FY14 Specialty Crop Block Grant Program- Farm Bill

North Dakota Department of Agriculture

Final Performance Report
USDA Agreement # 14-SCBGP-ND-0038

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Submitted: October 31, 2017
Resubmitted: January 5, 2018

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

Final Report

Partner Organization: National Sunflower Association

Project Summary

Downy mildew (DM), cited 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) pyramiding 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-two, we completed two backcrosses and advanced BC_4F_1 to BC_4F_2 generation, and mapped the DM R gene in RHA 468 to linkage group 1 of the sunflower genome.

Project Approach

I. Two backcrosses were made to three BC_2 populations derived from the crosses of CONFSCLR5/RHA 464 (carrying Pl_{Arg} and R_{12}), HA-R6 (R_{13a})/HA 458 (Pl_{17}), and HA-R6 (R_{13a})/HA-DM1 (Pl_{18}) and the BC_4 generation was obtained in 2015. In the early 2016, the selected double-resistant BC_4F_1 plants to rust and downy mildew were advanced to BC_4F_2 generation.

II. 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} is underway by marker-assisted selection, and the selected double R plants will be advanced to BC_4F_3 generation.

III. The downy mildew resistance gene in RHA 468 was mapped to linkage group 1 of the sunflower genome and temperately named as Pl_{468}. Both simple repeat sequence (SSR) and single nucleotide polymorphism (SNP) markers were developed to link to $Pl_{468.}$

IV. Molecular mapping of a novel DM R gene introduced from a wild species *H. argophyllus* PI 494578 is in progress. The BC_1F_2 and BC_1F_3 population were developed from the cross of HA 89/ PI 494578. One hundred and twenty BC_1F_2-derived BC_1F_3 families (30 seedlings from each of BC_1F_3 family, total 3,600 seedlings) will be inoculated with DM race 734 and recorded for

performance. Genomic DNA was isolated for 120 BC_1F_2 individuals and parents, and genotyping of BC_1F_2 population by genotyping-by-sequencing is underway.

Goals and Outcomes Achieved

Goals for 2015

a. Create BC_3 generation in spring greenhouse and BC_4 generation in fall greenhouse
b. Continue lab/greenhouse test of all generations for downy mildew and rust
c. Complete molecular mapping of the resistance gene in the line RHA 468
d. Begin the process of identifying molecular markers for a new DM resistance gene derived from *H. argophyllus*

Outcomes achieved

I. DM and rust tests in each backcross generation and two backcrosses

1. A total of 120 BC_2 seeds from the cross of HA-R6/HA 458 were germinated. Of them, 60 seeds with suitable length of root tip were inoculated with DM race 734. Twenty-four resistant plants were selected and further tested with DNA markers linked to the DM R-gene Pl_{17} in this population. The resistant plants were transferred to the greenhouse and backcrossed to the recurrent parent HA-R6 in the late February 2015 to produce BC_3.
2. Similarly, a total of 120 BC_2 seeds from the cross of HA-R6/HA-DM1 were germinated. Sixty eight seeds with suitable length of root tip were inoculated with DM race 734. Of them, 33 resistant plants were selected and further tested with DNA markers linked to the DM R-gene Pl_{18} in this population. The resistant plants were transferred to the greenhouse and backcrossed to the recurrent parent HA-R6 in the late February 2015 to produce BC_3.
3. A total of 280 BC_2 seeds from the cross of CONFSCLR5/RHA 464 were germinated. The 156 seeds with suitable length of root tip were inoculated with DM race 734. Of them, 52 DM resistant plants were selected and inoculated with rust race 336. Twenty two plants resistant to both downy mildew and rust were selected and further confirmed by DNA markers linked to the DM gene Pl_{Arg} and rust gene R_{12}, respectively. The double-resistant plants were transferred to the greenhouse and backcrossed to the recurrent parent CONFSCLR5 in the late February 2015 to produce BC_3.
4. A total of 320 BC_3 seeds from the cross of HA-R6/HA 458 were germinated. Of them, 238 seeds with suitable length of root tip were inoculated with DM race 734. The 92 resistant plants were selected and further tested with DNA markers linked to the DM gene Pl_{17} in this population. The resistant plants verified by DNA markers were transferred to the greenhouse and backcrossed to the recurrent parent HA-R6 in June 2015 to produce BC_4.
5. Similarly, a total of 140 BC_3 seeds from the cross of HA-R6/HA-DM1 were germinated. Of them, 112 seeds with suitable length of root tip were inoculated with DM race 734, and 36 resistant plants were selected and further tested with DNA markers linked to the DM gene Pl_{18} in this population. The resistant plants were transferred to the greenhouse and backcrossed to the recurrent parent HA-R6 in June 2015 to produce BC_4.

6. A total of 320 BC_3 seeds from the cross of CONFSCLR5/RHA 464 were germinated. The 135 seeds with suitable length of root tip were inoculated with DM race 734. Of them, the 56 DM resistant plants were selected and inoculated with rust race 336. Twenty plants resistant to both downy mildew and rust were selected and further confirmed by DNA markers linked to the DM gene Pl_{Arg} and the rust gene R_{12}. The double-resistant plants were transferred to the greenhouse and backcrossed to the recurrent parent CONFSCLR5 in June 2015 to produce BC_4.
7. A total of 71 and 215 BC_4F_1 seedlings derived from the crosses of HA-R6/HA 458 and HA-R6/HA-DM1, respectively were inoculated with DM race 734. The resistant plants were selected and further tested with DNA markers linked to the DM gene Pl_{17} and Pl_{18}. The selected resistant plants were transferred to the greenhouse and advanced to BC_4F_2 generation.
8. A total of 138 BC_4F_1 seedlings derived from the crosses of CONFSCLR5/RHA 464 were inoculated with DM race 734. The 40 DM resistant plants were selected and inoculated with rust race 336. Seventeen plants resistant to both downy mildew and rust were selected and further confirmed by DNA markers linked to the DM gene Pl_{Arg} and the rust gene R_{12}. The double-resistant plants were transferred to the greenhouse and advanced to BC_4F_2 generation.

II. Complete molecular mapping of the DM resistance gene in the line RHA 468

RHA 468 is a DM resistant male fertility restorer line. F_2 and F_3 populations were developed from the cross of HA-R8 and RHA 468. We completed downy mildew resistance phenotyping of the 190 F_3 families (30 seedlings from each of the family, total 5,700 seedlings) last year. Genomic DNA was isolated for 188 F_2 individuals and parents, and bulked segregant analysis located the DM R-gene to linkage group (LG) 1 of the sunflower genome. A total of 50 simple sequence repeat (SSR) markers previously mapped to LG1 of sunflower genome were used to screen between the two parents, HA-R8 and RHA 468, and 12 showed polymorphism. Subsequently, genotyping of 188 F_2 individuals was conducted using 12 polymorphic SSR markers. The DM R gene in RHA468 was mapped to the top end of LG1 of the sunflower genome and temperately named as Pl_{468}. To further saturate the region where Pl_{468} resides, a total of 64 single nucleotide polymorphism (SNP) markers were selected from LG1 SNP maps and screened between two parents. Thirty-seven SNP markers were found to be polymorphic and used to genotype F_2 population to identify SNP markers closely linked to Pl_{468}. The current genetic map consists of 12 SSR and 37 SNP markers, and the flanking markers delineated the DM resistance gene in an interval of 1.3 cM.

III. Begin the process of identifying molecular markers for a new DM resistance gene derived from *H. argophyllus*

The new downy mildew resistance was transferred from wild *Heliathus argophyllus* accession PI 494578 into cultivated sunflower. The cross between HA 89 and PI 494578 was made in 2012. To molecularly map this new gene, a BC_1F_2 population was obtained in 2014 and further advanced to BC_1F_3 generation in 2015. Phenotyping of downy mildew resistance in this BC_1F_3 population is underway to determine inheritance of DM resistance derived from *H. argophyllus* PI 494578. Genomic DNA was isolated for 120 BC_1F_2 individuals and parents. Genotyping of BC_1F_2 population is underway by the use of genotyping-by-sequencing methodology.

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 ensure 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 2016 NSA Research Forum was held on January 12 - 13, 2016 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. The lead researcher, Dr. Lili Qi, reported the molecular mapping of the DM resistance genes in sunflower and their impact on sunflower breeding programs. 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 23, 2016 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, will have an article summarizing this project in the March/April 2016 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

The undergraduates were provided to field, greenhouse, and lab experience that helped shape their careers. One undergraduate student matriculated to PhD programs. The training and knowledge gained by postdocs helped advance their careers. Seed company personnel were trained to use genetic information in a breeding context.

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Additional Information

Publications

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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.

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

Final Report

Partner Organization: North Dakota State University Carrington Research Extension Center

Project Summary

Fusarium root rot is a serious disease of field peas that cannot be managed effectively with existing tools; resistant varieties, fungicide seed treatments, and crop rotation have limited effectiveness. Field pea producers have long suggested that common herbicides may exacerbate the disease, but research rigorously assessing a possible link between specific herbicides and increased disease has been lacking. In this project, a field trial was conducted in Carrington, ND to rigorously quantify the impact on field pea root rot of common pre- and post-emergence herbicides applied to peas and long-residual herbicides applied the previous season to wheat. Root rot was severe in the trial, but Aphanomyces root rot – not Fusarium – was the primary disease. No herbicide treatments increased root rot severity, but application of two pre-emergence herbicides at high rates resulted in herbicide injury shortly thereafter and increased the foliar wilt symptoms associated with root rot during bloom and pod-fill. The results suggest that herbicide injury may reduce the tolerance of field peas to Aphanomyces root rot but provides no evidence that herbicides increase the severity of Aphanomyces root rot. It is unclear how herbicide usage may impact root rot when Fusarium is the primary disease.

The purpose of this project was to rigorously assess the impact of specific herbicides *(a)* applied pre- and post-emergence to peas and *(b)* applied to the previous wheat crop on field pea root rot and on the agronomic performance of peas under high root rot pressure. Specific objectives were to (1) identify the impact on Fusarium root rot of specific herbicides and herbicide programs used wheat/pea rotations and (2) develop herbicide usage guidelines for fields where the disease is a problem.

Root diseases have become a major limiting constraint on field peas in the past decade in North Dakota, and the disease has caused many producers in north-central and northwestern North Dakota to abandon field pea production in specific fields or across their entire operations over the past 5 years.

This was the first year of a multi-year effort to rigorously address this research question.

Project Approach

- The field trial was planted at an appropriate planting date (April 28). Conditions were very dry after planting, but an irrigation system was established over the trial. Pre-emergence herbicides were applied within 3 days of planting and activated immediately with overhead irrigation.
- Phytotoxicity associated with pre-emergence herbicide applications was assessed prior to applying post-emergence applications on June 3-4 when peas had 5 to 6 nodes. Applications of sulfentrazone (4.5 or 5.625 fl oz/ac Blanket 4F) with metribuzin (0.25 or 0.375 lb/ac Glory) but not sulfentrazone applied alone (4.5 fl oz/ac Blanket 4F; Figure 1) resulted in herbicide injury.

- The vigor of peas during early vegetative growth was assessed prior to applying post-emergence herbicides on June 3-4 when peas had 5 to 6 nodes. Application of the high rate of sulfentrazone plus metrizubin (5.625 fl oz/ac Blanket 4F + 0.375 Glory) resulted in reduced early-season vigor relative to other treatments (Figure 1).
- Plant height was assessed at bloom initiation. All herbicide treatments resulted in reductions in plant height (Figure 2).
- Root necrosis was assessed at full bloom and early pod development; the bottom inch of the epicotyl and top inch of the tap root was evaluated for root rot on approx. 50 plants per plot. None of the herbicide treatments applied pre-emergence or post-emergence to field peas or applied the previous year to the preceding rotational crop resulted in a statistically significant increase in root necrosis relative to the non-treated control (Figure 3).
- Nodulation associated with symbiotic Rhizobia, which is often reduced when Aphanomyces root rot is severe, was evaluated at full bloom and early pod development. Nodulation was poor in all treatments, but there was not enough evidence to conclude that herbicides exacerbated the problem (Figure 2).
- Root rot was severe in this trial, and *Aphanomyces euteiches,* causal agent of Aphanomyces root rot, was likely the predominant cause. Disease symptoms were highly characteristic of Aphanomyces root rot. *Fusarium* spp., the cause of Fusarium root rot, was successfully isolated from only 22% of roots exhibiting characteristic root rot symptoms, and *Rhizoctonia solani*, the cause of Rhizoctonia root rot, was isolated from only 2% of plants. Diseased tissues exhibited a predominant honey-brown to light-brown discoloration and were soft and sunken.
- The peas were desiccated with the herbicide paraquat on July 30, shortly before reaching full maturity, and were harvested on August 4. The pre-emergence herbicides sulfentrazone and metribuzin applied at a high rate and the post-emergence herbicides clethodim, sodium salt of bentazon, and ammonium slat of imazamox applied late resulted in a significant reduction in field pea yield (Figure 2). These treatments were not associated with increased root rot, and the observed yield reduction was likely caused by herbicide injury.
- Key results from this study have not been posted online, as it is difficult to generate rigorous recommendations from a single year of testing conducted in a single environment. Multi-location field trials are being conducted in 2016 to generate additional data. Results will be posted online in March 2017.
- The results from this study were shared with stakeholders at a field tour in July 2016, with an emphasis that the results are preliminary findings that need to be confirmed with additional testing. The audience at that field tour was 30 individuals.
- The findings from this study are likely crop-specific; they are of assistance to field pea producers but are unlikely to be directly applicable to other crops. It is unlikely that this research will confer any direct benefit to non-specialty crops.

Goals and Outcomes Achieved

Goal #1: Quantifying the impact of herbicides on Fusarium root rot in field peas.

- This study was successful at characterizing the impact of herbicides on Aphanomyces root rot of peas. Aphanomyces was not the root disease targeted in this study, but it is also very serious disease of peas. The results are very timely, as serious outbreaks of

Aphanomyces root rot have been reported in North Dakota and surrounding regions in the past two years.

- The results from this study suggest that herbicides commonly employed in wheat/pea rotations might not increase the severity of Aphanomyces root rot of field peas. However, when applied at a high rate that causes herbicide injury, some herbicides may make it more difficult for peas to tolerate high levels of Aphanomyces root rot, resulting increased levels of wilt symptoms typically associated with root rot, premature death, and reduced yield.
- Because Aphanomyces root rot was the primary disease in this study, this study was not successful at quantifying the impact of herbicides on Fusarium root rot.

Goal #2: Developing herbicide usage recommendations for fields where root rot is a problem.

- The results from this study strongly suggest that producers should be very careful to apply the pre-emergence herbicides sulfentrazone and metribuzin at or below the upper recommended rates relative to their soil pH, soil texture, and organic matter levels. Applied at slightly too high of a rate, this study suggests that these herbicides may reduce the ability of peas to tolerate Aphanomyces root rot.
- Multi-year, multi-environment data are needed before rigorous recommendations can be developed. This research is in progress.
- The target of 300 growers and crop advisors accessing information online that summarizes major results from this study will be met. Out of concerns that rigorous recommendations could not be made on the basis of a single year of testing, results from this study were not posted online until follow-up studies conducted in 2016 were completed. Results from the 2015 and 2016 field studies will be posted to the NDSU Carrington Research Extension Center website during a major upgrade to the plant pathology component of that website in March 2017. Once research results are posted, approx. 300 growers and crop advisors on an existing e-mail list will be notified that the website has been updated to include these and other research results, and page visits to the herbicide information will be tracked. This website is widely accessed by industry agronomists and producers, and the target outcome of 300 page views within a year of posting is expected to be reached.
- The target of disseminating results from this study to 400 growers and crop advisors attending extension meetings will be met. Out of concerns that rigorous recommendations could not be made on the basis of a single year of testing, dissemination of research results was largely delayed until data from follow-up studies conducted in 2016 was available. Research findings from this project and from follow-up research conducted in 2016 were presented to approx. 30 stakeholders at a field tour in Minot, ND in July 2016, will be presented to approx. 100 stakeholders (mostly farmers) at the MonDak Pulse Day in Williston, ND on Feb. 15, 2017, will be presented to approx. 140 stakeholders (mostly crop advisors and private-sector agronomists) at the NDSU Western Crop and Pest Management School in Dickinson, ND on Mar. 8, 2017, and will be presented to additional stakeholders at one to two field tours during summer 2017. Additional field trials addressing the objectives outlined in this project are planned for the 2017 growing season, and additional stakeholders will be reached when results from this study and from follow-up studies conducted in.

Beneficiaries

- Aphanomyces root rot is a widespread disease affecting field peas in most places where the crop is grown. This project is expected to benefit most 2,000+ the field pea producers who grow the crop on 600,000+ acres annually in the United States. It is also expected to benefit field pea producers in neighboring Canada.
- Results from this study suggest that commonly used pre-emergence herbicides, when applied at a rate slightly too high for the soil type, may reduce the ability of peas to tolerate Aphanomyces root rot. Producers who take extra caution with their application rates of these herbicides will benefit from this research through increased field pea yields in fields where Aphanomyces root rot is a problem.

Lessons Learned

- The goal of this research was to evaluate the impact of herbicides on Fusarium root rot, but Aphanomyces root rot was the primary disease that developed. While the results were informative, the primary goal of generating management recommendations for Fusarium root rot was not met. To generate results for Fusarium root rot, extra caution was taken when selecting sites for the research trials conducted in 2016. Research sites were selected that did not have a prior history of root rot in field peas, and the trials were inoculated with the Fusarium root rot pathogen using a methodology known to successfully establish the disease.
- The second lesson learned from this research is that additional work is needed to evaluate the impact of herbicides on Aphanomyces root rot. Aphanomyes root rot is also a severe problem in field pea production and, like Fusarium root rot, cannot be managed with any existing tools. In order to confirm that the findings from this research are replicable, follow-up research is needed targeting Aphanomyces root rot.

Contact Information

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Additional Information

- Figures illustrating key results follow.

Figure 1. Impact of herbicides applied pre- and post-emergence to field peas and applied the previous season to the preceding rotational crop on field pea establishment, vigor, phytotoxicity, and plant survival; Carrington, ND (2015). Plant populations were assessed by evaluated 9 meters of row per plot; vigor was estimated visually relative to the typical appearance of healthy, high-yielding peas at that growth stage; and herbicide damage (phytotoxicity) was estimated visually.

Details on herbicides applied: Glory, 75% metribuzin by weight (Makhteshim Agan of North America, Inc.; Raleigh, NC); Blanket 4F, sulfentrazone, 4 lbs ai/gal (Tenkoz, Inc.; Alpharetta, GA); Pursuit, ammonium salt of imazethapyr, 2 lbs ai/gal (BASF Corp.; Research Triangle Park, NC); Spartan Charge, carfentrazone-ethyl, 0.35 lbs ai/gal + sulfentrazone, 3.15 lbs ai/gal (FMC Corp.; Philadelphia, PA); Volunteer 2EC, clethodim, 2 lbs ai/gal (Tenkoz, Inc.; Alpharetta, GA); Basagran, sodium salt of bentazon, 4 lbs ai/gal (WinField Solutions, St. Paul, MN); Raptor, ammonium salt of imazamox, 1 lb ai/gal (BASF Corp.; Research Triangle Park, NC). All post-emergence herbicides were applied with 15 lbs ammonium sulfate per 100 gal and 1% v/v crop oil concentrate.

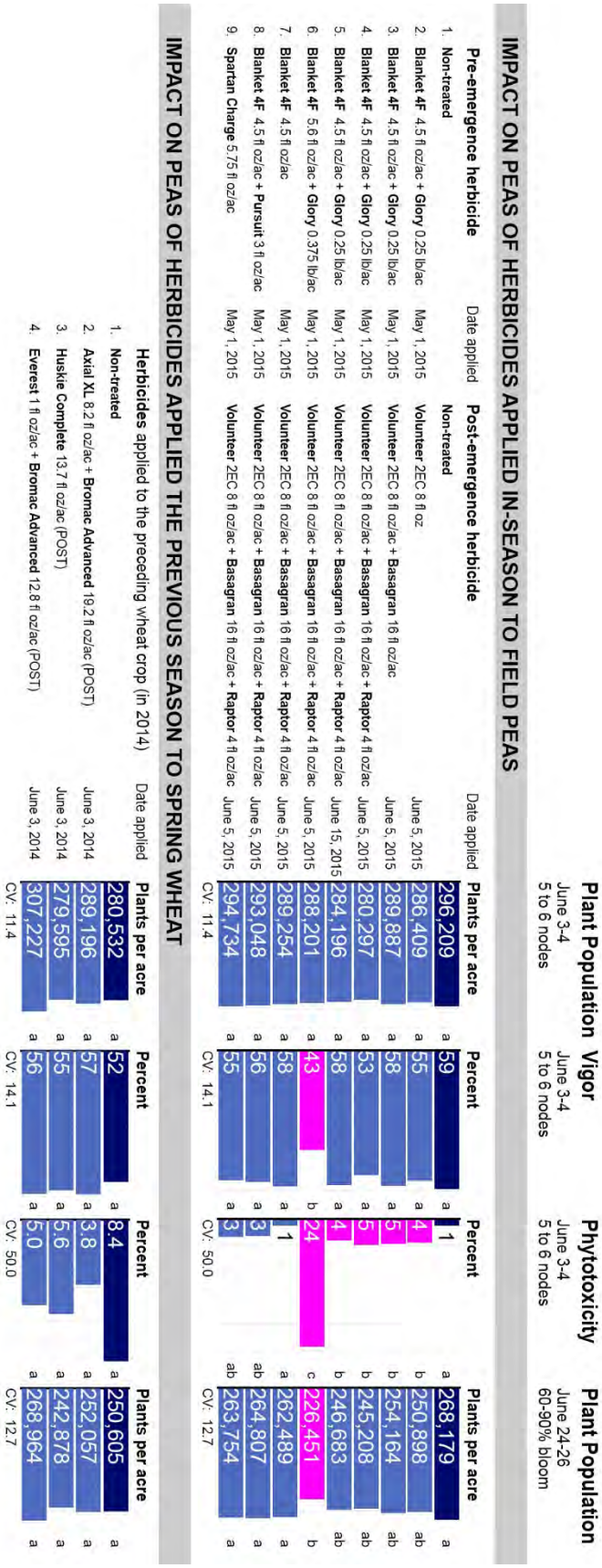
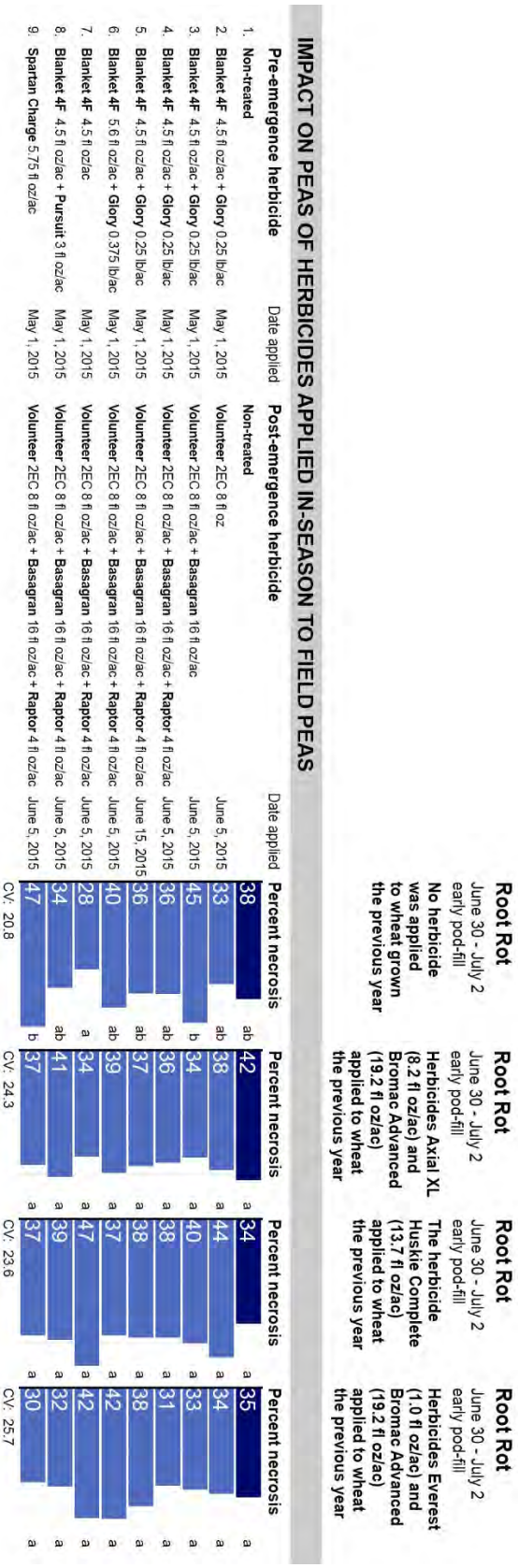


Figure 2. Impact of herbicides applied pre- and post-emergence to field peas and applied the previous season to the preceding rotational crop on wilted field pea plants, plant height, nodulation, and field pea yield; Carrington, ND (2015). The presence or absence of nodulation was assessed on the top 5 cm of the root mass (measured from the point of seed attachment) on an average of 50 roots per plot and a minimum of 42 roots per plot.

Details on herbicides applied: Glory, 75% metribuzin by weight (Makhteshim Agan of North America, Inc.; Raleigh, NC); Blanket 4F, sulfentrazone, 4 lbs ai/gal (Tenkoz, Inc.; Alpharetta, GA); Pursuit, ammonium salt of imazethapyr, 2 lbs ai/gal (BASF Corp.; Research Triangle Park, NC); Spartan Charge, carfentrazone-ethyl, 0.35 lbs ai/gal + sulfentrazone, 3.15 lbs ai/gal (FMC Corp.; Philadelphia, PA); Volunteer 2EC, clethodim, 2 lbs ai/gal (Tenkoz, Inc.; Alpharetta, GA); Basagran, sodium salt of bentazon, 4 lbs ai/gal (WinField Solutions, St. Paul, MN); Raptor, ammonium salt of imazamox, 1 lb ai/gal (BASF Corp.; Research Triangle Park, NC). All post-emergence herbicides were applied with 15 lbs ammonium sulfate per 100 gal and 1% v/v crop oil concentrate.

Figure 3. Impact of herbicides applied pre- and post-emergence to field peas relative to herbicides applied the previous season to the preceding rotational crop on field pea root rot; Carrington, ND (2015). Root rot was assessed as the percent of the bottom 2.5 cm of the epicotyl and top 2.5 cm of the tap root (measured from the point of seed attachment) exhibiting disease symptoms; in each plot, an average of 50 roots (and a minimum of 42 roots) were evaluated per plot.

Details on herbicides applied: Glory, 75% metribuzin by weight (Makhteshim Agan of North America, Inc.; Raleigh, NC); Blanket 4F, sulfentrazone, 4 lbs ai/gal (Tenkoz, Inc.; Alpharetta, GA); Pursuit, ammonium salt of imazethapyr, 2 lbs ai/gal (BASF Corp.; Research Triangle Park, NC); Spartan Charge, carfentrazone-ethyl, 0.35 lbs ai/gal + sulfentrazone, 3.15 lbs ai/gal (FMC Corp.; Philadelphia, PA); Volunteer 2EC, clethodim, 2 lbs ai/gal (Tenkoz, Inc.; Alpharetta, GA); Basagran, sodium salt of bentazon, 4 lbs ai/gal (WinField Solutions, St. Paul, MN); Raptor, ammonium salt of imazamox, 1 lb ai/gal (BASF Corp.; Research Triangle Park, NC). All post-emergence herbicides were applied with 15 lbs ammonium sulfate per 100 gal and 1% v/v crop oil concentrate.



Northern Plains Vegetable Variety Testing

Final Report

Partner Organization: Northern Plains Sustainable Agriculture Society

Project Summary

The Northern Plains Sustainable Agriculture Society's Farm Breeding Club (FBC), North Dakota State University, North Dakota Farmers Market & Growers Association, and the Entrepreneurial Center for Horticulture continued their collaboration, joined by Montana State University, 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. We continued to confer with market growers in identifying current standard cultivars and traits needed by growers for improved performance (e.g. heat tolerance, cold soil germination, taste, appearance, earliness). We employed replicated variety trials at NDSU in Fargo, ND as well as single-replicate, daughter sites at vegetable farms throughout North Dakota (ND). Data from these variety trials have been reported through field day tours, workshops at collaborator events, and through social media. We hope also to use the eOrganic Variety Trial Reports website. We have also assessed the value of the program to stakeholders through use of surveys and by soliciting feedback via social media and regular email communication. This model was replicated at Montana State University as well with daughter sites at vegetable farms in central Montana.

With the development of farmers markets and localized food systems, farmers seek varieties with agronomic traits suited to northern climates and quality traits sought by their customers. Growers need resilient varieties that perform despite suboptimal growing conditions; varieties that come through despite environmental challenges and adversity. However, there have not been replicated variety trials in North Dakota with many specialty crops for some time. This project addressed two of the NDDA 2014 funding priorities: 1) *Investing in specialty crop research, including organic research to focus on conservation and environmental outcomes*, specifically seeking out locally adapted varieties that perform well in our ecological niche. 2) Identifying the gaps in available adapted varieties and needs for *developing new and improved seed varieties and specialty crops.*

The **project goal** was *the identification of vegetable varieties that meet local climatic and environment challenges with highly marketable qualities, enhancing food security.*

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

» **Objective 2:** *Farmer-Scientist-Student-Consumer networks for sharing and collaborating on vegetable variety improvement with unique salable qualities.*

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

This project complimented and built on previous project work by adding a third year and a second state's scientifically replicated variety trial data to the data collected in 2013 and 2014, and it will be further enhanced by the final year of work in 2016/2017. Changes in environmental and climatic conditions can greatly influence variety performance. Therefore, it is important to

replicate variety trials across seasons and locations to identify the varieties that are dependable, despite variable conditions, providing scientifically reliable data. This project has not been submitted or funded by another Federal or State grant program.

Project Approach

- Set up surveys and communication: Growers for 2015 were identified in fall 2014 and winter 2015. An email list was set up for rapid communication with these growers in winter 2015 and was used all year. In late December, 2014 an online survey of growers was set up via Survey Monkey to get input about previous work and this year's list of crops. The Department of Agriculture, NPSAS, and the Entrepreneurial Center for Horticulture all shared the link with growers in the state in January. Project participants were also provided platforms for sharing observations, pictures, data, information, and questions through social media, the NPSAS newsletter, and an email group.
- Interviews with market farmers: Informal communications among our market growers (6) were used to help guide the project. We had 21 responses to our online survey about the most important market classes and traits of our specialty crops from both 2014 and 2015 work. Our focus in 2015 on bush, green, snap beans; nantes-type carrots; slicing cucumbers; romaine lettuce; and yellow, storage onions was based on these results and discussions with Mac Burgess at Montana State. We had 11 similar, paper surveys returned from among our 35 plus audience members at the 2015 NPSAS Winter Conference in January, 2015 (total conference attendance of 625). Average scores were 3.8 out of 5 for increased understanding of variety trials, 4 out of 5 for whether variety trials were useful, and 4.2 out of 5 for adopting the varieties that appeared to perform better. Scores were around 3.5 for interest in taking part in variety trials, but really only 7 of the 11 reported interest or strong interest in doing so. At the 2015 ND Local Foods Conference in Mandan, 19 surveys were returned from a workshop audience of about 30 (total conference attendance of 109). Sixteen of the 19 reported some or much increased knowledge about variety trials and ten reported an interest in conducting a variety trial. In April 2015, only 7 surveys were returned from an audience of 29 at the 2015 Garden Expo in Bismarck (total conference attendance in the many hundreds). Six reported a better or much improved understanding of variety trials, but only 2 reported an interest in taking part in them. Input on specific crops was lighter on the paper surveys but did support our crop choices. We decided that future surveys need to be shorter and more easily filled out.
- 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. Participating farmers have been learning to integrate trials into their standard farming practices, assess field conditions, layout trials, manage data collection, and utilize real data to evaluate the differences among varieties and their performance across seasons. We continued discussions of the project and our techniques at Farm Breeding Club meetings in March and November 2015 and onward via this next installment of the project. We tried adding web connections to enhance participation during both of these meetings, and this added two more collaborators to the list of participants each day. However, we were not able to get all of our widely spaced growers at the same meeting at the same time. We also had ongoing email contacts to alert growers to the needs for data collection, field day presentation, data submission, and billing invoice submission. The basics of how the project works have been presented along with our results at other meetings with other growers as well.

- Source seed for screening: We used mostly the same list of varieties for green beans, but came up with different lists for nantes-type carrots, slicing cucumbers, romaine lettuce, and yellow, storage onions as these were new classes upon which we focused. Some varieties from the previous two years were repeated if they seemed especially promising, and we again evaluated ten varieties of each type of specialty crop. Our growers made suggestions about their favorite varieties as the lists were finalized, and then seeds were ordered by NPSAS and MSU from a number of seed houses that provide untreated or organic seeds. NPSAS then distributed the seeds (along with stakes for each variety) to the North Dakota growers in February 2015.
- Project Participants: North Dakota project collaborators included the NPSAS Farm Breeding Club (FBC), Dr. Chiwon Lee, NDSU Plant Science Department, North Dakota Farmers Market & Growers Association (NDFMGA), Entrepreneurial Center for Horticulture (ECH), Organic Seed Alliance (OSA) and e-Organic. Farmer cooperators included: Marvin & Ilene Baker, North Star Farms, Carpio, ND; Annette & John Carlson, Morning Joy Farm, Cleveland, ND; Bill Bittle, Farm Manager, Entrepreneurial Center for Horticulture, Bottineau, ND; Glen Philbrick, Hiddendale Farm, Bismarck, ND; Eden McLeod, McClusky, ND; Marte Stensli and Bob Nelson, Tangle Tree Ranch, Doyon, ND; and Steve Zwinger, Prairie Seeds, Carrington, ND who also conducted additional trials at the Carrington REC.
- Variety evaluations on farm, at NDSU, and at MSU: NDSU, MSU, 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 (mother site) conducted replicated variety trials (4 replicates) of 5 priority market crops (ND selections: carrot, green bean, and cucumber; MT selections: onion and lettuce). Single replicates were conducted on at least three ND market farms (daughter sites) for each crop. In 2015 and 2016, Steve Zwinger planted ten varieties of onions in an organically managed, four replicate trial in Carrington. Transplants grown in NDSU greenhouses and then planted on the Carrington Research Extension Center organic research ground. Steve also planted single plots of ten green bean varieties and a 4 replicate, organic potato variety trial with thirteen specialty varieties at the Carrington Research Extension Center. Red Norland and Yukon Gold were the check varieties that were compared to 11 fingerling potatoes. Data from these trials have been compiled for presentation and are currently being analyzed along with the new data from NDSU and collaborator sites from 2016.
- Field tours and tasting events: There were several small field events at growers' locations in July and August, and also a large field event at NDSU in September of 2015. The project was also featured prominently during the Organic Field Tours held at the CREC in July of 2013 through 2016. Individual collaborators held tasting events with their friends, family, and coworkers at various times when produce was available.
- Compile variety trial results: Variety trial results came in from growers to NPSAS in November and December, 2015. Zhigang Wu at NDSU worked up the data collected in Fargo and Mac Burgess analyzed the data in Montana.
- Dissemination and outreach: The project disseminated critical, replicated trial data on traits of interest, production capacity, yield and quality of available varieties, to help farmers minimize risk and identify and develop new markets for quality 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 continues to be posted in the NPSAS Germinator Newsletter that is sent out to members. Video was shot during several field days in 2014 and again in 2015 by NPSAS staff. Finished videos were posted on YouTube: (https://www.youtube.com/watch?v=N5cI4pi_2vM, https://www.youtube.com/watch?v=Edb5OAk1MiI, https://www.youtube.com/watch?v=8YqHbF9jZYY, https://www.youtube.com/watch?v=P4181Yzut3o). They have received 65-174 views each since posting/reposting, and we have more video to process as part of the next project installment. 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 913 likes for the page. In addition the methods and results of the project have been presented during several workshops at the North Dakota Local Foods Conferences and at the Bismarck Garden Expo. As part of the final installment of the project we also planned a four hour preconference workshop based on these trials and presented it to the 2016 NPSAS Winter Conference in Aberdeen, SD. The NPSAS Farm Breeding Club hosted collaborating horticulture scientists Chiwon Lee and Mac Burgess, NDSU student Zhigang Wu, and NPSAS members Steve Zwinger, David Podoll, and Frank Kutka at this workshop for face-to-face networking. Farmers, research agronomists, students, and proponents of local and regional food systems discussed cultivar development and improvement, and variety trial collaboration. Chiwon Lee followed this up with a one hour workshop about specialty crop breeding and variety development a day later. We had about 35 members at each of the workshops.

- The background information and guidance we share on variety trials could be used for any type of crop, but we only present 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	Pre- and post-surveys; feedback via social media networks.

Much of the last year of our project will be following up with growers as the final data is released. However, as described in the activities section above, "We had 11 similar, paper surveys returned from among our 35 plus audience members at the 2015 NPSAS Winter Conference in January, 2015 (total conference attendance of 625). Average scores were 3.8 out of 5 for increased understanding of variety trials, 4 out of 5 for whether variety trials were useful, and 4.2 out of 5 for adopting the varieties that appeared to perform better."

