode on root disease of dry bean caused by fungi

Final Report

Project Summary

Soybean cyst nematode (SCN; *Heterodera glycines*) is a new threat to dry bean production in the North Dakota/Minnesota region where there are approximately 700,000 acres of dry bean planted every year. This region produces about 38 % of the US dry beans with a value of around 392 million dollars. SCN is a root pathogen that can seriously damage the growth and seed yield of the plant. The majority of bean varieties grown in the northern production area do not have resistance to SCN. Root rots caused by *Fusarium* species and *Rhizoctonia solani* are common problems in dry bean production and can be difficult to manage. SCN is known to interact with root infecting fungi of soybean resulting in greater damage to the root than caused by the individual pathogens. However, the interaction of SCN with fungal pathogens in dry bean has not been studied. Our research demonstrated that under greenhouse conditions, infection by SCN can cause greater root rot disease in dry bean when plants are growing in the presence of either *F. solani* or *R. solani*. In addition, our research indicated that SCN can reduce genetic resistance to root rot pathogens. Field experiments were established in 2016 and 2017 to determine if there was an interaction of SCN with fungal root rot pathogens in dry bean under field conditions. In 2016 disease pressure was high from *F. solani* and *R. solani* and most plants died before an interaction could be established. In 2017, in a field trial with *Fusarium solani*, there were no significant effects of SCN on disease development or yield parameters when compared to plants only infected with *F. solani*.

Project Approach

Root rot caused by *Fusarium* species and *Rhizoctonia solani* is a major disease of dry bean in North Dakota/Minnesota region. Soybean cyst nematode (SCN; *Heterodera glycines*) is now well established in North Dakota and Minnesota. Dry bean production is concentrated in the counties where SCN is now reported, especially in and near the Red River Valley. SCN spreads easily between fields and because it is a root infecting pathogen, it is not always easy to determine if a crop is infected with this nematode. In soybean, it is well known that SCN can interact with soil borne fungal pathogens by increasing root rot severity, thus complicating the management of this devastating disease. Within the bean classes, kidney beans are highly susceptible to soybean cyst nematode and the effect of this nematode on fungal root rot diseases is still unknown. Since SCN is a new threat in this area, growers have not had to consider SCN a management issue until just recently. Several greenhouse experiments were conducted to measure disease severity in kidney bean varieties challenged with root rot pathogens alone or in the presence of root rot pathogens and SCN. In addition, the effect of SCN on host resistance to root pathogens was tested. Isolates of two root rot fungal pathogens, *F. solani* and *R. solani* AG4, demonstrated to be virulent on dry bean were used in these evaluations. The general approach of this research was to use the moderately root rot resistant bean variety Rosie and the susceptible kidney variety Montcalm to examine the interaction of dry bean, root rot pathogens and SCN. Our research indicated that both Montcalm and Rosie are moderately susceptible to SCN Hg type 0 which is the most prevalent race in this region. The experiments were conducted under controlled conditions in the greenhouse in a water bath to maintain a constant temperature to insure consistent nematode infection and development within roots. Experiments under natural conditions were also conducted in micro-plots located on the

NDSU campus using different levels of SCN in presence and absence of *F. solani*. Inoculum concentration plays very important role in disease development, therefore, we conducted preliminary experiments to optimize the inoculation methodology and inoculum levels of fungal pathogens to obtain disease under our experimental conditions. Once inoculum levels were determined, interaction was studied in the presence of different levels of SCN.

For the Fusarium root rot/SCN interaction greenhouse experiments, each variety was inoculated three levels of SCN (0, 1,000 and 10,000 eggs per 100 cc of soil) and three levels of *F. solani* (0, 1,000 and 100,000 spores per gram of soil). Plants inoculated with the fungal pathogen, but in the absence of SCN, were considered as controls to make comparison with plants growing with both nematode and fungal pathogens. A split plot design was used where Fusarium treatments were whole plots and SCN treatments were subplots. The severity of root rot on 1-7 scale (Bilgi et al. 2008) was evaluated and compared to the controls six weeks after inoculation. The experiment was repeated three times in greenhouse with eight replications in each experiment. In Montcalm, a significant increase ($\alpha=0.05$) in disease severity was observed only at higher inoculum levels of Fusarium with both low and high levels of SCN in comparison to controls. In Rosie, a significant increase in disease severity was observed at low as well as high levels of Fusarium inoculum with low and high levels of SCN inoculum. Therefore, the data (Table 1) suggests that the SCN-Fusarium interaction has a significant impact on disease severity not only for a root rot susceptible variety, but also for a moderately resistant variety. One unusual observation was that plant height was significantly higher in the high Fusarium inoculum level compared to the non-inoculated plants in Rosie, but there was no significant effect of SCN on plant height when Fusarium was present. In Rosie there was no significant effect of SCN on root weight when SCN was present with the Fusarium. No significant differences in plant height or root weight were observed in Montcalm between the Fusarium treatments with and without SCN.

For the Rhizoctonia root rot/SCN interaction experiment, the variety Rosie was inoculated with three levels of SCN (0, 2,000 and 5,000 eggs per 100 cc of soil) and four levels of barley grains (0, 10, 25 and 75 grains per 100 cc of soil) infested with *R. solani* AG4. Plants inoculated with AG 4 alone were considered controls. This experiment was repeated two times in the greenhouse with ten replications each time. The severity of root rot on 1-9 scale (Muyolo et al. 1993) was evaluated and compared to the controls six weeks after of inoculation. A significant increase ($\alpha=0.05$) in root rot severity was observed with both low and high levels of SCN when 25 *Rhizoctonia* infested grains were used and compared to the controls in experiment 1. In experiment 2, a significant increase ($\alpha=0.05$) in root rot severity was observed with both low and high levels of SCN when 75 grains were used and compared to the controls. In neither experiment did SCN cause a significant reduction in plant height or root weight in the *Rhizoctonia* treatments. We did not use AG2-2 in the greenhouse experiments because preliminary experiments showed that AG2-2 was highly pathogenic and killed most plants before SCN would have time to interact with the plant and fungus.

Field experiments were conducted in both 2016 and 2017 with root rot pathogens and SCN. In 2016 the root rot pathogens were *F. solani*, *R. solani* AG4 and AG2-2 and the bean variety was Montcalm. Root rot was severe in 2016 and many plants died within weeks of planting; there were no significant effects of SCN on root rot or plant growth parameters. Therefore, modifications were made in the experimental design and the methods used in 2016 to plan the field experiment the following year. In 2017 the field research (Figure 1) focused only on *F. solani* using a spore inoculation method to infest the soil. A randomized complete block design was used and plants were inoculated with three levels of SCN (0, 1,000 and 5,000 eggs per 100 cc of soil) and two levels of fusarium (0, and 100,000 spores per cc of soil). The following growth parameters were evaluated in kidney bean varieties Rosie and Montcalm: plant height, pod weight, pod number, seed weight, and seed number. Comparisons were made between treatments of *Fusarium* plus SCN and *Fusarium* alone. In Montcalm, pod weight, pod number and seed weight were significantly reduced in the presence of *Fusarium*, but the addition of SCN not have a significant effect on these traits. In Rosie, no significant differences in pod weight, seed weight or seed number were observed between the treatments with *Fusarium* and *Fusarium* plus SCN. This may be due to the moderately resistant nature of Rosie and inoculum levels not sufficiently large enough to overcome the resistance under field conditions.

An article published in the NORTHARVEST BEANGROWER magazine will enhance understanding of SCN and its interaction with fungal pathogens and the risk associated with this disease complex: Soybean Cyst Nematode Threatens Dry Bean production. By Jessie Topp-Becker Vol 23 Issue 5 pp. 18-20. 2017. Another article about soybean cyst nematode was published in 2016: Berlin D. Nelson Jr. Soybean Cyst Nematode - A Threat to dry bean production. Annual Report of the Bean Improvement Cooperative, Vol 59:11.

In the original proposal, a reporting of the research results was to be given at an American Phytopathological Society annual meeting. However, the authors realized there were few significant results at that time and decided to present the results at the Bean Improvement Cooperative Meeting at East Lansing, MI, on October 29-31, 2017. The meeting was attended by dry bean breeders and pathologists, growers, seed dealers, county extension agents, crop consultants and others in the agricultural community who work with dry bean. A poster describing the research was prepared and presented at this meeting. The principal investigators on this project presented the poster and answered questions about the results from meeting attendees. The title of the poster was the following:
Shalu Jain, Periasamy Chitampalam, Juan M Osorno, Julie Pasche, Berlin D Nelson Jr. Interaction of *Fusarium solani* species complex and soybean cyst nematode on root rot severity in dry bean. BIC/NAPIA Meeting, East Lansing, MI, from Oct 29 - Nov 3, 2017. The poster is shown on the last page of this report. An abstract of this research will appear in the Annual Report of the Bean Improvement Cooperative, Vol 60, 2017.

Goals and Outcomes Achieved

In greenhouse experiments, dual infestation of soil with a high level of *F. solani* inoculum and SCN eggs at low or high levels caused severe root necrosis in comparison to high levels of *F. solani* alone in the moderately susceptible variety Montcalm. Results from this study indicate that if sufficient inoculum is present to cause root rot, SCN can increase root rot damage. In the moderately resistant variety Rosie, the addition of SCN to both low and high inoculum levels of *F. solani* spores increased disease severity. The results with Rosie suggest that SCN can reduce, or possibly neutralize genetic resistance to root rot pathogens in dry bean even at low inoculum levels. Further evidence that SCN will increase root rots was indicated by the greenhouse experiments with *Rhizoctonia.* The overall results of this research indicate that infestation of dry bean fields with soybean cyst nematode will potentially increase problems with root rot fungal pathogens. This research will help in understanding the role of SCN in the development of dry bean root rot caused by pathogenic fungi which could lead to better root rot management strategies. Our recommendation to bean growers is to closely monitor their fields for SCN infestation by using soil sampling and sending the samples to a qualified laboratory to determine the SCN egg levels. Keeping the SCN population low should help reduce the potential damage to dry bean from infection by SCN. Most dry bean varieties in the North Dakota-north Minnesota bean growing region have some susceptibility to SCN. Therefore, we also recommend developing and using SCN resistant dry bean varieties to minimize the potential damage from SCN and the interaction with fungal root rot pathogens.

Beneficiaries

The dry bean industry includes growers, crop scouts, agronomists, dry bean breeders, and agricultural scientists working on dry bean problems. Since soybean cyst nematode is a potential problem in North Dakota, Minnesota and Michigan, there are approximately 5,000 persons who will be beneficiaries of this new knowledge. Our research directly benefits bean growers as this project will increase awareness and knowledge of SCN identification, biology and management, plus an understanding of the potential damage to the bean crop in presence of the other root rot pathogens. The economic impact of this research cannot be directly measured since the generation of knowledge will have an impact in the future as more growers encounter fields infested with SCN.

Lessons Learned

Two major conclusions can be drawn from our research experiments. First, there was solid evidence from controlled experiments in the greenhouse that SCN can increase root rot disease of several fungal root rot pathogens and SCN may reduce or cancel genetic root rot resistance in bean varieties. Second, field experiments designed to demonstrate the effect of SCN on root rot fungal pathogens of dry bean were difficult to establish. This is most likely due to the numerous factors affecting the SCN-root rot interaction on the plants that we were unable to control. Further studies on the interaction between SCN and root rot pathogens on a wide range of inoculum levels and environments are needed under field conditions. Understanding how fungal pathogens and SCN interact at the cellular and molecular levels will also be useful in designing management strategies.

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Additional Information

Table 1 and Figure 1 are referenced in the project approach section. A copy of the poster presented at the BIC meeting in October 2017 is included here.

Table 1. Effect of SCN + *F. solani* on root rot of dry bean in a greenhouse experiment.

	Disease Severity	
Treatments	**Montcalm**	**Rosie**
A SCN0+Fus1	3.3	2.3
B SCN1+Fus1	3.8	3.8
C SCN2+Fus1	3.4	5.2
D SCN0+Fus2	2.6	4
E SCN1+Fus2	3.9	3.8
F SCN2+Fus2	4.9	3.8

Contrasts	**Montcalm**	**Rosie**
A vs. B	NS	**
A vs. C	NS	***
D vs. E	**	NS
D vs F	***	NS
B vs. C	NS	*
E vs. F	**	NS

*, **, and *** = significant at P = 0.05, 0.01 and 0.001, respectively. NS = not significant
SCN 0 = no nematode; 1 = low, 2 = high egg number
Fus 1 = low and 2 = high *Fusarium* inoculum level

Figure 1. Field experiment in 2017 consisting of microplots in Fargo, ND.

Poster presented at the BIC/NAPIA meeting on October 29 to November 3, 2017.

Interaction of *Fusarium solani* species complex and Soybean Cyst Nematode on Root Rot Severity in Dry Bean

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NORTH DAKOTA
NORTHARVEST BEANS

Background

- Fusarium root rot caused by members of *Fusarium solani* species complex (FSSC) is a major soil-borne fungal disease of dry edible beans.
- *Fusarium solani* and soybean cyst nematode (*Heterodera glycines*; SCN) are two root pathogens that thrive in analogous environments and can coexist in the North Dakota and Minnesota region, the largest producer of dry beans in USA.
- The crop is threatened by these two pathogens and any interaction between them may increase root rot damage.