» **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	Current project participants, project downloads and social media followers
Target	> ½ of the project attendees "opt in" to a listserve and/or interact via social media networks; core project participants contribute posts to the listserve and on social media.
Data Collection	Listserve and Facebook participants and contributors

We had good attendance at some events, like the NPSAS Winter Conference workshops, the North Dakota Local Foods Conferences, the Carrington Research Extension Center organic field days, and the NDSU specialty crop field day. Audiences numbered from about 20 to over 50. However, we also did not attract as many to the field events at the homes of individual growers in late summer. Sometimes we had no visitors, sometimes 2-4 would join us, but the best attendance was when the field day incorporated additional activities and was held as part of a larger NPSAS Summer Field Event when more than 20 visited the Hiddendale Farm near Turtle Lake. 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 good number of likes and shares. Personal connections seem to help a great deal with collaborators, while electronic communication appears much more useful for disseminating results. Big field events with multiple speakers seem much more useful for gathering the public than smaller tours.

» **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 NDFMGA surveys
Target	>3/4 of participants indicate increase in self-assessed knowledge level
Data Collection	Pre- & post-surveys; attendance; number of publication downloads, print copies distributed, and video views; feedback via social media.

Our surveys of growers at meetings and workshops (under interviews with market farmers in the Activities section) demonstrated that we met this target with most audiences. Good majorities of those who filled out surveys reported increased knowledge. Social media have not proven very useful for getting reports back from growers, however, they were very useful for getting survey participation when we needed input for choosing the crops to evaluate.

Beneficiaries

Primary beneficiaries of this project were commercial vegetable growers in North Dakota and Montana. 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 2013 Local Foods Directory there are at least 133 commercial vegetable growers in North Dakota. According to Montana Department of Agriculture's Food Infrastructure Map there are at least 115 commercial vegetable growers in Montana serving local commerce. The same resources also shows 50 active farmers markets in North Dakota and 65 in Montana. In North Dakota there are 27 CSA operations, 16 U-Pick farms, 15 roadside stands, and 42 mail order operations. There are likely

many more small home-based farm businesses that would benefit from this project. Innumerable home gardeners would also potentially benefit from this research.

Beneficiaries of this project who adopt new varieties based on information from this project should expect greater yields of higher quality vegetables when superior varieties are identified. Farmers should also benefit from enhanced consumer understanding of the differences among vegetable varieties and unique qualities available through local markets.

Interest in direct to consumer sales has increased three-fold from 1992 – 2007, going from $404 million to $1.2 billion. Sales grew twice as fast as total agricultural sales (105% vs. 48%). In 2008 US local food sales, including direct to consumer plus intermediated sales to retailers, restaurants, food service distributors, schools and institutions, were estimated to be $4.8 billion.

NASS statistics indicate total value to the economy of North Dakota vegetable production is $251 million while Montana's vegetable production is approximately $160 million. Much of this value is likely associated with field-scale vegetable crops not targeted by this project (i.e. potatoes). Even a modest increase in value of just 1% of North Dakota and Montana's vegetable crop production would be over $4 million. Coupled with the growth potential direct to consumer sales, this represents a significant return on investment.

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.

Direct grower input regarding their needs is key to guiding future variety development and improvement efforts. This project has been and continues to provide input and guidance into variety improvement and cultivar development by networking with public plant breeders at our land grant universities and providing data and evaluation back to the plant breeding process.

We had anticipated approximately 400 individuals would participate in our 8 field days; the pre-conference session and/or one or more conference workshops at the 2016 NPSAS Annual Winter Conference (600 attendees), Local Foods Conference (200 attendees), and Dakota Garden Expo (6000+ attendees). The number was about half of our expectation, with a total closer to 200 attendees largely due to low field day attendance. We anticipated distributing variety data results and fact sheets to 1,000 event participants. Handouts with data summaries taken by attendees were actually less than fifty, the total we printed out after seeing our actual audience sizes. It appeared that after the presentations most were satisfied and did not feel the need for a copy of the results. Variety trial reports, fact sheets, and videos were posted to NPSAS, YouTube, and Facebook pages. We anticipated reaching over 1,800 via newsletters, and 250 video views. Our Germinator newsletter has carried project information to our 400+ readers on a quarterly basis throughout the project, and we have had more than 250 video views. These carried the messages about project events, how the variety trials are performed, and what growers thought of the process.

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 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. This is our basic design. It is disappointing to have to have such a drastic insurance policy, but this is how variety trials for all crops get done on the northern plains and we were not surprised.
- We lost the Agriculture Director at NHSC in New Town due to staff changes for two years in a row. We were able to collaborate with the new Agriculture team there as well as the Agroecology team at United Tribes Technical College in 2016. Flexibility and ongoing communication was useful in getting and maintaining our grower participation.
- Coordinating meetings with all of the growers for training continued to be difficult. A more personal approach was considered for later work of this type to make sure we effectively reached and trained every grower. We have shared some of this training at public events about the project and so far have not found large numbers interested in taking part. This has been very disappointing, but those that have expressed interest have been very interested. Short, concise descriptions of the methods have been well received during workshops where significant project results or discussions of breeding were presented.
- 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. The NDSU field days were well attended and of course there were many things to see. Probably it would be better to visit with collaborators on a one-on-one basis in the summer and then have one, big event that would be very attractive to many from the general public.
- 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 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. 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.

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Additional Information

Some of the 2016 organic specialty crop work at the Carrington Research Extension Center. The variety trial for fingerling potatoes was nearby and as well managed.

NDSU specialty crop plots in July, 2015

Beans picked and evaluated at Dakota College at Bottineau in 2015.

Cucumbers picked and evaluated at Dakota College at Bottineau in 2015.

Lettuce grown by Glen Philbrick at Hiddendale Farm near Turtle Lake in 2015.

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

Final Report

Partner Organization: North Dakota State University Carrington Research Extension Center

Project Summary

Sclerotinia stem rot (white mold) is a critical production constraint for dry edible beans in North Dakota, and fungicides are widely employed for management of the disease. Fungicides are currently applied with a poor understanding of the number and timing of fungicide applications that optimize disease control, resulting in unnecessary applications. In this project, field trials were established under irrigation in Carrington, ND using two pinto and two navy bean varieties seeded to rows 14 and 28 inches apart. Sclerotinia disease levels were low in the navy beans due to severe bacterial blight but were much higher in the pinto beans, where bacterial blight was not severe. Fungicides were most effective when applied when 70 to 100% of plants had an open blossom and the first immature pods had developed irrespective of differences in canopy closure. A second fungicide application 10 to 14 days after the first resulted in improved Sclerotinia control only where bacterial blight and anthracnose were not severe. Fungicide application timing was tested in trials in which two sequential fungicide applications were made, and testing is currently in progress to evaluate whether optimal fungicide application timing may differ when a single fungicide application is made.

The goals of this project were (1) to improve Sclerotinia disease control in dry beans by identifying the optimal application timing for fungicides, (2) to help eliminate unnecessary fungicide applications by identifying the conditions under which a second fungicide application is most likely to be profitable, and (3) to develop improved recommendations for using fungicides to manage Sclerotinia in dry beans.

This project aims to address a disease that affects half or more of the dry bean acreage in North Dakota and Minnesota most years and is consistently identified as the most serious disease problem of dry beans in a survey of North Dakota and Minnesota farmers conducted annually by the NDSU Extension Service in cooperation with the Northarvest Bean Growers Association.

The work conducted as a part of this project represented the first year of a multi-year effort to address these research questions.

Project Approach

- The trials were planted with Western-grown certified disease-free seed procured in April.
- The field trials on planted May 26 and May 29, dates that fall within the recommended period for planting dry beans in central North Dakota.
- Supplemental overhead irrigation was delivered to the trials using micro-sprinkler irrigation systems consisting of rotating micro-sprinklers established on a 20-foot offset grid. Fungicide application timing was assessed under four irrigation regimes: intensive irrigation applied from the V3 to R1 growth stage (mid-vegetative growth to bloom initiation), intensive irrigation applied from the V3 to R3 growth stages (mid-vegetative growth until the

first pods reached full length), intensive irrigation applied from the V3 to R1 and R4 to R6 growth stages (mid-vegetative growth to bloom initiation and again from 50% of pods at full length until shortly before maturity), and from the R1 to R6 growth stages (bloom initiation until shortly before maturity). The response to one versus to sequential fungicide applications was assessed under three irrigation regimes: sufficient irrigation applied to reach water-holding capacity of the soil every time that the soil reached 50%, 65%, or 80% available water from the R3 to the late R6 growth stage (at least one pod at full length until shortly before maturity); no irrigation was applied prior to the R3 growth stage, as soil moisture never dropped below 50% available water prior to the R3 growth stage.

- Production of apothecia, small mushroom-like spore-producing structures of the Sclerotinia fungus, was assessed at full bloom and mid-pod development. Abundant apothecia occurred during full bloom (R2 and R3 growth stages), the period of maximum susceptibility to white mold, only when the high soil moisture was sustained during late vegetative growth and bloom initiation.
- Sclerotinia incidence and severity was assessed at mid pod development and shortly before maturity. Sclerotinia was most severe (30 to >60% of the canopy diseased, depending on how long wet conditions extended into the bloom period; Figures 1 to 4) and the response to fungicides was highest when sustained high soil moisture was observed during mid to late vegetative growth through bloom initiation. When wet conditions did not develop until mid pod-fill (at least one pod at full length), Sclerotinia was much less severe (< 20% of the canopy diseased; Figure 7).
- The dry beans were harvested shorty after maturity in September. Yields were generally maximized when fungicides were applied at 70 to 100% bloom and initial pod development irrespective of canopy closure at the time of the initial fungicide application (Figures 1 to 4). In all fungicide treatments, intensive irrigation at mid to late pod-fill sharply increased dry bean yields without increasing Sclerotinia disease severity.
- Results from this study have not yet been posted online, as it is difficult to draw rigorous conclusions from a single year of testing. Additional field trials are being conducted in 2016, and results will be posted online as soon as enough multi-year, multi-location data have been generated and rigorous recommendations can be developed.
- Preliminary results from this study were shared with stakeholders at a field tour in September 2015 and at a winter outreach meeting in December 2015. Approximately 80 people attended the September meeting and approximately 70 people attended the December meeting, of which at least half were farmers and the remainder crop consultants and agronomists representing private firms.
- The findings from this study are crop-specific; they are of assistance to dry bean producers but are unlikely to be directly applicable to other crops. As such, it is unlikely that this research will confer any direct benefit to non-specialty crops.

Goals and Outcomes Achieved

- <u>Project goal 1, output 1: Sclerotinia disease control and the likely profitability of one vs. two fungicide applications was assessed relative to plant architecture, row spacing, and canopy closure, and precipitation patterns.</u> Disease pressure was modest in this field trial due to severe root rot pressure in the pinto and navy beans, severe bacterial blight in the navy beans that developed after a 14-hour period during full bloom of sustained strong winds with gusts exceeding 40 miles per hour, and severe anthracnose in one of the pinto bean varieties

(Windbreaker) presumably due to seed-borne disease (despite the use of certified disease-free seed). Under these conditions, disease pressure was modest in the pinto bean varieties (upright vine or short vine architecture) and very low in the navy bean varieties (bush type architecture). Fungicide applications resulted in economically relevant reductions in Sclerotinia most consistently in 'Lariat' pintos, which lodged during early pod development; less consistently in 'Windbreaker' pintos, which did not lodge; and never in the navy beans due to low disease pressure (Figures 7, 8). Fungicide applications resulted in economically relevant reductions in Sclerotinia in pinto beans most consistently as the frequency of supplemental irrigation (a proxy for rainfall) increased and never in navy beans due to low disease pressure (Figures 7, 8). Row spacing and percent canopy closure when fungicides were applied had little impact on disease pressure and the likelihood that fungicide applications would confer an economically relevant reduction in disease. For the purposes of this discussion, a fungicide application was considered economically relevant if at least 7% of the canopy exhibited Sclerotinia in the non-treated control and a single fungicide application conferred at least a 33% reduction in disease. There was often a trend towards reduced Sclerotinia with a second fungicide application, but, under the low to moderate disease pressure observed in this trial, it is unlikely that the increased disease control associated with the second fungicide application was economically worthwhile in any of the dry bean varieties, row spacings, and irrigation regimes evaluated in this trial.

- Project goal 1, output 2: User-friendly report posted online. User-friendly figures illustrating key results have been generated (Figures 7, 8) for use in a user-friendly report summarizing key findings. The user-friendly report will be generated and post online in early summer 2016 before dry beans have entered bloom and before producers begin making decisions for fungicide usage.
- Project goal 1, output 3: Recommendations for optimal fungicide usage for control of Sclerotinia in dry edible beans. While it is not possible to generate rigorous management recommendations from a single field trial, the results from this project suggest that plant architecture and precipitation patterns are likely much more important determinants of the profitability of fungicide applications than row spacing or canopy closure.
- Project goal 2, output 1: Optimizing fungicide application timing relative to environmental conditions, plant architecture, and canopy closure. Optimal fungicide application timing for the control of Sclerotinia was consistent irrespective of differences in canopy closure, dry bean row spacing, and the timing and duration of supplemental irrigation applied to simulate different patterns of wet weather (Figures 1 to 5) and corresponded to 70% to 100% of plants with an open blossom and early pod development (longest pod less than 0.2 to 0.6 inches long) in the pinto bean cv. 'Lariat' and 100% of plants with an open blossom and pods up to full length in the pinto bean cv. 'Windbreaker'. The greater flexibility in fungicide application timing observed with the pinto bean cv. 'Windbreaker' may have been due to differences in plant architecture; 'Windbreaker' did not lodge, while the pinto bean cv. 'Lariat' lodged during early pod development. Disease pressure was very low in the navy beans (Figure 6), precluding a rigorous assessment of fungicide application timing in that market class.
- Project goal 2, output 2: Improved understanding of the relationship between Sclerotinia disease development and precipitation patterns, soil moisture fluctuations, plant architecture, and timing of canopy closure. One of the most significant findings from this study was that intensive irrigation applied at the R4 to R6 growth stages – after bloom is complete but a

critical period for seed development and pod-fill – significantly increased yield without increasing white mold (Figures 1 to 4). Follow-up research is needed to confirm this finding, but this has the potential to be very useful for irrigated producers who are concerned about white mold. For dryland producers, it is also relevant, as it suggests that the risk of losses to white mold are very low when weather favorable for the disease occurs late in crop development.

Outreach efforts: Due to relatively low disease pressure in two of three field trials conducted as a part of this project, it will be difficult to reach rigorous conclusions until additional testing is conducted. Consequently, results were not shared at Bean Day. Preliminary results were presented at a field tour in September 2015 and at a winter extension meeting in December 2015 and will be presented at future summer field tours and winter extension meetings in conjunction with results from follow-up studies.

Beneficiaries

This project benefitted the growers producing dry beans (1) in environments favorable for Sclerotinia and (2) under irrigation. The project will benefit the approx. 3,400 farmers producing dry beans in North Dakota, Michigan, and Minnesota and the approx. 3,000 farmers producing dry beans under irrigation in other states. It has benefitted these producers by generating an improved understanding of the optimal deployment of fungicides relative to rainfall patterns, canopy closure, and other variables.

The project has also benefitting the Northarvest Bean Growers Association (the commodity group representing dry bean growers in North Dakota and Minnesota) by providing insights into improving the management of white mold, a disease that is consistently identified as one of the most important production constraints in annual survey of dry bean producers sponsored by Northarvest.

Lessons Learned

In this study, fungicide application timing was tested with two sequential fungicide applications 13 to 14 days apart. When weather conditions are highly favorable for white mold for an extended period during bloom and early pod-fill, it is common for North Dakota and Minnesota producers to make two fungicide applications targeting white mold. However, when the perceived risk of disease is more moderate, a single fungicide application is often made.

Considerable latitude was observed relative to fungicide application timing in the research conducted as a part of this study, and it is unclear whether the same latitude would be observed if a single fungicide application were made. In multi-location, multi-environment fungicide application timing research targeting Sclerotinia in soybeans, delaying fungicide applications until full bloom and early pod development has consistently performed equal or better than applications made during bloom initiation. In soybeans, a single application is typically made when fungicides are applied targeting Sclerotinia, and all testing was conducted with a single fungicide application. Like dry beans, soybeans are most susceptible to Sclerotinia during full bloom and early to mid pod-fill (after a dense canopy has developed) and delaying the fungicide application until full bloom ensures that residual fungicide from that application persists throughout the period of maximum susceptibility. The application timing research conducted on dry beans, however, has been conducted with a focus on two sequential fungicide applications

(Figures 1 to 4). When two sequential fungicide applications are made, the persistence of residual fungicide from an initial fungicide application throughout the period of maximum susceptibility (full bloom and early to mid-pod development) is less important, as the second fungicide application ensures that the crop is protected once the fungicide residual drops below the level needed for satisfactory disease control (typically 2 weeks after application). Many North Dakota dry bean producers, particularly those towards the central part of the state, typically only make a single fungicide application targeting white mold. While considerable latitude was observed relative to the optimal timing of an initial fungicide application was observed when two sequential fungicide applications were made (Figures 1 to 4), the impact of fungicide application timing is likely to be much more pronounced when a single fungicide application is made. To provide rigorous recommendations on the application timing of fungicides for control of Sclerotinia on dry beans, application timing should be conducted for strategies involving both a single fungicide application and two sequential fungicide applications.

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Additional Information

- Figures illustrating major findings from this study are provided in the subsequent pages.

Figure 1. Response to fungicide application timing in 'Lariat' pinto beans seeded to rows 14 inches apart. To test the implications of different weather patterns on optimal fungicide application timing, intensive supplemental irrigation was applied at late vegetative growth to early bloom (V3 to R1 growth stage), at late vegetative growth to early pod (V3 to R3 growth stage), and at late vegetative growth and again at mid- to late pod-fill (V3 to R1 and R4 to R6 growth stages). In all treatments, 8 oz/ac of Endura (boscalid, 70% by weight; BASF Corp.) was applied in 15 gal water/ac. *Sclerotinia severity index is an estimate of the percent of the crop canopy that was diseased.*

Growth stage at 1st fungicide application: % of plants with an open blossom	% canopy closure	pod development	Fungicide applications	Irrigated June 29-July 17 (V3-R1)	Irrigated June 29-July 31 (V3-R3)	Irrigated Jun 29-Jul 17 (V3-R1) Aug. 8 - 18 (R4-R6)
				SCLEROTINIA SEVERITY INDEX (%)		
			Non-treated	41 a	55 c	41 a
73%	92%	no pods	July 16, July 30	20 a	38 ab	27 a
100%	97%	up 1 cm long	July 20, Aug. 3	25 a	31 a	28 a
100%	97%	up to 1.5 cm long	July 23, Aug. 5	32 a	47 bc	25 a
	100%	50% at full length	Aug. 3	32 a	50 bc	32 a
			Non-treated	34 a	52 c	31 a
				CV = 36.7	CV = 14.2	CV = 29.1
				YIELD (lbs/ac)		
			Non-treated	1990 a	1982 a	2234 a
73%	89%	no pods	July 16, July 30	2564 a	2416 a	2919 a
100%	92%	up 1 cm long	July 20, Aug. 3	2297 a	2403 a	3089 a
100%	97%	up to 1.5 cm long	July 23, Aug. 5	2280 a	2383 a	2731 a
	100%	50% at full length	Aug. 3	2320 a	2158 a	2848 a
			Non-treated	2598 a	2024 a	2722 a
				CV = 14.4	CV = 15.0	CV = 15.7

Figure 2. Response to fungicide application timing in 'Lariat' pinto beans seeded to rows 28 inches apart. To test the implications of different weather patterns on optimal fungicide application timing, intensive supplemental irrigation was applied at late vegetative growth to early bloom (V3 to R1 growth stage), at late vegetative growth to early pod (V3 to R3 growth stage), and at late vegetative growth and again at mid- to late pod-fill (V3 to R1 and R4 to R6 growth stages). In all treatments, 8 oz/ac of Endura (boscalid, 70% by weight; BASF Corp.) was applied in 15 gal water/ac. *Sclerotinia severity index is an estimate of the percent of the crop canopy that was diseased.*

Growth stage at 1st fungicide application: % of plants with an open blossom	% canopy closure	pod development	Fungicide applications	Irrigated June 29-July 17 (V3-R1)	Irrigated June 29-July 31 (V3-R3)	Irrigated Jun 29-Jul 17 (V3-R1) Aug. 8 - 18 (R4-R6)
				SCLEROTINIA SEVERITY INDEX (%)		
			Non-treated	36 a	59 c	41 c
73%	89%	no pods	July 16, July 30	21 a	29 a	16 ab
100%	92%	up 1 cm long	July 20, Aug. 3	23 a	32 ab	27 abc
100%	97%	up to 1.5 cm long	July 23, Aug. 5	30 a	46 abc	15 a
	100%	50% at full length	Aug. 3	32 a	49 bc	35 bc
			Non-treated	37 a	51 c	36 bc
				CV = 38.1	CV = 20.6	CV = 35.6
				YIELD (lbs/ac)		
			Non-treated	2056 a	1726 b	2354 b
73%	89%	no pods	July 16, July 30	2362 a	2374 a	2734 ab
100%	92%	up 1 cm long	July 20, Aug. 3	1984 a	2389 a	2530 ab
100%	97%	up to 1.5 cm long	July 23, Aug. 5	2139 a	2048 ab	2962 a
	100%	50% at full length	Aug. 3	1827 a	2031 ab	2580 ab
			Non-treated	2088 a	1778 b	2240 b
				CV = 13.4	CV = 10.5	CV = 10.2

Figure 3. Response to fungicide application timing in 'Windbreaker' pinto beans seeded to rows 14 inches apart. To test the implications of different weather patterns on optimal fungicide application timing, intensive supplemental irrigation was applied at late vegetative growth to early bloom (V3 to R1 growth stage), at late vegetative growth to early pod (V3 to R3 growth stage), and at late vegetative growth and again at mid- to late pod-fill (V3 to R1 and R4 to R6 growth stages). In all treatments, 8 oz/ac of Endura (boscalid, 70% by weight; BASF Corp.) was applied in 15 gal water/ac. *Sclerotinia severity index is an estimate of the percent of the crop canopy that was diseased.*

Growth stage at 1st fungicide application: % of plants with an open blossom	% canopy closure	pod development	Fungicide applications	Irrigated June 29-July 17 (V3-R1)		Irrigated June 29-July 31 (V3-R3)		Irrigated Jun 29-Jul 17 (V3-R1) Aug. 8 - 18 (R4-R6)	
				ERITY INDEX (%)		**SCLEROTINIA SEV**			
			Non-treated	36	a	51	c	31	a
99%	97%	up to 1-1.5 cm long	July 16, July 30	17	a	25	a	13	a
100%	98%	up to 3-5 cm long	July 20, Aug. 3	23	a	27	ab	18	a
100%	98%	up to full length	July 23, Aug. 5	24	a	28	ab	18	a
	100%	50% at full length	Aug. 3	26	a	39	abc	25	a
			Non-treated	25	a	42	bc	25	a
				CV = 39.4		CV = 21.0		CV = 41.7	
				YIELD (lbs/ac)					
			Non-treated	2774	a	2416	b	2841	a
99%	97%	up to 1-1.5 cm long	July 16, July 30	3167	a	2634	ab	3200	a
100%	98%	up to 3-5 cm long	July 20, Aug. 3	2931	a	2973	ab	3252	a
100%	98%	up to full length	July 23, Aug. 5	2659	a	3129	a	2922	a
	100%	50% at full length	Aug. 3	2799	a	2697	ab	2718	a
			Non-treated	2751	a	2783	ab	2753	a
				CV = 14.2		CV = 11.5		CV = 13.8	

Figure 4. Response to fungicide application timing in 'Windbreaker' pinto beans seeded to rows 28 inches apart. To test the implications of different weather patterns on optimal fungicide application timing, intensive supplemental irrigation was applied at late vegetative growth to early bloom (V3 to R1 growth stage), at late vegetative growth to early pod (V3 to R3 growth stage), and at late vegetative growth and again at mid- to late pod-fill (V3 to R1 and R4 to R6 growth stages). In all treatments, 8 oz/ac of Endura (boscalid, 70% by weight; BASF Corp.) was applied in 15 gal water/ac. *Sclerotinia severity index is an estimate of the percent of the crop canopy that was diseased.*

Growth stage at 1st fungicide application: % of plants with an open blossom	% canopy closure	pod development	Fungicide applications	Irrigated June 29-July 17 (V3-R1)	Irrigated June 29-July 31 (V3-R3)	Irrigated Jun 29-Jul 17 (V3-R1) Aug. 8 - 18 (R4-R6)
				/ERITY INDEX (%)	SCLEROTINIA SEV	
			Non-treated	41 b	57 c	34 a
99%	80%	up to 1-1.5 cm long	July 16, July 30	23 a	31 ab	20 a
100%	88%	up to 3-5 cm long	July 20, Aug. 3	26 ab	25 a	25 a
100%	93%	up to full length	July 23, Aug. 5	23 a	31 ab	25 a
	100%	50% at full length	Aug. 3	31 ab	48 bc	27 a
			Non-treated	27 ab	55 c	23 a
				CV = 27.1	CV = 25.3	CV = 44.2
					YIELD (lbs/ac)	
			Non-treated	2226 a	2046 bc	2267 b
99%	80%	up to 1-1.5 cm long	July 16, July 30	2513 a	2508 ab	2687 a
100%	88%	up to 3-5 cm long	July 20, Aug. 3	2547 a	2439 ab	2663 a
100%	93%	up to full length	July 23, Aug. 5	2552 a	2696 a	2686 a
	100%	50% at full length	Aug. 3	2283 a	2139 abc	2374 ab
			Non-treated	2333 a	1857 c	2305 b
				CV = 9.7	CV = 12.5	CV = 6.8

Figure 5. Response to fungicide application timing in Lariat and Windbreaker Pinto beans seeded to 14- and 28-inch rows; irrigated intensively from R1 to R6 growth stage (with sufficient frequency to maintain the soil continually moist 0.25 inches from the surface). In all treatments, 8 oz/ac Endura 70WG (boscalid, 70% by weight; BASF Corp.) was applied in 15 gal. water/ac. *Sclerotinia severity index is an estimate of the percent of the crop canopy that was diseased.*

Pinto bean cv. 'Lariat'

			14-inch row spacing			28-inch row spacing		
			Canopy closure (%)	Sclerotinia Sev. Index (%)		Canopy closure (%)	Sclerotinia Sev. Index (%)	
NON-TREATED CONTROL				6	a		6	a
July 16 and 30	**79%** bloom	pods up to **0.2 in.** long	**92%** canopy	1	a	**73%** canopy	3	a
July 20, Aug. 3	**100%** bloom	pods up to **0.6 in.** long	**97%** canopy	1	a	**83%** canopy	5	a
July 23, Aug. 5	**100%** bloom	pods up to **2.5 in.** long	**97%** canopy	3	a	**91%** canopy	5	a
				CV: 122.1			CV: 99.1	
				Yield (lb/ac)			Yield (lb/ac)	
NON-TREATED CONTROL				3070	a		2645	a
July 16 and 30	**79%** bloom	pods up to **0.2 in.** long	**92%** canopy	3299	a	**73%** canopy	2966	a
July 20, Aug. 3	**100%** bloom	pods up to **0.6 in.** long	**97%** canopy	3193	a	**83%** canopy	3056	a
July 23, Aug. 5	**100%** bloom	pods up to **2.5 in.** long	**97%** canopy	3124	a	**91%** canopy	2786	a
				CV: 17.3			CV: 13.7	

Pinto bean cv. 'Windbreaker'

			14-inch row spacing			28-inch row spacing		
			Canopy closure (%)	Sclerotinia Sev. Index (%)		Canopy closure (%)	Sclerotinia Sev. Index (%)	
NON-TREATED CONTROL				7	b		9	b
July 16 and 30	**50%** bloom	no pods	**89%** canopy	2	a	**83%** canopy	3	a
July 20, Aug. 3	**69%** bloom	pods up to **0.2 in.** long	**94%** canopy	1	a	**90%** canopy	2	a
July 23, Aug. 5	**93%** bloom	pods up to **0.6 in.** long	**95%** canopy	4	ab	**95%** canopy	3	a
				CV: 82.2			CV: 65.7	
				Yield (lb/ac)			Yield (lb/ac)	
NON-TREATED CONTROL				2977	a		2477	b
July 16 and 30	**50%** bloom	no pods	**89%** canopy	3067	a	**83%** canopy	2925	a
July 20, Aug. 3	**69%** bloom	pods up to **0.2 in.** long	**94%** canopy	3082	a	**90%** canopy	2960	a
July 23, Aug. 5	**93%** bloom	pods up to **0.6 in.** long	**95%** canopy	2904	a	**95%** canopy	3006	a
				CV: 17.2			CV: 12.7	

Figure 6. Response to fungicide application timing in Medalist and Navigator navy beans seeded to 14- and 28-inch rows; irrigated intensively from R1 to R6 growth stage (with sufficient frequency to maintain the soil continually moist 0.25 inches from the surface). In all treatments, 8 oz/ac Endura 70WG (boscalid, 70% by weight; BASF Corp.) was applied in 15 gal. water/ac. *Sclerotinia severity index is an estimate of the percent of the crop canopy that was diseased.*

Navy bean cv. 'Medalist'

			14-inch row spacing			28-inch row spacing		
			Canopy closure (%)	Sclerotinia Sev. Index (%)		Canopy closure (%)	Sclerotinia Sev. Index (%)	
NON-TREATED CONTROL				1	b		0	a
July 16 and 30	**39%** bloom	no pods	**93%** canopy	0	a	**69%** canopy	0	a
July 20, Aug. 3	**76%** bloom	pods up to **0.2 in.** long	**94%** canopy	0	a	**74%** canopy	1	a
July 23, Aug. 5	**98%** bloom	pods up to **0.6 in.** long	**95%** canopy	0	ab	**85%** canopy	0	a
				CV: 148.2			CV: 183.0	
				Yield (lb/ac)			Yield (lb/ac)	
NON-TREATED CONTROL				2279	a		2116	a
July 16 and 30	**39%** bloom	no pods	**93%** canopy	2366	a	**69%** canopy	2309	a
July 20, Aug. 3	**76%** bloom	pods up to **0.2 in.** long	**94%** canopy	2237	a	**74%** canopy	2207	a
July 23, Aug. 5	**98%** bloom	pods up to **0.6 in.** long	**95%** canopy	2355	a	**85%** canopy	2283	a
				CV: 11.4			CV: 11.3	

Navy bean cv. 'Navigator'

			14-inch row spacing			28-inch row spacing		
			Canopy closure (%)	Sclerotinia Sev. Index (%)		Canopy closure (%)	Sclerotinia Sev. Index (%)	
NON-TREATED CONTROL				0	a		2	a
July 16 and 30	**39%** bloom	no pods	**80%** canopy	0	a	**56%** canopy	0	a
July 20, Aug. 3	**76%** bloom	pods up to **0.2 in.** long	**78%** canopy	0	a	**62%** canopy	0	a
July 23, Aug. 5	**96%** bloom	pods up to **0.6 in.** long	**82%** canopy	0	a	**73%** canopy	0	a
				CV: 177.0			CV: 262.5	
				Yield (lb/ac)			Yield (lb/ac)	
NON-TREATED CONTROL				1621	a		1697	a
July 16 and 30	**39%** bloom	no pods	**80%** canopy	1925	a	**56%** canopy	1824	a
July 20, Aug. 3	**76%** bloom	pods up to **0.2 in.** long	**78%** canopy	1850	a	**62%** canopy	1847	a
July 23, Aug. 5	**96%** bloom	pods up to **0.6 in.** long	**82%** canopy	1838	a	**73%** canopy	1713	a
				CV: 21.5			CV: 10.3	

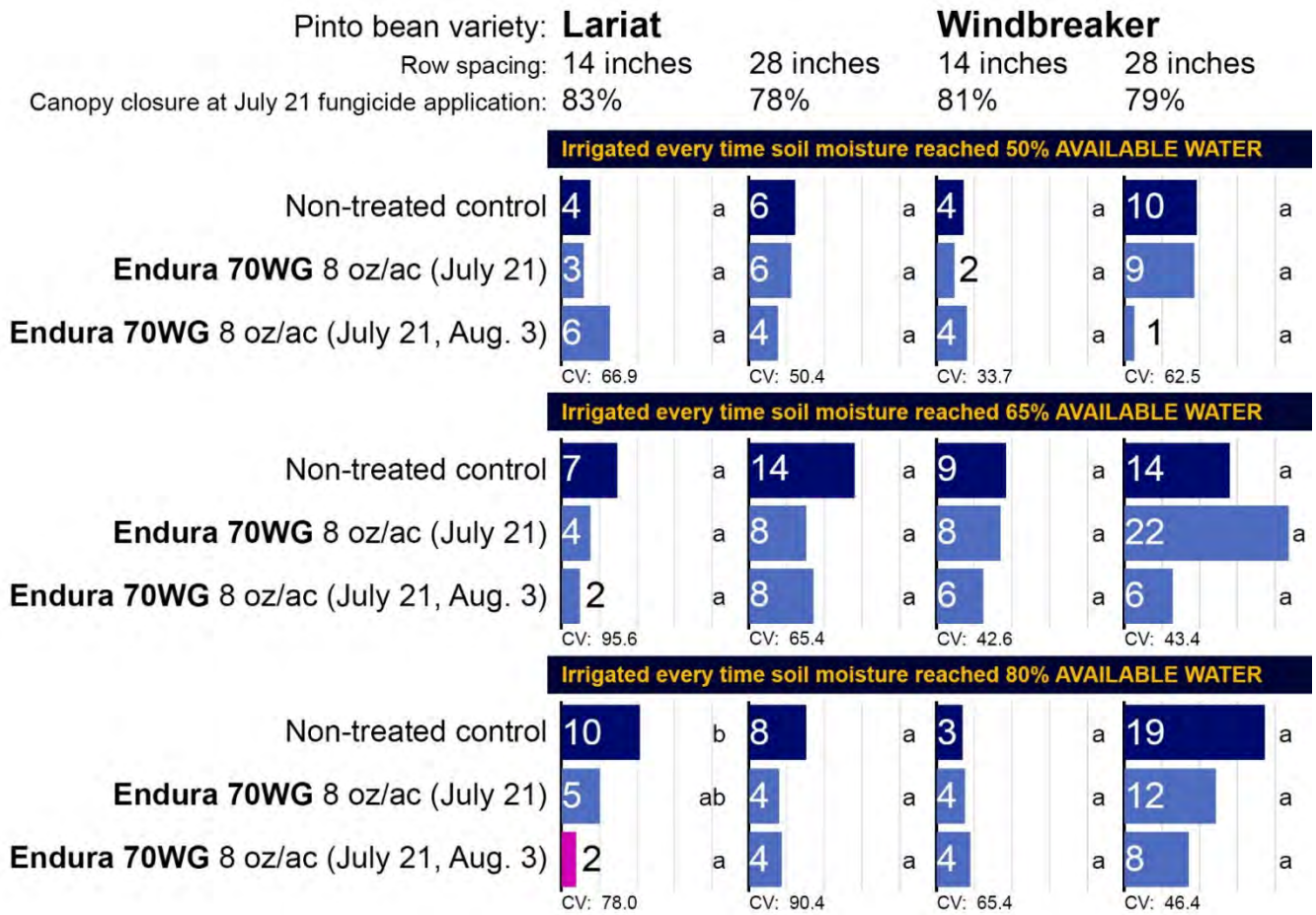


Figure 7. Changes in Sclerotinia pressure (percent of the canopy exhibiting disease) in response to fungicide application frequency in Lariat and Windbreaker pinto beans seeded to 14- and 28-inch rows; irrigated to 100% soil water-holding capacity every time that soil moisture reached 50%, 65%, or 80% water-holding capacity in the top 24 inches of the soil profile. *Fungicide applications on July 21 were conducted when an average of 51% and 71% of Lariat and Windbreaker pinto bean plants, respectively, had an open blossom.*

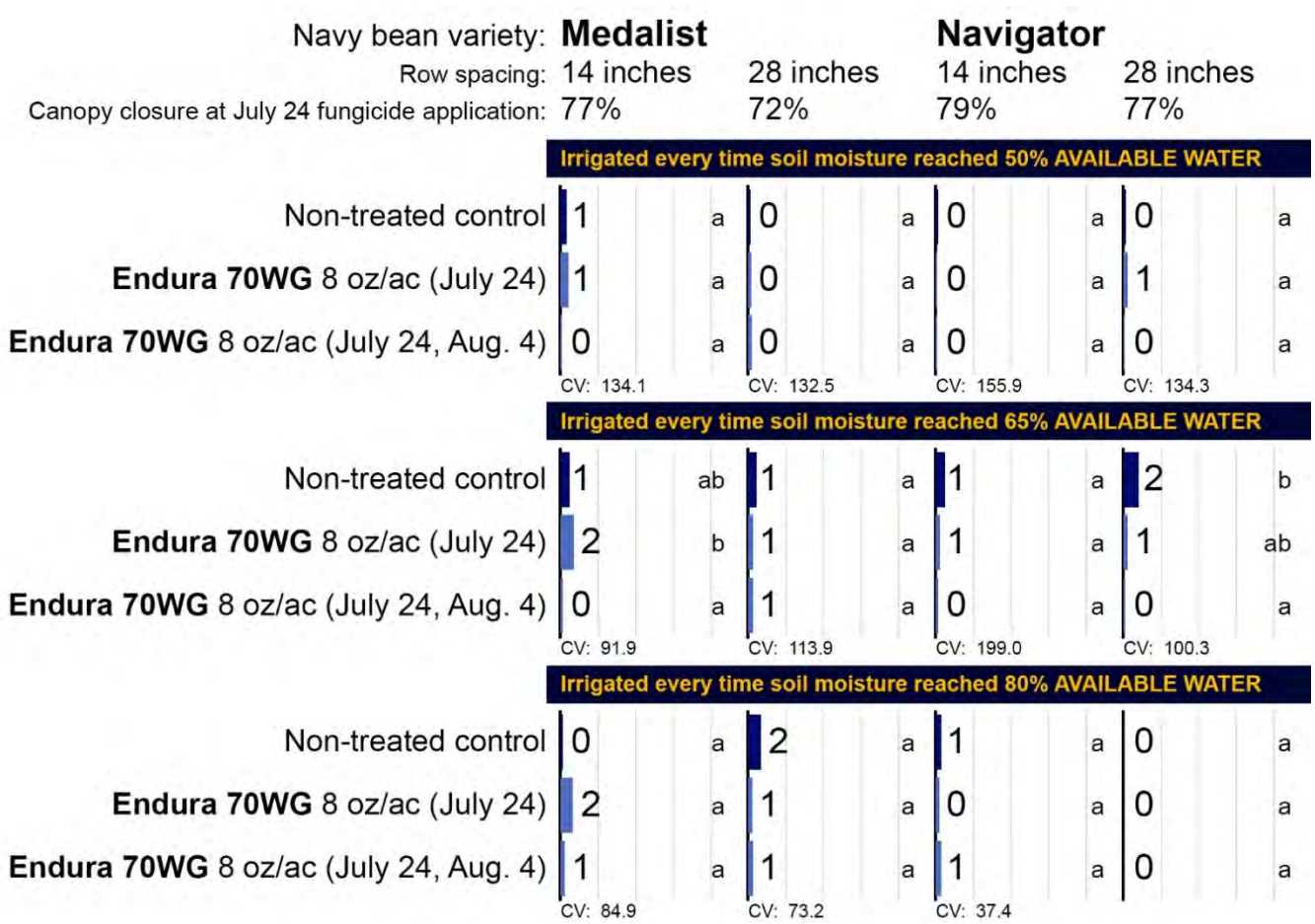


Figure 8. Changes in Sclerotinia pressure (percent of the canopy exhibiting disease) in response to fungicide application frequency in Medalist and Navigator navy beans seeded to 14- and 28-inch rows; irrigated to 100% soil water-holding capacity every time that soil moisture reached 50%, 65%, or 80% water-holding capacity in the top 24 inches of the soil profile. *Fungicide applications on July 24 were conducted when an average of 49% and 49% of Medalistt and Navigator navy bean plants, respectively, had an open blossom.*

Local Foods Initiative

Final Report

Partner Organization: North Dakota Department of Agriculture

Project Summary

Through this grant period many outcomes were achieved. First, meetings were convened across the state to identify challenges that specialty crop producers have. A final stakeholder meeting was held to discuss the findings and possible solutions to the ideas, challenges and questions brought up at the regional meetings. This grant also funded 19 community orchards across the state. In total, those orchards will have the opportunity to benefit more than 250,000 North Dakotans. Specialty crops were also promoted via over-the-air broadcasts at Farmers Markets. These broadcasts included topics about what crops were in season and differences in varieties. Producers also shared cooking and storing tips of specialty crops for consumers. These radio broadcasts helped to educate approximately 250,000 potential consumers across the state. This grant also promoted the use of specialty crops to hospitality businesses. Information was distributed to almost 700 restaurants across the state. Grants were offered to assist businesses to promote specialty crops in the food items they produced. This grant helped the North Dakota Department of Agriculture (NDDA) further its efforts to enhance the competitiveness of specialty crops focusing mainly on fruits and vegetables across the state.

This grant had many different projects with the same goal of enhancing the competitiveness of specialty crops.

- Meetings were convened with specialty crop growers in the cities of Grafton and Casselton with the purpose of understanding the challenges and needs those growers have. A final meeting was held with representatives from each of the regional meetings to identify common challenges and the next steps to help the growers in our state.
- The purpose of the restaurant grants was to help hospitality businesses promote the use of specialty crops, as well as educate them on the availability of locally-sourced specialty crops in North Dakota.
- The North Dakota community orchard project's purpose was to provide communities with the startup funds to plant and sustain an orchard in their community. This project built on previously funded projects by utilizing the same processes for grant selection developed in previously funded projects. It had also been identified in previous years that there were more communities that wanted assistance in starting a community orchard. These grant funds allowed the NDDA to immediately address those communities' needs.
- During this grant period, a professional photographer was hired to address the NDDA's need to have a diverse selection of high-quality specialty crop photos to use for many different marketing and promotion needs. Because of this grant, the NDDA has a very diverse selection of specialty crop photos that have been used and can still be used for many years to come.
- The purpose of the specialty crop radio promotion was to educate consumers about specialty crop production, and different types and varieties of specialty crops in the state. Specialty crop producers shared cooking and preservation tips during these live broadcasts.

This project was important and timely because there are many in the state that are hungry and knowledge of growing specialty crops is limited. North Dakota's population growth makes it even more important to connect new customers to those local specialty crops that are available here.

This project builds on previously funded projects, including the Hunger Free North Dakota Garden Project and North Dakota Community Orchard Grant Project by increasing the awareness of growing specialty crops to new communities and individuals. It also incorporates new activities, such as the photo library and radio promotion to increase awareness and promote specialty crops.

Project Approach

Coordinate the Hunger Free North Dakota Garden Project (HFNDGP). This was completed. The target for the 2015 growing season was 220,000 pounds. In 2015, more than 338,000 pounds of produce donations were recorded. This was well above our target. Another part of this activity was the awarding of HFNDGP grants. Working with North Dakota FFA and North Dakota State University Extension, more than 41 garden projects were funded and over 22,000 pounds of produce were donated across the state from these projects alone. The grant dollars used for these HFNDGP grants will help these projects be self-sustaining in the future. Needed supplies such as soil amendments, lumber for garden boxes and other needed gardening tools were purchased, allowing these projects to continue for many years without more grant funding.

Social Media – This activity is ongoing but during the time of the grant the number of likes on the NDlocalfoods Facebook page increased from 1,086 to 1,641.

Local Foods Conference – The Local Foods Conference was in Mandan on February 6 and 7, 2015. The conference had a pre-conference workshop showing fruit and vegetable producers how to use a record-keeping tool called Veggie Compass. Sessions included topics such as honey production, weed and animal control in your vegetable garden or fruit orchard, diseases, and labeling processed fruit and vegetable products. The Northern Plains Sustainable Ag Society (NPSAS) farm breeder club gave an update on their 2014 vegetable variety trials. A session on marketing hops was also discussed. The conference closed with a young gardener panel that shared the experiences they had growing their vegetable gardens and donating the produce to the Hunger Free North Dakota Garden Project. Based on data collected at registration, 58 percent of those attending were specialty crop producers.

Consensus Council – The regional meeting in Grafton was held March 10, 2015. Six producers attended the meeting along with five non-producers. The regional meeting in Casselton on April 8, 2015, had four producers present and three non-producers. The state meeting was held in Bismarck on April 22, 2015. Four producers attended along with six non-producers.

Community Orchard Project – This grant funded 19 community orchards across the state. Here is a summary of each project:

- Sitting Bull College, Fort Yates – Served as a teaching and learning space for local fruit production initiatives.
- Wahpeton Park District, Wahpeton – Expanded an existing community orchard, and supplied the local food pantry and school nutrition programs with harvested fruit.
- Munich Public Schools, Munich – Established to serve as a living classroom for hands-on learning initiatives.
- Richland 44 FFA, Colfax – Developed a school orchard and provided a living
- education lab.
- City of Sheyenne Tree Board, Sheyenne – Expanded and maintained the existing community and school orchard.
- City of Mylo, Mylo – Developed a community orchard to educate all ages about fruit production, fruit preservation and basic tree care.
- CHARISM Neighborhood Center, Fargo – Orchard provides neighborhood families and programs with fruit production to combat food insecurity.
- Go Bismarck Mandan Healthy Community Coalition, Bismarck – Developed multiple orchard locations to provide hunger relief, fruit production education and enhance community green space.
- Fargo Union Mission dba New Life Center – Created two orchard spaces to provide produce to guests and others in need.
- Valley City Beautification Commission – Helped jumpstart a larger beautification project in the city and provide learning opportunities for city civic groups.
- Pembina County Historical Society, Cavalier – Developed an orchard to encompass education, agriculture and nutrition through classes and workshops.
- Hankinson Public Schools, Hankinson – Established an orchard as a learning center for students and members of the community.
- Oberon School District #16, Oberon – Created an orchard to provide a living classroom for students to learn about horticulture and where their food comes from.
- Northwood Public Schools, Northwood – Planted an orchard to educate students on the growing cycle of fruit trees and bushes, to use the fruit in the school lunch program and to beautify the area.
- NDSU Dickey County Extension, Ellendale – Two orchard projects in Dickey County provided lessons for youth in planting, tending, harvesting, and preserving fruit and pollination.
- Lisbon Public Schools, Lisbon – Established an orchard to provide hands-on learning for elementary students on how to take care of the orchard and furnish fruit for breakfast and lunch programs.
- Edgeley Public Schools, Edgeley – Developed an orchard to be a teaching resource for the school and surrounding community.
- Cheney Middle School Team Thor Self-Contained Programs, West Fargo – Planted an orchard to benefit the school and community by providing learning opportunities for both.
- Legacy Elementary School, West Fargo – Established an orchard to teach students of all grade levels about where their food comes from and how to grow it. The fruit from the orchard will also be used for school snacks.
- Metigoshe Ministries, Bottineau – Developed an orchard to use as a hands-on learning center for campers, educating them on orchard care and where their food comes from.

Local Foods Restaurant Program – This activity had two parts. The first part was packets assembled by NDDA explaining what specialty crops were and information about procuring them from local sources. The NDDA partnered with the North Dakota Hospitality Association and mailed out almost 700 packets to hospitality business across the state. The packet of information that went out also contained information about a grant opportunity that was available through this grant to assist these businesses in promoting the specialty crops they use. From that initial mailing, we received many inquires but only one business qualified for the grant. A second effort was made to deli owners through the North Dakota Rural Grocers Association. Again, the NDDA received interest and even sent out notice of grant awards (NOGA) to two businesses but they did not sign the NOGA, so no funds were awarded to them.
Photo Library – This activity was completed with great success. A professional photographer was hired and specialty crop photos were taken on farms, at farmers markets, in a restaurant and at an orchard. The activity resulted in the NDDA getting more than 300 high-quality images of specialty crops that can be used for many years to come.

Specialty Crop media promotion – This activity focused on a billboard advertising campaign in the months of July and August. Billboards were placed within city limits of six urban communities including: Bismarck, Fargo, Grand Forks, Mandan, Minot and West Fargo. The billboards tagline was "Now in Season Fresh Fruits and Vegetables". The specialty crop billboard campaign ran for the months of July and August and the location of the billboard within the city was different each month. The billboard company estimates there was a total of 203,200 views per day. With a total view for the 60-day campaign at 12 million views.

With funds from the grant, we were able to run this campaign during the months of July and August in the summers of 2015, 2016 and 2017. In 2016 and 2017, we added the city of Dickinson. We estimate that during the three years, these billboards had more than 40 million views by motorists.

Second Printing of the Orchard Manual – This activity was started but not completed due to circumstances out of our control.

Goals and Outcomes Achieved

Consensus Council
Goals – To expand the knowledge and understanding of marketing specialty crops directly to consumers. To create regional working groups of specialty crop producers that can share best practices with each other. To develop a list of five statewide attainable action steps necessary in the upcoming year.
Through this grant, this goal was achieved by the NDDA directly getting input from the producers. The meeting facilitator used the survey as a starting point. Producers were able to provide feedback on the survey and share specific examples that relate to their operation at the regional meetings. At all the meetings, there were two common themes – producers have questions about regulations and they need more assistance with marketing. Below is the list of the five action steps identified at the state meeting.

1. Educate specialty crop producers and agents on new crop insurance..

2. Educate specialty crop producers on Good Agriculture Practices and how the new Food Safety Modernization Act rules will apply to them.
3. Offer more marketing training in different forms to specialty crop producers.
4. Divide the Farm to Market Guide into sections for marketing direct, at farmers markets, wholesale, to schools and nursing homes. Each section would be topic specific and written so the producer can understand it.
5. Distribute information to grocery stores to promote buying direct from specialty crop producers and work with the North Dakota Grocers Association.

Since these meetings, the NDDA has been focusing more of its efforts in these areas. An unexpected outcome from these meeting was the increased favorable rating the NDDA has received. To have NDDA staff drive across the state and hold meetings showed producers in our state the level of commitment the NDDA has to their overall success.

North Dakota Community Orchard Project

Goal – To increase and expand access to specialty crops by developing community orchards in 15 North Dakota communities. The orchards will be used to provide educational and nutritional knowledge and learning opportunities, as well as specialty crop consumption among children and adults.

This goal was surpassed. The NDDA awarded 19 projects grants and all of them completed their projects. Eighteen of the projects educated youth and will continue to be used as outdoor classrooms. Fourteen of the projects indicated that once their orchards start producing fruit, they will use that produce for hunger relief in their community. Fifteen of the projects are and will be used to directly impact families in the community.

Hunger Free ND Garden Grants

Goal - In partnering with the ND FFA, the goal was to award 10 specialty crop garden grants for youth organizations. The goal of these gardens was to show the values of donating to those in need, while provide learning opportunities and getting young people interested in pursuing future career goals related to specialty crops.

Through this grant, more than 41 garden projects were funded and over 22,000 pounds of produce were donated across the state from these projects alone. The NDDA also partnered with NDSU Extension to surpass the initial goal that was set. More than 2,600 youth and community members became engaged in the planning, creation, planting, growing, harvesting and donating of the produce that was grown in these gardens across the state.

Local Foods Conference

Goal – The previous year's specialty crop grower attendance was 72 percent. The goal is to strive for 75 percent attendance of specialty crop producers and to make the conference a "must attend" event for specialty crop producers. The outcome will be measured by surveys that specialty crop producers and attendees fill out stating how useful they feel the information will be to their specialty crop operation.

At the 2015 conference, 58 percent of the attendees identified themselves as specialty crop producers. This was below our goal. One of the possible reasons for this was the conference was held in a larger city and because of that, there were more specialty crop enthusiasts and

individuals that support specialty crop producers that attended. This group of people may garden at home but may not classify themselves as a producer.

Beneficiaries

The specialty crop stakeholders and groups include more than 150 specialty crop producers across the state and the agencies listed throughout this report that have a vested interest in helping this industry be successful in our state. These groups include but are not limited to state and local 4-H clubs and FFA chapters, the Great Plains Food Bank and its more than 200 partner agencies.

Community Orchard Grants
The community orchard grants benefited 250,329 people in 17 communities across North Dakota.

These communities all benefited by creating an orchard that was and will continue to be used as a living classroom, teaching young and old about specialty crops and how to care for them. Once these orchards start producing fruit, many of these projects indicated that they would donate the produce to charitable feeding organizations in their communities, benefiting even more people.

Hunger Free North Dakota Garden Project
The Hunger Free North Dakota Garden Project benefited 73,948 North Dakota residents (based off the Great Plains Food Banks estimates that 1 in 10 North Dakotans sought food assistance). Groups that benefited from this project include the Great Plains Food Bank, FFA, 4-H and more than 200 food pantries, soup kitchens and other food assistance organizations across the state.

NDFMGA & Local Foods Conference
The conference benefitted 42 specialty crop producers across the state who were in attendance. These producers benefited by attending presentations and breakouts sessions to learn how to better grow and market the specialty crops they raise. Other groups that had representation included North Dakota State University (NDSU), North Dakota Farmers Market and Garden Association, Dakota College at Bottineau, USDA Rural Development, FFA, 4-H, local health units, and the Bismarck-Mandan Convention and Visitors Bureau.

Lessons Learned

The first lesson learned came out of the community orchard grants. One of the grant recipients quit communicating with the department for a period after the grant was open. This caused a lot of issues related to administering their grant and the NDDA questioning if the project was going to be completed at all. In the end, the project was completed as outlined in their application. One of the potential solutions to prevent this from happening again is to ask for a second contact person or ask for a second phone number to reach the main contact person. The second lesson from this was the importance of keeping a communication log. The communication log allowed the NDDA to document the number of times the NDDA reached out to this grant recipient. The

communication log also proved valuable in explaining the situation to supervisors and upper management so a solution based on the facts could be determined.

One project not completed was the orchard manual. The lesson learned from this activity is that from the beginning all parties involved must fully understand the expectations of the project. For future projects, we learned that specific responsibilities need to be communicated at the start of the project.

Another lesson learned was the value of organization. This was especially important for the live broadcasts. This activity resulted in working with 15 radio stations that were hundreds of miles apart in different cities and most of the live broadcasts occurred within a month and a half. This involved working months in advance to secure times that not only worked for the stations but the specialty crop producers at the market as well. We also had to make sure the costs fell within budget. To manage all of this, a master spreadsheet was created that had all the important information in one place. The creation of a master spreadsheet is a tool that can be used for many different projects that have lots of details to manage.

Contact Information

Jamie Good
North Dakota Department of Agriculture
jgood@nd.gov
701-328-2659

Additional Information

Sample Orchard Grant Photos

CHARISM Orchard

City of Mylo

City of Sheyenne

Wahpeton Park Board

Hankinson Public School

Legacy Elementary School

Billboard advertising artwork

Hunger Garden Grants selected photos

Spray Bottles
FRONT PAGE TALENT

CARGILL
WYNDMERE FFA
Community Garden
GUIDELINES:
1) Everyone is welcome to enjoy the garden.
2) Anyone may weed or water
3) Produce not taken will be donated.
4) Any issues please report to the FFA program.

North Dakota Specialty Crop Export Expansion Project

Final Report

Partner Organization: North Dakota Trade Office (NDTO)

Project Summary

The North Dakota Trade Office (NDTO) will increase market visibility of the North Dakota specialty crop sector by developing future international markets. NDTO implemented a reimbursement program for approved participating companies in the North Dakota Specialty Crop Export Expansion Project. Specialty crop (SC) companies participated in NDTO led trade missions and one reverse trade mission to increase the U.S. and North Dakota's market share in each target country where companies met pre-qualified buyers, distributors and importers of specialty crops.

North Dakota is well-known domestically for being a top producer of peas, beans, lentils, and confectionary sunflowers; accounting for nearly 40% of the US market according to the Northern Pulse Growers Association. Globally, the U.S., and specifically North Dakota growers are relatively invisible, dwarfed by competitors, such as Canada and Australia. North Dakota producers want to increase their visibility and increase their market share averaging between 1% and 10% in four target countries. The North Dakota Trade Office (NDTO) is committed to expanding and increasing North Dakota trade, and we will assist companies with the development of long term expansion initiatives. NDTO will focus on building long-term success by 1) leading trade missions to Italy, Croatia, Colombia and Israel; 2) working with partners, such as the Foreign Ag Service (FAS) to identify prospective partners; 3) planning meetings and events to introduce North Dakota products to the target countries; and 4) conducting an inbound trade mission bringing buyers to North Dakota.

The objectives of the North Dakota Specialty Crop Export Expansion Project are:

1. Conduct 3 outbound trade missions to Italy-Croatia, Colombia, and Israel
2. Conduct one inbound trade mission for top prospects from each country to North Dakota
3. Meet prospective buyers in each of the four targeted countries
4. Increase the U.S. and North Dakota's market share in each country

Increased exports are vital to North Dakota's economy. Through expanded trade North Dakota creates new opportunities to sell products and services abroad. North Dakota growers of specialty crops are expecting an increase in production this year due to delayed planting conditions. The domestic market for their crops is limited. 97% of the world's consumers live outside the United States. The targeted countries are net importers of the products we grow. North Dakota leads the nation in the production of pulse crops, beans and confectionary sunflowers. These commodities are dependent on an export market, and with additional capacity; there is no better time to find new buyers, than the present.

This project was not built on a previously funded project.

Project Approach

- Work Plan: During the grant period NDTO conducted four (4) outbound trade missions, and one (1) inbound (reverse) mission.
- Trade Mission to Italy and Croatia
- Trade Mission to Colombia
- Reverse Trade Mission from Colombia
- Trade Mission to Egypt/Israel
- Trade Mission to India

- Trade Missions are used to develop new export markets for specialty crops. NDTO acted as the trade mission lead using a trade mission project plan and checklist. NDTO coordinated with the US Foreign Ag Service (USFAS) in country on logistics, interpreters, hotel, transportation in country etc. The market briefing, contacts and meetings with pre-qualified buyers, distributors and importers were arranged by USFAS in each country. The companies prepared brochures, descriptive literature and samples for each trade mission. A tour of the local market is also arranged.
- During the meetings, companies had the ability to share sample product, and discuss their individual specialty crops.
- NDTO handled the post mission work of surveying for results, data collection and reporting.

- Potential benefit to non-specialty crop products: The activities planned, including the companies involved and the prospective buyers were carefully vetted to ensure that NDTO mission was focused specifically and solely on allowable specialty crops.

- Trade Mission Model and Checklist:

Select Mission Destination:
6+ months prior to mission

Identify Date of Mission and Timetable

Reach Out to Companies and US FAS

Travel Logistics
Begin 2.5-3 months prior to mission

Itinerary/Agenda Building

North Dakota – XXXXXXXXX Trade Mission Specialty Crops

Date

Date	
--:--	• Arrival to XXX • Hotel Check-In
Date	
08:00 AM – 08:30 AM	
08:30 AM – 09:00 AM	
09:00 AM – 09:20 AM	
09:20 AM – 10:20 AM	• Introduction to Pulse Ingredients and Applications -
10:20 AM – 10:40 AM	• Break (Group Photo)
10:40 AM – 11:40 AM	• Nutritional Properties of Pulses -
11:40 AM – 12:00 PM	• Pulse Marketing Activities -
12:00 PM – 01:00 PM	• Lunch
01:00 PM – 02:00 PM	• Depart to meetings
02:00 PM – 04:00 PM	• Tour & Visit - Facility Tour
04:00 PM – 5:30 PM	• Depart to XXX
5:30 PM	• Dinner • Back to Hotel
Date.	
08:30 AM – 09:00 AM	• Depart to
09:00 AM – 09:30 AM	•
09:30 AM – 10:20 AM	• P

10:20 AM – 10:30 AM	• Break
10:30 AM – 12:00 PM	
12:00 PM – 01:00 PM	• Lunch
01:00 PM – 01:30 PM	
01:30 PM – 02:30 PM	
02:30 PM – 02:45 PM	• Break
02:45 PM – 05:45 PM	• 1-on-1 Meeting
05:45 PM – 07:00 PM	• Dinner a
07:00 PM –	• Depart to •
07:00 AM – 9:00 AM	
09:00 AM – 12:00 PM	• Visit & Meeting -
12:00 PM – 01:00 PM	
01:00 PM – 02:00 PM	• Lunch with
02:00 PM – 05:00 PM	• Visit & Meeting -
05:00 PM – 5:30 PM	•
06:30 PM –	• Dinner at
Date	
- 09:00 AM	
09:00 AM – 11:30 AM	
11:30 AM – 01:00 PM	
01:00 PM – 02:30 PM	

02:30 PM – 05:30 PM	• Visit & Meeting
05:30 PM – 07:00 PM	• Depart to
07:00 PM –	• Dinner
Date	
--:--	• Hotel Check-Out / Depart to Airport

Materials Review

Brochure Translation

Mission Logistics

Post Mission

- Evaluation summary/post mission survey

NDTO
NORTH DAKOTA TRADE OFFICE

INTERNATIONAL TRADE MISSION SURVEY

Please help us to understand how satisfied you are with this trade mission by completing our customer satisfaction survey. This information will remain confidential and used only in aggregate form to educate our team and the public regarding the success of the mission.

Please return this survey to the trade mission coordinator upon return from the trip or utilize mail, email, or fax number below to return the survey. If you have any questions please contact **Lindsey Warner at 701.231.1154**

Mail: Dept. 0510 PO Box 6050
Fargo, ND 58102

Fax: 701.231.1151

Email: lindsey@ndto.com

Please be assured mission results data will only be reported in aggregate.

Company Name:

Trade Mission Objective:

Please rate the following on a scale of 1 to 5 (excellent) as it relates to the trade mission:

Pre-event planning & communication	N/A	1	2	3	4	5
Overall Mission execution	N/A	1	2	3	4	5
Fulfillment of your company needs	N/A	1	2	3	4	5
Cost/benefit returns to your company	N/A	1	2	3	4	5
Quality of contacts or information	N/A	1	2	3	4	5
Increased knowledge of exporting	N/A	1	2	3	4	5
Travel logistics	N/A	1	2	3	4	5
Hotel accommodations	N/A	1	2	3	4	5
ND Trade Office performance	N/A	1	2	3	4	5

Overall, how well did the Trade Mission meet your objective on a scale of 1(poor) to 5 (excellent) 1 2 3 4 5
Comments

Would you participate in another trade mission? *Yes No*

If not, why?

Do you believe the state should fund future trade missions? *Yes No*

Are there countries you are specifically interested in with regard to future trade missions?

Has your company achieved actual sales or contracts as a result of this mission? *Yes No*

If yes, approximate how much? (Please provide estimated value)

Percentage increase in business:

Does your company anticipate an increase in export sales within the next year as a result of this activity? *Yes No*

If yes, approximately how much? (Please provide estimated value, confidential, used in aggregate)

Did this mission result in expansion to a new market for your company? *Yes No*

Did this mission yield contacts with new foreign buyers? *Yes No*

If yes, how many?

Did this mission result in any new distributorships being established? *Yes No*

If yes, how many?

Did this mission result in any new joint ventures being established? *Yes No*

Other comments/feedback concerning compliments or areas for improvement:

Thank you for taking the time to complete this evaluation!

Reverse Mission

Within 6 Months Following Trade Mission

Goals and Outcomes Achieved

Goal: $4.25 M in export sales through increased visibility of North Dakota Specialty crops in international markets
Benchmark: US Bureau of Census 2012/2013 export statistics
Target Markets: Croatia**,** Italy, Colombia, Egypt/Israel, India
Performance Measure:

The number of ND SC export companies that participate,
The number of meetings each company has on a trade mission or reverse mission
The value of export sales that result from these activities

Twelve specialty crop exporters increased the visibility of the North Dakota Specialty crop sector by traveling on five international trade missions to six countries meeting with 54 pre-qualified prospective buyers, distributors and importers of specialty crops. The result was $5.698 million dollars in sales of North Dakota specialty crops obtained through exporter trade mission surveys, US Bureau of Export statistics, and follow-up phone calls. In addition to the five trade missions, NDTO organized a reverse mission bringing 13 prospective buyers to North Dakota.

Activity	Month	# Participants	# Meetings	Export Sales
Italy/Croatia TM	Nov-14			

		3	**6**	**$1,035,000**
Colombia TM	**Apr-14**	**5**	**15**	**$2,764,885**
Egypt/Israel TM	**Jan-16**	**2**	**15**	**$0**
India TM	**Feb-16**	**2**	**12**	**$0**
Colombia /ND RM	**Aug-14**	**7**	**13**	**$1,898,310**
Total Export Sales				**$5,698,195**

Beneficiaries

- 2 new ND SC exporters were identified through outreach efforts for this grant and both participated in outbound trade missions with high interest from prospective buyers and sales or agreements being negotiated resulting from grant activities.
- The North Dakota Agricultural Statistics of USDA reported that in 2015 ND producers planted an additional 10,000 acres of dry edible beans from 650,000 ac res in 2014 to 660,000 acres in 2015.
- It is impossible for the NDTO to identify all the stakeholders that benefit from the completion of this projects accomplishments, the hundreds of growers, producers, carriers, and others benefit from the ripple effect increase in export sales has in our state economy. Directly, there are a core group of ten (10) SC export companies that benefit; they are pulse crop and / or dry bean processors and growers. These SC export companies receive the export sale, contract with the growers, clean and package the products, prepare the export, contract with the freight forwarders and carriers and implement the export process.

Lessons Learned

As expected, the only constant is that things always change. We have learned that during times of high prices the successful exporters will focus on meeting buyers and building relationships that will result in future sales. Specific examples are below.

- Buyers that were met during the Italy and Croatia Trade Mission in the fall of 2014 did not travel to ND for the reverse trade mission due to current pricing. With the value of the U.S. dollar at an all-time high, our products are more expensive than competitors.
- The Israel Trade mission was delayed by a few months from the expected date of November 2015 to January 2016. This delay was due to SC companies not being available to

travel to this market at the planned dates. The Israel Trade Mission was rescheduled based on availability of the participating SC companies.

- Due to current global situation, high ag commodity prices, high value of US dollar, terroristic concerns most companies, including SC companies have been careful where they travel to. That has significantly reduced the level of participation from our companies in these TM's, most are opting to stay home.
- The availability of competing product into Egypt/Israel from the Black Sea region (Russia/Ukraine) is impacting US sales. The Black Sea Region has low transportation costs and the low exchange rate compared to the dollar makes this region more favorable for buyers. Chickpeas were an in demand crop on this TM.
- The pulse industry in India market in the short run is flat. Pulses are not being bought from US markets due to high commodity prices and a high dollar value.
- One company, JM Grain's attendee on the Egypt/Israel TM built this trip, trade mission, into their travel plans, between shows. They did not depart from or return to their home state.

Contact Information

- Sharon May
- Telephone Number : 701-231-1158
- Email Address: sharon@ndto.com

Additional Information

- Press Release

Note: The Press Release references three delegations visiting North Dakota at the same time in August 2015, only one delegation, the delegation from Colombia was a SC delegation. The three reverse missions were published in a single press release as they happened at the same time. Each had its own Agenda and activities and to ensure media coverage of the missions.

North Dakota Hosts Business Leaders from Colombia, China in August

by NDTO Press Release

Posted on 8/20/2015

Three international delegations are visiting North Dakota this month in order to take part in reverse trade missions focused on the state's specialty crops and identity-preserved (IP) soybean markets. Two Chinese delegations and one Colombian delegation will tour the state, take part in educational events, and meet with North Dakota companies. The missions were organized through the cooperation of the North Dakota Trade Office, Northern Crops Institute, Northern Pulse Growers, Northarvest Bean Association, North Dakota Soybean Council, and North Dakota State University.

"Reverse trade missions give North Dakota companies the opportunity to show overseas buyers the amount of care and expertise that goes into our specialty crops and IP soybean products," said Dean Gorder, executive director of the North Dakota Trade Office. "These efforts are especially effective when preceded by an outbound trade mission, as we've done in Colombia and China. Now we get to return the hospitality to potential buyers that we've met abroad, show

them how our high quality crops are grown and processed, and where their food ingredients come from."

Colombia-North Dakota Specialty Crops Initiative

The Colombia reverse trade mission began on Monday with a focus on specialty crops and pulse ingredients such as peas, beans, lentils and confectionary sunflowers. The mission started off at the Northern Crops Institute where 17 Colombian delegates attended sessions on using pulse ingredients in baked goods, pasta, and snacks; pulse marketing activities; and quality characteristics of northern grown pulse. Participants continued on with presentations and one-on-one meetings with North Dakota companies. The mission will conclude tomorrow with visits to several company operations and a tour of the North Dakota Heritage Center in Bismarck.

The Colombia-North Dakota specialty crops initiative picked up momentum in April when five North Dakota pulse and dry bean processors toured Colombia and met with 20 potential specialty crop buyers. The delegation found success using the combined approach of holding wholesale buyer meetings as well as meetings with supermarket chain owners in order to sell ND pulses directly to Colombian consumers. Plans are in motion to return to Colombia in February 2016 to focus again on Colombian supermarkets and introducing the Colombian palate to ND pulses.

Discovery of Specific Starch Properties of NDSU Potato Germplasm for Nutritional and Industrial Applications

Final Report

Partner Organization: North Dakota State University

Project Summary

Amylose and amylopectin, the mostly resistant and soluble forms of starch, respectively, are two forms of starch present in the granule. In this project, we examined the effect of microwave steaming, a new cooking method, on soluble starch and resistant starch levels in potato tuber tissue; we determined that steaming is a more rapid and efficient means to cook tuber tissue for starch analysis, versus traditional baking or boiling methods. Using the steaming method, 219 clones from the North Dakota State University (NDSU) potato breeding program were evaluated soluble and resistant starch content. Unique clones were identified with varying levels of soluble and resistant starch compared to the Russet Burbank, Red Norland and Yukon Gold check cultivars. Physichochemical assessment of 12 unique clones, chosen based on their relatively high or low levels of soluble or resistant starch (six of each), compared to the three check cultivars, identified diverse granule sizes, pasting characteristics, gelatinization temperatures, and amylose and amylopectin molecular weight and abundance. Greenhouse-grown tubers were compared to field grown tubers, as a means to more efficiently screen potato genotypes, and were found to contain more soluble and resistant starch content than the field-grown tubers, implying that greenhouse-grown tubers cannot be used to screen genotypes for starch content. Unique genotypes may be of interest for utilization and manufacture of nutritional products, in bioplastics or other industrial application, pharmaceutical uses, and in novel foodstuffs.

Potatoes are approximately 80% water and 20% dry matter, with most of the dry matter being starch; however, genotypes vary for dry matter content, an environmentally sensitive trait. For example, Russet Burbank, the most widely grown cultivar in North America, has a mean specific gravity of approximately 1.074 in the Columbia Basin and approximately 1.091 in North Dakota, while Dakota Trailblazer (Farnsworth et al 2011) has a mean of 1.095 and 1.108 in WA and ND, respectively. Potato starch is composed of about 20-30% amylase (relatively small molecules of linear chains of glucose residues) with the amylopectin (has very large molecules containing branch chains of glucose residues) comprising the rest (Hoover 2001). Starch quality is important for the potato industry because the glycemic index is reduced in clones that have high proportion of amylase, texture and cooking requirements are affected by starch characteristics (Briant et al. 1945), and starch for industrial uses requires specific quality parameters. The North Dakota State University (NDSU) potato improvement team has actively developed improved potato cultivars and selections with high levels of starch and associated quality attributes suitable for French fry (other frozen) and chip processing; however, determination of specific starch profiles for this germplasm has not been explored.

The objectives of the work were to: 1) Evaluate parental genotypes and advancing selections for starch attributes, capitalizing on the genetic diversity contained within the NDSU potato breeding program; 2) Identify appropriate genotypes for fine screening of starch quality traits, based upon initial screening (objective 1), resulting in identification of clones suited for specific

innovative and novel end uses; and 3) Produce the prospective potato genotypes and work with industry and entrepreneurs to develop products (nutritional applications, pharmaceutical, snack foods, industrial, and others) as appropriate.

This project was a continuation of SCBG 12-25-B-1480. While an initial year of funding was sufficient to initiate the project, additional funding allowed us to validate the data collected, to conduct screening of a greater number of genotypes, as well as to compare field locations and production in the greenhouse to identify the best source of tuber material for evaluation and the rapid identification of potato genotypes with unique starch attributes.