Greenhouse Experiments

- Two red kidney bean genotypes, Rosie and Montcalm.
- Three levels (0, 1000, 10^5 spores/gm of sand respectively) of FSSC isolate 91-113-3.
- SCN Hg0 at three levels (0, 1000, 10,000 eggs/plant, respectively).
- Split plot with Fusarium as main plot and SCN as sub plot with eight replications. Plants were grown in greenhouse for 5 weeks at 27º C.

Fusarium root rot in Montcalm (without SCN). Untreated roots (a), roots with low level (1000 spores/gm of sand) of Fusarium infection (b), roots with high level (10^5 spores/gm of sand) of Fusarium infection (c).

Fusarium root rot in Rosie (without SCN). Untreated roots (a), roots with low level (1000 spores/gm of sand) of Fusarium infection (b), roots with high level (10^5 spores/gm of sand) of Fusarium infection (c).

Greenhouse Results

Effect of SCN + FSSC on root rot of dry bean

		Disease Severity¶	
	Treatments	Montcalm	Rosie
A	SCN0+Fus1	3.3	2.3
B	SCN1+Fus1	3.8	3.8
C	SCN2+Fus1	3.4	5.2
D	SCN0+Fus2	2.6	4
E	SCN1+Fus2	3.9	3.8
F	SCN2+Fus2	4.9	3.8

Contrasts	Montcalm	Rosie
A vs. B	NS	**
A vs. C	NS	***
D vs. E	**	NS
D vs F	***	NS
B vs. C	NS	*
E vs. F	**	NS

***, **, and *** = significant at P = 0.05, 0.01 and 0.001, respectively**
NS = not significant. Data from one of three experiments.

Disease severity was determined using a 1-to-7 disease rating scale where 1 = healthy roots with no lesion and 7 = pithy or hollow hypocotyl with very extended lesions, 80-100% root mass reduction, and functionally dead plant (Bilgi et al. 2008).

STATISTICAL ANALYSIS
The three experiments were analyzed separately. Plant height and root weight were subjected to analysis of variance using the Statistical Analysis System (SAS). Fishers protected least significant difference (LSD, P= 0.05) was used as a posteriori multiple comparison test for numeric data. Disease severity was subjected to nonparametric analysis using the LD_CI.sas and F1_LD_F1.sas macros according to the methods outlined by Shah and Madden (1994) for a split plot design. Contrasts were generated for biologically important comparisons of disease severity caused by Fusarium treatments with and without SCN.

Conclusions

Three greenhouse experiments demonstrated an interaction between SCN and Fusarium that increased disease severity in two different dry bean varieties in some, but not all treatments.

The level of Fusarium inoculum appears to be a critical factor. In Montcalm, the interaction was observed at the highest Fusarium level whereas in Rosie it was at the lowest level.

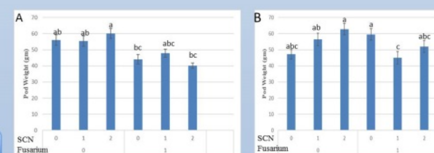
There were no significant effects on plant height or root weight.

Data from field experiments are still being analyzed.

Field Evaluations

- Two red kidney bean genotypes, Rosie and Montcalm
- Two levels (0 and 1) of *F. solani* (isolate 91-113-3) where 0 and 1 are 0 and 10^5 spores/gm of sand respectively.
- SCN Hg0 three levels (0, 1 and 2) where 0, 1 and 2 are 0, 10 and 50 eggs/gm of sand respectively)
- Randomized complete block design with six reps

Field micro-plots at North Dakota State University campus to study the effect of SCN-Fusarium interaction on growth parameters.



Effect of SCN - Fusarium interaction on Pod dry weight (gm) in Montcalm (A) and Rosie (B) variety.

- We are further evaluating other yield parameters from 2017 field data and plan to repeat this experiment in summer 2018.

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Studies on Cold Acclimation of Winter Legumes

Final Report

Project Summary

This project was specific to winter pea. The goal of this project was to increase farm profitability and sustainability by expanding crop rotation options for Midwest growers to include fall-sown winter peas. Fall-sown winter pea offer growers the opportunity to diversify their cropping systems and transfer a portion of their traditional spring field work to the fall. One of the most significant advantages to winter crops is that fields which typically remain wet in the spring can be sown in the fall. These otherwise unsuitable fields can be productive rather than lying fallow or producing suboptimal crops. Growers in the Midwest region have repeatedly expressed interest in having the opportunity to grow fall-sown peas and alleviate the pressure of planting in an increasingly narrow window of opportunity in the spring. Successful field trials in ND in 2009 and 2010 showed that production of winter pea in the Midwest is possible. Success of the 2011 season is based on visual observations of survival in field trials at both Fargo and Minot, ND, where significant differential winter kill among genotypes and within segregating populations was observed.

The specific objectives of this project were to characterize the agronomic and genetic components of winter pea production such that optimum agronomic practices and superior varieties can be developed. Gaining a greater understanding of the genetic basis for winter hardiness coupled with DNA marker technology will provide pea breeders with additional tools and techniques for more efficient selection of improved varieties. Successful completion of this project will provide growers in the Midwest a fall-sown legume to add to their rotations, a set of best management practices for fall-sown legume production, and improved winter pea breeding lines and varieties.

Project Approach

Objective 1: Genetically characterize the key phenotypic and metabolic mechanisms required for winter survival. Two recombinant inbred line (RIL) populations designed for the study of winter hardiness were developed and screened in the field and greenhouse for winter survival. Population 1 was derived from the cross, ’Shawnee’/‘Melrose’ and population 2 was derived from the cross ‘Medora’/’Melrose’. Population 1 and 2 were comprised of 286 and 265 RILs, respectively. Both populations were sown into standing stubble at Fargo and Minot, ND, in replicated trials in small plots comprised of 3 rows 2.1 m long spaced 19 cm apart. A targeted stand density of 12 plants m^{-2} was achieved using a sowing density of 8 plants per linear foot of row which equates to 14 plants m^{-2}. Fall stand counts morphological characters such as leaf size, branching, number of nodes and length of internodes were recorded based on 5 plants from the center row.

Each RIL line was evaluated in controlled conditions in a growth room for photoperiod sensitivity and response to temperature. Individual seeds for each RIL were sown in 10 cm square pots filled with Sunshine LC1 potting medium. Population size prohibits evaluation of all RILs simultaneously; therefore, each population was divided into subsets of 25-30 RILs. Parents of the respective population were included with each set to provide an internal control across sets. Each subset included four replicate pots. Growth room conditions were set at 25/20 °C day/night temperature, 10/14h day/night photoperiod and 50% humidity during the evaluations. Date of first flower was recorded. RILs flowering at the same time or earlier than the spring parent (Medora or Shawnee) were considered to have spring growth habit

Objective 2: Identify DNA markers suitable for marker assisted selection of improved germplasm through genetically mapping the traits identified in Objective 3 on the Pisum genetic map. A genetic map was developed for both Population 1 and 2 using available STMS and SNP markers. DNA was extracted using standard laboratory protocols and PCR was performed using 30 cycles including the appropriate annealing temperature for each specific primer pair. Polyacrylamide gels were used where necessary to distinguish polymorphism based on small band size differences; however, agarose gel electrophoresis was used for polymorphism that could be distinguished with lower resolution. Linkage groups (LG) in the Population 1 and 2 maps were anchored using common loci from previously published maps. Genetic maps will be created using JoinMap 4.0 and QTL analysis were performed using QTL Cartographer 2.5.

Courtney Holdt, a graduate student working on the project, presented a poster presentation titled Genetic Studies of Winter Hardiness in Pea at the Legumes for a Sustainable World: 2nd International Legume Society Conference on October 11, 2016. She also presented an oral presentation at the annual ASA, CSSA, SSSA meeting on November 2, 2016.

Goals and Outcomes Achieved

Objective 1: Genetically characterize the key phenotypic and metabolic mechanisms required for winter survival. Results from Hartley's test for homogeneity proved that analysis of the area under the injury curve (AUIC) values could be combined for both the studies using both mapping populations ('Shawnee'/'Melrose' and 'Medora'/Melrose'). Combined ANOVA proved variation due to sets, genotypes within sets and the run x set x genotype interaction did not have a significant impact on differences in AUIC among lines. Variation due to genotype was significant for both mapping populations.

We observed that survival greater than 75% was achieved for 7% and less than 1% of lines from 'Shawnee'/'Melrose' and 'Medora'/Melrose' populations, respectively, in the 2015-2016 field study. A negative correlation was seen between greenhouse results (AUIC values) and field survival using the 'Shawnee'/'Melrose' (-0.38) and the 'Medora'/'Melrose' populations, respectively. This indicates that the winter hardiness detected by the greenhouse protocol does relate to actual winter hardiness in the field. This is an important result as this shows we can screen for winter hardiness in the greenhouse multiple times per year while field tests can be only done once per year.

Objective 2: Identify DNA markers suitable for marker assisted selection of improved germplasm through genetically mapping the traits identified in Objective 3 on the Pisum genetic map. Results from quantitative trait analysis detected a total of five QTL across three linkage groups. Single QTL for winter hardiness were identified in linkage groups III, II, and 6; and single QTL for field survival were found in linkage groups III and 6 (Figure 1). Results from this study will allow for use of molecular marker-assisted selection to develop pea varieties with improved winter hardiness.

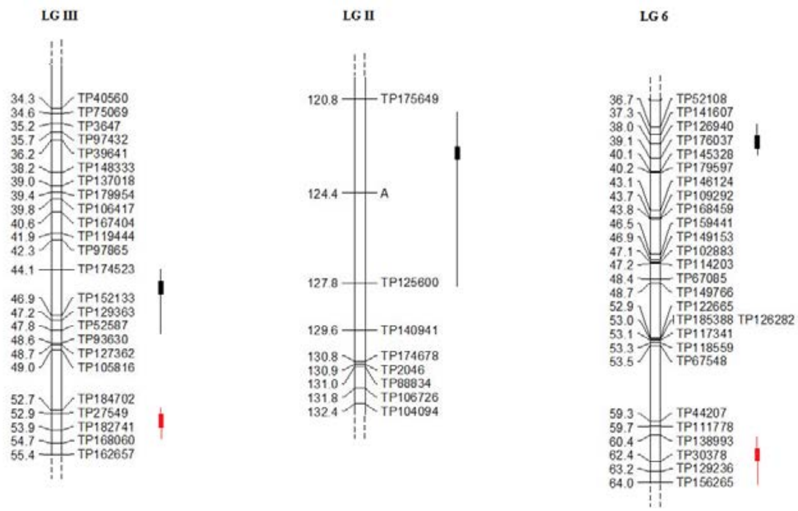

Figure 1. QTL for winter hardiness (black) and field survival (red) identified for the 'Shawnee'/'Melrose' population with Qgene and supported by QTL IciMapping on LG1, LG4 and LG6.

Courtney Holdt presented a poster presentation titled *Genetic Studies of Winter Hardiness in Pea* at the Legumes for a Sustainable World: 2nd International Legume Society Conference on October 11, 2016. She also presented an oral presentation at the annual ASA, CSSA, SSSA meeting on November 2, 2016. The title of the paper she presented was *Evaluation of Winter Hardiness in Pea (Pisum sativum L.).* The abstracts and related citations are listed under the Additional Information section.

Beneficiaries

- A new method of greenhouse screening for identification of winter hardy pea varieties was developed as part of objective 1. This method will allow for testing of winter hardiness by pea breeders to be done multiple times per year vs. only once per year using field testing.
- DNA markers identified as part of objective 2 will allow pea breeders to develop winter pea varieties with improved winter hardiness using marker-assisted selection.
- The overall beneficiary for both objectives will be growers. They will have new winter pea varieties in the future with improved winter hardiness. There are an estimated 600 producers in North Dakota that will benefit.

Lessons Learned

The goals of this project were achieved. Lessons learned included:

- A greenhouse methodology developed to identify consistent differences in freeze injury among lines was validated.
- The correlation between the greenhouse and field survival was -0.38 for the first population and -0.64 for the second population.

- A total of five QTL were identified across three linkage groups. Single QTL for winter hardiness were identified in linkage groups III, II, and 6; and single QTL for field survival were found in linkage groups III and 6.
- Results from this study will allow for use of molecular marker-assisted selection to develop pea varieties with improved winter hardiness.