Project Approach

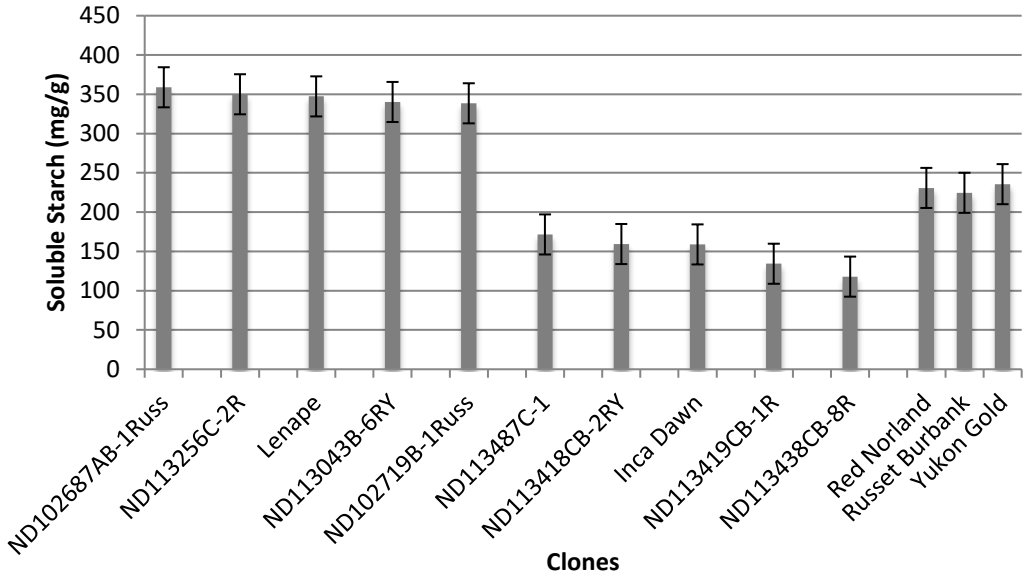
Activity one was to evaluate potato clones grown in the field utilizing the Megazyme procedure. Two hundred nineteen clones were grown at two locations (Absaraka, ND, and Baker, MN) in 2014; both were non-irrigated sites. From Baker, 199 clones were investigated, and 43 genotypes from Absaraka were examined. Of the genotypes analyzed from Absaraka, 23 of the clones were also studied from Baker. Three check cultivars (Red Norland, Russet Burbank, and Yukon Gold) were used for each set of clones analyzed. Ziploc® Zip'n Steam bags were used as a cooking method for our study. Two tubers of each clone were washed, peeled, cut into identically sized pieces (2.5 cm^2), placed into a Ziploc® Zip'n Steam bag, and microwaved (1200W) for 4 minutes on high (steamed). Following cooking, cooked tuber tissue was riced and mixed. Soluble starch (SS) and resistant starch (RS) were determined using the Megazyme Resistant Starch Assay (K-RSTAR, Megazyme International Ireland, Ltd, Co. Wicklow, Ireland) kit. A modified miniaturization of the assay was utilized (Raatz et al. 2016). Samples of potato were analyzed in triplicate. Unique clones within the NDSU Potato Breeding program were discovered with significantly higher and lower levels of soluble starch (Table 1). Our statistical model indicated that clones grown at Absaraka, ND, did not differ in their SS or RS levels. Additional clone data (ie. 219 clones, 2 locations) can be found in: Krabbenhoft, L.K., 2016. Identification of specific starch profiles in North Dakota State University potato germplasm for nutritional and industrial utilization. MS Thesis. North Dakota State University. 118p.

Table 1. Unique potato clones found within NDSU germplasm resources compared to control varieties, for soluble and resistant starch, grown at Baker, MN, in 2014.

	Clone	Soluble Starch (mg/g)		Clone	Resistant Starch (mg/g)
Highest 5	ND102687AB-1Russ	358.9	a*	ND113517ABC-9	152.1
	ND113256C-2R	350.0	ab	ND102822CAB-1	143.1
	Lenape	347.3	ab	ND113163-1	137.5
	ND113043B-6RY	340.2	b	ND102549TB-2Russ	129.4
	ND102719B-1Russ	338.5	b	ND113517ABC-6	127.9
Lowest 5	ND113487C-1	171.6	d	ND113438CB-8R	54.0
	ND113418CB-2RY	159.4	de	ND102990B-3R	49.1
	Inka Dawn	158.9	de	ND113060-1	47.4
	ND113419CB-1R	134.3	ef	ND102903-1R	46.0
	ND113438CB-8R	117.9	f	ND102921C-3	40.9
Controls	Red Norland	230.8	c	Red Norland	75.0
	Russet Burbank	224.6	c	Russet Burbank	81.4
	Yukon Gold	235.6	c	Yukon Gold	91.3
	Mean	246.6		Mean	85.8
	LSD (α = 0.05)	18.6		LSD[a] (α = 0.05)	8.9

[*]*Different letters* signify *means* are *significantly different* using PROC MIXED (α ≤ 0.05).
[a]differences between clones for resistant starch were not detected, so a protected mean square was used.

The control varieties, Red Norland, Russet Burbank, and Yukon Gold, were not shown to differ significantly in their SS levels.



- *Figure 3.1. Unique clones grown at Baker, MN with highest or lowest levels of soluble starch (mg/g) based on dry weight. Error bars represent ±SE. LSD for soluble starch (mg/g) = 18.6.1*

Different market classes of potato were evaluated in our studies, including dual-purpose, frozen processing, chip processing, specialty, germplasm, fresh, and flake. Significant differences were found for SS and RS among market types grown at both locations. Although studies have been performed that examine the starch profiles of various clones, no known research has been performed on the starch profiles between specific market types. Mean starch profiles for market classes grown at Baker, MN, and Absaraka, ND, are presented in Table 2. Frozen and flake market types were not grown at Absaraka, ND. From Baker, MN, there were 14 dual-purpose, 19 frozen processing, 34 specialty, 41 germplasm, 45 fresh, 45 chip processing, and one flake market class analyzed. From Absaraka, ND, eight dual-purpose, 11 specialty, one germplasm, 11 fresh, and 12 chip processing market genotypes were analyzed.

Table 2. Average soluble and resistant starch levels between market classes and growing locations.

	Location			
	Baker, MN		Absaraka, ND	
Market Class	Soluble (mg/g)	Resistant (mg/g)	Soluble (mg/g)	Resistant (mg/g)
Dual purpose	266.8[b*]	74.6[bc]	212.0[a]	92.4[b]
Frozen	260.4[b]	93.8[ab]	na[**]	na
Specialty	255.5[b]	79.9[ab]	177.5[a]	99.7[ab]
Germplasm	245.1[b]	84.1[ab]	200.6[a]	95.9[b]
Fresh	244.6[b]	78.6[ab]	195.6[a]	103.4[ab]
Chip	257.8[b]	104.6[a]	229.6[a]	119.0[a]
Flake	323.6[a]	53.3[c]	na	na

[*] *Different letters* signify *means* are *significantly different* using Tukey's Range Test ($\alpha \leq 0.05$).

[**]The frozen and flake market classes were not analyzed from genotypes grown at Absaraka.

Our results indicate that clones within the chip processing market class have a significantly higher level of RS, suggesting that higher levels of amylose may contribute to improved chip color and quality. Potato clones used for frozen products possess similar qualities as chip processing market clones, although higher sugar content can be tolerated (Smith 1977). Starch from potato is preferred by the food industry due to the low level of lipids and protein, and thus good paste clarity (Glicksman 1969). Potato starch dextrins are advantageous over other sources of starch for adhesives due to their remoistenability and rheology, resulting in a desirable direct tack (Zobel et al. 2009). The textile industry also produces better products with potato starch, due to its film properties (Kerr 1950). Potato, tapioca, and waxy maize starch have desirable traits in oil drilling technology, because of their excellent fluid loss properties (Kraak 1992). Potato starch also is preferred as a precoat on filters due to large granule size (Zobel et al. 2009).

For the second project activity, starch samples from 12 diverse potato clones, compared to three commercial varieties (Red Norland, Russet Burbank, and Yukon Gold), were isolated, and their physicochemical properties investigated. Clones were selected based on their unique SS and/or RS levels, six were chosen based on their relatively high or low levels of SS, and six were chosen due to their relatively high or low levels of RS (Table 3). The research objective established a foundation of fine chemistry research to further explain the unique SS and/or RS

content that these clones elicit. Starch granules from the pith (center) and near the cortex (outer flesh) of the tubers were examined by Scanning Electron Microscopy. Granules ranged in length from 3 µm to 92 µm (Table 4); starch granule length is important for specific industrial applications, such as filters, and aids in explaining starch digestion. Granule numbers and size distributions are presented in Table 5. Pasting profiles were examined by rapid visco analyzer (RVA), revealing different pasting profiles for clones. Gelatinization characteristics, studied using differential scanning calorimetry (DSC), exhibited different gelatinization parameters (Tables 6 and 7). Isolated starch samples were examined by high performance size exclusion chromatography (HPSEC), to determine amylopectin and amylose molecular weights, and abundance of both. Amylopectin and amylose percentages ranged from 77.5% to 83.7% and 16.3% to 22.5%, respectively (Table 8). Differences in amylopectin and amylose molecular weight also were found. Our results indicate that diverse potato clones within the NDSU breeding program possess starch with unique physicochemical characteristics.

Table 3. Clones selected for physicochemical assessment based on soluble and resistant starch levels.

	Clone	Soluble Starch (mg/g)		Clone	Resistant Starch (mg/g)
Highest 3	ND102687AB-1Russ	358.9	a*	ND113517ABC-9	152.1
	Lenape	347.3	ab	ND102549TB-2Russ	129.4
	ND113508C-4	337.4	b	ND113517ABC-6	127.9
Lowest 3	ND113487C-1	171.6	d	ND113060-1	47.4
	Inka Dawn	158.9	de	ND102903-1R	46.0
	ND113438CB-8R	117.9	f	ND102921C-3	40.9
Controls	Red Norland	230.8	c	Red Norland	75.0
	Russet Burbank	224.6	c	Russet Burbank	81.4
	Yukon Gold	235.6	c	Yukon Gold	91.3
	Mean	246.6		Mean	85.8
	LSD ($\alpha = 0.05$)	18.6		LSD[a] ($\alpha = 0.05$)	8.9

**Different letters* signify *means* are *significantly different* using PROC MIXED ($\alpha \leq 0.05$).

[a]differences between clones for resistant starch were not detected, so a protected mean square was used.

Table 4. Starch granule size distribution for raw potato tissue near the cortex and pith of the tuber.

	Starch granule sizes (µm)							
	Length of cells cortex (µm)		Width of cells cortex (µm)		Length of cells pith (µm)		Width of cells pith (µm)	
Clone	Mea	Range	Me	Range	Mea	Range	Mea	Range
ND102687AB-	28.8	12.0-	22.	7.0-	21.0	11.0-	16.0	10.0-
Lenape	23.5	4.0-	19.	4.0-	34.8	7.0-	26.2	7.0-
ND113508C-4	36.7	12.0-	25.	3.0-	34.6	12.0-	24.9	11.0-
ND113487c-1	24.9	12.0-	18.	9.0-	24.6	9.0-	17.5	7.0-
Inka Dawn	13.2	3.0-	11.	3.0-	13.9	4.0-	13.5	5.0-
ND113438CB-	30.9	15.0-	24.	16.0-	37.9	30.0-	26.4	21.0-
ND113517ABC	20.7	5.0-	13.	5.0-	22.1	6.0-	15.3	4.0-
ND102549TB-	26.5	13.0-	17.	9.0-	20.7	12.0-	20.4	7.0-
ND113517ABC	34.0	10.0-	22.	12.0-	22.0	4.0-	17.3	3.0-
ND113060-1	23.8	5.0-	17.	6.0-	25.7	7.0-	16.7	7.0-
ND102903-1R	21.5	6.0-	15.	4.0-	23.1	8.0-	15.6	5.0-
ND102921C-3	28.5	8.0-	19.	9.0-	25.2	12.0-	17.1	8.0-
Red Norland	23.1	8.0-	17.	7.0-	25.0	10.0-	16.0	7.0-
Russet Burbank	26.1	6.0-	17.	6.0-	20.8	4.0-	15.5	4.0-
Yukon Gold	22.6	4.0-	17.	6.0-	25.9	6.0-	20.9	5.0-
Mean	17.6		17.		24.4		17.9	

Table 5. Size distribution of starch granules near the cortex for raw potato tissue of unique clones compared to industry standards (Red Norland, Russet Burbank, and Yukon Gold).

	Total number granules	% of Total granule sizes						
		Length near cortex (µm)						
		<10	10-19	20-29	30-39	40-49	50-59	60-69
ND102687AB-1Russ	17	0	35	35	6	6	18	0
Lenape	34	26	18	15	26	9	6	0
ND113508C-4	23	0	22	22	22	13	9	13
ND113487c-1	17	0	29	47	12	6	6	0
Inka Dawn	53	30	51	15	2	2	0	0
ND113438CB-8R	13	0	31	15	38	0	15	0
ND113517ABC-9	35	11	43	23	23	0	0	0
ND102549TB-2Russ	34	0	16	63	11	11	0	0
ND113517ABC-6	25	0	16	20	28	32	4	0
ND113060-1	52	10	33	29	13	15	0	0
ND102903-1R	30	7	43	23	23	3	0	0
ND102921C-3	25	4	12	44	20	16	4	0
Red Norland	24	4	29	50	4	13	0	0
Russet Burbank	21	10	24	14	48	5	0	0
Yukon Gold	33	14	45	24	10	21	0	0

Table 6. Gelatinization behavior based on Rapid Visco Analyzer (RVA) of starches from 12 potato clones with unique soluble or resistant starch levels, compared to industry standards (Red Norland, Russet Burbank, and Yukon Gold).

Clone	RVA* Pasting characteristics[b]					
	PV* (cP**)	HPV* (cP)	BKD* (cP)	CPV* (cP)	STB* (cP)	PT (min)
ND102687AB-1Russ	5505[g***]	3814[a]	1691[j]	5417[a]	1603[a]	3.8[cde]
Lenape	7227[b]	3840[a]	3387[c]	4763[c]	923.0[ef]	3.7[e]
ND113508C-4	7315[ab]	2674[g]	4642[a]	3486[g]	812.0[fgh]	3.3[h]
ND113487c-1	4885[i]	2668[g]	2217[h]	3662[f]	994.5[de]	3.9[bcd]
Inka Dawn	1151[k]	831.0[k]	320.0[k]	1272[k]	441[i]	4.7[a]
ND113438CB-8R	2028[j]	1637[j]	391.5[k]	2069[j]	432.5[i]	4.0[b]
ND113517ABC-9	6126[d]	3131[c]	2995[d]	4194[d]	1063[d]	3.4[gh]
ND102549TB-2Russ	4825[i]	2855[e]	1970[i]	4224[d]	1369[b]	3.8[de]
ND113517ABC-6	7370[a]	3694[b]	3677[b]	4911[b]	1218[c]	3.8[cde]
ND113060-1	5740[f]	3056[d]	2684[f]	3886[e]	830.0[fgh]	3.9[bc]
ND102903-1R	5328[h]	2582[h]	2746[f]	3318[h]	737.0[h]	4.0[b]
ND102921C-3	5955[e]	3029[d]	2926[de]	3914[e]	886.0[efg]	3.3[h]
Red Norland	5656[f]	2781[f]	2876[e]	3523[g]	742.5[h]	3.5[fg]
Russet Burbank	6360[c]	2763[f]	3598[b]	3555[fg]	792.5[gh]	3.5[f]
Yukon Gold	4796[i]	2261[i]	2535[g]	3089[i]	828.0[fgh]	3.3[h]
Mean	5351	2774	2577	3686	911.4	3.7
LSD	99.6	64.01	88.04	122	106.9	0.11

* RVA = rapid visco analyzer, PV = peak viscosity, HPV = hot paste viscosity, BKD = breakdown, CPV = cold paste viscosity, STB = setback, PT = peak time.
** cP = centipoise
***Values with different letters are significantly different ($\alpha \leq 0.05$) using Fischer's LSD.

Table 7. Gelatinization temperatures and enthalpies, based on starches from 12 potato clones with unique soluble or resistant starch levels, compared to industry standards (Red Norland, Russet Burbank, and Yukon Gold).

Clone	Thermal properties				
	Onset (°C)	Peak (°C)	Conclusion (°C)	Temperature Range (°C)	Enthalpy (J/g)
ND102687AB-1Russ	68.5[a*]	73.3[a]	79.3[a]	10.9[b]	11.3[bcd]
Lenape	68.7[a]	72.3[b]	77.3[b]	8.6[cd]	13.6[a]
ND113508C-4	61.0[i]	66.9[f]	72.9[g]	11.9[a]	12.7[abc]
ND113487c-1	66.0[de]	69.6[ef]	75.6[cde]	9.6[c]	11.4[bcd]
Inka Dawn	67.9[b]	72.8[b]	78.6[a]	10.7[b]	5.39[e]
ND113438CB-8R	67.0[c]	71.4[c]	79.6[a]	12.6[a]	7.3[e]
ND113517ABC-9	66.4[d]	70.0[de]	74.7[ef]	8.3[d]	10.0[d]
ND102549TB-2Russ	63.9[h]	70.5[d]	76.7[bc]	12.9[a]	11.2[cd]
ND113517ABC-6	66.2[d]	70.2[d]	75.6[cde]	9.4[c]	14.9[a]
ND113060-1	65.5[ef]	70.2[d]	76.2[cd]	10.7[b]	13.4[ab]
ND102903-1R	64.4[g]	69.0[f]	75.3[de]	10.9[b]	12.7[abc]
ND102921C-3	69.0[ef]	73.5[f]	73.5[g]	8.0[d]	12.7[abc]
Red Norland	67.4[bc]	71.1[c]	76.1[cd]	8.8[cd]	11.1[cd]
Russet Burbank	65.3[f]	69.1[f]	73.9[fg]	8.6[cd]	10.6[cd]
Yukon Gold	67.5[b]	71.6[c]	76.2[cd]	8.7[cd]	12.7[abc]
Mean	66.1	70.5	76.1	10.0	11.4
LSD	0.5	0.6	1.1	0.9	2.0

[*] Values with different letters are significantly different ($\alpha \leq 0.05$) using Fischer's LSD.

Table 8. High performance size exclusion chromatography (HPSEC) parameters of starches from 12 potato clones with unique soluble or resistant starch levels, compared to industry standards (Red Norland, Russet Burbank, and Yukon Gold).

Clone	Amylopectin %	Amylose %	Amylopectin $M_w \times 10^7$	Amylose $M_w \times 10^6$
ND102687AB-1Russ	83.70[a*]	16.30[g]	1.76[e]	2.92[c]
Lenape	79.77[f]	20.23[b]	1.38[j]	1.80[g]
ND113508C-4	81.63[b]	18.37[f]	2.10[b]	2.33[d]
ND113487c-1	79.79[f]	20.21[b]	1.20[l]	1.30[k]
Inka Dawn	80.36[d]	19.64[d]	2.04[c]	1.54[i]
ND113438CB-8R	81.10[c]	18.90[e]	1.74[f]	1.40[j]
ND113517ABC-9	79.75[f]	20.25[b]	1.35[k]	1.17[n]
ND102549TB-2Russ	81.24[c]	18.76[e]	1.07[n]	1.27[l]
ND113517ABC-6	81.26[c]	18.74[e]	1.65[g]	1.09[o]
ND113060-1	81.76[b]	18.24[f]	1.64[h]	3.35[b]
ND102903-1R	80.40[d]	19.60[d]	2.45[a]	6.72[a]
ND102921C-3	80.48[d]	19.52[d]	1.02[o]	1.56[h]
Red Norland	80.01[e]	19.99[c]	1.17[m]	2.02[e]
Russet Burbank	77.46[g]	22.54[a]	1.49[i]	1.23[m]
Yukon Gold	79.65[f]	20.35[b]	1.78[d]	1.89[f]
Mean	80.56	19.44	1.59	2.11
LSD	0.18	0.18	0.02	0.01

[*] Values with different letters are significantly different ($\alpha \leq 0.05$) using Fischer's LSD.

Diverse soluble (SS) and/or resistant starch (RS) levels were discovered in the NDSU potato breeding program germplasm. Activity one (Table 1) provides information regarding SS and RS levels when tubers were grown in the field. Our third project activity was to determine if clones grown in the greenhouse vary in their SS or RS concentration compared to clones grown in the field. Screening tubers of clones grown in the greenhouse versus the field could possibly provide a more rapid and efficient assessment. A total of 48 clones were analyzed between the field locations of Baker, MN, Absaraka, ND, and from in the greenhouse. Results indicated that growing environment significantly impacts SS and RS content. Greenhouse-grown clones contained significantly reduced levels of SS and RS compared to field grown tubers, indicating that there are environmental factors that dramatically impact the starch profiles within the clones. The findings suggest that greenhouse-grown tubers should not be used to analyze SS or RS content, due to the large variation in the starch profile compared to field-grown tubers.

Table 9. Comparison of soluble and resistant starch levels (based on dry weight) for clones grown at Baker, MN, Absaraka, ND, and in the greenhouse.

	Location[a]		
	Baker, MN	Absaraka, ND	Greenhouse

Clone	Soluble Starch (mg/g)	Resistant Starch (mg/g)	Soluble Starch (mg/g)	Resistant Starch (mg/g)	Soluble Starch (mg/g)	Resistant Starch (mg/g)
Dakota Pearl	238.0[abcd*]	77.4[ab]	200.5[b]	99.9[ab]	171.5[a]	30.5[de]
Dakota Ruby	261.0[abc]	74.0[ab]	163.1[b]	108.1[ab]	177.5[a]	24.3[ef]
Dakota Russet	273.0[ab]	90.9[a]	202.8[b]	87.2[b]	171.5[a]	40.9[cd]
Russet Norkotah	231.9[abcd]	56.7[bc]	170.9[b]	117.0[ab]	132.0[b]	18.7[ef]
Romanze	224.5[bcd]	41.0[c]	164.0[b]	91.1[b]	183.2[a]	26.7[a]
ND102775C-5RR	220.7[cd]	94.3[a]	179.3[b]	127.8[a]	91.6[c]	13.8[f]
ND113224C-3Russ	203.6[d]	61.5[bc]	367.7[a]	101.6[ab]	132.0[b]	56.0[b]
ND7743C-2RS	279.9[a]	77.2[ab]	153.8[b]	94.4[b]	104.6[c]	53.9[bc]
Mean	241.6	71.6	200.3	103.4	143.1	41.1
LSD	51.5	29.1	60.4	32.3	21.0	13.7

[*]Values with different letters are significantly different (α=0.05) using Fischer's LSD.

Activities (4 & 5) to increase genotypes to facilitate evaluation by potential consumers occurred in 2016 near Williston, ND. Additionally, we are in communication with several industries that use starch in development of products. We are hoping they will evaluate materials in 2017 or beyond for their specific uses.
The final activities were dissemination of research results to grower/industry representatives. This occurred at field days, the Northern Plains Research Reporting Conferences (including February 2017), amongst other events as reported in our quarterly and annual reports. We are currently preparing manuscripts for refereed journal publication.

Goals and Outcomes Achieved

The goal of the project was to evaluate diverse germplasm developed and maintained by the NDSU potato breeding program for starch attributes, including total starch, resistant starch, and determination of the amylase/amylopectin ratio.

Our findings demonstrate the diverse soluble starch (SS) and resistant starch (RS) content and characterizations found in the NDSU) potato breeding program germplasm. Some genotypes may provide adequate starch characteristics that can be utilized in various industrial applications, such as biodegradables, pharmaceuticals, textiles, and filters. The development of clones with high or low levels of amylose or amylopectin is desired for certain applications. Our project determining the feasibility of a more efficient cooking method, microwave steaming, to cook tuber material for starch analysis, screened clones present in the NDSU potato breeding program for unique starch profiles, examined 12 unique genotypes for fine chemistry attributes using scanning electron microscopy (SEM), rapid visco analyzer (RVA), differential scanning calorimetry (RVA), and high performance size exclusion chromatography (HPSEC), and

determined that genotypes grown in the greenhouse differed from field-grown genotypes for SS and RS content.

Per the performance measure, benchmark, and target in our research proposal, starch content in potato genotypes is highly variable, ranging from 11-30% in potato cultivars, to 4-40% in wild potato species (Jansen et al. 2001). Genotypes with high amylose content are desirable due to their low glycemic index and high fiber content. Behall and Hallfrisch (2002) demonstrated that diets containing more than 50% amylose significantly reduced plasma glucose and insulin levels. Starch quality parameters for other end uses (ie. bioplastics, pharmaceutical) dictate quality measures for specific utilization. We established our benchmark as identification of genotypes with high levels of amylose. And a target of identification of even a single genotype with higher levels of amylase than Russet Burbank would allow us to study heritability of this trait and potentially develop a molecular marker for rapid screening of germplasm in the future.
Our screening efforts identified clones that fit these parameters and now are seeking industry partners, and further research collaborations with other US researchers in order to provide healthier food options to consumers, particularly diabetics.

In summary, microwave steaming was shown to not influence SS and RS levels. The microwave steam method was used evaluate genotypes in the NDSU potato breeding program and genotypes with uniquely high, or low, levels of SS or RS were identified. Distinct starch profiles were also indicated for market types, suggesting that the end-use of a genotype depends on the starch profile. Twelve clones were further analyzed for their starch granule morphology, pasting properties, gelatinization characteristics, and starch molecular weight and chain length distribution; clones displayed varying granule size distributions, and exhibited significantly different parameters for fine chemistry characteristics. Clones with unique starch characteristics may provide appropriate attributes for specific industrial or nutritive product applications. In order to examine large numbers of genotypes for starch attributes, rapid assessment and efficiency are desired. Greenhouse grown tuber tissue was compared to field grown, for SS and RS content. Results indicated that clones grown in the greenhouse contained significantly lower levels of SS and RS than field-grown clones, suggesting that environmental conditions impact the starch profile. The duration of storage may have been a factor contributing to the altered starch profile, since greenhouse tubers were stored for approximately four months; whereas, field-grown tubers were stored for 12-16 months prior to analysis. Research and development for starch-based applications should examine clones grown from the field, as most applications desire high starch content. For nutritive purposes, a low-starch potato is preferred. Environmental conditions, including soil fertility, rainfall, temperature, and storage conditions (duration, temperature, and humidity), have been shown to impact the starch profile. The need for further assessment of the environmental and storage factors that influence starch profiles among the germplasm should be emphasized. Further research is needed to determine the factors that impact starch profiles in clones with applicability to food and industrial products. Unique clones in this study may provide insight into breeding and developing potato cultivars for specific starch profiles in order to obtain optimum starch characteristics for specific product applications. And the potential for development of molecular markers to speed selection in early generations of potato breeding and development programs.

Beneficiaries

Potato producers (seed and commercial), and the potato industry involved in snack production (extruded snacks, gluten-free, etc.), bioplastics utilizers, health/diet food product manufacturing (gluten-free for example), and pharmaceutical coatings makers are potential beneficiaries, along with consumers.

The project has the potential to benefit all potato producers in North Dakota (and beyond). There are approximately 60 certified seed potato producers and 250 commercial potato producers in the Northern Plains Potato Growers Association membership. All may benefit if unique genotypes are adopted by industrial and food manufacturers for specific products, due to the additional production and marketing of seed and commercial potatoes, and the potential for increased value of the crop by providing an outlet for new and improved products, potentially employing more individuals and increasing farmgate value. Based on the product potential from our findings, several utilizers of starch could expand production of previous and new products which may lead to an increase in jobs as an indirect impact. Additionally, adoption of unique genotypes identified in this project or in the future, may impact market share for areas such as ND and MN where potato dry matter is higher than other production areas such as WI and WA.

Lessons Learned

Several positives were identified from our project, including the ability to be able to use microwavable steam bags in assessment. A negative could be the large amount of time that large numbers of genotypes require for analysis, despite our efforts to identify reliable and rapid assessment techniques.

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Additional Information

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Screening and Developing Lentil Cultivars Tolerant to Sulfentrazone

Final Report

Partner Organization: North Dakota State University

Project Summary

Lentil production is hindered by the lack of safe and effective herbicides. Sulfentrazone is an effective broadleaf herbicide used in other legume crops, but not in lentil due to marginal crop safety. It is well documented that some cultivars of legume crops such as soybean and dry bean differ in sensitivity to sulfentrazone. Therefore, it is likely that lentil cultivars also may respond differently. If more tolerant lentil cultivars could be identified or developed, sulfentrazone would provide significantly better weed control than currently labeled herbicides.
This project evaluated lentil cultivar sensitivity to sulfentrazone through field, greenhouse, and laboratory studies. Cultivars representing different market classes (e.g., green vs. red) and ancestry were evaluated. Field and greenhouse studies showed that four cultivars were more tolerant to sulfentrazone than 11 other cultivars. These cultivars will be evaluated further in field trials and in the pulse breeding program. If sufficient tolerance can be found naturally in lentil cultivars or developed through the breeding program, lentil farmers would benefit from being able to apply sulfentrazone for better broadleaf weed control.

Lentil production is hampered by the lack of safe and effective herbicides. Sulfentrazone has been the most widely used herbicide in dry pea and chickpea due to its activity on kochia and wild buckwheat. However, lentil tolerance to sulfentrazone is marginal and injury can be moderate to severe in sandy, high pH, low organic matter soils.
It is well documented that some cultivars of legume crops such as soybean and dry bean differ in sensitivity to sulfentrazone. Therefore, it is likely that lentil cultivars also may respond differently. If more tolerant lentil cultivars could be identified or developed, sulfentrazone would provide significantly better weed control than currently labeled herbicides.
This project evaluated lentil cultivar sensitivity to sulfentrazone through field, greenhouse, and laboratory studies. Cultivars representing different market classes (e.g., green vs. red) and ancestry were evaluated.
Another objective was to develop reliable greenhouse or laboratory methods for determining sensitivity that agree with field results. Field studies to determine cultivar sensitivity are labor intensive and time consuming. Reliable greenhouse or lab methods would reduce the time and expense needed to determine sensitivity of newly released cultivars.

Project Approach

- This project is part of the thesis work of Tiffany Walter, graduate student. Tiffany is currently in the data analysis and writing phase. Upon completion, the information will be disseminated through the NDSU website, NPGA website, NPGA newsletter, and Western Society of Weed Science. If interested in receiving the information when available, contact the project contact.
- Field studies were conducted in 2014 and 2015 at two locations each year representing different soil types. Six cultivars were evaluated for tolerance to 1X and 2X (4 and 8 fl oz/A) rates of sulfentrazone. Data were collected on crop density, height, visual injury, and yield.

- Lentil tolerance was highly influenced by soil characteristics. Less injury was observed in soils with lower pH, higher organic matter, and loam soil. More injury was observed in higher pH, lower organic matter, and lighter soil.
- The cultivar CDC Sedley tended to be more tolerant to sulfentrazone than other cultivars in the field trials.

- A greenhouse study was conducted to evaluate 15 lentil cultivars for tolerance to five rates of sulfentrazone using two soil types. Data collected included lentil emergence, height, and biomass.
  - The cultivar CDC Sedley and three NDSU experimental lines consistently showed more tolerance to sulfentrazone than the other 11 cultivars in both soil types.
- These studies were summarized at summer field tours for farmers in Minot and Williston as well as winter meetings in Minot, Williston, and Wolf Point. The field tours in Minot and Williston had 80 and 75 attendees, respectively. Attendance was as follows for the winter meetings: Minot, 150 attending; Williston, 80 attending; Wolf Point, 100 attending.
- End products will include a thesis and a journal publication.

Goals and Outcomes Achieved

- Our primary goal was to find one or more lentil cultivars with higher tolerance to sulfentrazone. We achieved that goal as we observed four cultivars that stood out as showing greater tolerance to sulfentrazone.
- A second goal was for field and greenhouse results to be in agreement as to which cultivars are more tolerant. Our field and greenhouse results were in agreement showing that CDC Sedley was more tolerant and that cultivars Maxim and Richlea were more sensitive. CDC Sedley generally was less impacted as indicated by measurements on density, height, visual injury, and yield.
- The Benchmark for lentil visual injury in previous field studies was about 20-60%. Our Target was to find a cultivar that would show only about 15% early-season visual injury in the field and then recovering to less than 10% injury. The CDC Sedley injury ranged from 10-46% compared to generally much higher injury for other cultivars tested in the field.
- We observed two cultivars in the greenhouse test that showed even more tolerance than CDC Sedley. These two cultivars were not included in the field test, but will be included in future field tests.
- We recommend that CDC Sedley and the three experimental lines be evaluated further in future field tolerance trials as well as the pulse breeding program.

Beneficiaries

- Lentil producers will benefit from this research.
- About 400 lentil producers will benefit.
- If sufficient tolerance can be found naturally in lentil cultivars or through a breeding program, lentil farmers would benefit from being able to apply sulfentrazone for better broadleaf weed control.

Lessons Learned

- One of the challenges with conducting crop tolerance trials with soil-applied herbicides is the variable nature of soils across a field. Soil characteristics in fields are not always uniform. Thus, to use sulfentrazone in lentil or even other crops, a farmer should soil sample in multiple places in the field to determine how much variability there is in soil pH, organic matter, and texture.
- The mutagenicity test is much more difficult when using soil-applied herbicides compared to postemergence herbicides. There is a greater likelihood of false-positives for tolerance with soil-applied herbicides as several factors are involved as to whether the herbicide will injure the crop (such as soil characteristics, rainfall amount, timing of rainfall, etc.).

Contact Information

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Validation of biochemical markers to predict sugar end development under field conditions

Final Report

Partner Organization: North Dakota State University

Project Summary

A potato trial was planted in Inkster, ND on June 10, 2015 to evaluate different processing potato cultivars. The 10 cultivars planted in this trial were Russet Burbank, Pinnacle, Manistee, Umatilla, Accumulator, Nicolet, Lamoka, Snowden, Atlantic, and MegaChip. Atlantic was the best performing cultivar tested when compared across yield parameters (Table 1). It had the greatest amount numerically of tubers from 6-10 oz, 10-14 oz, total yield, marketable yield, and percent tubers >6 oz. Pinnacle and Snowden tended to have a large number of undersized tubers (< 4 oz). Too small potatoes are can be a problem for chip processors. Pinnalce and Snowden averaged 34-42% of tubers undersized. However, this may have been because the stem number (Table 2) was high on these cultivars. This research indicates in ND that Atlantic produces a higher marketable yield on average than many other cultivars. Further work would needs to be done to evaluate new developed chipping cultivars to determine the agronomic qualities in North Dakota.

The objective of this project was to evaluate the agronomic growth and yield of ten potato cultivars grown in North Dakota. This is important because potatoes represent the largest vegetable crop produced in Minnesota and North Dakota and nearly 70% of this crop is processed to form French fries and potato chips. As competition of high quality and high producing potatoes is necessary for potato growers to be economically sustainable, this research has evaluated some cultivars that could be suitable for processing in North Dakota.

Project Approach

This trial was planted at Inkster, ND on June 10, 2015 with a 2-row hand assisted planter. This irrigated site is common to planting different processing potato cultivars. There were 10 cultivars planted in this trial, Russet Burbank, Pinnacle, Manistee, Umatilla, Accumulator, Nicolet, Lamoka, Snowden, Atlantic, and MegaChip. Each plot was 1 row wide (3 feet) by 25 feet long. Stand and stem number was counted on July 10. Normal agronomic practices were used to grow the crop. Tubers were harvested on 15 October 2015 and graded thereafter for yield and assessed any internal or external problems.

There were differences in the graded yield data. Atlantic was the best performing cultivar tested when compared across yield parameters (Table 1). It had the greatest amount numerically of tubers from 6-10 oz, 10-14 oz, total yield, marketable yield, and percent tubers >6 oz. Pinnacle and Snowden tended to have a large number of undersized tubers (< 4 oz). Too small potatoes are can be a problem for chip processors. Pinnalce and Snowden averaged 34-42% of tubers undersized. However, this may have been because the stem number (Table 2) was high on these cultivars. This research indicates in ND that Atlantic produces a higher marketable yield on average than many other cultivars, but because of a shorter season and only one year of data more research needs to be conducted.

Dr. Robinson completed the agronomic evaluation and harvest of the research plots. Data from this project were shared on the NDSU/U of M Potato Extension website www.ag.ndsu.edu/potatoextension and in the Valley Potato Grower magazine. Social media outlets such as Twitter (@spudology) and the NDSU Potato Facebook page were used to share this information to growers and encourage them to read the full report on the NDSU/U of M Potato Extension website.

Funds for this project were specifically for the potato research project and not used in any other crop. The sole purpose of this project was to evaluate ten different potato cultivars. Further work would need to be done to evaluate new developed chipping cultivars to determine the agronomic qualities in North Dakota.

Table 1. Graded yield of 10 processing cultivars grown at Inkster, ND in 2015. Within columns a significant difference is indicated by a different letter at p=0.05.

	<4 oz		4-6 oz	6-10 oz		10-14 oz		>14 oz	Total yield		Total marketable		>6 oz		>10 oz
	Cwt/a												%		
Russet Burbank	47	c	81	145	a-d	51	ab	19	343	ab	296	abc	62	a	20
Pinnacle	153	a	106	88	d	18	b	2	366	ab	214	c	30	c	6
Manistee (MSL292-A)	94	abc	103	102	bcd	21	b	3	322	b	228	bc	39	abc	8
Umatilla	98	abc	111	121	a-d	22	b	1	354	ab	256	bc	42	abc	7
Accumulator	70	bc	94	174	ab	48	ab	12	398	ab	328	ab	58	ab	15
Nicolet	84	abc	112	149	a-d	37	ab	23	406	ab	321	abc	51	abc	15
Lamoka	80	abc	112	168	abc	30	ab	9	399	ab	319	abc	52	abc	10
Snowden	120	ab	114	94	cd	17	b	9	354	ab	234	bc	34	bc	8
Atlantic	53	bc	108	203	a	61	a	25	450	a	397	a	64	a	19
MegaChip	63	bc	82	147	a-d	44	ab	13	349	ab	286	abc	58	ab	16

Table 2. Stand and stem count of 10 processing cultivars grown at Inkster, ND in 2015. Within columns a significant difference is indicated by a different letter at p=0.05.

	Stand		Stem	
	Number			
Russet Burbank	22	ab	76	cde
Pinnacle	24	a	95	abc
Manistee (MSL292-A)	22	ab	61	de
Umatilla	21	ab	70	cde
Accumulator	22	ab	115	a
Nicolet	24	a	85	bcd
Lamoka	20	b	52	e
Snowden	22	ab	105	ab
Atlantic	23	ab	74	cde
MegaChip	22	ab	77	b-e

Goals and Outcomes Achieved

The North Dakota field agronomic trial was completed, as outlined in the grant. Unfortunately, the University of Minnesota portion that would have evaluated the cold sweetening and sugar ends of these cultivars was not accomplished. This was because the lead PI in Minnesota, Christian Thill unexpectedly passed away and the University of Minnesota did not accept the Minnesota specialty crop block grant award. Based on our data generated from this project, we learn that Atlantic was a good cultivar in a shorter season environment. However, because this is only one year of data it is difficult to draw strong conclusions. At least two years of data would be more acceptable for field research information.

Visual demonstration of the plots was shared with the potato community at the Inkster field day on 20 Aug 2015 and discussed at the International Crop Expo Potato section on 15 February 2016. Because the Minnesota portion of this grant was not funded, the Minnesota field day and winter meetings were not held. Field day attendance was 200 persons and at the winter meeting the attendance was 120 persons.

Beneficiaries

The stakeholders that would benefit from this research are those who grow potatoes for the processing markets. As stated before, this is about 70% of the potato growers in the state or about 120 growers. Because the fully project was not realized, the benefits are minimal. There was some data generated to help make decisions from an agronomic stand point, but it would have been more valuable if there were more data that evaluated the cold sweetening and sugar ends of these cultivars.

Lessons Learned

We were able to accomplish what we set out to do. It could have been better if the research study could have been conducted over two growing seasons to provide more consistent data.

Contact Information

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Additional Information

Image of the processing potato agronomy trial from Inkster, ND on July 16, 2015.

NDSU/U of M website with publication:
https://www.ag.ndsu.edu/potatoextension/documents/ResearchonChippotatotrialresults.pdf

Valley Potato Grower magazine link:
http://www.valleypotatogrower.com/flipbookMayJune2016mag/flipbook/

Contribution of Nematode Populations and Soil Properties in Root Rot of Peas

Final Report

Partner Organization: North Dakota State University

Project Summary

Molecular techniques were developed to quantify *Fusarium* species and nematode populations in association with field pea plant and soil samples collected in 2014 and 2015, and the interaction among micro-organisms, soil factors and nematodes was evaluated. Seven economically important groups of plant-parasitic nematodes were detected and molecular assays were developed to quantify two of these nematode species. Pin nematodes were predominant among samples taken. Levels of stunt and spiral nematodes were moderate, and root-lesion, dagger and root-knot nematodes were low. Three multiplex QPCR assays were developed to quantify seven *Fusarium* species. *F. avenaceum, F. culmorum, F. graminiarum* and *F. acuminatum* were the most common species colonizing diseased pea roots. Models generated for the associations between plant-parasitic nematodes and soil properties indicate that the iron generally increased with pin nematodes and the organic matter and cation exchange capacity were related to decreases in stunt nematodes. Root rot severity was most closely associated with soil potassium, but pH, cation exchange capacity and ammonia levels also had a significant effect. Four scientific presentations have been made, five presentations were given to growers, extension professionals and others in the industry. One scientific manuscript has been submitted for publication and four more are in preparation.

In 2006, 610,000 acres of field peas were produced in North Dakota. However, the average acreage was reduced to nearly 300,000 from 2012 to 2015. The field pea industry in North Dakota raised concerns that reductions in acres over the past several years would continue if root rot management options are not identified. In 2016, commodity prices led North Dakota producers to plant 640,000 acres to field peas, but concerns over root rot remain high. Root health is affected by numerous factors, and many are not readily apparent due to the complex interactions with soil, plants and other organisms. Previous research conducted on field peas in North Dakota indicates that the root rot complex consists of numerous *Fusarium* species (Chittem et al., 2015). There currently are no effective management practices available to North Dakota field pea producers. Cultivars display a range of sensitivities to these pathogens because genetic resistance to the *Fusarium* species complex is controlled by the action of many genes. Unfortunately, the level of resistance in cultivars currently grown in North Dakota is not sufficient to provide protection under high levels of disease pressure. Seed treatment fungicides are commonly used by dry pea growers, however, these fungicides typically only provide disease suppression for the first 3 to 5 weeks of the growing season and root rotting fungi are in contact with growing roots throughout the season.

The detection of *Fusarium* species associated with root rot of dry peas is currently conducted using time- and labor-intensive plating assays. This is a major limiting step to the number of fields and plants that can be surveyed. Beyond sample collection and processing, plating assays require incubation on nutrient media for 2 to 4 weeks to allow pathogen growth and further fungal isolation and identification that can extend another 4 to 6 weeks. Additionally, discerning among *Fusarium* species is a difficult process requiring trained personnel and soil-associated

non-pathogenic fungi make it difficult to accurately quantify fungal infection. Real-time PCR has been utilized successfully to quantify *Fusarium* interactions with other hosts (Muraosa et al., 2014; Nicolaisen et al., 2009). This technique allows for much more rapid (2 to 3 days) sample processing, resulting in a large increase in the number of plants that can be evaluated in a given time. Additionally, real-time PCR allows for quantification based on the number of fungal cells in a given amount of host tissue.

Plant-parasitic nematodes are important components of root rot complexes that restrict productivity of field peas. The nematode surveys in Idaho in 2008 revealed that two species of lesion nematodes (*Pratylenchus neglectus* and *P. thornei*) and one species of pin nematode (*P. hamatus*) were prevalent in rhizosphere soil and pea roots. These nematodes reduced plant height by 50 to 70% and yield compared to controls (Riga et al., 2008). Previous surveys conducted in Canada revealed that pea root rot severity in all fields were positively and significantly correlated with densities of stunt nematode (*Tylenchorhynchus* spp.) in soil and the incidence of *F. solani* root infections increased significantly with densities of three nematodes (*Tylenchorhynchus* spp., *Helicotylenchus* spp., *Paratylenchus* spp.) in soil (Celetti et al., 1990). The exact biological role of these nematodes in the pea root rot disease complex is unclear. Nematodes have appeared to initiate the occurrence of fungal diseases by wounding root tissue in wheat (Smiley and Nicol, 2009).

Very limited information exists on the occurrence of nematodes on field peas in North Dakota. Nematode surveys conducted in 2004 and 2005 indicated that stunt and pin nematodes were predominant. The population density for stunt nematodes was as high as 22,180/kg of soil, which clearly exceeds damage thresholds reported (Patel and Thakar, 1989). Lesion, spiral, root-knot (*Meloidogyne* spp.), dagger (*Xiphinema* spp.), and lance (*Hoplolaimus* spp.) nematodes were also reported (K. Merrifield; unpublished data). However, nematode species was not determined, and nematodes have not been monitored in North Dakota field peas during the past decade. The new NDSU nematology program surveyed selected pea fields in collaboration with the pulse crop pathology program, determined nematode population densities and their distribution, and quantified key nematode species in fields using a combination of molecular and morphological methods. This proposed project has set the foundation for a nematology research program in field peas in North Dakota.

Research dedicated to the association of soil properties, the incidence of soil-borne pathogens, nematode populations and root rot under field conditions has been limited. *F. solani* f. sp. *glycines*, *Heterodera glycines* nematode density, and soil properties have been reported to influence soybean sudden death syndrome severity under field conditions (Rupe et al., 1993; Scherm et al., 1998). Similar studies on other soil-borne pathogens have also reported associations between soil properties and disease incidence (Broders et al., 2009; Zitnick-Anderson et al., 2014). These associations have not been investigated in North Dakota field peas. The purpose of this study is to support a collaborative effort between pathology and nematology and use knowledge obtained on the distribution and density of individual nematode species, soil properties and *Fusarium* species to determine risk and apply management practices to minimize crop damage in field peas. The objectives of this project were to optimize, validate and utilize molecular procedures to identify nematode species associated with dry peas in North Dakota, optimize, validate and utilize real-time PCR assays for the quantification of six *Fusarium* species in dry pea roots and determine how soil properties, including texture, organic matter and metals, nematode type and density, and *Fusarium* species and quantity, affect root rot in dry pea fields.

Project Approach

- Process and send soil samples for nematode enumeration and soil property analysis (Dec 2014)
  - Forty soil samples collected in summer 2014 were sent to the NDSU soil testing lab for property analysis and to Western Laboratories, Parma, ID for nematode enumeration at the genus level using microscopy.
- Extract, identify and count nematodes from selected plant roots (Dec 2014 to Feb 2015)
  - Eleven fields were selected and re-sampled in 2015 for nematode species identification and population determination.
- Present preliminary results at the Northern Pulse Growers Association Annual Meeting - 224 meeting registrants (Jan 2015)
  - Preliminary results were presented to growers, extension professionals and other industry stakeholders in an oral presentation given by Julie Pasche at the Northern Pulse Growers Association Annual Convention in January, 2015.
- Extract DNA from root tissue (Feb to Mar 2015)
  - DNA was extracted from roots of 132 plants inoculated in the greenhouse with single *Fusarium* species and combinations of the seven *Fusarium* species of interest.
  - DNA was extracted from 196 root samples collected from commercial field peas.
- Optimize and validate PCR assays for *Fusarium* species (Jan to Apr 2015)
  - Previously developed molecular methods for the quantification of *Fusarium* species were not adequately specific or sensitive; therefore, novel real-time QPCR primers were developed for seven *Fusarium* species. Assays using with real-time QPCR primer sets were validated using DNA extracted from pure cultures of *F. graminearum, F. culmorum, F. sporotrichiodes, F. solani, F. redolens, F. acuminatum* and *F. avenaceum*.
  - Taqman hybridization probes were designed to correspond to amplicons produced from PCR assays of each species. Assays consisting of primers and probes were validated using DNA extracted from pure cultures of each *Fusarium* species and field pea plants inoculated in the greenhouse.
  - Three multiplex real-time QPCR assays have been developed to quantify seven (target = 6) *Fusarium* species, therefore this portion of the Measurable Expected Outcomes was exceeded. Assay number one quantifies *F. avenaceum and F. culmorum*, assay two quantifies *F. graminearum* and *F. sporotrichiodes* and assay three quantifies *F. solani, F. redolens* and *F. acuminatum*.
  - The specificity of all three multiplex real-time QPCR assays was evaluated against 11 micro-organisms including non-target *Fusarium* species and other root rotting and foliar pathogens of pea. Only target genes from organisms of interest were amplified, no cross-reaction was observed.
  - All target *Fusarium* species were amplified from the respective roots inoculated under greenhouse conditions with single *Fusarium* species or combinations of the seven target species.
- Optimize molecular procedures to differentiate nematode species (Jan to Apr 2015)
  - DNA was extracted from single nematodes. The D2-D3 expansion region of 28S rRNA and ITS region of rDNA were amplified and PCR products were purified and sequenced or cloned using pGEM-T easy vector and sequenced.
- Quantify *Fusarium* species in root tissue using real-time QPCR (May to Jul 2015)

- Secondary validation of real-time QPCR assays was performed using DNA extracted from naturally diseased root tissue collected (192 plants) from the field compared to *Fusarium* species obtained using traditional plating methods.
- Analyses of *Fusarium* species colonizing root tissue collected from pea fields using plating methods was completed and species were identified using traditional morphological methods.
- Results from the three multiplex real-time QPCR assays corresponded to, and therefore, were validated by results of *Fusarium* populations determined using the plating method on 192 pea roots from field samples.
- Results indicate that *F. avenaceum, F. culmorum, F. graminiarum* and *F. acuminatum* were the most common species colonizing diseased pea roots. *F. avenaceum* and *F. acuminatum* were recovered from approximately 95% of fields samples, while *F. culmorum* and *F. graminearum* were recovered from 35% and 40%, respectively.

- Identify plant-parasitic nematodes to species using molecular and morphological methods (May to Jul 2015)
  - Molecular methods were used to identify nematodes isolated from soil and roots to species.
  - The comparative sequence analysis identified the root-lesion nematode species as *Pratylenchus scribneri*, the dagger nematode as *Xiphinema rivesi*, the stubby root nematode as *Paratrichodorus allius*, the pin nematode as *Paratylenchus nanus*, and the spiral nematode as *Helicotylenchus digonicus*.
  - The stunt nematodes recovered had less than 90 % identity to any published *Tylenchorhynchus* species and therefore were submitted to the taxonomist Dr. Zafar Handoo (USDA-ARS Nematology Laboratory, Beltsville, MD) for species identification. The stunt nematodes were identified as *Quinisulcius acutus* and *Tylenchorhynchus agri*, which have not been previously reported in North Dakota.
  - The root-knot nematode population was too low to obtain ample specimens for species identification.
  - A total of seven nematode species were characterized from soils collected from field peas in North Dakota, this is an increase of three nematode species over the proposed target of the determination of the four species.
- Travel to field sites for nematode and plant sampling (May to Sept. 2015)
  - Thirty-five soil samples were processed and assayed for nematodes at the NDSU nematology lab using the sugar centrifugal flotation method. Fifty-seven additional soil samples were sent to Western Laboratories, Parma, ID for nematode identification and enumeration at the genus level using microscopy.
  - Eighty soil samples were collected in 2015 and analyzed for property analysis by the NDSU soil testing laboratory.
- Resample selected fields and assay plant and soil samples; confirm nematode identity and distribution and compare nematode densities (Jun to Dec 2015)
  - Forty-eight plant samples were collected from the fields and nematodes were extracted from the plant roots grown in 2015 using the Whitehead tray method.
  - Seven economically important groups of plant-parasitic nematodes were detected including root-knot, root-lesion, stunt, spiral, pin, dagger, and stubby root nematodes. Pin nematodes were predominant, stunt and spiral nematodes were moderate, and root-lesion, dagger, root-knot, and stubby root nematodes were low.

- Pin, spiral and stunt nematodes were observed in one-third of the total soil samples. Pin nematode represented the highest density among identified groups.
- A new group of nematode "without stylet", morphologically close to pin nematode was observed. These nematodes were identified as the pin nematode *Paratylenchus nanus* type B by the Senior Nematologist, Dr. Sergei Subbotin in California Department of Food and Agriculture.
- Corresponding location data indicated that pin nematode populations were consistently the highest in McHenry County. Other nematode groups were variable among sampled counties.

- Report preliminary results to the Pulse Crops Working Group- 12 meeting attendees (Aug 2015)
  - Preliminary results were reported to the Pulse Crops Working Group held in conjunction with the American Phytopathological Society (APS) Annual Meeting in Pasadena, CA in August, 2015 by Julie Pasche.
- Analyze data to examine relationships between nematode densities, *Fusarium* species, soil properties and root rot (Aug to Dec 2016)
  - The associations between plant-parasitic nematodes (7 groups) and soil properties (17 categories) were analyzed. The models indicate that increased iron content in soil generally was related to an increase in population densities of pin nematodes and the organic matter and cation exchange capacity was related to a decrease in populations of stunt nematodes.
  - Surveyed fields were placed into seven groups based on geographic location and evaluated for the likelihood of isolating each *Fusarium* spp. using canonical correspondence analysis. Based on this analysis, *F. acuminatum*, *F. avenaceum*, *F. redolens* and *F. oxysporum* were associated with numerous geographic groups. However, *F. sporotrichiodes* and *F. culmorum* were most closely associated with samples collected from Ward, Renville and Bottineau counties, *F. graminiarum* was associated with samples from McKenzie and Dunn counties, and *F. solani* was associated with samples from Divide, Williams, Burke and Mountrail counties.
  - Associations among the seven geographic locations and soil properties were examined using multiple regression analysis. These analyses indicate that electro-conductivity and soil pH were important factors in grouping locations in 2014, while cation exchange capacity and electro-conductivity appear to be useful in separating groups in 2015. Unfortunately, not all seven *Fusarium* spp. were isolated from an ample number of fields to perform similar in-depth association analyses.
  - While the results of these analyses do not provide a direct comparison between soil properties and the likelihood of isolating *Fusarium* spp., they do provide an indication of these relationships which can be further evaluated as more data is collected or in evaluations conducted under controlled conditions.
  - Pearson's correlation analysis determined a significant ($\alpha = 0.05$) positive (both factors increase) association between root rot severity and soil pH ($r = 0.2403$; $P = 0.0318$), and a significant negative correlation (as root rot increases the other factor decreases) with ammonia ($r = -0.4406$; $P = 0.0190$), potassium ($r = -0.2868$; $P = 0.0099$) and copper ($r = -0.4356$; $P = 0.0205$).
  - Principle component analysis was utilized to further elucidate the significant associations between root rot severity and soil properties determined significant via Pearson correlation analysis. This analysis revealed that potassium levels in the soil most influenced root rot severity.
- Present results at the Northern Pulse Growers Association Annual Convention 240 meeting registrants (Jan 2016)

- Preliminary results were presented to growers, extension professionals and other industry stakeholders by Julie Pasche at the Northern Pulse Growers Association in January, 2016.

- Prepare reports and technical manuscripts (Jan to Mar 2016)
  - All quarterly and annual reports were completed and submitted to the SCBGP as indicated in the guidelines.
  - An abstract entitled "Plant-parasitic nematodes on field pea in North Dakota" was presented at the American Phytopathological Society (APS) Annual Meeting in August, 2015 in Pasadena, CA.
  - An abstract entitled "Comparison of two nematode extraction techniques" was presented at the annual meeting of the Society of Nematologists in July, 2015 in East Lansing, MI.
  - Manuscript preparation for the multiplex QPCR assays to quantify seven *Fusarium* species will be submitted to the APS Journal Plant Disease in early July, 2016.
  - A manuscript describing one species-specific conventional PCR assay and one real-time PCR assay developed and utilized for identifying the root-lesion nematode *Pratylenchus scribneri* was submitted to the APS Journal Plant Disease.
  - A manuscript describing one species-specific conventional PCR assay and one real-time PCR assay were developed and utilized for identifying the stubby root nematode *Paratrichodorus allius* is being prepared and will be submitted to the APS Journal Plant Disease.
  - Additional manuscripts describing the relationships among the factors evaluated in the research are in preparation for submission to the APS journal Plant Disease.
- Share results with extension professionals (Jan to Mar 2016 – actually ongoing throughout)
  - This was accomplished through interaction at grower field days and annual meetings, as well as through personal interactions.
  - Preliminary results were presented and the NDSU Research and Extension Center Conference in Fargo on March 3, 2016 by Julie Pasche with 25 meeting attendees.
- Present results at the North American Pulse Growers Association (NAPIA) meeting- 81 meeting registrants (Nov 2016 –occurred in Nov 2015, the date was incorrect in the proposal)
  - Preliminary results were presented to the scientific community at the North American Pulse Improvement Association (NAPIA) in November, 2015.

Goals and Outcomes Achieved

Goal #1: identify nematode species in association with pea roots using previously developed molecular methods
Outcome for goal #1: Seven nematode species were identified using molecular methods. This surpasses the target of using molecular methods to identify four nematode species.

Target = 4; Outcome = 7

Goal #2: Quantify six *Fusarium* species from pea roots using previously developed real-time PCR assays.
Outcome for goal #2: Effective conversion of previously developed assays for the detection of *Fusarium* spp. in field pea was not possible, therefore, novel real-time QPCR assays were developed to for seven *Fusarium* spp. colonizing field pea root tissue. These assays were validated using *Fusraium* spp. in pure culture, field pea plants inoculated in the greenhouse, and

naturally infected plants obtained from commercial fields. This surpasses the target of developing assays for six species.
Target = 6; Outcome = 7

Goal #3: Determine associations among soil properties, nematode populations, *Fusarium* species and root rot of peas.
Outcome for goal #3: Numerous statistical analyses were performed to facilitate identifying associations among the parameters evaluated in this research. Some *Fusarium* species were more commonly found in specific geographical locations. Meaningful soil characteristics including cation exchange capacity, pH and electro-conductivity were determined to best describe the seven geographic locations. Root rot was increased with increasing pH, and decreasing potassium, ammonia and copper in the soil. Pin nematodes were found to increase with increasing iron, and decreasing cation exchange capacity and organic matter was related to a decrease in populations of stunt nematodes.
Target = 3; Outcome = 7

Beneficiaries

The economic impact of field pea production in ND reached a high of $141 million in 2007 (NASS 2016). This is in addition to the benefit field peas provide as a rotational crop. While root rot is a major issue in field pea production, some areas of the state more commonly see losses than others. No wide-spread survey of disease associated yield loss has been conducted; therefore, it is difficult to estimate the economic impact on a state-wide basis. There are approximately 2,500 field pea producers in North Dakota, although not all will benefit from the results of this research.
Field peas have been important specialty crops in North Dakota since production started to rapidly increase around 1999. Planted acres of dry pea increased nearly 10-fold from approximately 65,000 acres in 1999 to 610,000 in 2006 (NASS 2016). In 2009, acres planted decreased to less than 500,000, and hit a low of 85,000 in 2011. While the drastic decrease in 2011 was greatly due to major flooding in pea growing regions, a general decline was occurring prior to that time. Through field observations, prior research and discussions with growers and other industry personnel, a hypothesis has been formed that the general decline may be largely due to root rotting pathogens. During the peak of production, some growers did not follow 4-year crop rotation recommendations. This practice has led to the build-up of pathogen populations, most notably root rotting pathogens. Numerous factors affect producers planting decisions, however, there is concern that the survival of the pea industry in North Dakota will be greatly influenced by the ability to manage root rot. The results of this research provide field pea growers with information on the specific type of root rotting pathogens, fungal and nematode, present in their production region. This is the first step towards the development of management strategies, including the development of resistant varieties.

Lessons Learned

- Goals and outcome measures were achieved and exceeded. This is in great part to the effort, communication and cooperation of all scientists involved in the studies.

- Additional data is needed to intensively evaluate the association between *Fusarium* species and soil properties. We did not envision the large number of samples required to collect an ample number of some species. However, analyses are ongoing and some associations may be uncovered in this area with those species recovered most frequently. Alternatively, the data collected as part of this research will be coupled with additional data collected in the future to elucidate these associations.

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Additional Information

- Other activities not outlined in "Project Activity"
  - Preliminary results were presented to the scientific community and other industry stakeholders by Julie Pasche at the Pulse Crops Working Group Annual meeting in March, 2015-12 meeting attendees.
  - Preliminary results were presented to growers, extension professionals and other industry stakeholders in an oral presentation by Julie Pasche at the Pulse USA Trade Show in November, 2015 (32 meeting attendees) and the RD Offutt Company agronomy meeting (17 meeting attendees) on February 10, 2016

Research on Vegetable Production in Controlled Environment Systems

Final Report

Partner Organization: North Dakota State University

Project Summary

Production of lettuce and other leafy green vegetables has been increasing in the United States especially for local consumption. Vegetable production under controlled environment conditions has distinctive advantages over field production in supplying produce year-round, reducing water consumption, local supply of fresh and quality product. In this research, two different hydroponic culture systems were utilized to study some of the common problems associated with greenhouse production. Sixty some cultivars of lettuce were evaluated for culture on NFT (nutrient film technique) system to identify superior cultivars. Leaf edge burns of lettuce, a common physiological disorder, were studied for possible control. The pattern of nitrate (NO_3^-) accumulation in leaf tissues of lettuce as influenced by nutrient solution was studied. The influence of artificial lights on the growth of lettuce was also studied. Outcome of this research will enhance year-round supply of fresh leafy green vegetables for local and regional consumption as well as the understanding of plant growth and development under intensive production system. This project did not build on a previously funded project with the SCBGP.

Project Approach

The controlled environment production of vegetable crops has distinctive advantages over the conventional production system: 1) year-round supply, 2) high quality produce for local food systems, 3) nutrient dense vegetables that are free of agricultural chemicals and microbial contamination, 4) efficient use of water and land. For example, to produce a head of lettuce, only 1 gallon of water is needed by the NFT (nutrient film technique) hydroponic system, as compared to 150 gallons of water needed for field production in California. More than 80% of vegetables consumed in ND and surrounding region comes from California. With the renewed interest in growing vegetables for farmer's markets and local food systems, greenhouse production of vegetables, especially leafy greens, for local and regional consumption is rapidly increasing throughout the country. Growing conditions in ND have a comparative edge with abundant sunlight, clean air and relatively cheap energy source, compared to that of eastern Canada where a large portion of tomato, bell pepper and cucumber that we consume is grown. While several businesses are now actively pursuing hydroponic production in the state, the technical know-how as well as growing experience are largely lacking. The purposes of this research were to: 1) identify superior cultivars of lettuce for hydroponic culture; 2) solve problems associated with physiological disorders; 3) develop ways to reduce leaf nitrate content,4) study efficacy of using artificial lights for winter production, and 5) study the efficacy of air injection to lettuce leaf canopy for increased photosynthesis and yield.

1. Evaluation of lettuce cultivars for hydroponic culture

Sixty four cultivars of lettuce were cultured on NFT system and their performance was evaluated in three different experiments (see the research progress report attached). Cultivars that showed physiological disorders like leaf edge burns and early bolting were eliminated during the following experiments. In general, butterhead type of lettuce including Rex RZ was free of leaf burns and bolting problem. The high ranked 10 cultivars in overall rating and bolting resistance were 11C3487, 3SX820, 3SX821, 3SX822, Pomegranate Crunch, Vivian, Winter Density, Butterhead, Refugio, and Rex RZ. Among those cultivars, Rex RZ, 11C3487, 3SX820, 3SX821 and 3SX822 had not shown only high plant fresh weight but also high brix (Table 4). In the experiment, bolting in the lettuce cultivars was worse than former experiment and it might be caused by calcium deficiency. General commercial fertilizer for hydroponic cultivation consists does not contain Ca^{2+} and Mg^{2+}, because the tap water for using hydroponic culture usually has enough amounts for plant growth. However, this experiment had been performed for six weeks and the calcium concentration in the nutrient solution was not enough for lettuce growth. Additionally, this outer leaf marginal necrosis can be occurred at low humidity and high nutrient solution rate. High salt in the nutrient solution may damage to the plant tissue, and low humidity surround circumstance can promote evaporation between plant stoma and the atmosphere in the greenhouse. Those various combinations may bring critical outer leaf marginal burn during the experiment.

2. Strategies for reducing leaf nitrate concentration in lettuce

High tissue concentration of nitrate (NO_3^-) in leafy greens like lettuce and spinach is considered harmful for human health. Nitrate when ingested is converted to nitrite (NO_2^-) and then nitrosamines. Nitrosamines, having a chemical structure [$R^1N(-R^2)-N=O$] with a nitro group bonded to an amine, are known as chemical carcinogens especially stomach and intestinal cancers. While Europe has regulations on the upper limits of nitrate in vegetables, no such mandate is enforced in the United States. We have evaluated nitrate contents of lettuce grown in hydroponic system. We have tested the influence of the ratio of ammonium (NH_4^+) to nitrate (NO_3^-) concentrations in the nutrient solution to the accumulation of nitrate (NO_3^-) in the leaf tissues of lettuce. While preliminary results of the experiment were published (see appendix), the ways by which leaf nitrate levels can be reduced are being investigated. The influence of the composition of the nutrient solution mineral contents on the pattern of nitrate accumulation in the leaf tissues as well as the influence of artificial lights are also being studied.

3. Influence artificial lighting on growth of lettuce

Three different lights (HPS, LEP, LED) were used were evaluated for their influence on the growth and quality of lettuce cultured hydroponically and in root substrate. The yield and quality of vegetable crops grown under artificial light were compared. Our preliminary findings were: 1) the quality of lettuce grown under plasma light (LEP) was better than that of lettuce grown under LED (light emitting diodes) or high pressure sodium light (HPS), 2) the efficiency of lighting in terms of utility cost was best with LED followed by LEP and HPS lamps, 3) photosynthetic efficiency during culture period varied by cultivars of lettuce, 4) yield of lettuce was higher when artificial light was used

compared to natural light available. Currently, the pattern of nitrate accumulation in the leaf tissues of lettuce as influenced by artificial lights is under investigation.

4. Ion balancing for hydroponic culture nutrient solutions

Preparation of nutrient solutions containing different ratios of ammonium and nitrate forms of nitrogen was optimized for hydroponic culture. These solutions will be used for future studies on the influence of different forms of nitrogen on the performance of lettuce. A system for balancing cation and anion nutrients for irrigation water containing minerals has been developed. This system will be used for recommending proper use of macro nutrients for irrigation waters locally available. Along with the optimization of nutrient content, pH adjustment of irrigation water by using acid injection system. Current and future research includes how to handle irrigation water containing high levels of total dissolved salts or high pH values.

5. Boron deficiency and toxicity symptoms on lettuce

Leaf margin burns are the most commonly occurring physiological disorder symptom during hydroponic culture of lettuce. We suspected this physiological disorder was caused by deficiency or excess amount of boron (B) in irrigation water. We grew 2-3 different cultivars of lettuce with nutrient solutions containing elevated levels of boron as well as nutrient solution containing no boron. When boron concentration in the nutrient solution was higher than 0.2 μM B, toxicity symptoms developed with severe necrotic leaf margin forming at 2.0 μM B (see Fig 13). When the nutrient solution contained no boron, the development of deficiency symptom developed on newly formed leaves that became brownish without full expansion (see Fig 14). The toxicity and deficiency symptoms of boron were different from the commonly observed leaf margin burns that are usually restricted to leaf older, fully expanded leaves. The necrotic (browning) tissues developed on mature leaves by excess amount of boron were more widely spread forming irregular shape as compared to the very thin necrotic leaf margin burns commonly observed. Therefore, the common leaf margin burns that occur more uniformly may have been due to environmental conditions such as low humidity or the fluctuation of temperature extremes.

6. Performance of grafted tomatoes in the greenhouse

Most fruit bearing vegetables grown in the greenhouse in Japan and Korea are from grafted plants. Grafted plants are more tolerance to disease pressure and produce higher yields compared to non-grafted own root plants. In order to test the efficacy of grafting, we made seedling grafting using Torero (beefsteak tomato, indeterminate) and Cannonball (NDSU release, determinate) tomatoes as scion cultivars and Maxifort (from Johnny's Selected Seeds) and Blocking (Korean) tomatoes as rootstocks. The grafted plants are now grown in the greenhouse and their performance will be compared with those of non-grafted plants. Findings of this research will be used to formulate recommendations for greenhouse tomato growers in the U.S.

7. Publication of research findings in scientific journals

In 2016, findings our research were presented at the annual conference of the American Society for Horticultural Science (see References). Two manuscripts based on research findings are now being prepared for publication in scientific journals.

Information generated by six different areas of research were communicated with the local and regional growers as well as future producers of vegetables. Significant contributions with the research with NDSU includes:

a. *Selection of lettuce cultivars for hydroponic culture.*
The best performing cultivars of lettuce as well as those that had bolting problem during hydroponic NFT culture were identified. Local growers are now using the cultivars identified for having desirable growth characteristics.

b. *Strategies for reducing nitrate (NO_3^-) levels in lettuce.*
The influence of nutrient solution containing different ratios of ammonium (NH_4^+) and nitrate (NO_3^-) forms of nitrogen on leaf nitrate contents was determined.

c. *Influence of artificial lights on growth and quality of lettuce.*
The efficacy of using artificial lights (HPS, LED, LEP) was determined for selected cultivars of lettuce. Information obtained will be used for cultural recommendations for producing quality crops while minimizing utility costs.

d. *Modification of nutrient solutions for lettuce culture.*
A system of balancing macronutrient ions (NH_4^+, Ca^{++}, Mg^{++}, K^+, NO_3^-, SO_4^{-2}, $H_2PO_4^-$) during preparation of hydroponic nutrient solutions according to water analysis was developed.

e. *Influence of boron (B) on the development of leaf edge burns in lettuce.*
The toxicity and deficiency symptoms of boron (B) for growing lettuce were characterized with attention to the occurrence of leaf edge burns.

f. *Performance of grafted tomatoes in the greenhouse.*
Two different cultivars of tomato granted onto two different rootstocks were grown in the greenhouse. Unfortunately, data were not obtained due to insect damage. We are currently growing a new set of grafted plants and results will be reported later.

Goals and Outcomes Achieved

Goals	Outcomes achieved
1. Evaluation of lettuce cultivars for hydroponic culture	1. Three sets of experiments were carried out to evaluate performance of 64 cultivars of lettuce. Good data were obtained for grower recommendation. (About 85% goals achieved)
2. Strategies for reducing leaf nitrate concentration in lettuce	2. Influence of different ratios of ammonium (NH_4^+): nitrate (NO_3^-) in the nutrient solution on leaf tissue nitrate content were tested.

	Research ongoing. (About 50% goals achieved)
3. Influence of artificial lighting on growth of lettuce	3. Three different lights (LEP, LED, HPS) were evaluated for their efficacy for use in lettuce culture in 3 different experiments. (About 60% work completed)
4. Ion balancing for hydroponic nutrient solutions	4. Tested and revised for optimum nutrient solutions for hydroponic culture with emphasis on ion balancing using irrigation water containing various minerals. (80% work completed)
5. Boron deficiency and toxicity symptoms on lettuce	5. Boron toxicity and deficiency symptoms of lettuce were induced and characterized for grower use. (About 70% goals achieved)
6. Performance of grafted tomatoes in the greenhouse	6. Grafted tomato plants are currently being evaluated. Need replicated trials. (About 30% of work accomplished)
7. Publications of research findings in scientific journals	7. Outcomes of research has been presented at the annual meetings of ASHS. Two manuals are being prepared for publication in scientific journals.

This project had three goals: To select best performing cultivars of leave lettuce including bib and Romaine lettuces for hydroponic culture, to solve the occurrence of physiological disorder symptoms such as leave margin burns and distortion on new growth in lettuce grown in the greenhouse, and to optimize nutrient solution for growing lettuce, tomato, and other vegetables in the greenhouse. The list of best performing lettuce cultivars including bib lettuce (i.e., Rex RZ) and Romaine lettuce (i.e., Parris Island) has been generated. The cause and remedy for lettuce leaf margin burn symptoms has not been determined; however, our progress toward solving the problem has been suggested. The method of optimizing nutrient solutions for growing lettuce and other vegetables has been tested. Attention was made on how to balance macronutrient ions when using raw waters with known mineral analysis.

The manual of growing lettuce hydroponically is currently in process and will be completed for distribution in 2018.

The following have been published in scientific journals:

Abstracts published:

a. Kim, C., L.J. Cihacek, and C.W. Lee. 2016. Changes in nitrate contents of hydroponically grown leaf lettuce as influenced by the ratio of ammonium and nitrate forms of nitrogen in nutrient solution. HortScience 51(9): S197.

b. Choi, S., C. Kim, Z. Wu, and C.W. Lee. 2016. Influence of artificial lights on nitrate and sugar contents of leaf lettuce. HortScience 51(9): S287.

c. McCollum, C., C. Kim, and C.W. Lee. 2016. Evaluation of lettuce cultivars for hydroponic culture. HortScience 51(9): S385.

Articles in preparation:

a. Influence of the ratio of ammonium and nitrate forms of nitrogen in the nutrient solution on the tissue nitrate content of hydroponically grown lettuce (*HortScience*).

b. Performance of leaf lettuce cultivars grown hydroponically using the nutrient film technique (NFT) (*HortTechnology*).

c. Effect of nitrate to ammonium ratios on growth, nitrate accumulation and chlorophyll-a fluorescence in lettuce plants (*J. ASHS*).

d. Growth and photosynthesis of selected lettuce cultivars under field and greenhouse conditions (*J. ASHS*).

e. Variation in photosynthetic pigments and anthocyanin contents of selected green and red leaf lettuce cultivars (*HortScience*).

New findings in the elvaulation of lettuce cultivars for hydroponic culture are included in the report attached.

The influence of artificial lights on lettuce growth was tested for plasma light (LEP, full spectrum), light emitting diodes (LED, blue and red lights), and high-pressure sodium (HPS, selected spectra) lamps were adjusted to have about similar light intensity (200 $\mu mol \cdot m^{-2} \cdot sec^{-1}$) during the experiment. Results are included in the report attached.

Our target to evaluate and select leaf lettuce for hydroponic culture can be found within the Project Approach section. Tastes of leaf lettuce Tastes of leaf lettuce grown hydroponically were tested by a panel of 4 people (data not included in the report). Nutritional qualities were not tested due to insufficient funds. We do not have recommendations as of yet, but we are currently working on a continuation project to fulfil this target. A cultural manual is currently being written and will be available in 2018.

Beneficiaries

Reports of research progress have been made available for local as well as regional vegetable growers through presentations during field days, conferences, and workshops held by grower groups (i.e., NPSAS, local food groups, high tunnel growers) as well as telephone calls and interactions at famers markets. Listed below are those who communicated with us most frequently.

Larry Schumacher, Geomato Co.
Chad Hove, Hove Greenhouse, Jamestown, ND
Jim Brown, CropKing Hydroponics
Bill Erbes, Bill Erbes Farms
John Skarie, Lakeview Greenhouses

Beneficiaries include the participants in oral and poster presentations given during the ASHS and NPSAS annual conferences, local workshops and invited talks:

2016 annual conference of ASHS (Atlanta, GA)

Oral presentation (65 persons) and two poster presentations (55 persons)

2016 annual conference of NPSAS (Aberdeen, SD)

Two workshops (75 persons)

2016 field day at NDSU (35 participants)

2016 Carrington Res and Ext Center field day (150 persons)

2016 Tour of greenhouses to see ongoing research at NDSU by students and local citizens (160 persons)

2017 Local Foods conference held in Mandan, ND (60 participants)

2017 NDSU High Tunnel conference workshop (30 participants)

Total beneficiaries (2016-2017): 630

Lessons Learned

1. The first experiment always fails. If it works, it is a miracle!
2. What a joy to work with many inspired people young or old!