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Additional Information

1. Genetic Studies of Winter Hardiness in Pea (*Pisum sativum* L.)

Holdt, C. and K. McPhee. 2016. Genetic Studies of Winter Hardiness in Pea. Poster presented at: 2nd International Legume Society Conference, Troia, Portugal. 11-14 October. p.22

ABSTRACT
Production of dry pea has increased in North Dakota and other states in the Great Plains due to their high protein content and ability to symbiotically fix atmospheric nitrogen. Pea production in southern states is typically a fall-sown crop while northern states traditionally grow spring-sown peas. Fall sowing allows the pea crop to avoid high summer temperatures during the bloom period and increase seed production. Inclusion of fall-sown pea in crop rotations would benefit northern states like North Dakota, however; the upper Midwest states experience extreme cold winter temperatures that limit the use of winter peas. This research aims to aid the development of winter hardy peas by developing improved screening methods to identify increased levels of winter hardiness. An RCBD with six and four replicates was used to evaluate 62 germplasm lines and 160 RILs, respectively. Two-week-old seedlings were given a four-week acclimation period at 4°C and subjected to the freezing cycle. The freeze chamber ramped down from 3°C to -8°C at a rate of 1°C/hr and increased at the same rate after a one hour freeze at -8°C. Individual plants were given an injury score every three days during the 21-day recovery period. Injury scores were used to calculate an Area Under the Injury Curve value for each replicate plant. Phenotypic data for winter hardiness will be used to conduct QTL analysis using a SNP based map of the Shawnee/Melrose recombinant inbred line population. Statistically significant differences in injury among lines were detected (P<0.01) among the 62 germplasm lines. Results of the QTL analysis will be presented. Development of improved protocols and molecular tools to detect winter hardiness in controlled environment will increase the productivity of breeding programs focused on winter peas.

2. Evaluation of Winter Hardiness in Pea (*Pisum sativum* L.)

Holdt, C. and K. McPhee. 2016. Evaluation of Winter Hardiness in Pea (*Pisum sativum* L.). Paper presented at: ASA, CSSA, and SSSA Annual Meetings, Phoenix, AZ. 2-6 November.

ABSTRACT
Production of dry pea has increased in North Dakota and other states in the Great Plains due to their high protein content and ability to symbiotically fix atmospheric nitrogen. Pea production in

southern states is typically a fall-sown crop while northern states traditionally grow spring-sown peas. Fall sowing allows the pea crop to avoid high summer temperatures during the bloom period and increase seed production. Inclusion of fall-sown pea in crop rotations would benefit northern states like North Dakota, however; the upper Midwest states experience extreme cold winter temperatures that limit the use of winter peas. This research aims to aid the development of winter hardy peas by developing improved screening methods to identify increased levels of winter hardiness. An RCBD with six and four replicates was used to evaluate 62 germplasm lines and 160 RILs, respectively. Two-week-old seedlings were given a four-week acclimation period at 4°C and subjected to the freezing cycle. The freeze chamber ramped down from 3°C to -8°C at a rate of 1°C/hr and increased at the same rate after a one hour freeze at -8°C. Individual plants were given an injury score every three days during the 21-day recovery period. Injury scores were used to calculate an Area Under the Injury Curve value for each replicate plant. Phenotypic data for winter hardiness will be used to conduct QTL analysis using a SNP based map of the Shawnee/Melrose recombinant inbred line population. Statistically significant differences in injury among lines were detected ($P<0.01$) among the 62 germplasm lines. Results of the QTL analysis will be presented. Development of improved protocols and molecular tools to detect winter hardiness in controlled environment will increase the productivity of breeding programs focused on winter peas.

Project Title: **Towards the Advancement of Genetic Resources for Dry Edible Bean Resistance to Common Bacterial Blight**

NOGA# **15-338**

Final Report

Partner Organization

North Dakota State University

Project Summary

North Dakota dry bean production has an average value of nearly $325 million/year (USDA-NASS 2014). The 640,000 acres of dry beans planted in 2014 represented more than double that of the planted acres in the second highest bean producing state, Michigan. Over the last more than a decade, on average, North Dakota has produced greater than 35% of the total US dry beans. Common bacterial blight (CBB) of dry bean can result in up to 40% yield reductions due to loss of photosynthetic area. However, CBB severity does not reach high levels which limit seed yield and quality in all fields. North Dakota dry bean growers typically do not rank CBB among the most important diseases (Knodel et al, 2012 to 2017); however, CBB was the most commonly observed disease (60 to >90%) in NDSU surveys of over 155 North Dakota dry bean fields conducted from 2013 to 2017. While White Mold is generally considered by growers as the most damaging disease of dry beans in North Dakota (Knodel et al, 2012 to 2017), it was observed in 40 to 50% of the same surveyed fields. We believe this may be due, in some cases, to the generally even distribution of CBB and what may appear as moderate to low level of infection that are not alarming to growers; however, these levels of infection may still limit yield and seed quality. The use of certified seed can reduce CBB severity, but measures to manage disease progression have minimal efficacy after it is established in a field. Varieties vary in resistance to CBB, but few commonly grown varieties have ample resistance. Preliminary genotypic and phenotypic data indicate that resistance genes not linked to the three major markers (SAP6, SU91 and BC420) used for marker assisted selection may be present in NDSU breeding material. The goal of this research was to use genome wide association mapping to determine the presence of previously described and undescribed CBB resistance loci in NDSU breeding material from dry bean market classes grown in North Dakota. Additionally, releasing varieties with increased CBB resistance is an important goal of the NDSU dry bean breeding project. The project described here aids in advancing this objective.

This project detected previously described and undescribed CBB resistance loci in NDSU breeding lines. A unique feature of this project is the genotyping database was generated for the NDSU breeding lines. This database was used in this project to associate markers with CBB resistance loci and identify the presence of the loci in each of the screened breeding lines. In the future, this database can be used to identify and track the genetic background of breeding lines adapted to North Dakota growing conditions. The database also can be, and currently is being, used to associate markers with other traits of interest including other disease resistance loci, quality traits, and agronomic traits. In the future, genomic areas of interest can be converted into markers easily incorporated into the breeding program for use in marker assisted selection. This is advantageous for the NDSU breeder since the markers identified are representative of their breeding material and will accelerate the development of CBB resistant cultivars. We do not know of another dry bean breeding program in the US with this level of genomic information available to them.

Project Approach

The research outlined below was performed during this granting period as outlined in the proposal 'Workplan'.

Dry bean lines from the NDSU breeding program were grown in a large growth room at 26°C degrees under a 16 hour photoperiod with 70% humidity. Three replicates and two samples per replicate were used to evaluate lines for reaction to CBB at both the monofoliate and trifoliate growth stage. The abaxial leaf surface was inoculated at 10 (monofoliate) and 21 (trifoliate) days post-planting using an air brush sprayer (O'Boyle et al., 2007; Singh and Munoz, 1999; Tryphone et al., 2012). The bacterial inoculum was prepared from 2 to 3 day old cultures diluted to 1 x 10^7 CFU/ml (Duncan et al., 2012) in a 12.5 mM potassium phosphate buffer (pH 7.1) (Mutlu et al., 2008). Disease reactions were evaluated 14 days post-inoculation (dpi) using a 1 to 9 scale (Aggour et al., 1989) where 1 is no visible reaction and 9 is highly susceptible (Table 2; Figure 1). Lack of emergence and other plant growth irregularities resulted in incomplete phenotypic data for some lines (Tables 2 & 3). Disease reactions were averaged across replicates and histograms were generated with JMP Genomics. Lines were classified as resistant (≤3), moderate (3.1 to 5.5), and susceptible (>5.5) at both growth stages.

Phenotypic CBB reaction evaluated at both the mono- and trifoliate growth stages under controlled conditions ranged from resistant to highly susceptible for dry bean breeding lines provided by Dr. Juan Osorno (Table 1; Figure 1). Overall, mean disease severity of breeding lines belonging to the Andean genetic background at the monofoliate (3.4) and trifoliate (3.5) stages were similar (Figure 2). Breeding lines belonging to the Middle American gene pool also generally displayed similar levels of monofoliate resistance as trifoliate resistance with a score mean of 4.5 and 4.5, respectively. A similar pattern was observed when the lines were separated by race and market class (Figure 2) except for the small red market class which displayed an average resistance score of 2.6 for monofoliate and 3.5 for trifoliate screening. In all market classes, some individual lines were classified in different reaction categories based on growth stage. Lines were identified from most market classes with CBB resistance; however, relatively fewer lines from the navy and black market classes tended to have CBB resistance. Results from phenotypic evaluations will be shared with the breeding program to enable crosses made with CBB resistance in mind.

Phenotypic data also was used in genome wide association mapping to identify regions corresponding to CBB resistance. DNA was extracted, sequencing libraries were generated and libraries were sequenced. Sequences from lines from nine market classes in various stages of varietal development were analyzed and single nucleotide polymorphisms (SNPs) were identified using several bioinformatics tools (Table 1).

Table 1. Gene pool, race, market class and stage of development of varieties and NDSU breeding lines evaluated for CBB resistance and sequencing.

Gene pool / Race	Variety	Advanced	Preliminary	Total
	Developmental stage			
Race Durango	16	71	344	431
Great Northern	6	19	78	103
Pink	1	4	24	29
Pinto	5	31	170	206
Small red	4	17	72	93
Race Mesoamerican	4	62	216	282
Black	4	42	140	186
Navy	0	20	76	96
Total Middle American	20	133	560	713
Race Nueva Granada	9	4	126	139
Dark Red Kidney	4	0	61	65
Light Red Kidney	4	4	45	53
White Kidney	1	0	20	21
Total Andean	9	4	126	139

Figure 1. Disease reaction rating scale (1 to 9) based on % of leaf area affected for CBB in dry bean used to identify the resistant and susceptible phenotypes of breeding lines.

Table 2. Common bacterial blight reaction by market class of breeding lines evaluated at the monofoliate stage under controlled conditions. Lines inoculated were classified as resistant (≤3), moderate (3.1 to 5.5), and susceptible (>5.5).

	Resistant	Moderate	Susceptible	*Missing data
Durango	131	206	62	32
Great Northern	15	67	17	4
Pink	6	21	1	1
Pinto	34	107	42	23
Red	76	11	2	4
Mesoamerican	29	138	98	17
Black	26	104	47	9
Navy	3	34	51	8
Middle American	160	344	160	49
Nueva Granada	62	75	2	0
Dark Red Kidney	38	26	1	0
Light Red Kidney	16	37	0	0
White Kidney	8	12	1	0
Andean	62	75	2	0

*Phenotypic data from lines with less than 2 plants were not included in the analyses.

Table 3. Common bacterial blight reaction by market class of breeding lines evaluated at the trifoliate stage under controlled conditions. Lines inoculated were classified as resistant (≤3), moderate (3.1 to 5.5), and susceptible (>5.5).

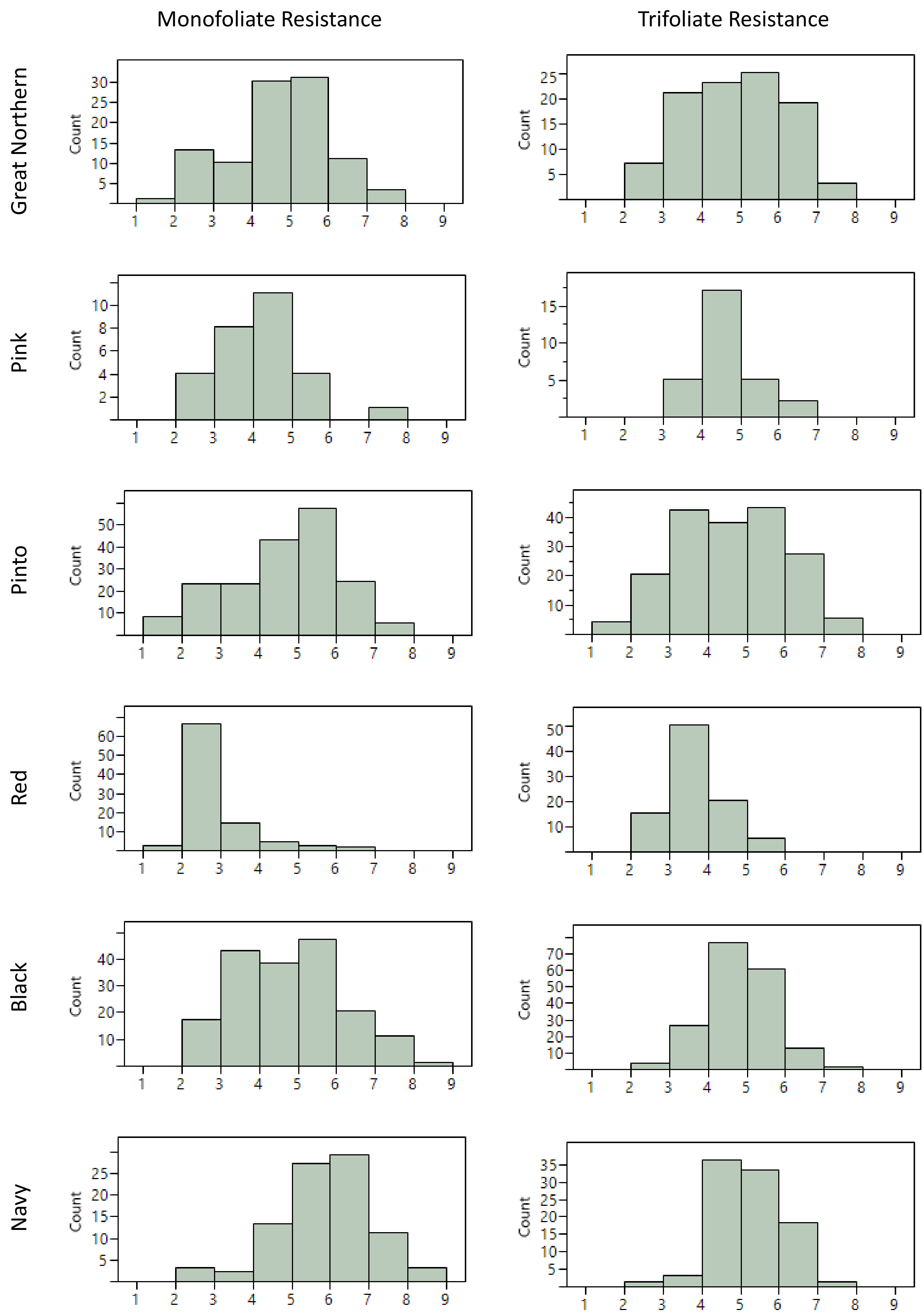
	Resistant	Moderate	Susceptible	*Missing data
Durango	78	231	87	35
Great Northern	14	50	34	5
Pink	1	25	3	0
Pinto	29	101	49	27
Red	34	55	1	3
Mesoamerican	11	211	48	12
Black	9	149	20	8
Navy	2	62	28	4
Middle American	89	442	135	47
Nueva Granada	45	94	0	0
Dark Red Kidney	17	48	0	0
Light Red Kidney	22	31	0	0
White Kidney	6	15	0	0
Andean	45	94	0	0

*Phenotypic data from lines with less than 2 plants were not included in the analyses.

One primary genomic region was highly associated with CBB resistance in lines from the Andean gene pool based on results from genome wide association mapping. Association mapping of the Middle American gene pool data indicated multiple genomic regions were highly associated with CBB resistance. Evaluation of four models (general linear, effect of population structure, effect of individual relatedness, and effect of both population structure and individual relatedness) produced varied results. The best model for monofoliate resistance in the both gene pools, according to MSD values, was the model reflecting both population structure and individual relatedness and the model incorporating only individual relatedness for trifoliate resistance. Each trait in both gene pool data sets had four to six highly associated SNPs, all located on chromosome 10 near the 41.7 Mb region. The strongly associated SNPs from the Andean data set were all on chromosome 10. A lower p-value threshold (0.005) identified additional regions in the Andean data set that may contain candidate genes for trifoliate CBB resistance including regions on chromosome 3, 4, 5, 6, 8, 10 and 11 and chromosomes 7 and 11 for monofoliate CBB resistance (Figure 3). The strongly associated SNPs from the Middle American data set were on chromosome 3, 4, 6, 7, 9, 10 and 11 for monofoliate resistance and chromosomes 4, 5, 7, 8, 9, 10, and 11 for trifoliate resistance (Figure 4).

Several SNPs identified in this study strongly associated with trifoliate resistance in the Middle American population are co-located with SU91 on chromosome 8 at 62.8 Mb. SNPs located between 41 and 42 Mb identified as being highly associated with CBB resistance in both the Andean and Middle American populations for both monofoliate and trifoliate resistance coincide with previously described marker SAP6 on chromosome 10 at 41.0 Mb. No SNPs identified with CBB resistance in either population are co-located with previously described marker BC420 on chromosome 7 at 17.0 Mb. Phenotyping and genotyping data were compiled into a single electronic location for accessible use in further projects. Data were analyzed using several statistical packages including JMP Genomics and GAPIT in R.

This work did not directly benefit commodities other than specialty crops.



Monofoliate Resistance
Trifoliate Resistance
Great Northern
Pink
Pinto
Red
Black
Navy
Count

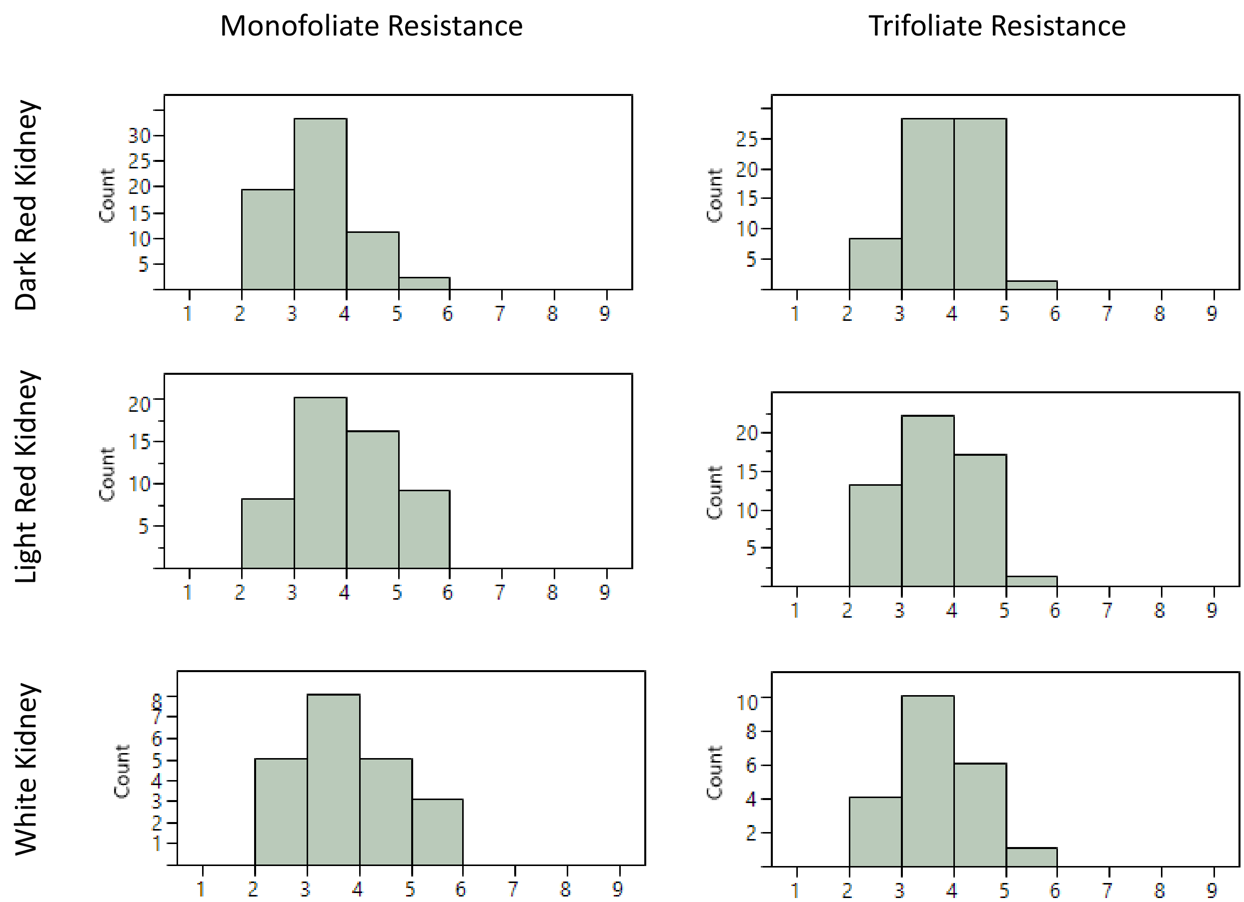


Figure 2. Lines from nine market classes in the NDSU breeding program were evaluated for phenotypic reaction to common bacterial blight at both monofoliate and trifoliate plant stages.

<u>Goals and Outcomes Achieved</u>

The overall outcome of this proposal was the generation of genotypic data for 852 lines from the NDSU breeding program. This resource is not only valuable for the identification of resistance to CBB, but also can be used to identify genetic regions for other traits of interest (agronomic and disease / pest / stress resistance) and markers associated with these traits. This will aid in marker assisted selection and integration of desirable traits. Additionally, this will and reduce resources needed for greenhouse and field evaluations for each breeding generation.

The performance goals and measureable outcomes were completed as outlined in the proposal. The goal of this research was to use genome wide association mapping to determine the presence of previously described and undescribed CBB resistance loci in NDSU breeding material from dry bean market classes grown in North Dakota. These goals were accomplished through the CBB phenotyping and sequencing 852 lines from the NDSU breeding program. The performance measures outlined in the proposal were to select 800 advanced and preliminary lines from the NDSU breeding program (**benchmark = 0, target >800**); genotype (sequence) 700 selected advanced and preliminary lines from the breeding program (**benchmark = 0, target > 700**); produce greater than 30,000 genetic markers, (**benchmark =3, target > 30,000**); and identify additional CBB resistance loci **(benchmark 3, target = 4)** present within the breeding program.

No genotypic information was available for lines in the NDSU breeding program and no comprehensive CBB phenotyping had been undertaken prior to initiating this research project. During this project, 852 NDSU breeding lines were phenotyped for CBB reaction. The CBB resistance level ranged from resistant to highly susceptible in the breeding lines of the nine market classes (Figure 1 and 2). The 852 lines were genotyped, leading the identification of 30,438 SNPs identified for lines in the Andean gene pool (mainly kidney beans) and 41,998 SNPs identified for lines in the Middle American gene pool (pinto, navy, black, great northern, red and pink). The SNP marker data set can be further exploited by the breeding program to identify SNP markers associated with other traits of interest including disease resistance to other pathogens. Dry bean rust and soybean cyst nematode phenotypic evaluation of many of these same lines has already commenced and the genotypic information generated in the current project will allow genome wide association mapping to be conducted for resistance to these diseases and pests.

The SNP data set allowed the association of CBB resistance to SNP markers with a known chromosomal location. The primary region associated with CBB resistance in the Andean gene pool was near the same chromosomal location as the SSR marker SAP6 on chromosome 10. Other resistance loci may be found on chromosomes 3, 4, 5, 6, 8, 10, and 11, but these loci need further validation as they were not as strongly associated with resistance (Figure 3).

We have confirmed the association of resistance with SNPs near the chromosomal locations of SAP6 and SU91 in the Middle American gene pool. We did not identify any SNPs associated with CBB resistance near the chromosomal location of BC420, the third marker commonly used in MAS. However, we did identify multiple additional SNP markers associated with resistance in the Middle American gene pool on chromosomes 3, 4, 5, 6, 7, 8, 9, 10, and 11 (Figure 4). A total of 11 chromosomal loci were associated with CBB resistance in the NDSU breeding program, far exceeding the targeted identification of 4 loci. The new SNP markers will undergo validation and conversion to easily scored markers useful for MAS for CBB resistance.

Beneficiaries

There are over 1500 dry bean producers in North Dakota and nearly 500 in Minnesota that are members of the Northarvest Bean Growers Association that could benefit from this project. The NDSU breeding program directly serves these growers by breeding for dry beans in market classes important to both states and, as such, this research on improving genetic resources will benefit growers in both states. The research proposed here is not a continuation of another project, nor has it been submitted for another Federal or State grant program.

Lessons Learned

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Additional Information

Research results were presented to the Northarvest Bean Growers and two professional scientific meetings (see citations below).

- Simons KJ, Lamppa RS, McClean PE, Osorno JM, and Pasche JS. SNPs Identified for CBB Resistance in Dry Bean. Presented at: Bean Improvement Cooperative. October 29 to 31, 2017. East Lansing, MI.

- Simons KJ, Lamppa RS, McClean PE, Osorno JM, and Pasche JS. New SNP Associated with Common Bacterial Blight Resistance in Dry Edible Bean Breeding Lines. Presented at: International Conference on Legume Genetics and Genomics. September 18 to 22, 2017. Siofok, Hungary.