Several experiments had to be rerun due to irregularities in temperature control in the old greenhouse that was used for this research. Spread of thrips and other insect pest was also a problem especially with the gravel covered greenhouse floor. A better nutrient solution injection system is needed for the NFT culture of leafy greens.

Certain experiments were not carried out as planned, due to lack of instruments needed to measure photosynthesis (i.e., LI-COR meter) effectively. For future research, we will seek other external funding sources to purchase an ion-chromatography system (for nitrate measurements) and additional LED grow lights.

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Additional Information

See attachments:

1. Research progress report (2016-1017)
2. Publications (2016)

Annual Report Performance Report
January 1, 2016 - March 31, 2017

Title: Research on Vegetable Production in Controlled Environment Systems
Project NOGA Number: 14-207 (14-SCBGP-ND-0038)

Chiwon W. Lee
Department of Plant Sciences, North Dakota State University

Annual Performance Report Section	**Corresponding State Plan Project Proposal Section(s)**
Activities Performed	**Work Plan**
1. Lettuce cultivar evaluation and selection. Sixty (60) commercial cultivars of lettuce were grown with NFT system. Fresh weight, produce quality, bolting, leaf nitrate content, sugar content and physiological disorders were evaluated in two separate experiments.	1. Evaluation of lettuce cultivars for hydroponic culture. *Expected Measurable Outcomes*: Best cultivars will be identified for recommendation to hydroponic lettuce growers.
2. Strategies for reducing tissue nitrate (NO_3^-) levels in leafy vegetables. Nitrate (NO_3^-) absorption and accumulation in lettuce and basil tissues as influenced by the combination of ammonium (NH_4^+) and nitrate (NO_3^-) forms of nitrogen in hydroponic solution were studied.	2. Research to reduce tissue nitrate (NO_3^-) contents in lettuce. *Expected Measurable Outcomes*: Influence of nutrient solution ammonium (NH_4^+) and nitrate (NO_3^-) forms of N will be characterized. Light and other environmental factors influencing tissue NO_3^- will be known.
3. Influence artificial lighting on growth of lettuce. Three different lights (HPS, LEP, LED) were used were evaluated for their influence on the growth and quality of lettuce in hydroponic and substrate culture systems.	3. Influence of artificial lights on lettuce culture. *Expected Measurable Outcomes*: Information on the effect of light supplementation on growth of lettuce and herbs.
4. Nutrient balancing for hydroponic culture. Preparation of nutrient solutions containing different ratios of ammonium and nitrate forms of nitrogen was optimized for hydroponic culture.	4. Modification of nutrient solution for hydroponic culture. *Expected Measurable Outcomes*: Materials and procedures for preparing nutrient solutions containing different ratios of fertilizer ions.
5. Influence of boron (B) deficiency on lettuce. Boron deficiency symptoms on lettuce were characterized using hydroponic culture.	5. Boron (B) deficiency symptoms characterized. *Expected Measurable Outcomes*: Identification of boron deficiency symptoms and their remedy.
6. Performance of grafted tomatoes in the greenhouse. Due to greenhouse space limitations, this experiment was not carried out. Tomato seedlings are now grown for grafting culture in the greenhouse in 2017.	6. Performance of grafted tomatoes in the greenhouse. *Expected Measurable Outcomes*: Growth and yield of grafted tomato plants in the greenhouse will compared with non-grafted tomatoes.
7. Disseminate information on research findings. Results of experiments were communicated as posters and oral presentation at the annual ASHS conference in Atlanta, GA.	7. Findings of research will be published in scientific journals. *Expected Measurable Outcomes*: Manuscripts on research findings are being prepared for submission to HortScience.

Research Activities Performed

1. Lettuce cultivar evaluation and selection

a. Experiment 1

Seeds of sixty cultivars of lettuce were planted a Rockwool cube and seedlings were transplanted onto the channel of hydroponic NFT system in two weeks. The cultivar name and seed source are listed in Table 1. During the experiment, 60 cultivars leafy lettuce have been given 20-20-20 commercial fertilizer as 200 $mg \cdot L^{-1}$ concentration. Each unit contained four 6-foot long troughs which were slightly slanted to allow nutrient solution flow into the sump bucket (5-gallon size). The nutrient solution was continuously applied to the plants (3 plants per cultivar) and was collected into the bucket for recirculation. To compensate the loss of nutrient solution by plant absorption and transpiration, each sump bucket was replenished with new solutions as needed (Fig. 1).

Table 1. List of selected lettuce cultivars used in the experiment.

Cultivar	Seed source	Cultivar	Seed source
1. Aerostar	High Mowing	31. Looseleaf Blend	Burpee
2. Ansar	High Mowing	32. Nevada	High Mowing
3. Arctic Crisp	Burpee	33. Oakleaf	Agassiz
4. Arroyo	High Mowing	34. Outredgeous	Johnny's Selected
5. Baby Romaine	Livingstone	35. Parris Island Cos	High Mowing
6. Balfour	High Mowing	36. Parris White	Burpee
7. Bibb	Johnny's Selected	37. Parris Island	Livingstone
8. Black Seeded Simpson	Livingstone	38. Pomegranate Crunch	High Mowing
9. Boulder	High Mowing	39. Prizehead	Agassiz
10. Buttercrunch	Livingstone	40. Red Oak	Livingstone
11. Butterhead	Johnny's Selected	41. Red Romaine	Livingstone
12. Caesar's Favorite	Burpee	42. Refugio	High Mowing
13. Coastal Star	High Mowing	43. Rex Rz	Rijk Zwaan
14. Emerald Oak Lettuce	High Mowing	44. Rhazes	High Mowing
15. Encino	High Mowing	45. Romaine	Johnny's Selected
16. Four Seasons	Burpee	46. Rosalita	Burpee
17. Galactic	High Mowing	47. Rouge d'Hiver	Burpee
18. Giant Caesar	Burpee	48. Salad Bowl	Agassiz
19. Grand Rapids	Ferry-Morse	49. Silvia	Burpee
20. Grand Rapids TBR	Agassiz	50. Simpson Elite	Livingstone
21. Green Ice	Agassiz	51. Slobolt	Livingstone
22. Green Star	High Mowing	52. Summer Crisp	Johnny's Selected
23. Green Summer Crisp	Johnny's Selected	53. Tango	High Mowing
24. Green Towers	High Mowing	54. Tennis Ball	Livingstone
25. Iceberg A	Burpee	55. Vivian	Burpee
26. Igloo	Burpee	56. Winter Density	High Mowing
27. Jericho	Fedco Seeds	57. 11C3487	3 Star Seed Co.
28. Lettony	High Mowing	58. 3SX820	3 Star Seed Co.
29. Little Caesar	Burpee	59. 3SX821	3 Star Seed Co.
30. Lollo	Johnny's Selected	60. 3SX822	3 Star Seed Co.

Fig. 1. Hydroponic culture of 60 leaf lettuce cultivars. A-seedling germinated on Rockwool cubes were transplanted to NFT system with nutrient recirculation: pictures were taken 10 days (A), three weeks (B), and four weeks (C) after seedling placement.

At the time of harvesting, plant growth parameters (size and shape), chlorophyll content (using a SPAD meter), and leaf color were recorded. Leaf tissue sugar content was determined by the digital refractometer or a Brix meter. The tissue sap pH and nitrate concentration were also determined using a portable pH and nitrate meter. The results of these measurements are presented in Tables 2 and 3).

Results

The high ranked 10 cultivars in overall rating and bolting resistance were 11C3487, 3SX820, 3SX821, 3SX822, Pomegranate Crunch, Vivian, Winter Density, Butterhead, Refugio, and Rex RZ. Among those cultivars, Rex RZ, 11C3487, 3SX820, 3SX821 and 3SX822 had not shown only high plant fresh weight but also high brix (Table 4).

In the experiment, bolting in the lettuce cultivars was worse than former experiment and it might be caused by calcium deficiency. General commercial fertilizer for hydroponic cultivation consists does not contain Ca^{2+} and Mg^{2+}, because the tap water for using hydroponic culture usually has enough amounts for plant growth. However, this experiment had been performed for six weeks and the calcium concentration in the nutrient solution was not enough for lettuce growth. Additionally, this outer leaf marginal necrosis can be occurred at low humidity and high nutrient solution rate. High salt in the nutrient solution may damage to the plant tissue, and low humidity surround circumstance can promote evaporation between plant stoma and the atmosphere in the greenhouse. Those various combinations may bring critical outer leaf marginal burn during the experiment.

Table 2. Some of the quality parameters of lettuce cultivars grown hydroponically measured 4 weeks after seedling transplanting.

No.	Cultivar	Plant fresh weight (g)	Plant dry weight (g)	pH	Nitrate (mg/l)[z]	% Sugar[y] (Brix)	Chloro-phyll[x] (SPAD)	Leaf margin burns[w]
1	Aerostar	150.3	14.9	6.2	4,700	5.4	3.3	5.0
2	Ansar	158.7	13.5	6.1	6,900	4.4	3.3	5.0
3	Arctic Crisp	89.0	10.8	6.1	5,700	6.6	1.0	5.0
4	Arroyo	141.0	14.3	6.1	4,000	5.0	4.2	5.0
5	Baby Romaine	95.0	9.0	6.2	4,000	5.4	3.7	5.0
6	Balfour	88.3	9.7	6.1	11,000	7.1	3.0	5.0
7	Bibb	109.3	10.8	6.1	14,000	3.4	2.0	5.0
8	Black Seeded Simpson	170.3	15.6	5.3	14,000	4.0	3.0	5.0
9	Boulder	103.3	9.4	6.0	14,000	4.9	4.5	5.0
10	Buttercrunch	135.3	13.7	6.0	12,000	6.3	4.2	5.0
11	Butterhead	132.0	12.4	6.4	20,000	6.0	4.0	5.0
12	Caesar's Favorite	108.7	10.8	6.2	16,900	7.4	3.3	5.0
13	Coastal Star	167.0	15.1	6.0	22,000	6.6	4.0	5.0
14	Emerald Oak	130.3	12.3	5.5	20,000	5.3	2.0	5.0
15	Encino	134.7	13.5	6.3	5,200	5.4	4.5	5.0
16	Four Seasons	61.7	6.2	6.1	26,000	6.0	2.7	5.0
17	Galactic	77.0	8.2	6.4	17,000	5.0	4.3	5.0

18	Giant Caesar	168.0	16.7	4.7	22,000	4.8	3.7	5.0
19	Grand Rapids TBR	98.3	9.4	5.9	19,000	5.7	4.0	5.0
20	Green curled buffer	44.7	4.4	5.2	18,000	7.7	4.0	5.0
21	Green Ice	112.3	11.2	4.5	20,000	5.2	4.0	5.0
22	Green Star	154.0	14.7	6.1	12,000	10.3	4.2	5.0
23	Green Towers	83.0	7.3	5.9	13,000	6.7	4.0	5.0
24	Iceberg A	97.3	9.0	6.0	7,100	6.1	3.3	5.0
25	Igloo	122.3	11.8	5.9	6,000	5.0	3.0	5.0
26	Jericho	156.7	15.6	5.7	11,000	3.8	3.0	5.0
27	Lettony	145.3	15.1	5.8	6,200	3.8	4.0	5.0
28	Little Caesar	135.7	12.7	6.0	12,000	4.1	3.7	5.0
29	Lollo	72.7	6.0	5.6	21,000	5.0	5.0	5.0
30	Looseleaf Blend	137.0	13.5	5.8	10,000	3.9	2.7	5.0
31	Nevada	111.0	11.1	5.9	14,000	3.9	2.3	5.0

[z]Measured by Horiba nitrate meter.
[y]Percent sugar was measured by a refractometer.
[x]Measured by a SPAD meter.
[w]Leaf edge burn was scored from 1 to 5, with 1 showing the highest leaf edge burn and 5 showing the lowest leaf edge burn.

Table 3. Some of the quality parameters of lettuce cultivars grown hydroponically measured 4 weeks after seedling transplanting (continued).

No.	Cultivar	Plant fresh weight (g)	Plant dry weight (g)	pH	Nitrate (mg/l)	% Sugar (Brix)	Chlorophyll (SPAD)	Leaf margin burns*
32	Oakleaf	102.3	10.4	6.1	22,000	6.5	3.7	5.0
33	Outredgeous	113.0	11.0	6.4	24,000	7.3	5.0	5.0
34	Parris Island	143.3	15.3	6.0	14,000	4.6	3.7	5.0
35	Parris Island Cos	126.7	12.3	6.0	14,000	5.9	2.7	5.0
36	Parris White	131.7	12.8	5.8	20,000	5.3	4.0	5.0
37	Pomegranate Crunch	79.0	7.6	5.8	18,000	5.1	5.0	5.0
38	Prizehead	87.3	8.7	6.1	19,000	4.2	4.0	5.0
39	Red Oak	81.3	8.2	6.3	30,000	5.3	4.5	5.0
40	Red Romaine	48.7	5.0	6.1	18,000	6.0	4.2	5.0
41	Refugio	40.7	3.8	5.5	31,000	6.3	4.7	5.0
42	Rex Rz	154.0	14.6	6.3	17,000	5.2	4.5	5.0
43	Rhazes	73.7	6.8	5.5	17,000	6.8	4.5	5.0
44	Romaine	179.0	18.2	6.2	12,000	7.5	4.5	5.0
45	Rosalita	104.3	10.9	6.3	12,000	7.4	4.5	5.0
46	Rouge d'Hiver	87.7	9.2	6.3	12,000	6.0	4.8	5.0
47	Rouge Red	84.0	10.1	6.3	9,900	4.2	4.5	5.0

48	Salad Bowl	152.0	14.4	4.7	6,600	3.5	4.0	5.0
49	Silvia	89.0	8.8	4.7	13,000	5.3	4.0	5.0
50	Simpson Elite	144.3	13.3	6.1	16,000	4.1	4.0	5.0
51	Slobolt	218.7	21.8	5.3	18,000	3.8	4.3	5.0
52	Summer Crisp	132.0	12.5	5.0	14,000	5.1	3.0	5.0
53	Tango	87.3	8.6	5.3	19,000	4.9	1.0	5.0
54	Tennis Ball	75.3	8.2	5.2	16,000	4.9	2.7	5.0
55	Vivian	170.7	17.9	6.9	20,000	4.6	3.7	5.0
56	Winter Density	75.3	7.4	6.6	6,000	6.1	4.0	5.0
57	11C3487	106.3	11.8	6.1	23,000	8.0	4.2	5.0
58	3SX820	109.7	11.1	6.1	22,000	5.8	5.0	5.0
59	3SX821	119.0	10.2	6.1	18,000	4.2	5.0	5.0
60	3SX822	125.7	13.0	6.2	21,000	6.9	4.0	5.0

[z]Measured by Horiba nitrate meter.
[y]Percent sugar was measured by a refractometer.
[x]Measured by a SPAD meter.
[w]Leaf edge burn was scored from 1 to 5, with 1 showing the highest leaf edge burn and 5 showing the lowest leaf edge burn.

Table 4. Characterization of lettuce cultivars grown in NFT system.

	Cultivar	Height (cm)	Width (cm)	Plant color[z]	Plant shape[y]	pH	Brix (%)	Chloro-phyll (SPAD)	Bolt (1-5)	Leaf margin burns (1-5)	Leaf mold (1-5)	Overall rating (1-5)
1	Aerostar	23.3	25.7	DG	U	6.2	5.4	59.0	5.0	3.3	5.0	3.3
2	Ansar	26.3	24.7	DG	U	6.1	4.4	56.2	5.0	3.3	5.0	3.5
3	Arctic Crisp	77.7	19.0	DG	U	6.1	6.6	54.9	1.0	1.0	5.0	1.0
4	Arroyo	28.0	24.3	DG	U	6.1	5.0	66.8	4.3	4.2	5.0	4.0
5	Baby Romaine	21.0	16.3	G	U	6.2	5.4	51.6	5.0	3.7	5.0	3.8
6	Balfour	12.7	20.0	G	H	6.1	7.1	57.2	5.0	3.0	5.0	3.0
7	Bibb	22.0	23.7	DG	U	6.1	3.4	47.9	5.0	2.0	5.0	2.0
8	Black Seeded Simpson	53.3	24.3	LG	U	5.3	4.0	27.5	1.0	3.0	5.0	2.0
9	Boulder	15.3	25.0	G	H	6.0	4.9	50.4	5.0	4.5	5.0	4.0
10	Buttercrunch	24.7	22.7	G	U	6.0	6.3	45.8	4.0	4.2	5.0	3.5
11	Butterhead	16.3	23.3	LG	LM	6.4	6.0	36.9	5.0	4.0	5.0	4.5
12	Caesar's Favorite	21.7	23.3	DG	U	6.2	7.4	62.8	5.0	3.3	5.0	3.3
13	Coastal Star	26.0	26.3	DG	U	6.0	6.6	59.2	4.7	4.0	5.0	4.0
14	Emerald Oak	28.7	21.0	G	U	5.5	5.3	47.4	3.0	2.0	5.0	2.0
15	Encino	19.3	30.0	LG	M	6.3	5.4	31.9	4.5	4.5	5.0	4.0
16	Four Seasons	13.3	25.0	GR	M	6.1	6.0	30.6	5.0	2.7	5.0	2.3
17	Galactic	21.0	27.0	R	M	6.4	5.0	27.6	5.0	4.3	5.0	4.0
18	Giant Caesar	28.7	28.3	G	U	4.7	4.8	45.4	5.0	3.7	5.0	3.7
19	Grand Rapids TBR	24.7	26.0	LG	U	5.9	5.7	35.7	3.0	4.0	5.0	3.0
20	Green curled buffer	12.0	27.3	G	LM	5.2	7.7	44.1	5.0	4.0	5.0	3.0

21	Green Ice	16.0	30.0	DG	M	4.5	5.2	37.4	5.0	4.0	5.0	4.0
22	Green Star	26.3	31.7	DG	U	6.1	10.3	42.9	4.0	4.2	5.0	3.5
23	Green Towers	25.3	19.7	DG	U	5.9	6.7	5.8	5.0	4.0	5.0	3.5
24	Iceberg A	23.7	24.3	LG	H	6.0	6.1	41.0	4.0	3.3	5.0	3.0
25	Igloo	20.3	27.3	DG	M	5.9	5.0	63.9	5.0	3.0	5.0	3.0
26	Jericho	19.0	29.0	G	U	5.7	3.8	45.1	5.0	3.0	5.0	2.3
27	Lettony	24.0	26.7	DG	U	5.8	3.8	60.1	4.3	4.0	5.0	4.0
28	Little Caesar	18.3	19.3	DG	U	6.0	4.1	44.1	5.0	3.7	5.0	3.3
29	Lollo	16.3	20.7	G	M	5.6	5.0	39.5	4.3	5.0	5.0	4.0
30	Looseleaf Blend	53.3	20.0	LG	U	5.8	3.9	27.8	1.0	2.7	5.0	1.3
31	Nevada	17.7	18.3	G	U	5.9	3.9	28.4	5.0	2.3	5.0	3.5
32	Oakleaf	17.0	24.3	LG	M	6.1	6.5	33.2	5.0	3.7	5.0	3.2
33	Outredgeous	18.0	33.0	RG	M	6.4	7.3	40.9	4.7	5.0	5.0	4.0
34	Parris Island	23.3	19.7	DG	U	6.0	4.6	47.1	5.0	3.7	5.0	3.7
35	Parris Island Cos	23.0	18.7	DG	U	6.0	5.9	48.2	5.0	2.7	5.0	3.0
36	Parris White	22.3	21.3	DG	U	5.8	5.3	48.1	5.0	4.0	5.0	4.2
37	Pomegranate Crunch	20.7	21.0	RG	U	5.8	5.1	45.8	5.0	5.0	5.0	5.0
38	Prizehead	18.7	23.3	LGR	M	6.1	4.2	25.8	5.0	4.0	5.0	3.3
39	Red Oak	17.0	28.3	RG	M	6.3	5.3	35.7	5.0	4.5	5.0	4.0
40	Red Romaine	28.0	30.3	RG	M	6.1	6.0	52.4	4.3	4.2	5.0	3.5
41	Refugio	19.7	28.3	R	M	5.5	6.3	39.5	5.0	4.7	5.0	4.5
42	Rex Rz	17.3	24.7	G	LM	6.3	5.2	40.2	5.0	4.5	5.0	4.5
43	Rhazes	20.0	20.3	RG	U	5.5	6.8	47.5	5.0	4.5	5.0	4.0
44	Romaine	29.7	26.0	DG	U	6.2	7.5	61.2	5.0	4.5	5.0	4.5
45	Rosalita	27.0	28.7	GR	U	6.3	7.4	50.8	4.0	4.5	5.0	3.5
46	Rouge d'Hiver	23.7	25.0	RG	U	6.3	6.0	47.7	4.0	4.8	5.0	3.5
47	Rouge Red	19.0	28.0	GR	M	6.3	4.2	32.0	5.0	4.5	5.0	4.0
48	Salad Bowl	22.0	35.3	LG	M	4.7	3.5	26.4	4.7	4.0	5.0	4.0
49	Silvia	29.0	27.3	GR	U	4.7	5.3	79.1	4.0	4.0	5.0	3.0
50	Simpson Elite	60.7	18.3	LG	U	6.1	4.1	52.3	1.0	4.0	5.0	1.0
51	Slobolt	30.3	38.7	LG	M	5.3	3.8	23.7	5.0	4.3	5.0	3.7
52	Summer Crisp	19.3	22.7	LG	U	5.0	5.1	35.5	5.0	3.0	5.0	3.5
53	Tango	68.3	19.7	G	U	5.3	4.9	46.3	1.0	1.0	5.0	1.0
54	Tennis Ball	24.7	18.3	LG	U	5.2	4.9	27.1	3.0	2.7	5.0	2.0
55	Vivian	28.7	28.0	DG	U	6.9	4.6	47.9	5.0	3.7	5.0	5.0
56	Winter Density	19.7	17.0	DG	U	6.6	6.1	35.4	4.7	4.0	5.0	5.0
57	11C3487	14.7	25.0	LG	LM	6.1	8.0	35.3	5.0	4.2	5.0	5.0
58	3SX820	10.0	24.7	LG	LM	6.1	5.8	35.3	5.0	5.0	5.0	5.0
59	3SX821	13.0	22.7	LG	LM	6.1	4.2	43.1	5.0	5.0	5.0	5.0
60	3SX822	13.7	24.7	LG	LM	6.2	6.9	32.0	5.0	4.0	5.0	5.0

[z]Plant color: DG-dark green, LMG-lime green , LG-light green, G-green, G-R-green-red, DR-dark red, G-green, R-G-red-green.
[y]Plant shape: LM-low mounded, U-upright, P-pyramidal, LV-low vase shape, M-mounded, V-vase, H-head.

Fig. 2. Lettuce cultivars grown with NFT system.

Fig. 2. Lettuce cultivars grown with NFT system (continued).

Fig. 2. Lettuce cultivars grown with NFT system (continued).

Fig. 2. Lettuce cultivars grown with NFT system (continued).

b. Experiment 2

In the second experiment of cultivar evaluation, eight cultivars of lettuce were evaluated for their performance under hydroponic culture: Cosmoplia RZ, Flandria RZ, Freckles Romaine, Parris Island, Rex RZ, Vivian, 11C3487, and 3SX820 (Table 5). Plants were cultured on the NFT system as the first experiment.

Seeds of eight cultivars of lettuce which selected from the last experiment were planted on Rockwool cubes and seedlings were transplanted onto the channels of hydroponic NFT system in two weeks. During the experiment the average daytime light intensity was 249.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ or 19,860 lux. The greenhouse ambient air temperature ranged from 23.8 and 22.3 °C. Plants were grown with 200 ppm N nutrient solution prepared with a 20-20-20 commercial analysis fertilizer. Each unit contained four 6-foot long troughs which were slightly slanted to allow nutrient solution to flow into the sump bucket (5-gallon size). The nutrient solution was continuously applied to the plants (3 plants per cultivar) and collected into the bucket for recirculation. To compensate the loss of nutrient solution by plant absorption and transpiration, each sump bucket was replenished with new solutions as needed. At the time of harvesting (Fig. 3), plant growth parameters (size and shape), chlorophyll content (using a SPAD meter), and leaf color were recorded. Leaf tissue sugar content was determined by a digital refractometer (Brix meter). The tissue sap pH and nitrate concentration were also determined using a portable pH meter and nitrate meter (Horiba meter). The experiment was conducted from May 2 to June 2, 2016. The results of these measurements are presented in Table 2.

Table 5. List of 8 selected lettuce cultivars and their seed suppliers used in the experiment.

Cultivar	**Seed source**
1. Cosmoplia RZ	Johnny's Selected
2. Flandria RZ	Rijk Zwaan
3. Freckles Romaine	High Mowing
4. Parris Island	Livingstone
5. Rex Rz	Rijk Zwaan
6. Vivian	Burpee
7. 11C3487	3 Star Seed Co.
8. 3SX820	3 Star Seed Co.

Fig. 3. Plants of 8 lettuce cultivars grown with NFT system. Picture was taken at the time of harvesting.

Results

When evaluated after 4 weeks of culture, plants of four different cultivars showed bolting problems. Compare with Cos cultivars (Cosmopolia RZ, Rex RZ, 11C3487 and 3SX820), Romaine cultivars (Frenkles Romaine, Parris Island, and Vivian) readily showed bolting problems. As a result, we concluded that Cos cultivars are more suitable for cultivation in the hydroponic system. Among the Romaine cultivars, Parris Island showed the worst bolting rate, it means that Parris Island is not appropriate for the NFT system. Rex RZ and Vivian had low growth index than other cultivars had to be replaced during the experiment due to a mechanical problem with the NFT system.

The leaf edge burns with a different degree of symptom expression had shown in Table 3. The appearance of leaf margin burns perhaps was due to the bouts of temperature surge and dry air caused by the high intensity of sunlight and accumulated heat from the summer temperature. Even the outdoor temperature and sunlight were higher than winter seasons, after the installation of shading clothe (50% shading) over the plants, the temperature and light intensity were maintained more or less stable. In general, flower stalk formation was less of problem and the incident of leaf margin burns was minimal. The highest ranked 3 cultivars in overall rating and bolting resistance were Cosmopolia RZ, Flandria RZ, and Freckles Romaine. Those cultivars had shown high plant fresh biomass (Table 6).

In this experiment, we found that the best cultivars used for the NFT system were Cosmopolia RZ, Rex RZ, 11C3487 and 3SX820. Cultivars Cosmopolia RZ, Rex RZ, Flandria RZ, Freckles Romaine, and Vivian did not show the problem of tip burn (Table 7). Plants grown for this experiment developed far less problem of physiological disorders. This may have been due to less light intensity and lower ambient temperatures with under the shade cloth cover. Better nutrient formations may also be needed for the prevention of leaf tip burns.

Table 6. Some of the quality parameters of lettuce cultivars grown hydroponically measured 4 weeks after seedling transplanting.

	Cultivar	Plant fresh weight (g)	Plant dry weight (g)	pH	Nitrate (mg/l)[z]	% Sugar[y] (Brix)	Chloro-phyll[x] (SPAD)	Leaf margin burns[w]
1	Cosmopolia RZ	181.0	7.0	6.4	15,000	3.5	53.2	5.0
2	Flandria RZ	142.3	5.5	6.7	16,000	2.5	32.5	5.0
3	Freckles Romaine	203.0	23.2	6.3	9,000	2.6	76.5	5.0
4	Parris Island	186.3	10.6	6.6	8,000	3.4	41.8	3.0
5	Rex RZ	44.3	3.0	6.4	17,000	3.4	39.8	5.0
6	Vivian	41.0	2.3	6.9	9,000	5.2	50.5	5.0
7	11C3487	163.3	7.1	6.5	23,000	2.5	35.6	4.8
8	3SX820	170.3	8.1	6.4	22,000	4.4	30.6	4.0

[z]Measured by a Horiba nitrate meter.
[y]Percent sugar was measured by a refractometer.
[x]Measured by a SPAD meter.
[w]Leaf edge burn was scored from 1 to 5, with 1 showing the highest leaf edge burn and 5 showing the lowest leaf edge burn.

Table 7. Plant quality parameters of the eight lettuce cultivars grown for 4 weeks with the nutrient recirculation system (NFT).

	Cultivar	Height (cm)	Width (cm)	Plant color[z]	Plant shape[y]	pH	Brix (%)	Chloro-phyll (SPAD)	Bolt (1-5)	Leaf margin burns (1-5)	Leaf mold (1-5)	Overall rating (1-5)
1	Cosmopolia RZ	17.0	26.7	G	LM	6.4	3.5	53.2	4.0	5.0	5.0	5.0
2	Flandria RZ	15.3	23.7	G	LM	6.7	2.5	32.5	5.0	5.0	5.0	5.0
3	Freckles Romaine	21.0	25.0	DG	U	6.3	2.6	76.5	5.0	5.0	5.0	5.0
4	Parris Island	29.0	22.7	DG	U	6.6	3.4	41.8	2.0	3.0	5.0	2.5
5	Rex Rz	8.7	18.7	G	LM	6.4	3.4	39.8	3.0	5.0	5.0	4.0
6	Vivian	18.7	15.0	DG	U	6.9	5.2	50.5	5.0	5.0	5.0	5.0
7	11C3487	10.0	20.3	G	LM	6.5	2.5	35.6	4.5	4.8	5.0	5.0
8	3SX820	12.7	22.7	G	LM	6.4	4.4	30.6	4.0	4.0	5.0	4.5

[z]Plant color: DG-dark green and G-green.
[y]Plant shape: LM-low mounded and U-upright.

Fig. 4. Plants of eight different lettuce cultivars harvested after 4 weeks of culture on NFT system. A-harvested lettuces of six different cultivars harvested, B-comparison of lettuce plants harvested except Rex Rz and Vivian that had trouble on the NFT system.

2. Strategies for reducing tissue nitrate concentrations

a. Experiment 1

High concentration of nitrate in vegetables can be a serious threat to human health. Usually nitrate in the vegetable is relatively non-toxic, but approximately 5% of all ingested nitrate is converted in saliva and the gastrointestinal tract to nitrite, a more toxic form of nitrogen. The only chronic toxic effects of nitrate are those resulting from the nitrite formed by its reduction by bacterial enzymes. Nitrite and *N*-nitroso compounds, which form when nitrite binds to other substances before or after ingestion (for example, the amines derived from proteins), are toxic and can lead to severe health problems in humans. Thus, the assessment of the health risk of nitrate to humans should encompass the toxicity of both nitrite and *N*-nitroso compounds (Santamaria P., 2006).

Santamaria P. 2006. Nitrate in vegetables: toxicity, content, intake and EC regulation. J. Sci. of Food Agric. 86:10-17.

An experiment was conducted to determine if changes in the ratio of the two different forms (NH_4^+, NO_3^-) of nitrogen can influence tissue nitrate content. Rockwool-grown seedlings of leaf lettuce cv. Rex Rz were anchored and grown on Styrofoam boards inside tubs containing 5 different N solutions: 1) 100% NH_4^+, 2) 75% NH_4^++25% NO_3^-, 3) 50% NH_4^++50% NO_3^-, 4) 25% NH_4^++75% NO_3^-, 5)100% NO_3^- at total 12 of meq/liter N. All fertilizer solutions contained modified levels of other macronutrients from a Hoagland solution for reducing nitrate concentration in the lettuce. When lettuce plants were harvested after 4 weeks of culture, tissue nitrate levels were higher when the nutrient solution contained increasing percentage of NO_3^- in relation to NH_4^+ in general. The growth of the plants was the greatest when it was grown with 1:1 mixture of NH_4^+ and NO_3^- (solution 3, Figs. 6 and 7). Tissue nitrate content in this treatment was about 4,000 ppm higher compared to solution 2 (Figs. 8 and 9).

Fig. 5. The lettuce grown in the five different NH_4^+:NO_3^- ratio nutrient solution in hydroponic tub cultures for 4 weeks: A-at the time of anchoring seedlings on tub culture, B-four weeks later, C-close up of tub cultures, D-at the time of harvesting plants.

Difference in biomass yield between the solution 3 and solution 2 was statistically not significant. As tissue NO_3^- levels increased, plant sap pH values increased and the leaf chlorophyll index became lower. It appears that a hydroponic solution containing 75% NH_4^++25% NO_3^- can effectively be used for growing lettuce to lower leaf nitrate levels without sacrificing yield and produce quality.

Fig. 6. Rex RZ lettuce grown with various combinations of ammonium (NH_4^+) and nitrate (NO_3^-) in the nutrient solution.

Fig. 7. Rex RZ lettuce grown with various combinations of ammonium (NH_4^+) and nitrate (NO_3^-) in the nutrient solution (picture taken after removal of root system).

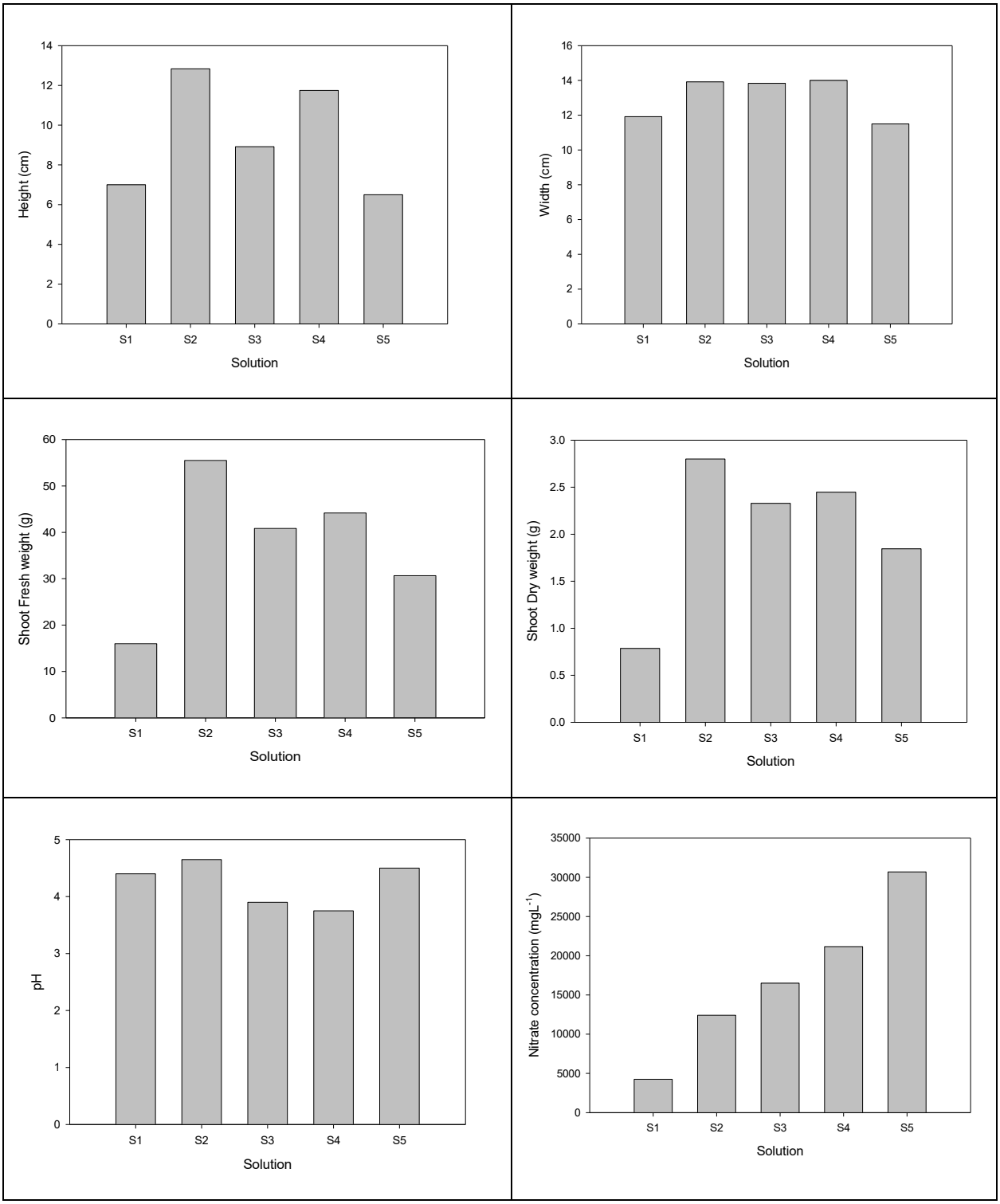


Fig. 8. Influence of five different nutrient solution in the "Rex RZ" lettuce growth and nitrate concentration in the plant tissue (S1, NH_4:NO_3 100:0; S2, NH_4:NO_3 75:25; S3, NH_4:NO_3 50:50; S4, NH_4:NO_3 25:75; S5, NH_4:NO_3 0:100).