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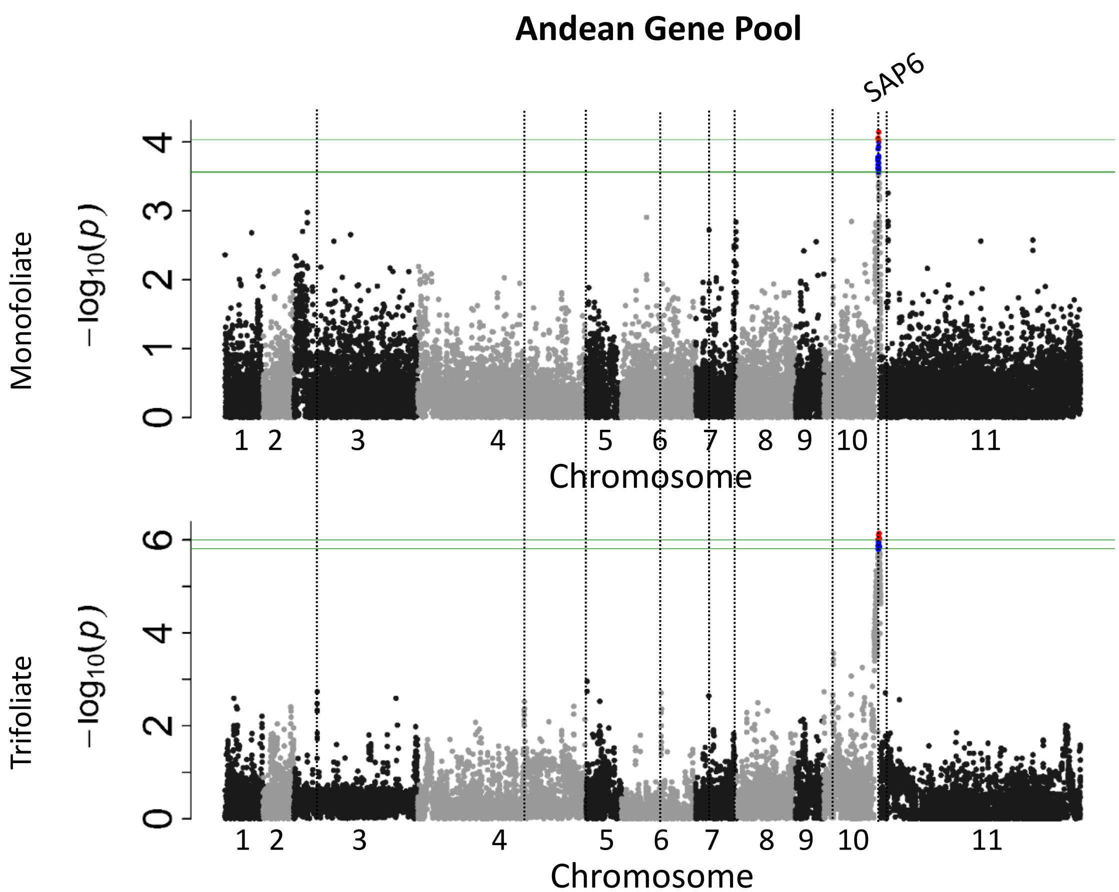

Figure 3. Manhattan plot showing CBB resistance a set locations across each of the 11 chromosomes found in dry bean Andean gene pool. The most significantly associated region with CBB resistance is found on chromosome 10 which encompasses the SAP6 SSR marker. Several other regions on chromosomes 3, 4, 5, 6, 7, 8, 10, and 11 are weakly associated with resistance and require further validation.

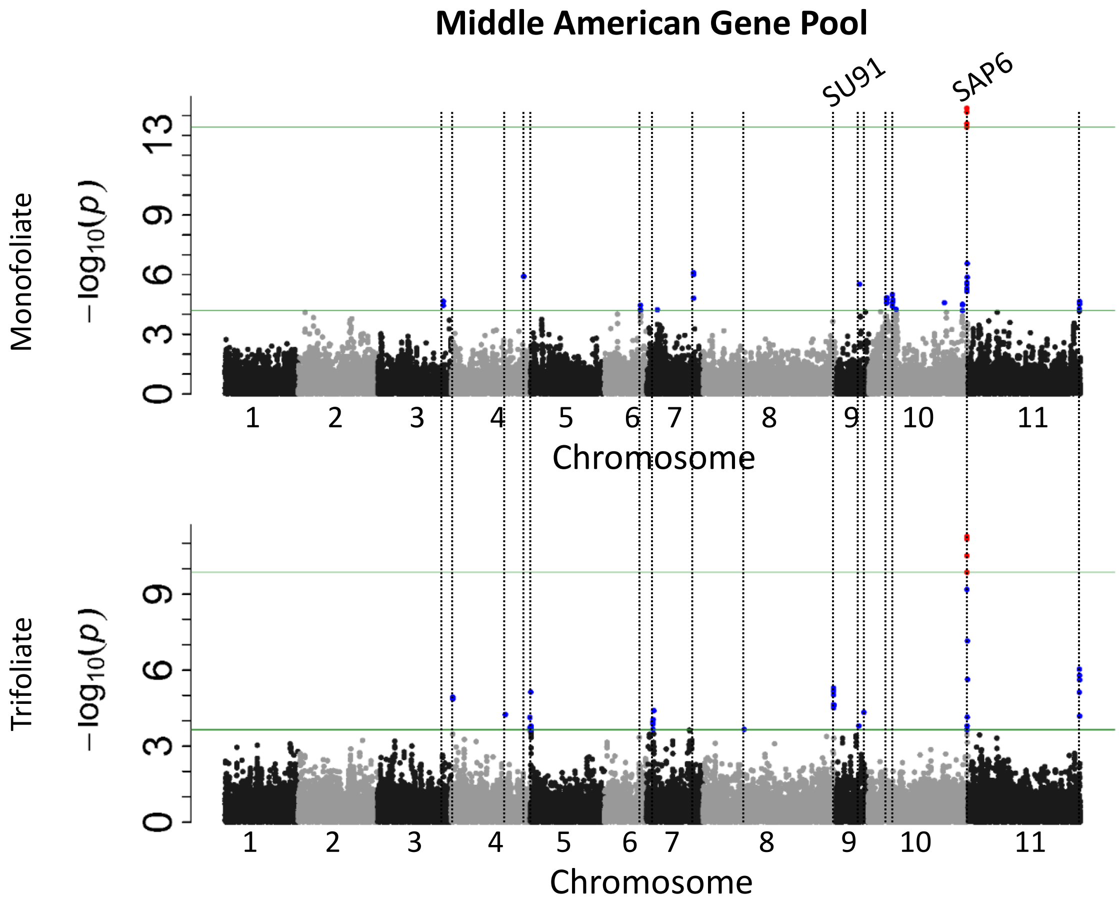

Figure 4. Manhattan plot showing CBB resistance a set locations across each of the 11 chromosomes found in the dry bean Middle American gene pool. The most significantly associated region with CBB resistance is found on chromosome 10 which encompasses the SAP6 SSR marker. Also identified on chromosome 8 was a region encompassing the SU91 marker. The remaining associated markers are found on chromosomes 3, 4, 5, 6, 7, 9, 10, and 11.

Project Title
Expanding local crop opportunities in North Dakota through season extension using high tunnels.

NOGA#
15-339

Final Report

Partner Organization

Project Summary

- North Dakota's growing season is short. High tunnels extend the season by adding protection from the late spring and early fall frosts. High tunnels are also used to protect the crop from detrimental weather events and can help moderate daily temperature fluctuations. High tunnels are a relatively inexpensive alternative to traditional greenhouses; semi-permanent and easy to install; and do not require electricity, although they can have varying degrees of automation depending on individual grower needs.
- High tunnels are used within the state, however, there is a lack of published research on North Dakota high tunnel production, nor is there a comparable source of information from other states as ND's climate varies widely. Aside from conducting publishable research specific to North Dakota's growing conditions, a separate goal of this project was to create a public listserv specifically for ND high tunnel growers. As a result, an NDSU delegated listserv was created as well as a public Facebook page in which users can post pictures, share ideas and ask questions.
- This project was not built on a previously funded SCBGP fund, however, research on high tunnels will continue to be done in the tunnels that were built for this project.

Project Approach

- During the entirety of this grant period from beginning to end, two high tunnels were constructed on the west and east sides of the state; traditional and non-traditional crops were grown in both tunnels; a Facebook group and listserv forum specifically for North Dakota high tunnel growers was created as part of the community of practice revolving around this project; and finally, extending the season in North Dakota with the use of high tunnel technology was showcased as a result of this project. Another favorable development that began as a result of this project was a webinar series which focused on high tunnel-specific issues. The webinars were hosted by team member NDSU Extension Horticulturalist Dr. Esther McGinnis and aired from November until April. The videos were recorded and archived online, and to date they have had 650 participants and/or views.
- This project did not benefit commodities outside of the specialty crop block entities.
- The original partners on this project were the Williston Research Extension Center, the

Plant Science department at NDSU and a subcontract with Dakota College at Bottineau. These three groups represented three vastly different locations around the state. However, after the first season, the contributions from Dakota College at Bottineau were less than satisfactory and thus the subaward was terminated. The project carried on in the second season utilizing only two locations, Williston and Fargo. The teams from both Williston and Fargo worked together to complete the goals and objectives of this project. Both teams contributed equally in the overall accomplishments and completion of this project.

Goals and Outcomes Achieved

- The expected measurable outcomes that have been achieved have been the education of 75 specialty crop producers on high tunnel recommendations as well as to have 100 members involved in the community of practice surrounding this grant project which includes the Facebook page and email listserv. In total there are 156 subscribers to the NDSU high tunnel listserv and 156 members on the North Dakota High Tunnels Facebook page.
- The third expected measurable outcome was to have 50 specialty crop stakeholders adopt vegetable and cut flower recommendations from the results of this project. As researchers, we feel that it is not practical to make recommendations for growers based on only two years' worth of data. Instead, growers have been given information on which crops to grow and management practices pertaining to high tunnels in general rather than specific cultivar recommendations.
- Surveys were distributed to the members of the North Dakota Farmers Market and Growers Association at their annual meeting in both 2016 and 2018. Thirty-seven participants took the needs assessment survey on high tunnel production and the survey results indicated a need for more research on the following areas: disease management, soil fertility and nutrition, high tunnel ventilation, crop rotation, and marketing.

Beneficiaries

- Specialty crop groups that benefited from this project and the continuation of further research include North Dakota vegetable and fruit producers that may participate in their local Community Supported Agriculture (CSA), farmers markets and farm to table operations.
- It is estimated that over 700 direct, in-person interactions were achieved because of field days, workshops, private tours and oral presentations. It is also estimated that approximately 700 electronic interactions were achieved due to the online webinars given throughout the duration of this project.
- In addition to public tours and online webinars, curiosity has also generated a lot of interest and has prompted various people to stop by and inquire about the project. This project has been the feature of articles written in the *Sidney Herald*, *Bismarck Tribune*, *Fargo Forum* and the *Williston Herald*. The articles have also been picked up by various website news such as an international website, HortiDaily, the Morning Ag Clips and was also ran by other news outlets such as the Ag Round Up, Farm and Ranch Guide and The Prairie Star.
- Specific interactions:
  - 2016 Construction Field Day. April 29th, 2016. Absaraka, ND. 40 participants learned and participated with hands on experience building a high tunnel.
  - 2016 Field Days-

        - August 17th, 2016. Absaraka, ND 110 participants. High tunnel project presented by grad student and researchers, Dr. Hatterman-Valenti and Dr. McGinnis.
        - July 15, 2016. Nesson Valley (Ray, ND) 46 participants. High tunnel project presented by grad students, Kyla Splichal and Jacob Kluza as well as researcher, Dr. McGinnis.
- 2017 Field Days-
        - August 9th, 2017. Absaraka, ND 70 participants (inclement weather reduced attendance). High tunnel research presented by grad student Jacob Kluza and researcher Dr. Hatterman-Valenti
        - July 14, 2017. Nesson Valley 90 participants. High tunnel research presented by grad student, Kyla Splichal.
        - July 25th, 2017. Yard and Garden Open house field day. Grad Student Jacob Kluza presented high tunnel cut flower production to 15 attendees.
- Private Tours-
    - North Dakota Water Education Foundation. July 20, 2016. Kyla Splichal gave oral presentation to 20 participants on Irrigation Tour.
    - Private tour in Alamo, ND August 9th, 2016. Nesson Valley-Kyla Splichal was invited to join a private high tunnel tour and present information about NDSU High Tunnel research.
    - Ray Garden Club. July 6th, 2017. Nesson Valley-Grad student, Kyla Splichal gave tour in high tunnel. 25 attendees were allowed to look at vegetables and cut flowers.
    - Leadership Williston. April 11th, 2018. Nesson Valley-Kyla Splichal gave oral presentation on high tunnel production and season extension to 15 attendees. Leadership Williston is a partnership with the Chamber of Commerce's Partners in Education and Leadership Committee to "Train and Empower Williston's Future Leaders."
- Workshops:
    - NDSU Workshops-Fargo, ND.
        - March 24-25th, 2017. Grad Student Kyla Splichal a part of a high tunnel construction panel for question and answer. Also gave a presentation about the NDSU high tunnel vegetable trials. Approximately 85 in attendance both days.
        - April 28th 2018. Researcher Dr. Hatterman-Valenti presented NDSU high tunnel results with vegetables and cut flowers to approximately 70 participants.
    - American Society of Horticulture Science annual meeting. Waikaloa, HI. September 17-22nd, 2017. Both grad students orally presented their respective research projects, high tunnel vegetable production and cut flowers. Attendance of each were 15 and 20 respectively. Researcher Dr. Hatterman-Valenti presented on infrared heat strips to extend the spring and fall season even further in a high tunnel..
    - MonDak Ag Summit. November 15th, 2017. Kyla Splichal presented on high tunnel vegetable production to 25 area producers and interested

attendees from the Montana, North Dakota region in Sidney, MT.
    - Building Local Food Markets Season Extensions event. Dickinson, ND. April 7th, 2018. Kyla Splichal presented NDSU vegetable high tunnel production to 27 attendees.
- Webinars:
    - High Tunnel webinar series hosted by Dr. Esther McGinnis which consisted of 7 online webinars that ran from November 2017 to April 2018. All topics were high tunnel specific. Total participants was 648 at the conclusion of the series.
    - Field to Fork webinar series-February 21st, 2018. Kyla Splichal presented "A Snapshop of NDSU's High Tunnel Research Project." With 63 live attendees at the time of presentation. Presentation is recorded and available to the public at any time.

Survey results distributed at the North Dakota Farmers Market and Growers association annual meetings in 2016 and 2018 indicated the need for more research on the following topics: disease management, soil fertility and nutrition, high tunnel ventilation, crop rotation, and marketing.
A survey given at the Season Extension Event in Dickinson, ND (April 7th 2018) indicated more research needed on organic versus conventional production, produce safety training, conservation practices, the Community Supported Agriculture CSA business model, produce pricing and market demand.

Lessons Learned

- Insect pests and disease pathogens can be a major issue in high tunnels if not dealt with immediately. Under field conditions, pest populations can be more readily controlled via natural predators and other weather-related events. Under the protection of a high tunnel, the environment that is created under the plastic is extremely conducive to rapid insect reproduction and thus an explosion of pest populations in a short amount of time.
- Diseases can also thrive under high tunnel conditions. If all three components of the disease triangle (host, pathogen, environment) are present an outbreak will occur. A lesson learned in both disease and insect issues is the importance of weekly, if not daily scouting in the high tunnel. Diligently looking for insects and pests on a regular basis will save an enormous amount of time and economic loss in the end.

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Additional Information

- N/A

Project Title
Dry Bean Cultivars' Response to Rhizobium Inoculation for Yield, Protein Content and Nitrogen Fixation Potential

NOGA#: 15-340

Final Report

Partner Organization
North Dakota State University

Project Summary

- ***Purpose of the project***

  Inoculation of dry bean (*Phaseolus vulgaris* L.) seeds with nodulating bacteria (*Rhizobium phaseoli*) is not a common practice in North Dakota and Minnesota. Growers are reluctant to use inoculant because application of common peat- or granular-based medium pose a problem for the air-seeder as they clog the pores of the seeder. Application of a liquid inoculant is compatible with the air-seeder. Increasing biological nitrogen fixation (BNF) potential of dry bean using compatible cultivars and suitable inoculation medium has potential to increase the production, grain quality and economic profitability by reducing the dependency on chemical fertilizer.

- ***Importance and timeliness of the project***

  Cost of chemical fertilizer is a significant input in production systems. During 1998-2014, the cost of urea increased from $181 to $537 per metric ton. Considering the modest cost of inoculation (approximately $2-$3/acre) compared to the potential benefits in yield and N inputs to a cropping system, producers are well advised to seriously consider inoculation of their legume crops in all circumstances. However, growers are not adopting the inoculation in their management practice mainly due to the inconvenience in application of inoculant carrier on seed. Current developments in inoculation carrier allow farmer to apply inoculant medium in furrow with an extender. This study will determine the success of in furrow inoculant application on dry bean BNF potential. Moreover, dry bean genotypes differ significantly in their symbiotic association with *Rhizobium*, growth, seed yield and other yield components. Screening of cultivars to find out the dry bean cultivars with higher and rhizobium strain will encourage the adoption of inoculation in practice. With increasing cost of commercial nitrogenous fertilizers, utilization of inoculants to increase the biological N fixation potential of dry bean could reduce the production cost and increase economic profitability. This project will also provide preliminary/baseline data to compare and quantify the amount of N fixed by different dry bean cultivars. Selection of N-efficient cultivar will further reduce the cost of N-fertilizer for the following crop as well.

Project Approach

➢ ***Work Plan***

This on-farm field experiment was conducted with four cultivars of each Pinto, Black, and Kidney beans with and without BNF inoculants. The experiment was laid out in a split plot randomized complete block design with inoculation as main plot and cultivars as subplot factor with four replications. Each plot consisted of 4 rows of 12 ft. length. Initial soil samples were collected in order to quantify for nitrogen, phosphorus, and potassium levels. No fertilization was done. Dry bean seeds were treated with different types of inoculation. Chemicals were applied to protect the crop from pathogens, insects, and weeds. During the growing season, plant subsamples were collected from the border rows at three growth stages, (i) V_3 stage or third trifoliate (ii) R_2 stage or late flowering. At harvest, the middle two rows were harvested for yield and quality parameters. In addition, above and below ground (root) sampling was done to determine biomass and nodule count and weight. After harvest, surface soil samples were analyzed for nitrogen content to estimate the nitrogen additions from BNF. The data was analyzed using SAS 9.4 statistical software and research outcomes were presented in symposium and meetings.

Goals and Outcomes Achieved

➢ ***Project activities***

- A field research was conducted to evaluate the response of dry bean cultivars to two different types (peat and liquid) of commercial *Rhizobium* inoculants for improved nitrogen fixation potential and yield, at Prosper, ND in 2016.
- Plots were laid out following a split plot randomized complete block design, where inoculation was the main plot treatment and the cultivars were used as sub plot treatment.
- Plants samples were collected at V_3 and R_2 stage. Plant samples were prepared following their standard protocol and sent to UC Davis for ^{15}N analysis. %Ndfa (percent N derived from atmosphere) were calculated and the amount of N_2-fixation were estimated.

➢ ***Research outcomes***

- No significant differences in %Ndfa were found among the market classes or the cultivars among the market classes. Pinto showed highest %Ndfa at both the growth stages; 76% in V_3 stage and 82% in R_2 stage (Table 1). Among cultivars, Wind breaker (84%) and Montcalm (92%) showed highest %Ndfa at V_3 and R_2 stage respectively.
- Significant differences in amount of N_2 fixed were found among the market classes. Black fixed highest amount of N_2 (113 kg N ha^{-1}), followed by black, pinto and kidney. No significant variations were found among the inoculation treatments or cultivars among the market classes.

- Significant variations were found among inoculation types and among the market classes. Inoculation could not increase bean yield significantly. Pinto (1321 kg ha^{-1}) had the highest bean yield followed by navy (1113 kg ha^{-1}), black (1093 kg ha^{-1}) and kidney (671 kg ha^{-1}).

➢ ***Research presentations***

- Research outcomes were presented as an oral presentation titled 'Biological Nitrogen Fixation in Dry bean cultivars inoculated with *Rhizobia*' in the annual ASA-CSSA-SSSA meeting, Tampa, 2017
- An original research manuscript titled 'Determining Symbiotic Nitrogen Fixation in Dry Bean Cultivars using Ureide Method and Isotope Dilution Techniques' was submitted to the peer reviewed journal 'Archives of Agronomy and Soil Science' for publication
- The research outcomes were also presented as a poster at 'SNRS Symposium, NDSU' in December 2016

Beneficiaries

➢ Dry bean growers of four major market classes, Pinto, Navy, Black, and Kidney of any region in United States will be benefitted from the project outcomes. Approximately 2000 dry bean growers of ND and MN will be directly benefitted.

➢ ***Potential economic impact of the project***

Current recommendation suggests use of 70 lb N/ac in the case of without inoculation and 40 lb N/ac with inoculation. Inoculation will save the cost of 30 lb N (~ 8.84/acre). Cost of inoculation is approximately $3.35/ac. Inoculation will save $5.49/ac. Moreover, nitrogen credit for the next crop is around 40 lb N/ac ($12/acre). Inoculation can save dry bean growers at least $18.50/acre.

Lessons Learned

➢ *Rhizobia* inoculation could not significantly increase nitrogen fixation or bean yield. The native *Rhizobia* strains seemed to be more effective in symbiosis than the supplied *Rhizobia* strains, and probably that is why we could not get any response to inoculation.

➢ The liquid inoculant could not perform better than or similar to the peat inoculant.

Contact Information

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Table 1. Variations in mean percent nitrogen derived from atmosphere (%Ndfa) of sixteen dry bean cultivars within Pinto, Navy, Kidney and Black bean market classes under different *Rhizobia* inoculation treatments at two different growth stages in Prosper, ND (2016)

Market Class	Cultivar	V_3 stage				R_2 stage			
		Inoculation			Cultivar (Mean)	Inoculation			Cultivar (Mean)
		Control	Liquid	Peat		Control	Liquid	Peat	
Pinto	La Paz	77.6	63.2	79.4	73.4	81.3	71.4	81.1	83.8
	Lariat	78.5	75.2	82.8	78.8	78.2	81.4	70.0	75.9
	ND307	90.8	63.4	80.9	78.4	85.9	74.5	84.3	82.2
	Wind breaker	85.3	53.7	84.1	74.3	97.7	75.6	79.1	87.5
Mean			76.2				82.3		
Navy	Avalanche	67.7	77.1	83.3	76.0	76.0	85.2	84.8	81.1
	Ensign	77.7	75.8	65.8	73.1	71.0	84.9	79.0	79.4
	Medalist	66.1	58.0	79.6	67.9	68.3	72.3	83.1	78.0
	T9905	54.4	71.7	67.1	64.4	87.2	74.6	86.0	81.3
Mean			70.4				79.9		
Kidney	Foxfire	64.0	75.2	72.5	70.6	70.6	87.6	79.7	80.2
	Montcalm	72.8	67.7	60.8	67.1	78.0	75.5	91.8	80.6
	Pink Panther	78.4	76.4	73.0	75.9	79.5	83.7	81.9	78.3
	Redhawk	77.9	60.2	72.1	70.1	75.6	71.4	85.2	78.7
Mean			70.9				79.5		
Black	Eclipse	65.4	64.2	82.8	70.8	75.1	82.1	88.0	75.9
	Loreto	67.7	73.8	74.4	70.0	72.3	69.9	79.5	74.6
	Zenith	65.0	69.3	77.7	70.7	85.7	82.2	78.7	81.5
	Zorro	72.2	52.8	77.3	67.5	85.2	82.3	82.4	80.0
Mean			70.2				78.0		
Inoculation (Mean)		72.6A	67.4B	75.9A		79.2	78.4	82.2	
Inoculation			**				NS		
Market Class (MC)			NS				NS		
Inoculation* MC			*				NS		
Cultivar (MC)			NS				NS		
Inoculation* Cultivar (MC)			NS				*		

Means followed by same uppercase letter are not statistically significant among market classes at $P \leq 0.05$ under a specific growth stage;
* Treatment main effect statistically significant at $P \leq 0.05$; ** treatment main effect statistically significant at $P \leq 0.01$; NS: treatment main effect statistically non-significant

Table 2. Variations in mean nitrogen fixed (kg ha^{-1}) and mean bean yield (kg ha^{-1}) of sixteen dry bean cultivars within Pinto, Navy, Kidney and Black bean market classes under different Rhizobia inoculation treatments in Prosper, ND (2016)

		N_2-fixed (Kg ha^{-1})				Bean Yield (Kg ha^{-1})			
		Inoculation			Cultivar (Mean)	Inoculation			Cultivar (Mean)
Market Class	Cultivar	Control	Liquid	Peat		Control	Liquid	Peat	
Pinto	La Paz	100	76.0	85.4	87.2	1456	1307	1332	1365
	Lariat	109	112	72.3	97.7	1302	1320	1549	1391
	ND307	103	58.9	104	88.7	960	1136	1018	1038
	Wind breaker	118	57.6	90.0	88.5	1685	959	1713	1452
Mean		90.5bc				1312a			
Navy	Avalanche	91.2	61.4	88.2	80.3	1334	1017	1101	1151
	Ensign	134	69.6	126	110	1175	843	1026	1015
	Medalist	85.9	86.3	111	94.5	1171	854	1045	1023
	T9905	75.2	116	122	104	1356	1025	1406	1262
Mean		97.2ab				1113b			
Kidney	Foxfire	72.4	67.9	41.3	60.6	858	382	105	448
	Montcalm	86.3	115	91.8	97.7	1192	688	638	839
	Pink Panther	77.3	35.1	58.3	56.9	1268	594	356	739
	Redhawk	104	61.2	74.7	80.1	931	530	512	658
Mean		79.8c				671c			
Black	Eclipse	106	101	119	109	838	1225	1088	1050
	Loreto	118	143	112	124	1249	1127	1430	1269
	Zenith	79.8	104	148	111	1174	922	859	985
	Zorro	111	95.4	118	108	1120	1032	1048	1067
Mean		113a				1093b			
Inoculation (Mean)		98.2	85.0	97.7		1191A	935B	1014B	
Inoculation		NS				**			
Market Class (MC)		**				**			
Inoculation * Market Class		NS				*			
Cultivar (MC)		NS				NS			
Inoculation * Cultivar (MC)		NS				NS			

Means followed by same lowercase letter(s) are not statistically significant ($P < 0.05$) among the market classes; Means followed by same uppercase letter are not statistically significant ($P < 0.05$) among different inoculation treatments;

* Treatment main effect statistically significant at $P < 0.05$; ** treatment main effect statistically significant at $P < 0.01$; NS: treatment main effect statistically non-significant

Project Title

'Using Fungi and No-till to Enhance Organic Vegetable Production in North Dakota'

NOGA#

15-341

Final Report

Partner Organization

None

Project Summary

Tillage is relied on for weed control but can degrade soil quality. Eliminating tillage could provide important ecosystem services that result from improvements in soil microbial community function – particularly mycorrhizae fungi – which, in turn, could improve plant uptake of nutrients and water. Arbuscular mycorrhizae fungi (AMF) inoculants have been developed to enhance soil microbial community dynamics in organic systems where tillage is used.