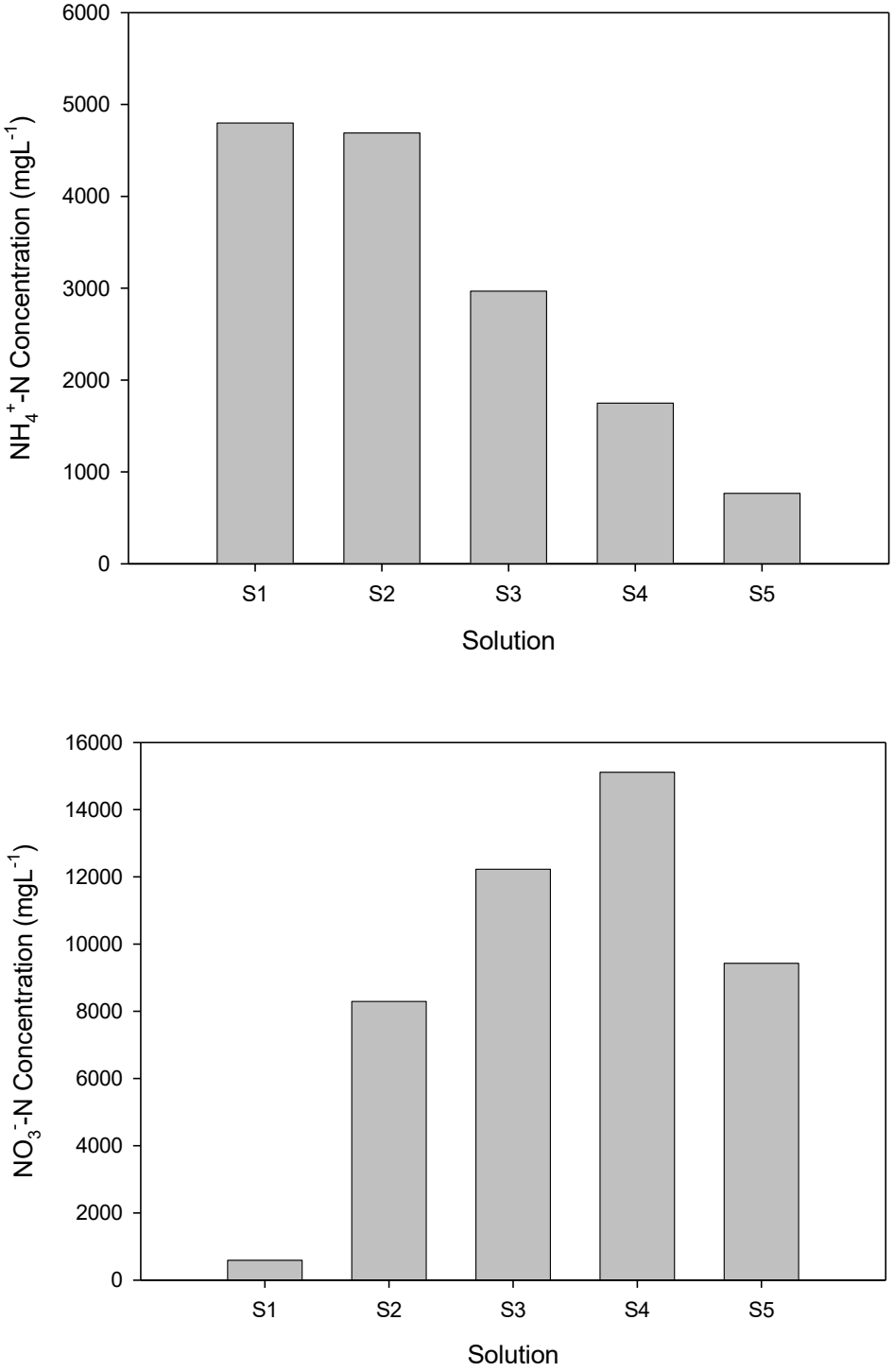


Fig. 9. Concentration of NH_4^+-N and NO_3^--N in the plant tissue (S1=NH_4^+:NO_3^- 100:0, S2=NH_4^+:NO_3^- 75:25, S3=NH_4^+:NO_3^- 50:50, S4=NH_4:NO_3 25:75; S5, NH_4^+:NO_3^- 0:100).

b. Experiment 2

This experiment was conducted to determine if changes in the ratio of the two different forms (NH_4^+, NO_3^-) of nitrogen in the nutrient solution can influence tissue nitrate content.

Rockwool-grown seedlings of lettuce (Rex RZ, Rijk Zwaan) and basil (supplied by Gertens Garden) were anchored on Styrofoam board floated on nutrient solution inside a tub (Fig. 10). Plants were grown in the Exolite-covered greenhouse with light intensity varying from 320 to 900 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (30,000 - 50,000 lux) depending on the weather conditions. The temperature of the greenhouse was set at 70 ºF (day) and 60 ºF (night). The leaf temperature measured by an infrared thermometer varied from 26 °C to 28 °C.

Five different nutrient solutions used for treatments are as follows:

Solution 1: 100% NH_4^++0% NO_3^-
Solution 2: 75% NH_4^++25% NO_3^-
Solution 3: 50% NH_4^++50% NO_3^-
Solution 4: 25% NH_4^++75% NO_3^-
Solution 5: 0% NH_4^++100% NO_3^-

All solutions had a total of 12 of meq/liter N and the standard concentrations of other macronutrients as a modification of the Hoagland solution (Table 18). The experiment had conducted from May 2 to June 2, 2016 (Fig. 5). At the time of harvesting, plant growth parameters (size and shape), chlorophyll content (using a SPAD meter), and leaf color were recorded. Leaf tissue sugar content was determined by a digital refractometer or Brix meter. The tissue sap pH and nitrate concentration were also determined using a portable pH and nitrate meter (Horiba meter).

Results

Compared to last nitrate experiments, nitrate concentration in the plant tissue had a similar tendency in plant growth rate which decreased with higher nitrate concentrations in the fertilizer solution (Tables 8 and 9). The growth of basil had a similar result as that of Rex RZ (Table 9). Chlorophyll index (SPAD), pH, and sugar content (Brix reading) in the plant tissue had no significant differences as nitrate (NO_3^-) to ammonium (NH_4^+) ratios in the nutrient solution changed (Tables 8 and 9).

During the experiment, the greenhouse where the experiment was conducted had increased temperature and light intensity which caused more physiological disorders in the Rex RZ lettuce and Basil. As a result, algae growth in the nutrient solution was more noticeable. In this experiment, plant root and shoot growth rates were comparatively lower than those of previous experiments (Figs. 13 and 14). However, the nitrate concentration in the tissue and growth rate of the plants had a similar tendency as a result of increasing nitrate (NO_3^-) to ammonium (NH_4^+) ratio in the nutrient solution (Figs 11 and 12).

Fig. 10. The lettuce grown in the five different NH_4^+: NO_3^- ratio nutrient solutions in hydroponic tub cultures for 5 weeks at the time of harvest: Seedlings anchored on Styrofoam boards.

Table 8. Plant growth parameters of Rex RZ grown five different nutrient solutions in the hydroponic tub for 4 weeks.

Treatment	Height (cm)	Width (cm)	pH	Brix (%)	Chlorophyll (SPAD)	Shoot fresh weight (g)	Shoot dry weight (g)
NH_4^+ 100: NO_3^- 0	9	15	6.7	5.3	24.2	15.3	0.9
NH_4^+ 75: NO_3^- 25	8.3	13.0	6.3	3.9	26.1	9.7	0.7
NH_4^+ 50: NO_3^- 50	8.7	10.3	6.1	4.6	27.8	10.7	0.7
NH_4^+ 25: NO_3^- 75	4.7	8.0	6.0	2.6	28.9	7.0	0.6
NH_4^+ 0: NO_3^- 100	4.3	10.3	6.3	4.2	32.6	8.7	0.5

Table 9. Plant growth parameters of basil grown five different nutrient solutions in the hydroponic tub for 5 weeks.

Treatment	Height (cm)	Width (cm)	pH	Brix (%)	Chlorophyll (SPAD)	Shoot fresh weight (g)	Shoot dry weight (g)
NH_4^+ 100: NO_3^- 0	4.3	10.3	6.3	4.2	32.6	8.7	0.8
NH_4^+ 75: NO_3^- 25	7.8	8.0	6.1	4.6	31.1	4.3	0.4
NH_4^+ 50: NO_3^- 50	10.0	9.8	6.0	3.0	24.7	7.0	0.6
NH_4^+ 25: NO_3^- 75	7.7	7.3	6.2	3.1	16.8	3.0	0.3
NH_4^+ 0: NO_3^- 100	5.7	6.0	5.9	3.9	23.2	3.0	0.3

Fig. 11. Rex Rz lettuce plants grown hydroponically in solutions containing: 1) 100% NH_4^+, 2) 75% NH_4^++25% NO_3^-, 3) 50% NH_4^++50% NO_3^-, 4) 25% NH_4^++75% NO_3^-, 5)100% NO_3^- at total 12 of meq/liter N.

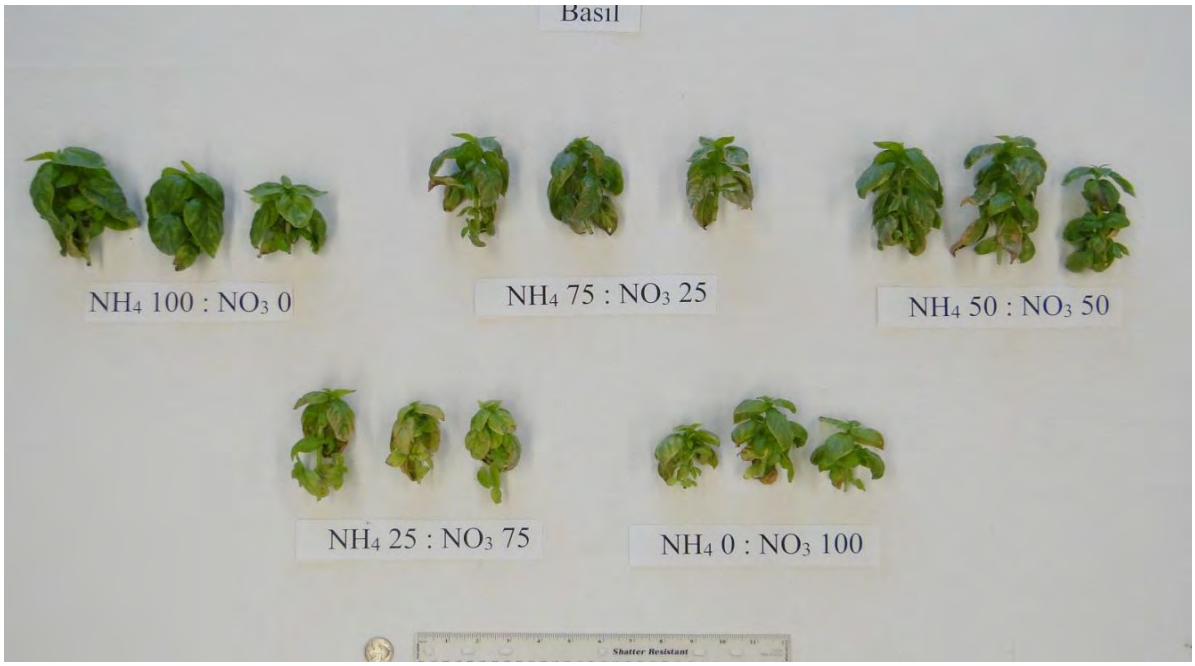


Fig. 12. Basil plants grown hydroponically in solutions containing five different combinations of NH_4^+ and NO_3^- forms of nitrogen for 4 weeks.

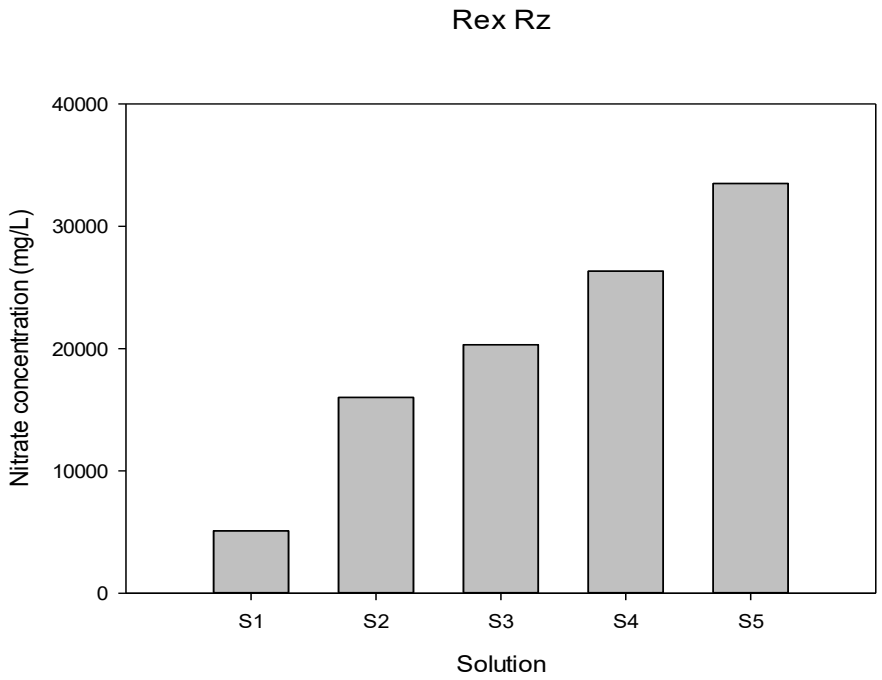


Fig. 13. Plant tissue nitrate concentrations in the Rex RZ as influenced by five different combinations of NH_4^+ and NO_3^- forms of nitrogen: S1 (100% NH_4^+), S2 (75% NH_4^+, 25% NO_3^-), S3 (50% NH_4^+, 50% NO_3^-), S4 (25% NH_4^+, 75% NO_3^-), S5 (100% NO_3^-).

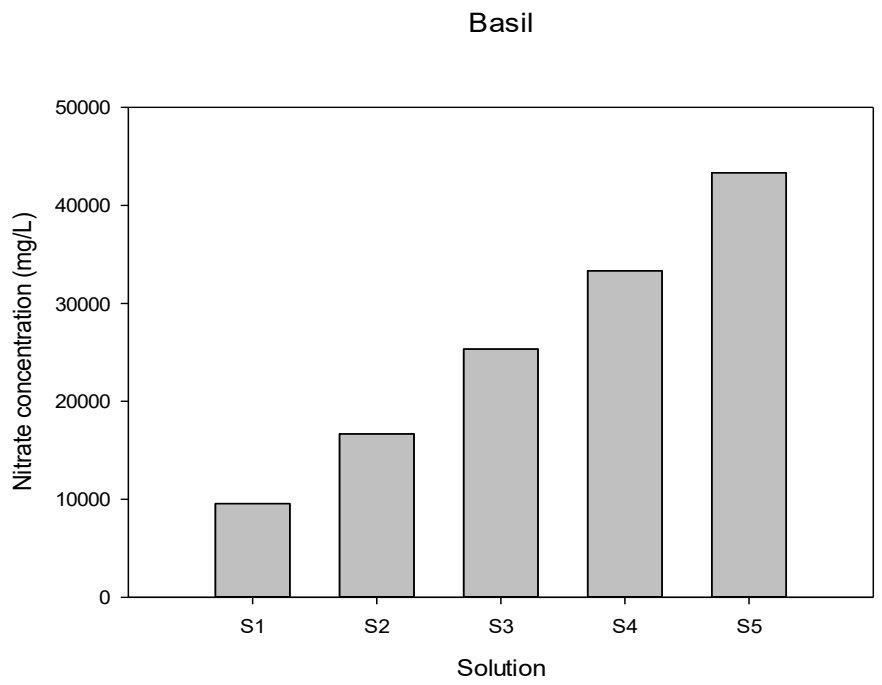


Fig. 14. Plant tissue nitrate concentrations in the Basil as influenced by five different combinations of NH_4^+ and NO_3^- forms of nitrogen: S1 (100% NH_4^+), S2 (75% NH_4^+, 25% NO_3^-), S3 (50% NH_4^+, 50% NO_3^-), S4 (25% NH_4^+, 75% NO_3^-), S5 (100% NO_3^-).

3. Influence of artificial lighting on the growth of lettuce

a. Light Experiment 1

The influence of artificial and supplemental lighting on the growth of leaf lettuce was carried out in the the greenhouse. In this experiment, lettuce (cultivar Parris Island) were grown in hydroponic tubs inside 1m x 1m x 1.5 m chambers (Figs. 15 and 16) with three different light sources: light emitting diode (LED, combination of blue and red spectra), light emitting plasma (LEP) lamp, and high pressure sodium (HPS) lamps. Plants were also grown under natural light outside the growth chamber with and without supplemental lights of LEP and HPS on the greenhouse bench. The average light intensity measured in lux and photon flux density ($\mu mol \cdot m^{-2} \cdot s^{-1}$), ambient temperature, and leaf temperatures measured during the experimental period are shown in Table 10. The lettuce cultivars were transplanted in pot filled with commercial root media at February 29, 2016. Among the different treatment, fertilizer solution nitrogen (N) concentration was 100 mg/liter during the experiment by using a 20-20-20 commercial analysis fertilizer.

Fig. 15. Light cages used for artificial lights LEp, LED, and HPS lamps for growing lettuce hydroponically.

Table 10. Average light intensity in lux, photon flux density, and air temperature of the experimental chambers with and without artificial lights.

	Light intensity (lux)	Photon flux density ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Air temperature (°C)	Leaf temperature (°C)
LED (light emitting diode)	5,040	89	31.4	32.1
Natural light	8,460	142	20.7	23
Supplementary light by LEP	2,000	352.3	20.1	29
Supplementary light by HPS	33,200	400	22.4	28.5
LEP (light emitting plasma)	25,700	389	33.7	35.5
HPS (high pressure sodium)	42,900	505	36	40.3

Fig. 16. Lettuce grown in hydroponic tubs and root substrate (BM mix) inside artificial lights with: A-light emitting plasma (LEP), B-LED light, C-high pressure sodium (HPS) lamps; D- plants grown under natural light in the greenhouse.

Table 11. Influence of various artificial lights on growth of Parris Island transplanted in BM root media.

Treatment	Height (cm)	Width (cm)	pH	Nitrate (mg/L)	Brix (%)	Chlorophyll (SPAD)	Shoot Fresh weight (g)	Shoot dry weight (g)
Natural light	20.2	8.3	5.6	6,567	3.3	32.8	26.3	2.8
Supplemental light HPS	13.7	24.7	5.2	8,400	4.2	53.8	114.0	17.1
Supplemental light LEP	15.7	23.7	5.5	8,033	2.0	37.8	140.0	18.0
LEP	22.0	18.5	6.4	4,900	2.3	37.0	107.0	14.5
LED	23.8	7.2	7.1	5,300	1.5	33.8	51.7	2.1
HPS	14.5	21.0	7.0	3,700	2.9	47.8	187.0	30.2

Fig. 17. Parris Island lettuce grown under artificial lights (LEP, LED, HPS) in comparison to that grown under natural sunlight in the greenhouse.

Fig. 18. Parris Island lettuce grown under artificial lights (LEP, LED, HPS) in comparison to that grown under natural sunlight in the greenhouse.

Fig. 19. Parris Island lettuce grown under artificial lights (LEP, LED, HPS) in comparison to that grown under natural sunlight in the greenhouse.

b. Experiment 2

Light sources used for this experiment were:

High pressure sodium lamp (HPS)
Light emitting diode lamp (LED, blue and red spectra combined)
Light emitting plasma lamp (LEP)

Seedlings of Rex RZ lettuce and basil were grown in either root substrate (BM mix) or hydroponically under the three different light sources (Fig. 20). Artificial lighting was done inside three different chambers constructed with cotton fabrics that transmitted less than 5% of natural light. Chamber dimensions were 1m x 1m x 1.5 m (Fig. 20). Plants were grown hydroponically as well as in pots (Figs. 21 and 22). The distance from the lamp to plant level was 0.8 m, 0.6 m, and 0.9 meter for LEP, LED, HPS lights, respectively. Plants were grown for 4 weeks using a nutrient solution containing 200 ppm N prepared with a commercial 20-20-20 fertilizer. Plants were also grown outside the lighting chamber under natural light conditions for growth comparisons.

The high-pressure sodium lamps (HPS) provided highest light intensity as well as highest temperature (Table 12). Light intensity under HPS and LEP was similar at the distance specified. LED lights provided the lowest light intensity (89 $\mu mol \cdot m^{-2} \cdot s^{-1}$) as compared to LEP (389 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and HPS (505 $\mu mol \cdot m^{-2} \cdot s^{-1}$).

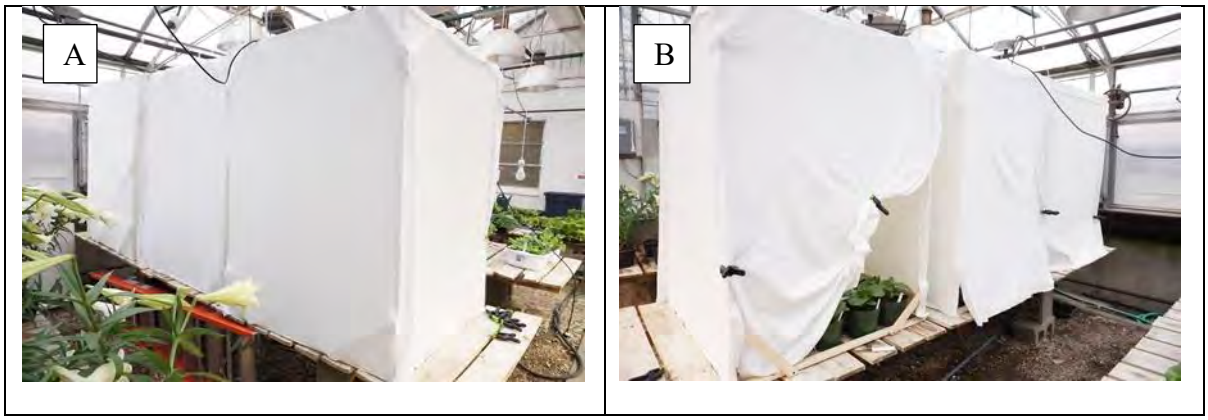


Fig. 20. Light cages used for artificial lights LEP, LED, and HPS lamps for growing lettuce and basil.

Table 12. Environmental conditions used for artificial lighting experiments for growing lettuce and basil.

Light Source	Lux	ppfd[z] ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Air temperature (°C)	Leaf temperature (°C)
LED (light emitting diode)	8,270	129.7	24.6	21.8
Natural light	19,860	249.5	23.8	22.3
LEP (light emitting plasma)	22,200	316.1	28.0	25.0
HPS (high pressure sodium)	29,000	519.0	26.7	28.8

[z]Light measurement by photosynthetic photon flux density (*ppfd*).

Results

While the fresh weight yield of lettuce was highest under HPS lamps (Tables 13 and 14), all plants had leaf margin burns when grown under HPS lamps, perhaps due to the combination of high temperature and high light intensity. Since lettuce require not more than 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the distance between the lamp and plants must be increased. Biomass yield under LED was the lowest (Table 14).

The shape, color, and general appearance of lettuce grown under LEP were much more desirable than that grown under either LED or HPS lamps (Figs. 21, 22, 23, 25). Tissue nitrate concentration was highest when both lettuce and basil plants were grown under LEP (Tables 13) in substrate culture.

When plants were grown hydroponically in tubs, both Rex RZ lettuce and basil had the highest nitrate levels under high pressure sodium lamps (HPS) (Table 16).

Close-up pictures of Rex RZ lettuce and basil grown in substrate culture under natural light, LEP, LED, and HPS lamps are presented in Fig. 23. Rex RZ lettuce and basil grown hydroponically in tubs under natural light, LEP, LED and HPS are shown in Fig. 24.

In both Rex RZ lettuce and basil, leaf tissue nitrate contents were higher when plants were grown hydroponically compared to substrate culture (Tables 13, 14, 15, 16). The nitrate concentrations of the leaf extract ranged from 9,000ppm to 38,000 ppm when measured by a portable Horiba nitrate meter. The accuracy of the Horiba nitrate meter is questionable.

Fig. 21. Lettuce and basil plants grown under natural light (A), LEP (B), LED (C) and HPS (D).

Fig. 22. Lettuce and basil plants grown hydroponically under natural light (A), LEP (B), LED (C) and HPS (D) for 4 weeks.

Table 13. Influence of various artificial lights on growth of Rex RZ transplanted in BM root media.

Treatment	Height (cm)	Width (cm)	pH	Nitrate (mg/L)	Brix (%)	Chlor. (SPAD)	Shoot Fresh weight (g)	Shoot dry weight (g)
Natural light	14	27	6.4	10000	2.6	28.4	103.7	4.9
LEP	14.3	33.7	6.3	18667	2.5	29.1	123.7	6.5
LED	12.0	34.7	6.6	10333	1.8	22.5	105.0	4.2
HPS	14.0	35.0	6.3	10700	4.2	26.4	175.7	12.0

Table 14. Influence of various artificial lights on growth of Rex RZ lettuce hydroponic culture.

Treatment	Height (cm)	Width (cm)	pH	Nitrate (mg/L)	Brix (%)	Chlor. (SPAD)	Shoot Fresh weight (g)	Shoot dry weight (g)
Natural light	15.7	23.3	6.5	20333	0.8	34.3	106.7	5.1
LEP	15.7	30.7	6.5	32333	2.9	26.6	88.3	5.0
LED	15.7	32.7	6.7	14333	1.8	26.6	75.7	2.8
HPS	13.3	29.7	6.2	42333	3.2	28.2	105.7	6.5

Table 15. Influence of various artificial lights on growth of basil transplanted in BM root media.

Treatment	Height (cm)	Width (cm)	pH	Nitrate (mg/L)	Brix (%)	Chlor. (SPAD)	Shoot Fresh weight (g)	Shoot dry weight (g)
Natural light	10.0	12.3	5.9	11000	2.8	35.0	5.0	0.5
LEP	13.7	14.7	6.2	22000	3.3	30.4	7.7	0.6
LED	7.7	13.7	6.4	9367	3.7	29.8	3.3	0.3
HPS	9.7	14.0	6.2	11667	2.5	32.4	6.3	0.6

Table 16. Influence of various artificial lights on growth of basil in hydroponic tub culture.

Treatment	Height (cm)	Width (cm)	pH	Nitrate (mg/L)	Brix (%)	Chlor. (SPAD)	Shoot Fresh weight (g)	Shoot dry weight (g)
Natural light	16.0	9.0	6.1	16000	2.4	33.5	15.3	1.1
LEP	10.0	8.0	6.3	28000	4.8	32.2	7.0	0.6
LED	9.3	8.0	6.2	11000	2.0	34.5	5.3	0.4
HPS	10	9.3	6.0	38500	4.6	36.8	9.0	0.8

Fig. 23. Rex RZ lettuce grown with root substrate (BM Mix) under artificial lights (LEP, LED, HPS) for four weeks.

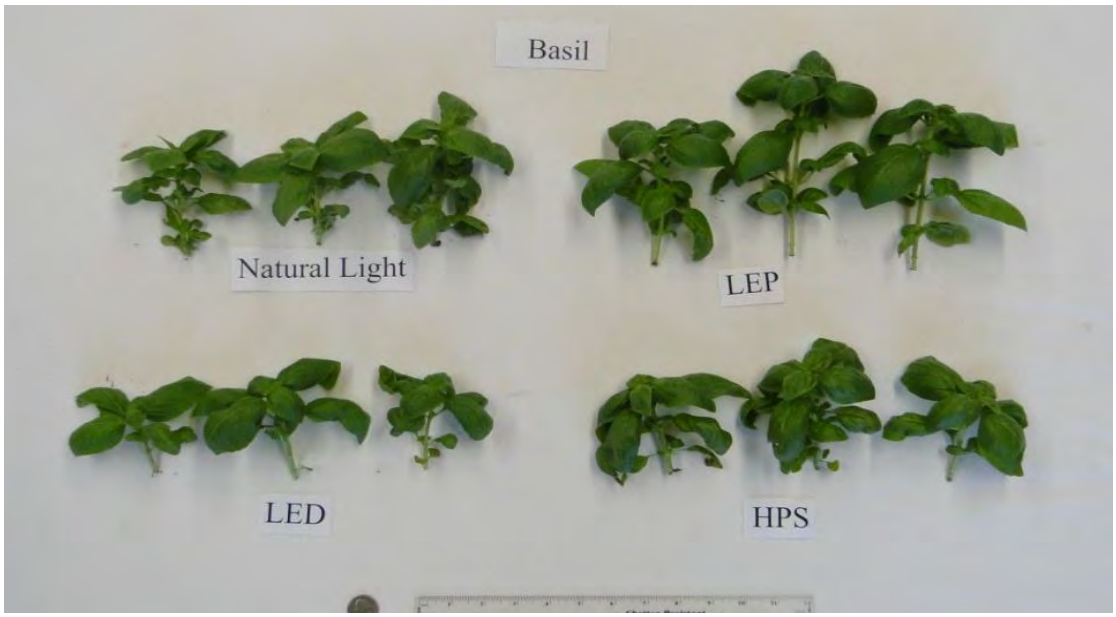


Fig. 24. Basil grown with substrate culture under artificial lights (LEP, LED, HPS) for four weeks.

Fig. 25. Rex RZ lettuce grown on hydroponic tubs under natural light, LEP, LED, and HPS for four weeks.

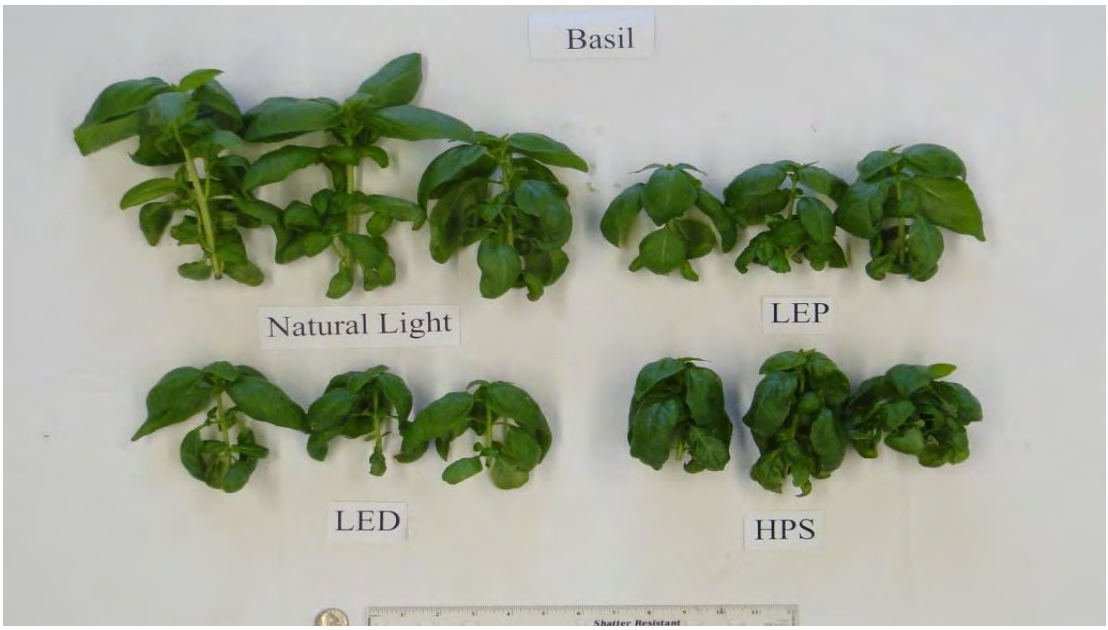
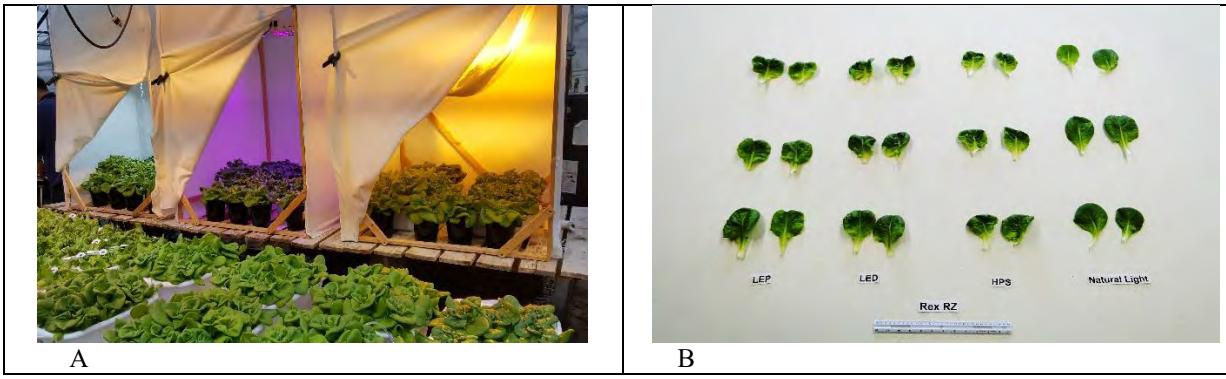


Fig. 26. Basil grown on hydroponic tubs under natural light, LEP, LED, and HPS for four weeks.

c. *Light Experiment 3*



A B

Fig. 27. Growth of lettuce under three different artificial lights LEP (light emitting plasma), LED (light emitting diode), HPS (high pressure sodium): A-three compartments used for lighting experiment, B-lettuce grown with different artificial light sources only or as supplemental lights.

Table 17. Equations and glossary of terms used by the JIP-test for the analysis of Chl a fluorescence transient OJIP emitted by dark-adapted photosynthetic samples.