This project was aimed at determining the impact that no-till farming practices and an AMF inoculant have on vegetable crop growth/health in farmer-developed, small-scale organic vegetable production systems. We hypothesized that vegetable crop growth/health would be superior under no-till, and that it would be enhanced by the application of an AMF inoculant under conventional-till because of the negative effects of tillage on AMF community function

This project was timely because small-scale vegetable production systems are becoming more prevalent in ND with increased consumer interest in consuming fresh, locally produced fruit and vegetables. Weed control and nutrient deficiencies are the two greatest challenges identified by organic vegetable growers when surveyed regionally and nationally. Tillage is used by most organic vegetable growers to control weeds, but tillage disrupts the soil microbial community – particularly AMF. This, in turn, can exacerbate nutrient deficiencies since AMF improve uptake of phosphorus, other nutrients, and water by most vegetable crops. In addition, tillage can degrade soil physical properties and expose bare soil to erosion. These unintended impacts negatively affect environmental quality and natural resources, ultimately reducing profitability when vegetables are grown using organic practices. Adoption of organic no-till practices when growing vegetables can create soil environments where AMF and other micro-organisms are protected from the severe habitat disruption that occurs when tillage is used. In addition, use of vegetative mulches in no-till systems can provide excellent control of many weeds and release nutrients to vegetable crops as the mulch material decomposes. In conventional-till environments, AMF population dynamics are degraded when soils are disturbed, and the application of an AMF inoculant could enhance recovery. If adoption of no-till practices or use of microbial inoculants can enhance nutrient uptake and plant

growth/health, then when coupled with soil aggregation and other possible improvements, vegetable crop and soil quality should be enhanced.

This project was a new project, and did not build on any previous SCBGP projects. A sister project was submitted and did receive funding from a private funding source (The Ceres Trust Research Fund). Weed community, soil quality, vegetable yield, crop quality and economic data will be collected in that project, but vegetable chlorophyll and porometer measurement to assess crop plant responses to tillage and AMF treatment were not part of this project. This SCBGP project was initially funded for one year, but we requested and were granted a no-cost extension that allowed us to collect data for two years.

- Did the grantee provide a background for the initial purpose of the project, which includes the specific issue, problem, or need that was addressed by this project?
- Did the grantee establish the motivation for this project by presenting the importance and timeliness of the project?
- If the project built on a previously funded project with the SCBGP or SCBGP-FB, did the grantee describe how this project complimented and enhanced previously completed work?

Project Approach

Activities and Tasks:

To test our hypotheses, table beet, winter squash, sugar snap pea, and onion were grown in tilled and no-till (mulched) plots with and without an organically certified, AMF inoculant at two certified-organic locations in North Dakota (Dickinson and Absaraka) during 2016 and 2017. Impact of tillage and inoculant application on stomatal conductance and chlorophyll content of vegetable crops were determined periodically as indicators of plant growth/health. As part of the sister project funded by The Ceres Trust, we also quantified the weed community, crop yield, AMF colonization of crop roots, and numerous chemical, physical, and biological measures of soil health.

Pre- and post-surveys were distributed to vegetable growers attending summer field days and were used to quantify the knowledge gained about no-till and microbial inoculants in organic vegetable production in North Dakota. Adoption of these practices by organic vegetable growers was documented to demonstrate impact (i.e., outcomes) of this project in the state. Via these surveys, contacts with two producers were forged and the research team (Dr. Gramig and her graduate student) visited both producers' operations to learn more about their practices and to advocate incorporating mulching into their systems, which they did. Dr. Gramig also traveled separately to Aneta, ND to present ideas from this research to a group of community gardeners.

Research results were presented at MOSES Organic Agriculture Conference in Feburary 2017 and at the ASA-CSSA-SSSA Annual Meeting in October 2017. Field day talks about the research were given at the NSDU Horticulture Research Farm in August of 2016 and 2017.

Research Results:

Onion:

At Absaraka, onion chlorophyll was greater in mulched plots than in tilled plots (347 vs. 310 mg/m^2). Also, onion chlorophyll was greater in 2016 than in 2017 (355 vs. 302 mg/m^2). Regarding the mulch effect, typically the mulch was associated with increased soil N, so that could translate into greater leaf chlorophyll. Regarding the year effect, because the plots were fertilized with poultry manure only once in 2015, by 2017 the soil N probably had decreased quite a bit. But since the mean onion chlorophyll in mulched plots was roughly equal to the mean onion chlorophyll value in 2016 (347 vs. 355 mg/m^2), one might conclude that the mulch compensated for the lack of fertilizer additions through the duration of the study.

At Dickinson, mulched plots were associated with greater onion leaf chlorophyll than tilled plots (343 vs. 325 mg/m^2). Curiously, AMF inoculant was associated with decreased onion leaf chlorophyll compared to non-inoculated plots (322 vs. 344 mg/m^2). During 2017, onion chlorophyll was greater than during 2016 (365 vs. 300 mg/m^2). Mulch was associated with increased soil N, which could increase leaf chlorophyll as with these results. The effect of the AMF inoculant is perplexing and not easily explained. AMF typically impacts plant access to phosphorus (a relatively immobile soil nutrient) more than access to nitrogen (which is highly mobile in the soil profile). Also the increase in leaf chlorophyll during 2017 is also not very easily explained. We would expect the opposite pattern to occur because the plots were only fertilized during 2015.

At Absaraka, onion stomatal conductance decreased during 2017 compared to 2016 (489 vs. 605 $mmol/m^2s^1$). This may be attributed to the fact that 2017 was much drier than 2016. The plots were irrigated during 2017 to compensate, but success was partial. Neither the tillage treatment nor the AMF inoculant influenced onion stomatal conductance.

At Dickinson, onion stomatal conductance was much greater in 2016 than in 2017 (589 vs. 306 $mmol/m^2s^1$). One explanation for this observation is that the soil was drier in 2017 than in 2016. Mulch did not impact stomatal conductance for onions.

At Absaraka, during 2016 and 2017, onion yield was much greater in mulched plots compared to tilled plots (496 vs. 321 g per bulb in 2016 and 488 vs. 246 g per bulb in 2017). This effect could be due to many factors, among them conservation of soil moisture and contributions to soil nitrogen by the alfalfa mulch. However, the nitrogen effect may be more important because (1) mulch did not affect onion stomatal conductance and (2) mulch did not enhance yield during 2015. During 2015, soil nitrogen levels were likely more even among tilled and mulched plots because all the plots were fertilized with composted poultry manure at the start of the growing season. And the mulch would not have had much time to decompose and add more N to the soil.

At Dickinson, within each year, onion yield was always greater in mulched plots. The differences were quite substantial (74 % greater in 2015, 57% greater in 2016, and 368% greater in 2017). Mulch could have increased onion yield by contributing nitrogen or conservation soil moisture, or both. Since stomatal conductance was not affected by mulch whereas chlorophyll was greater for onion grown in mulched plots, soil nitrogen may be a more important factor than soil water. However, onions are notoriously sensitive to water deficits, so both factors may be important.

Beet:

Note: During 2017, the beet crop failed at Dickinson due to drought conditions.

At Absaraka, beet leaf chlorophyll was greater during 2016 than during 2017. Also, tillage was associated with increased plant chlorophyll. I would expect the year effect because we only applied fertilizer during 2015. So by 2017 N was probably running a bit low. Another reason could be the irrigation in 2017 leached some N away from the crop roots. However, why would chlorophyll be reduced with the mulch? This result is counter to what we would expect because the mulch should have added some N to the soil, which would increase chlorophyll. At Dickinson, no treatment effect impacted beet leaf chlorophyll.

Beet stomatal conductance was not influenced by any treatment factor at either site. At Absaraka, mulched plots were associated with greater per-plant beet yield than tilled plots (305 vs. 225 g per plant). Since stomatal conductance was not altered by the mulch, this result is more likely related to soil nitrogen differences caused by the mulch decomposing and adding nitrogen to the soil. At Dickinson, none of the treatments impacted per plant beet yield at Dickinson, but the tillage effect was marginally insignificant. However, beet yield in mulched plots tended to be greater in the mulched plots than the tilled plots.

Pea:

At Absaraka, pea leaf chlorophyll was not influenced by mulch or AMF inoculant. Pea chlorophyll was greater during 2016 compared to 2017 (309 vs. 283 mg/m^2). This result could be attributed to the reduction in soil nitrogen over time as N from the 2015 fertilization declined over time. The effect of AMF on pea leaf chlorophyll was marginally insignificant. There was a slight trend for AMF inoculant to be associated with greater leaf chlorophyll compared to non-inoculated plots. Neither tillage nor AMF inoculant impacted pea leaf chlorophyll at Dickinson.

At Absaraka, an interactive effect of year, tillage, and AMF inoculant was found. During 2017 in tilled plots only, AMF inoculant was associated with decreased pea leaf stomatal conductance, however this effect was marginally significant ($p = 0.0481$). No other treatments or treatment combinations influenced pea stomatal conductance.

At Dickinson, pea plants grown in tilled plots exhibited greater stomatal conductance than plants grown in mulched plots (353 vs. 459 mmol m^2s^1). This result runs counter to what one might expect. Mulches typically conserve soil moisture, which should lead to less water stress and greater stomatal conductance.

At Absaraka, during 2015 and 2017, pea yield was not affected by the mulch or the AMF inoculant. During 2016, however, mulched plots were associated with increase in pea yield compared to tilled plots (94 vs. 53 g per plant). This difference is due to a difference in the number of pods that were produced by each plant.

At Dickinson, pea yield per plant was greater for peas grown in mulched plots compared to plants grown in tilled plots (39 vs. 31 g per plant). The number of pods per plant were also greater for mulched than tilled plots. Since peas can fix nitrogen, one explanation is that the mulch helped to conserve soil moisture at the dry Dickinson site, thereby either extending the flowering period or increasing plant size which in turn resulted in more fruits per plant.

Squash:

At Absaraka, squash leaf chlorophyll was not influenced by the treatment or AMF inoculant. At Dickinson, squash leaf chlorophyll was much greater during 2017 than 2016 (319 vs. 187 mg/m^2). However, squash leaf chlorophyll was not influenced by either mulch or AMF inoculant.

At Absaraka, squash leaf stomatal conductance was not influenced by mulch or AMF inoculant. At Dickinson, squash leaf stomatal conductance was greater for plants grown in mulched than tilled plots during 2017 only (659 vs. 427 $mmol/m^2s^1$).

At Absaraka, squash yield was slightly greater for plants grown in mulched plot than for plants grown in tilled plots (16 vs. 13 kg per plant). AMF inoculant did not affect squash yield. At Dickinson, squash plants grown with mulch had greater yield than plants grown in tilled plots (2.9 vs. 1.7 kg per plant).

Summary and Recommendations:

In general, at both sites, alfalfa mulch was associated with increased crop yield for three of the four crops grown in this study (onion, pea, and squash). Beet yield was not influenced by the mulch treatment. The increase in yield associated with mulch would not be attributable to weed suppression by the mulch as we removed weeds to separate the weed effect from the mulch effects on soil factors and crop growth. The yield increase could be due to mulch conserving soil moisture, contributing to soil nitrogen, or both. In general, stomatal conductance, which is an indication of plant water stress, was not affected much by the mulch treatment. But leaf chlorophyll was often greater for plants grown with mulch compared to plants grown in the tilled plots. Alfalfa hay contains considerable nitrogen, which is released made available for plant uptake as the mulch decomposes. Soil tests performed as part of the sister project associated with this study indicated that soil nitrogen increased with the mulch treatment. Results from the sister study also confirmed that, as one might expect, the alfalfa mulch provided considerable weed suppression. Therefore, we showed that using alfalfa mulch benefits vegetable production in at least two ways: (1) mulch suppresses weeds, thereby increasing yields and saving labor, and (2) mulch adds nitrogen to the soil, which also increases yield of many crops. Some labor is required to apply the mulch and growers must either purchase or grow the hay, which is another expense. We need not quantify these expenses for this study, so this would be an important consideration for future studies. Growers must be convinced that a tactic not only produces good results but also adds to their bottom line.

Almost no measure of plant performance or crop yield was impacted by the AMF inoculant. Inoculants such as the one we investigated are widely touted to growers. In some instances where soils are highly depleted of natural endemic AMF species or in areas where phosphorous is the most limiting resource for plant growth, AMF inoculants may have a place. But for this region of the country, where phosphorous typically is not limiting, these inoculants probably have limited if any benefit in most situations.

Outreach Results:

During the field day held in 2016, we conducted surveys to assess prior knowledge about and subsequent interest in trying no-till mulch and AMF inoculants in organic vegetable production systems as a result of field day participation. Twelve participants completed the survey. Three of twelve participants had not heard of using no-till mulch approaches before the field day. Five of twelve participants had not tried no-till mulch systems. All participants had a favorable view of using no-till mulch approaches prior to the field day. After the field day presentation, seven of the participants indicated that they would like to try no-till mulch approaches. Regarding use of AMF inoculant in vegetable production systems, half the participants had not heard of using AMF before the field day. Eight participants had not tried using AMF before. Six of the participants had a favorable view of using AMF prior to the field day, the other six had no opinion because they had never heard of AMF before. At the end of the field day, four participants wanted to try AMF and two additional participants said they might be willing to try AMF. From these surveys, we connected with two producers who grow vegetables: Ross and Amber Lockhart of Heart and Soil Farm and Bill Miller, who runs the community garden in Aneta, ND. Dr. Gramig and her graduate student traveled to visit both these producers and talked to them about how our research results would benefit their operations. Both expressed interest in trying the approaches. Dr. Gramig traveled again to Aneta to give a presentation to the community about organic management of weeds, which focused primarily on conveying the advantages of using no-till and mulch in vegetable production.

The research and outreach activities outlined above fulfill the main experimental and outreach activities, as stated in the grant work plan, as well as subsequent reports. Moreover, some of the outreach activities (Dr. Gramig's second trip to Aneta) go beyond the original plans for outreach.

- Were the activities and tasks performed during the entire grant period briefly summarized? This section should discuss the tasks provided in the Work Plan or the approved project proposal. This includes significant results, accomplishments, conclusions and recommendations, as well as favorable or unusual developments.
- If the overall scope of the project benefitted commodities other than specialty crops, did the grantee indicate how project staff ensured that funds were used to solely enhance the competitiveness of specialty crops?
- Did the grantee detail the significant contributions and role of project partners in the project?

Goals and Outcomes Achieved

GOALS:

(1) Increase the knowledge of organic vegetable growers on the plant growth/health benefits that result from adopting no-till practices and using an AMF inoculant when growing vegetables in organic conventional-till systems
(2) Adoption of no-till practices by organic vegetable growers in North Dakota

OUTCOMES:

(1) Via numerous presentations made at scientific conferences, grower meetings, and field days, approximately 250 scientists and growers gained new knowledge about using no-till practices in small-scale production systems. See outreach results in above section for specific results from the surveys we conducted to measure outcomes.

(2) No-till practices including mulching were adopted by at least one producer, Heart and Soil Farm, and several community gardeners in Aneta, ND.

Documented adoption was somewhat lower than we anticipated, but some adoption instances may not have been documented because some participants did not want to fill out surveys. Overall the project was successful because we were able to gather scientific evidence that supports the use of alfalfa mulch and no-till for organic vegetable crop production. We also documented that expensive commercial AMF inoculants are likely not worth the expense to growers because they provided few if any measurable benefits.

- Did the grantee supply the activities that were completed in order to achieve the performance goals and measurable outcomes identified in the approved project proposal or subsequent amendments?
- If outcome measures were long term, was a summary of the progress made towards this achievement provided?
- Did the grantee provide a comparison of actual accomplishments with the goals established for the reporting period?
- Did the grantee clearly convey completion of achieving outcomes by illustrating baseline data that has been gathered to date and showing the progress toward achieving set targets?
- Did the grantee highlight the major successful outcomes of the project in quantifiable terms?

Beneficiaries

Beneficiaries of this project include all meeting and field day attendees who learned more about no-till vegetable production as well as the benefits of AMF. Total attendees were approximately 250 people. Specifically these beneficiaries include the owners of Heart and Soil Farm and the community garden in Aneta ND. Certainly others benefitted too, but this wasn’t documented because many meeting and field day attendees did not want to participate in follow-up visits. Another beneficiary was the graduate student who worked on this project, as well as the summer student workers who worked on this project. Through the project, all these students gained new knowledge about no-till production methods and AMF fungi. We do not have an assessment of the economic impact of the project. However, since weeds are one of the biggest problems that organic producers face, documented alternative means of controlling weeds that also increase yield through nutrient additions and prevention of water loss will have some economic benefits.

- Did the grantee provide a description of the groups and other operations that benefited from the completion of this project's accomplishments?
- Did the grantee clearly state the number of beneficiaries affected by the project's accomplishments and/or the potential economic impact of the project?

Lessons Learned

The primary lesson learned through conducting this project is that implemented a no-till deep mulch system that uses alfalfa hay mulch has numerous benefits. We expected that the mulch would effectively suppress weeds-that wasn't the true question. What we hoped to show is that mulching and no-till practices provide benefits to vegetable crop production beyond weed suppression. Indeed, we saw that for most crops, no-till with mulch led to yield increases that were not due to weed suppression, but rather were probably due to soil health benefits including nutrient addition and prevention of water loss. A formal economic lifecycle analysis would have been a good addition to this study. Some producers felt that applying the mulch would be too time-consuming. Therefore, a formal cost/benefit analysis would have been helpful to demonstrating not only the efficacy of the approach, but also the economic benefits. Another lesson learning is that AMF inoculants do not provide measurable benefits to vegetable crops in all environments. Most soils contain endemic populations of AMF. Therefore, why would one need to supplement these populations? Products containing AMF have in recent years become more prevalent and usually are sold to growers on the basis of claims of great benefit. Our research provides evidence that in some situations these products are likely without benefit. This knowledge will save growers from spending money on products that are unnecessary.

- Did the grantee offer insight into the lessons learned by the project staff as a result of completing this project?
- Did the grantee provide any unexpected outcomes or results that were an effect of implementing this project?
- If goals or outcome measures were not achieved, did the grantee identify and share the lessons learned to help others expedite problem-solving?

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- Email Address

Additional Information

Beamer KP, **Gramig GG**, Carr PM. 2017. Weed management and soil quality outcomes of non-chemical weed control tactics. ASA-CSSA-SSSA Annual Meeting. Tampa, FL. 246-4.

The student working on this project will complete his MS degree during August 2018. We expect one peer-reviewed publication to result from this work.

- Did the grantee provide any additional information available (i.e. publications, websites, photographs) that is not applicable to any of the prior sections?

NDTO's Specialty Crop Expansion Beyond Borders 2015

Final Report

Project Summary

The following information describes the results of the project coordinated by the North Dakota Trade Office (NDTO) to expand markets for regional specialty crops beyond the borders of the United States. The results of the project address the selection of the target markets (and alternatives if impediments negatively impact target market selection), educational programs on the health benefits of pulses as a protein source, any increases in export sales and the development of business opportunities that appeal to the market. A comprehensive evaluation incorporated the use of market research, survey results, and export statistics with a focus on the increase of export sales dollars to the target markets.

The United Nations named 2016 as the International Year of the Pulse (IYOP) with the hope of positioning pulses as the primary source of protein and essential nutrients. Plant -based protein sources, such as pulses, are a dietary staple in the South American countries of Colombia and Peru. Pulse crops are locally grown. However, with Colombia in a state of El Niño severe drought and the government's effort to fight food inflation with a temporary elimination of import tariffs on basic food items, including dry beans and lentils, imports were on the rise in 2015. Peru has traditionally been an importer of lentils and green split peas.

Imports into these countries have been dominated by Canadian suppliers. Pulse products from the U.S., and specifically the Upper Midwest are less well known than our Canadian competitors, averaging market share between 1% and 10%.

Among consumers, the competition is based on quality, price, and service with appearance and taste equally important considerations. Colombia has the 5th largest packaged food market in Latin America and the 27th largest retail food market in the world, with 75% of the Colombian population residing in urban areas. In both Colombia and Peru, how consumers shop and where they shop is changing as many households are now dual-income. Convenience stores, food service and the restaurant sector are also expanding. Peru was named by World Travel Awards as the World's Leading Culinary Destination from 2001 to 2016, spurring an increase in restaurants and high-quality food ingredients for consumers who prefer healthy foods locally produced.

This project helped to confirm the market opportunities in Colombia and Peru through trade missions, Better For You Food Events and reverse trade missions. The Better For You Food Ingredients events demonstrated the different ways to incorporate pulses into traditional dishes to an audience of food processors and manufacturers. This project builds upon the success of NDTO's 2014 SCBG grant project which included a trade mission to Bogota, Colombia by expanding from a local (North Dakota) project to a regional (upper Midwest) project and also expanding into other key urban areas of Colombia and Peru. The trade missions are followed up by reverse trade missions back to the U.S. for the delegates to observe harvest and learn more about the industry. These trade missions are critical for regional SC producers to cultivate important business relationships with the international customers and long-term partnerships. Additionally, they create momentum for immediate purchases of a variety of pulses.

Project Approach

A reimbursement program was implemented for approved participating companies to offset the high cost of developing these international markets. NDTO organized and conducted a trade mission to Colombia, led by ND Agriculture Commissioner Doug Goehring, and a Better For You Food Ingredients event March 1 thru 9, 2016, in the cities of Bogotá and Medellín. Seven SC exporters participated in the mission, meeting and collaborating with six Colombian stores to hold in-store promotions. The Better For You Food Ingredients event was attended by approximately 75 guests from 30 Colombian companies. The program featured North Dakota State University's Dr. Juan Osorno presenting on peas, lentils, dry edible beans, pulse flour and borage. Following the educational portion of the program, a renowned Colombian chef conducted a live presentation of Colombian recipes using U.S. regional specialty crops.

The Colombian Reverse Trade Mission was held August 21-23, 2016 in the cities of Fargo and Bismarck, North Dakota with farm tours and stops throughout the region and an educational component, Pulse Nutrition and Applications. Ten (10) SC exporters participated in the reverse trade mission to cultivate business relationships with the thirteen (13) Colombian food buyers. Northern Crops Institute in Fargo, ND was the site of the educational component, Pulse Nutrition and Applications. The program was presented by Natsuki Fujiwara, Food Scientist and Dr. Clifford Hall, North Dakota State University professor. The program included topics for peas, lentils, dry edible beans, pulse flour and borage such as 'Pulses in Snack Applications' and 'Quality Characteristics and Parameters of Northern Grown Pulse'. The farm tours enabled the Colombian buyers to experience firsthand the traceability from field to table, including harvest, storage, and packaging. Reported export sales following this activity were $2,825,443.

The Peru Trade Mission was organized and conducted by NDTO April 22-27, 2017, led by ND Agricultural Commissioner Doug Goehring, and a Better For You Food Ingredients event was held in the city of Lima. Four (4) SC exporters participated in the mission meetings and collaborated with eight Peruvian buyers while touring the largest and most popular Peruvian supermarkets and gourmet stores. They also toured Peru's large importers of dried legumes and food ingredients. The Better For You Food Ingredients event was held April 25 and was attended by approximately 75 Peruvian buyers. The program was presented by Northern Crop Institute's Food Scientist Natsuki Fujiwara and focused on peas, lentils, dry edible beans, chickpeas, and pulse flour. Following the educational portion of the program, a Peruvian chef conducted a live presentation of local Peruvian dishes with U.S. regional specialty crop ingredients.

The Peruvian Reverse Trade Mission was held August 14-18, 2017 in the cities of Fargo, Minot, Richardton and Bismarck, North Dakota with farm tours and stops throughout the region and an educational component, Pulse Nutrition and Applications. Eight (8) SC exporters participated in the reverse trade mission to cultivate business relationships with the eight (8) Peruvian food buyers. Northern Crops Institute in Fargo, ND was the site of the educational component, Pulse Nutrition and Applications. The program was presented by Natsuki Fujiwara, Food Scientist. The program included topics for peas, lentils, dry edible beans, pulse flour, chickpeas and borage such as 'Pulses in Snack Applications' and 'Quality Characteristics and Parameters of Northern Grown Pulse'. The farm tours enabled the Peruvian buyers to experience firsthand the traceability from field to table; including harvest, storage, packaging. Reported export sales following this activity were $3,131,325.

Goals and Outcomes Achieved

- Increase in export sales
  - Actual export sales of $ 14,893,563 as of September 30, 2017. The projected economic impact was increased export revenue of $7.25 M. We exceeded that by double above what was projected.
  - ROI = 81.9

- Conduct 3 outbound trade missions and 1 reverse trade mission
  - NDTO conducted 2 outbound trade missions and 2 reverse trade missions. The SC exporters changed their mind about the planned TM to Mexico and India due to the high commodity prices along with already having contacts in these markets. The SC exporters requested we identify new export markets.

- Strengthen the export market for upper Midwest regional pulses
  - US market share of the pulse market into Colombia increased 63% in 2016 ($10 M) over 2015 U.S. market share. Canada's market share dropped in 2016 to 50% while the U.S. had captured 21% of the import market.

- Monitor performance
  - NDTO conducted surveys of the participating SC companies following each activity; quarterly and annually. In addition, NDTO utilized federal sources of statistical data, such as Census, to obtain results.

Beneficiaries

The target beneficiaries for this project were the fourteen (14) SC exporters who were direct recipients of the reimbursement program. Indirectly, 82.7 jobs were impacted or created based on the federal criteria of 1 job for every $180,000 in export revenue. Specifically, the increase in SC exports also benefits the transportation and logistics community, packaging and cartage companies, state and local economies, local businesses services and has created business expansion for smaller companies, contract growers, etc.

Lessons Learned

The challenge for this project is identification of a target market at the time of writing. International market conditions can change day to day. At the start of the project, Mexico was a market of opportunity. However, with time, most of the SC exporters were interested in identifying a new and yet unknown market. India was another market that was subject to low commodity prices, high transport costs, and lack of interest by the SC exporters. In order to overcome these obstacles, NDTO conducted more research to understand consumption trends and markets where our competition, Canada, has a significant market share. Further research was obtained through outreach to USDA's Foreign Ag Service.

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