Parameter	Definition
Extracted and technical fluorescence parameters	
F_t	Fluorescence at time t after onset of actinic illumination
F_0	Minimal fluorescence intensity at 50 us
F_{150}	
F_{300}	
F_J	Fluorescence intensity at the J-step (at 2ms)
F_M	Maximal fluorescence intensity all RCs closed
T_{max}	Time to reach F_M in ms
$F_v = (F_t - F_0)$	Variable fluorescence at time t
$F_v = (F_M - F_0)$	Maximal variable fluorescence
$V_t = (F_t - F_0) / (F_M - F_0)$	Relative variable fluorescence at time t
V_J	Relative variable fluorescence at 2 ms
Area	Total complementary area between fluores. curve and F_M
$(dV/dt)_0$ or $M_0 = 4\ (F_{300\ \mu s} - F_{50\ \mu s})/(F_M - F_{50\ \mu s})$	Approx. initial slop (in ms^{-1}) of the fluorescence transient normalized on F_v
$S_m = \text{Area} / (F_M - F_0)$	Energy needed to close all RCs (multiple TO)
S_s	smallest S_m, Q_A reduced only once (w/ DCMU, single TO)
$S_m / t_{Fmax} = [Q_A / Q_{A\ total}]_{av} = [1 - (Q_A^- / Q_{A\ total})]_{av}$	Average redox state of QA in the time span from 0 to t.
$= 1 - B_{av}$	Average fraction of open RCs during the time needed to complete to closure
$N = S_m / S_s = S_m \cdot [(dV/dt)_0] / V_J = S_m \cdot M_0 \cdot (1 - V_j)$	How many times Q_A reduced from 0 to t_{Fmax}
Specific energy fluxes at time zero	

$ABS/RC = M_0 (1/V_J) / \varphi_{P0}$	Absorption flux (of antenna Chls) per RC
$TR_0/RC = M_0 \cdot (1/V_J)$	Trapping flux (leading to Q_A reduction) per RC
$ET_0/RC = M_0 (1/V_J) \cdot \psi_{E0}$	Electron transport flux (further than Q_A^-) per RC
$RE_0/RC = M_0 (1/V_J) \cdot \psi_{E0} \cdot \delta_{R0}$	Electron flux reduding end electron acceptors at the PSI acceptor side per PC
Quantum yields and efficiencies	
$\varphi_{pt} \equiv TP_t / ABS = [1-(F_t/F_M] = \Delta F_t / F_M$	Quantum yield for primary photochemistry at any time t
$\varphi_{po} \equiv TP_0 / ABS = [1-(F_0 / F_M)]$	Maximum quantum yield for primary photochemistry
$\psi_{E0} \equiv ET_0 / TP_0 = (1 - V_J)$	Efficiency/probability for electron transport (ET), i.e. efficiency/probability that an electron moves further than Q_A^-
$\varphi_{E0} \equiv ET_0 / ABS = [1-F_0 / F_M)] \cdot \psi_{E0}$	Quantum yield for electron transport
$\delta_{R0} \equiv RE_0 / ET_0 = (1-V_I) / (1-V_J)$	Efficiency/probability with which an electron from the intersystem electron carriers moves to reduce end electron acceptors at the PSI acceptor side (RE)
$\varphi_{R0} \equiv RE_0 / ABS = [1-F_0 / F_M)] \cdot \psi_{E0} \cdot \delta_{R0}$	Quantum yield for reduction of end electron acceptors at the PSI acceptor side (RE)
$\gamma_{RC} \equiv Chl_{RC} / Chl_{total} = RC / (ABS + RC)$	Probability that a PSII Chl molecule function as RC
$RC / ABS = \gamma_{RC} / (1- \gamma_{RC}) = \varphi_{po} (V_J / M_0)$	Q_A^- reducing RCs per PSII antenna Chl (reciprocal of ABS/RC)
Performance indexes (products of terms expressing partial potentials at steps of energy bifurcations	
$PI_{ABS} = [\gamma_{RC} / (1- \gamma_{RC})] \cdot [\varphi_{P0} / (1- \varphi_{P0})] \cdot [\psi_0 / (1- \psi_{E0})]$	Performance index (potential) for energy conservation from exciton to the reduction of intersystem electron acceptors
$PI_{total} = PI_{ABS} \cdot [\delta_{R0} / (1- \delta_{R0})]$	Performance index (potential) for energy conservation from exciton to the reduction of PSI end acceptors

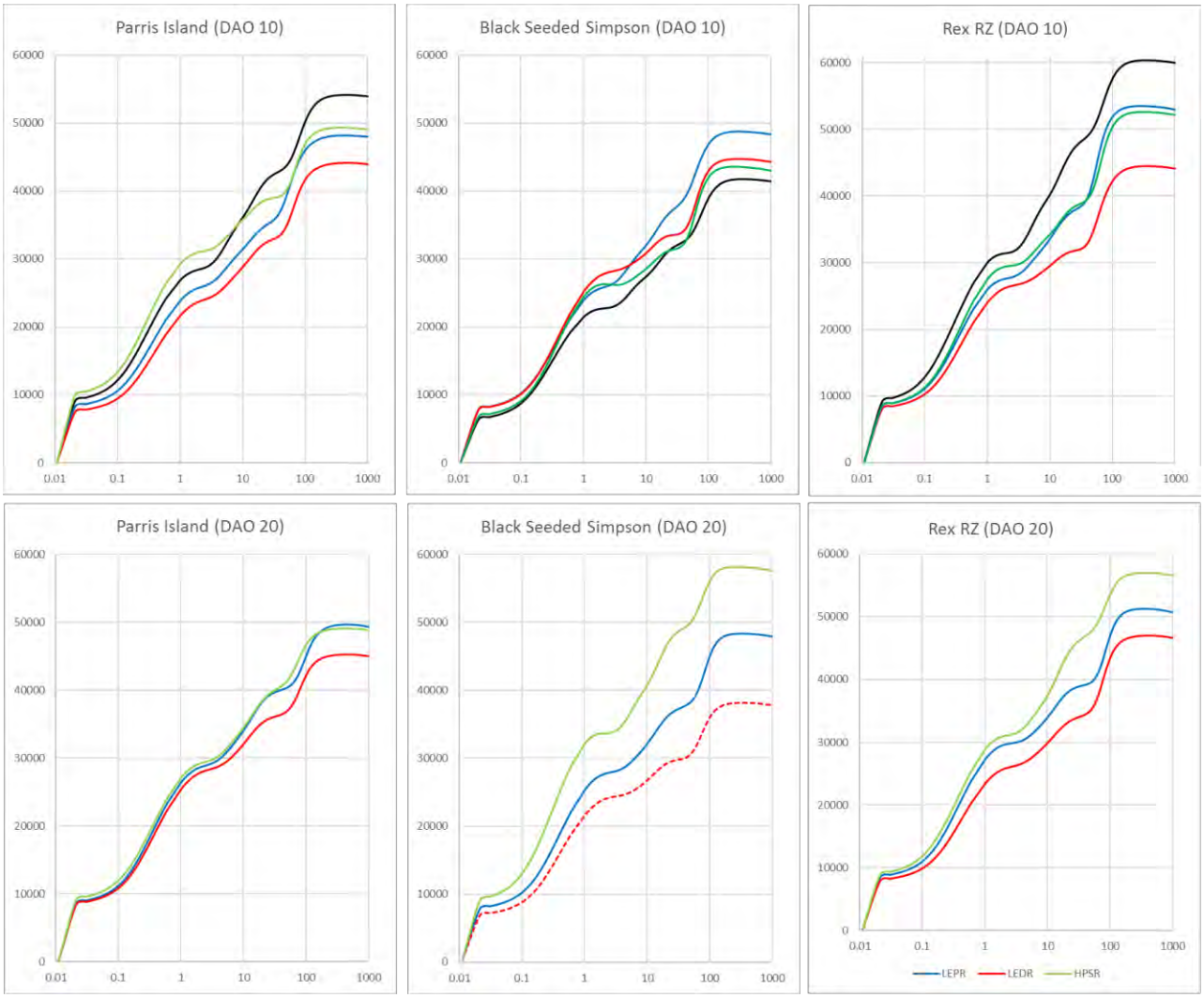
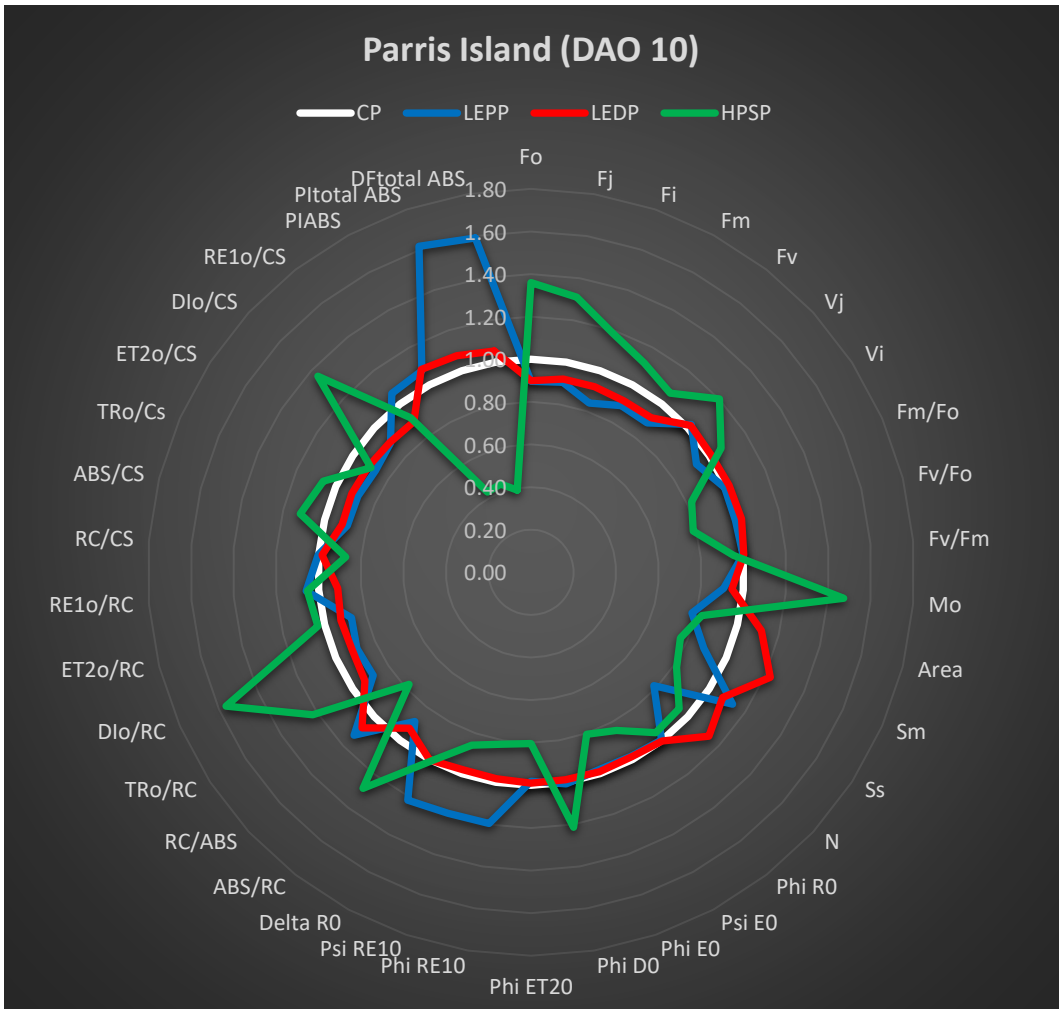


Fig. 28. The OJIP chlorophyll a fluorescence transient curve (log time scale) in lettuce leaves of cvs. Parris Island, Black Seeded Simpson, and Rex RZ hydroponically grown under different light sources, LEP (blue line), LED (red line), HPS (green line), and Natural Light (black line). Before the measurements, leaves were dark-adapted for at least 30 min. Chlorophyll a fluorescence measurements were made on fully expanded leaves still attached to the plants 10 and 20 days after onset of different light treatments (DAO). Three plants from each cultivar and light conditions and 3 measurements per plant were taken (9 replicates).

Table 18. Selected photosynthetic parameters deduced by the JIP-test analysis of Chl a fluorescence transients.

	Parris Island				Black Seeded Simpson				Rex RZ			
	Natural Light	LEP	LED	HPS	Natural Light	LEP	LED	HPS	Natural Light	LEP	LED	HPS
Fo	9674	8678	7802	10611	6835	8276	8239	7282	9888	9005	8489	9023
Fj	28257	25573	23505	30779	22532	25546	27522	26124	31225	27417	26041	29225
Fi	42170	35438	32659	38736	31769	37398	33385	31337	47818	38142	31763	38576
Fm	54002	48035	43955	49118	41549	48672	44558	43418	60185	53350	44272	52413
Fv	44329	39357	36152	38508	34713	40397	36318	36136	50297	44345	35784	43389
Vj	0.42	0.43	0.44	0.53	0.45	0.44	0.54	0.52	0.42	0.42	0.49	0.46
Vi	0.73	0.68	0.69	0.73	0.72	0.71	0.68	0.66	0.75	0.65	0.65	0.67
Fm/Fo	5.59	5.54	5.63	4.64	6.11	5.88	5.39	5.97	6.12	5.93	5.26	5.84
Fv/Fo	4.59	4.54	4.63	3.64	5.11	4.88	4.39	4.97	5.12	4.93	4.26	4.84
Fv/Fm	0.82	0.82	0.82	0.78	0.84	0.83	0.81	0.83	0.84	0.83	0.81	0.83
Mo	0.84	0.76	0.72	1.06	0.87	0.75	0.85	0.89	0.85	0.76	0.81	0.82
Area	21331738	16631524	18559304	15364346	12129972	18689686	10767784	9124985	16854613	12949898	12962511	13389733
Sm	477.03	422.91	518.82	397.55	347.54	568.45	304.44	255.23	339.82	294.05	362.68	309.48
Ss	0.50	0.57	0.61	0.50	0.52	0.59	0.64	0.59	0.50	0.55	0.61	0.58
N	953.56	748.23	850.11	803.81	665.27	936.65	474.53	434.11	680.04	534.44	593.10	538.28
φ_{P0}	0.82	0.82	0.82	0.78	0.84	0.83	0.81	0.83	0.84	0.83	0.81	0.83
ψ_{E0}	0.58	0.57	0.56	0.48	0.55	0.56	0.46	0.48	0.58	0.58	0.51	0.54
φ_{E0}	0.48	0.47	0.46	0.37	0.46	0.47	0.38	0.40	0.48	0.49	0.41	0.45
φ_{D0}	0.18	0.18	0.18	0.22	0.16	0.17	0.19	0.17	0.16	0.17	0.19	0.17
φ_{ET20}	0.48	0.47	0.46	0.37	0.46	0.47	0.38	0.40	0.48	0.49	0.41	0.45
φ_{RE10}	0.22	0.26	0.26	0.21	0.24	0.24	0.26	0.28	0.21	0.29	0.28	0.27
ψ_{RE10}	0.27	0.32	0.31	0.27	0.28	0.29	0.32	0.34	0.25	0.35	0.35	0.33
δ_{R0}	0.46	0.56	0.56	0.57	0.52	0.50	0.70	0.70	0.43	0.59	0.69	0.60
ABS/RC	2.44	2.16	2.01	2.58	2.30	2.10	1.96	2.06	2.41	2.20	2.03	2.11
RC/ABS	0.41	0.46	0.50	0.39	0.44	0.48	0.52	0.49	0.42	0.46	0.50	0.48
TRo/RC	2.00	1.77	1.65	2.02	1.92	1.73	1.60	1.71	2.02	1.83	1.64	1.75
DIo/RC	0.44	0.39	0.36	0.56	0.38	0.36	0.36	0.35	0.40	0.37	0.39	0.36
ET2o/RC	1.16	1.01	0.93	0.96	1.05	0.98	0.75	0.82	1.16	1.06	0.83	0.93
RE1o/RC	0.54	0.57	0.52	0.54	0.55	0.49	0.49	0.57	0.50	0.63	0.57	0.56
RC/CS	22158	22222	21869	19073	17926	23110	22605	21047	24870	24378	21950	24884
ABS/CS	54002	48035	43955	49118	41549	48672	44558	43418	60185	53350	44272	52413
TRo/Cs	44321	39362	36154	38514	34709	40399	36314	36136	50296	44357	35787	43378
ET2o/CS	25732	22464	20447	18346	19009	23126	17034	17294	28974	25942	18232	23183
DIo/CS	9681	8673	7801	10604	6840	8273	8243	7282	9889	8993	8485	9035
RE1o/CS	11836	12599	11295	10377	9780	11276	11166	12082	12363	15215	12513	13833
PI_{ABS}	2.62	2.82	3.05	1.31	2.76	3.21	2.03	2.23	3.02	3.28	2.39	2.81
$PI_{total\ ABS}$	2.26	3.66	3.94	1.72	2.96	4.05	5.82	5.54	2.49	4.88	5.18	4.62
$DF_{total\ ABS}$	0.35	0.56	0.59	0.23	0.47	0.48	0.70	0.73	0.35	0.67	0.69	0.62


Parris Island (DAO 10)
CP
LEPP
LEDP
HPSP
Fo
Fj
Fi
Fm
Fv
Vj
Vi
Fm/Fo
Fv/Fo
Fv/Fm
Mo
Area
Sm
Ss
N
Phi R0
Psi E0
Phi E0
Phi D0
Phi ET20
Phi RE10
Psi RE10
Delta R0
ABS/RC
RC/ABS
TRo/RC
DIo/RC
ET2o/RC
RE1o/RC
RC/CS
ABS/CS
TRo/Cs
ET2o/CS
DIo/CS
RE1o/CS
PIABS
PItotal ABS
DFtotal ABS
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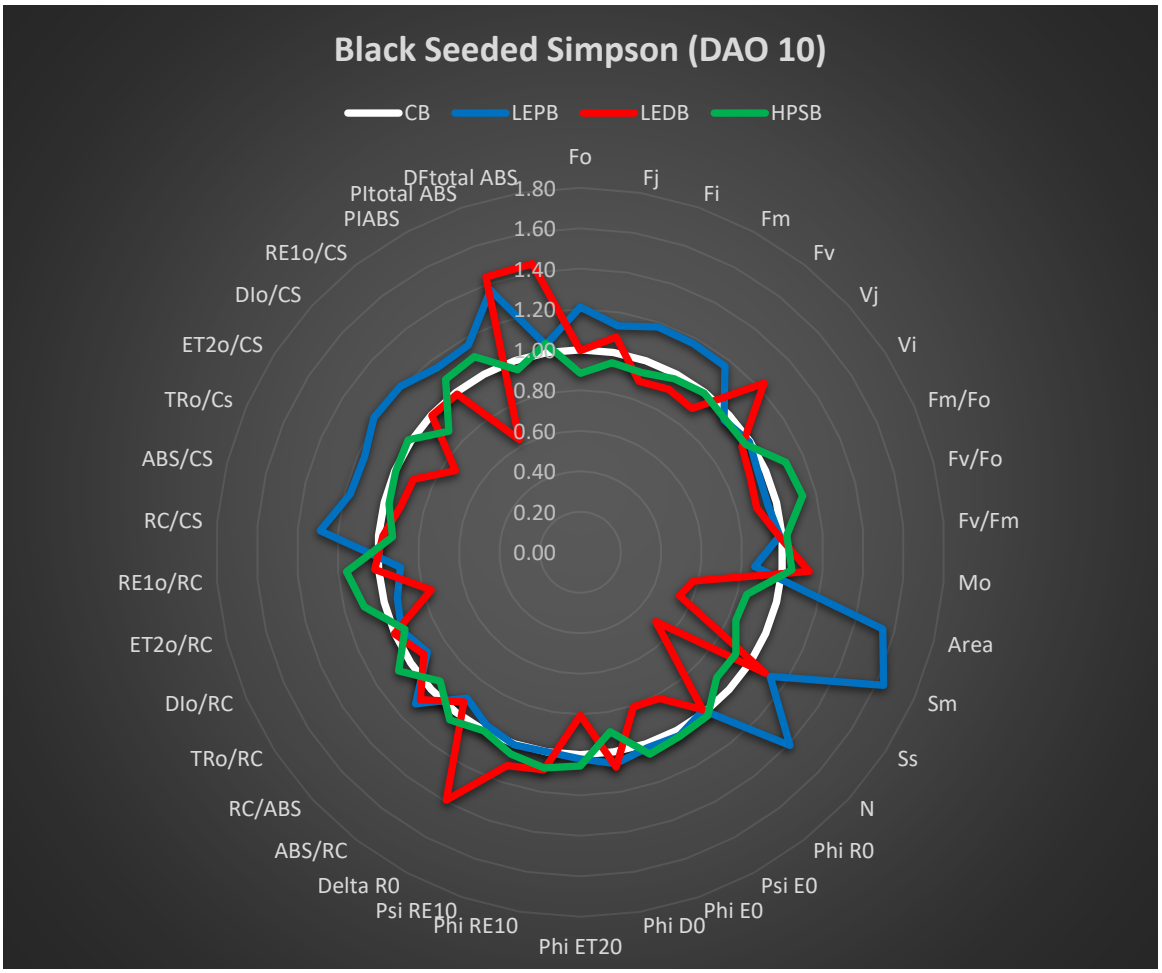
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00


Black Seeded Simpson (DAO 10)
CB
LEPB
LEDB
HPSB
Fo
Fj
Fi
Fm
Fv
Vj
Vi
Fm/Fo
Fv/Fo
Fv/Fm
Mo
Area
Sm
Ss
N
Phi R0
Psi E0
Phi E0
Phi D0
Phi ET20
Phi RE10
Psi RE10
Delta R0
ABS/RC
RC/ABS
TRo/RC
DIo/RC
ET2o/RC
RE1o/RC
RC/CS
ABS/CS
TRo/Cs
ET2o/CS
DIo/CS
RE1o/CS
PIABS
PItotal ABS
DFtotal ABS
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1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00

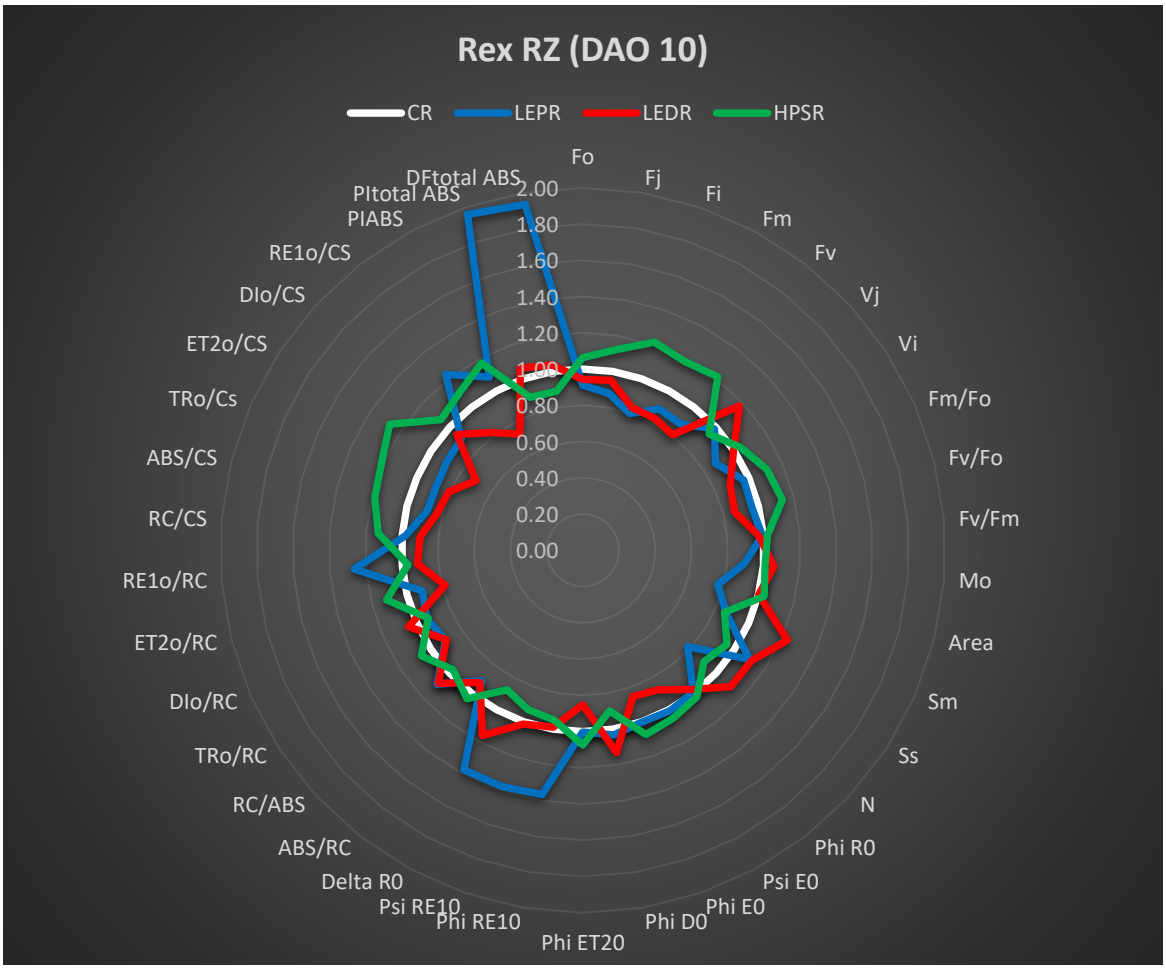

Fig. 28. Spider plots of JIP parameters deduced from Chl a fluorescence OJIP transient curves in the leaves of 3 cultivars, Parris Island (A), Black Seeded Simpson (B), and Rex RZ (C), grown hydroponically (DAO 10) under different light conditions, LEP (blue circle), LED (red circle), and HPS (green circle). Leaves of 3 cultivars grown under natural light were used as controls (white circle). The value of the control is set as 1.

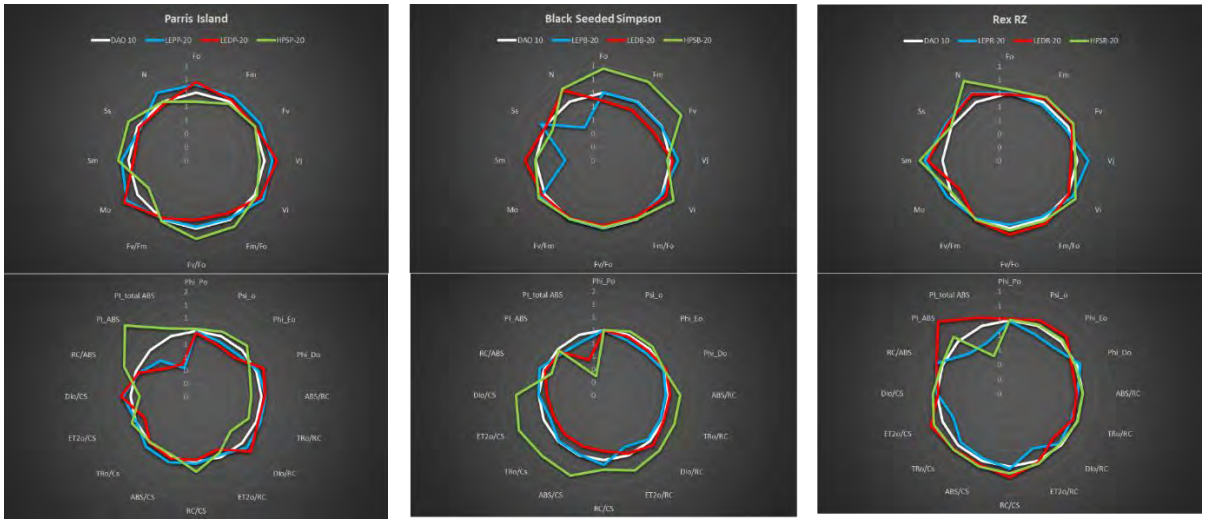

Fig. 29. Spider plots of JIP parameters deduced from Chl a fluorescence OJIP transient curves in the leaves of 3 cultivars, Parris Island (A), Black Seeded Simpson (B), and Rex RZ (C), grown hydroponically under different light conditions, LEP (blue circle), LED (red circle), and HPS (green circle). The data from DAO 10 were used as controls to compare ones from DAO 20. The value of the control (white circle) is set as 1.

Table 19. Effect of light source on growth of lettuce cultivars measured 4 weeks after seedling transplanting.

Cultivar	Light Source	Length (cm)		Width (Cm)	Fresh Weight (g)			Dry Weight (g)		Final pH in used solution	SPAD	Brix
		Shoot	Root		Total	Shoot	Root	Shoot	Root			
Parris Island	Natural Light	21.6	14.8	22.1	51.8	39.0	12.8	1.6	2.1	6.5	40.9	2.5
	LED	17.7	19.6	17.0	77.4	54.4	23.0	2.9	2.1	7.5	47.4	4.3
	LEP	20.0	19.2	18.7	85.6	58.9	26.7	2.6	2.4	7.6	44.6	2.6
	HPS	20.2	20.7	18.8	84.9	57.4	27.4	2.2	2.0	7.8	41.0	3.5
Black Seeded Simpson	Natural Light	31.2	20.2	19.1	49.9	38.2	11.7	1.8	2.0	6.3	40.9	3.6
	LED	22.4	24.6	18.1	83.1	54.0	29.1	2.8	2.3	6.4	21.8	4.2
	LEP	25.4	31.7	20.3	84.6	52.6	32.0	3.8	2.4	4.0	19.7	3.8
	HPS	23.2	29.9	25.2	88.3	56.9	31.4	3.1	2.6	7.2	14.3	3.7
Rex RZ	Natural Light	15.8	26.7	22.6	50.0	38.1	11.9	1.4	2.1	8.0	30.9	3.1
	LED	15.6	29.1	16.4	72.0	49.0	23.0	2.2	2.0	7.6	35.1	3.9
	LEP	16.2	28.1	19.2	78.9	55.6	23.3	2.7	2.3	7.3	31.6	2.7
	HPS	14.0	31.7	16.4	78.9	53.4	25.4	1.6	2.1	7.2	30.5	3.9

Analysis of OJIP fluorescence transients by the JIP-test

Chlorophyll a fluorescence transients of the dark-adapted leaves of 3 lettuce cultivars under different light sources at DAO 10 and 20 are shown, on logarithmic time scale up to 1 s, in Fig x. All curves show the typical OJIP at the O, J, I and P steps.

At DAO 10, either lower levels of maximum variable fluorescence (F_V) in Parris Island and Rex RZ or higher levels of F_v in Black Seeded Simson were observed when compared to the control plants grown under natural light (Table x). Significant decreases in F_v/F_0 ratio were observed for Parris Island/HPS, Black Seeded Simpson/LED, and Rex RZ/LED combinations compared to the control. This could indicate the participation of the antenna apparatus in the down regulation of photosynthesis.

With regard to the parameters that describe the yield and efficiency of the electron transport chain, either maximal photochemical quantum yield ($F_V/F_M = \varphi_{po}$), the quantum yield of electron transport between PSII and PSI ($ET_0/ABS = \varphi_{EO}$) and the efficiency that an electron moves further than QA- were affected by the among cultivars grown under different light sources. Combinations of Parris Island with HPS, Black Seeded Simpson and Rex RZ with either LED or HPS showed lower values when compared to the control.

The specific energy flux ratios of PSII in almost all combinations of cultivars/light sources were lower than ones in the control except for the Parris Island/HPS combination including ABS/RC and DI_0/RC. On the other hand, the specific energy flux ratios of phenomenological fluxes per CS in Black Seeded Simpson with LEP, LED and HPS were higher when compared to the control.

In general, performance index (PI) describes the energy conservation between photons absorbed by PSII and the reduction of intersystem electron acceptors (PI_{ABS}) and the reduction of PSI end acceptors (PI_{TOTAL}).

The highest values of PI_{ABS} were obtained from dark-adapted leaves grown under either LED or LEP for Parris Island or Black Seeded Simpson and Rex RZ, respectively, mainly due to higher RC/ABS values. In addition, significant increases in values of PI_{TOTAL} were observed compared to ones of PI_{ABS}, indicating that efficiencies with which an electron from the intersystem electron carriers moves to reduce end electron acceptors at the PSI acceptor side could be improved by utilizing artificial light sources.

4. Nutrient balancing for hydroponic culture

A Commonly used fertilizer in greenhouse with hydroponic culture is 20-20-20 commercial fertilizer based on Nitrogen concentration. Although current fertilizer can bring moderate income to farmers, for high quality lettuce growth, modification of the hydroponic solution is needed. In addition, the nutrient solution for lettuce growth should not only contain Ca and Mg ions, but also be considered a nitrate, potential carcinogen, in harvested plant tissue. For demanding those conditions, the nutrient solution should be formulated optimum concentration of macro-nutrients as shown below:

Macronutrients (meq/L): 5 Ca^{2+}, 2 Mg^{2+}, 6 K^{+}, 12 N (NH_4^{+}+NO_3^{-}), 2 SO_4^{2-}, 2 $H_2SO_4^{-}$

This solution is a modification of the original Hoagland solution which was used for hydroponic culture of vegetables and floricultural crops for many decades. Additional tests may be needed to further fine-tune the concentrations of macronutrients such as Ca^{2+}, NH_4^{+}, NO_3^{-} for growing leafy vegetables like lettuce, the instructions provided here will be a good guide line. Procedures for preparing nutrient solutions are shown as below:

a. Preparation of macronutrient solutions using good-quality water (deionized water)

In this experiment, for controlling all factors that can affect on the total macronutrient concentration in the final solution, the chemical amounts were negligible in the water, deionized water was used. The main object of this nutrient balancing was figuring out the optimum nutrient solution considering ammonium and nitrate ratio in the solution. To achieve the object, the nutrient solutions were formulated as 100 % NH_4^{+} + 0 % NO_3^{-}, 75 % NH_4^{+} + 25 % NO_3^{-}, 50 % NH_4^{+} + 50 % NO_3^{-}, 25 % NH_4^{+} + 75 % NO_3^{-}, and 0 % NH_4^{+} + 100 % NO_3^{-}, respectively (Table 3 and 4). The total standard meq/L was 32, but for fostering lettuce growth, each nutrient solution were modified from 46 to 52 meq/L. Also, the initial pH and EC were considered moderate range to attain an adequate condition for lettuce growth. Each pH and EC was shown Table 20.

Table 20. Change of pH and EC in the nutrient solutions containing different ratios of ammonium (NH_4^{+}) and nitrate (NO_3^{-}) forms of nitrogen.

	Ammonium (NH_4^{+}): nitrate (NO_3^{-}) ratio				
	100:0	75:25	50:50	25:75	0:100
Initial pH	6.2	5.8	6.0	6.1	5.9
pH after 2 weeks	5.3	5.3	4.3	5.7	5.9
Initial EC (dS/cm)	2.3	2.1	2.6	2.1	2.1
EC after 2 weeks (dS/cm)	2.4	2.4	2.8	2.4	2.3

Table 21. Nutrient ion balancing for the five different NH_4:NO_3 ratio nutrient solution prepared with deionized water based on Hoagland nutrient solution.

Solution 1. 100 % NH_4^+ + 0 % NO_3^-

	Ion concentration (meq/liter)												
Nutrient	H^+	Ca^{2+}	Mg^{2+}	K^+	NH_4^+	Na^+	NO_3^-	SO_4^{2-}	$H_2PO_4^-$	Cl^-	HCO_3^-	OH^-	Total
Required		6	2	6	2		10	4	2				32
Needed	0	4	3	4	12	0	0	6	11	2	4	0	46
1. $(NH_4)_2SO_4$					3			3					3
2. $NH_4H_2PO_4$					7				7				7
3. NH_4Cl					2				`	2			2
4. $MgSO_4$ $7H_2O$			3					3					3
5. KH_2PO_4				4					4				4
6. $CaCO_3$		4									4		4
Total	0	4	3	4	12	0	0	6	11	2	4	0	46

Solution 2. 75 % NH_4^+ + 25 % NO_3

	Ion concentration (meq/liter)												
Nutrient	H^+	Ca^{2+}	Mg^{2+}	K^+	NH_4^+	Na^+	NO_3^-	SO_4^{2-}	$H_2PO_4^-$	Cl^-	HCO_3^-	OH^-	Total
Standard		6	2	6	2		10	4	2				32
Solution 2	0	6	4	5	9	0	3	7	9	1	4	0	48
$NH_4H_2PO_4$					7				7				7
NH_4NO_3					1		1						1
NH_4Cl					1				`	1			1
$Ca(NO_3)_2$ $4H_2O$		2					2						2
KH_2PO_4				2					2				2
$MgSO_4$ $7H_2O$			4					4					4
K_2SO_4				3				3					3
$CaCO_3$		4									4		4
Total	0	6	4	5	9	0	3	7	9	1	4	0	48

Solution 3. 50 % NH_4^+ + 50 % NO_3^-

Nutrient	Ion concentration (meq/liter)												
	H^+	Ca^{2+}	Mg^{2+}	K^+	NH_4^+	Na^+	NO_3^-	SO_4^{2-}	$H_2PO_4^-$	Cl^-	HCO_3^-	OH^-	Total
Standard		6	2	6	2		10	4	2				32
Solution 3		7	6	4	6	2	6	10	7	0	0	2	50
$NH_4H_2PO_4$					3				3				3
NH_4NO_3					3		3						3
$Ca(NO_3)_2\ 4H_2O$		3					3						3
$CaSO_4\ 2H_2O$		4						4					4
KH_2PO_4				4					4				4
NaOH						2						2	2
$MgSO_4\ 7H_2O$			6					6					6
Total	0	7	6	4	6	2	6	10	7	0	0	2	50

Solution 4. 25 % NH_4^+ + 75 % NO_3^-

Nutrient	Ion concentration (meq/liter)												
	H^+	Ca^{2+}	Mg^{2+}	K^+	NH_4^+	Na^+	NO_3^-	SO_4^{2-}	$H_2PO_4^-$	Cl^-	HCO_3^-	OH^-	Total
Standard		6	2	6	2		10	4	2				32
Solution 4	0	7	4	7	3	5	9	6	7	0	0	4	52
NH_4NO_3					3		3						3
$Ca(NO_3)_2\ 4H_2O$		1					1						1
$CaSO_4\ 2H_2O$		2						2					2
KH_2PO_4				3					3				3
$CaH_4(PO_4)_2$		4							4				4
$Mg(NO_3)_2\ 6H_2O$			4				4						4
$NaNO_3$						1	1						1
NaOH						4						4	4
K_2SO_4				4				4					4
Total	0	7	4	7	3	5	9	6	7	0	0	4	52

Solution 5. 0 % NH_4^+ + 100 % NO_3^-

Nutrient	Ion concentration (meq/liter)												
	H^+	Ca^{2+}	Mg^{2+}	K^+	NH_4^+	Na^+	NO_3^-	SO_4^{2-}	$H_2PO_4^-$	Cl^-	HCO_3^-	OH^-	Total
Standard		6	2	6	2		10	4	2				32
Solution 5	0	8	6	7	0	2	12	3	6		0	2	46
KNO_3				3			3						3
$Ca(NO_3)_2\ 4H_2O$		3					3						3
$Mg(NO_3)_2\ 6H_2O$			6				6						6
KH_2PO_4				3					3				3
$CaH_4(PO_4)_2$		3							3				3
$CaSO_4\ 2H_2O$		2						2					2
NaOH						2						2	2
K_2SO_4				1				1					1
Total	0	8	6	7	0	2	12	3	6	0	0	2	46

Table 22. Chemicals used to prepare a nutrient solution containing 100 % NH_4^+ + 0 % NO_3^- used in the tissue nitrate experiment.

Chemical	Effect on acidity	Mol. weight	Equiv. weight	g/L
$(NH_4)_2SO_4$	very acid	132.15	66.1	0.198
$NH_4H_2PO_4$	acid	115.04	115.04	0.805
NH_4Cl	acid	53.5	53.5	0.107
$MgSO_4\ 7H_2O$	neutral	246.5	123.25	0.370
KH_2PO_4	basic	120.1	120.1	0.480
$CaCO_3$	basic	100.1	50.1	0.200

Table 23. Chemicals used to prepare a nutrient solution containing 75 % NH_4^+ + 25 % NO_3^- used in the tissue nitrate experiment.

Chemical	Effect on acidity	Mol. weight	Equiv. weight	g/L
$NH_4H_2PO_4$	acid	115.04	115.04	0.805
NH_4NO_3	acid	80.05	80.05	0.080
NH_4Cl	acid	53.5	53.5	0.054
$Ca(NO_3)_2\ 4H_2O$	basic	236.16	118.1	0.236
KH_2PO_4	basic	120.1	120.1	0.240
$MgSO_4\ 7H_2O$	neutral	246.5	123.25	0.493
K_2SO_4	neutral	173.26	87.13	0.261
$CaCO_3$	basic	100.1	50.1	0.200

Table 24. Chemicals used to prepare a nutrient solution containing 50 % NH_4^+ + 50 % NO_3^- used in the tissue nitrate experiment.

Chemical	Effect on acidity	Mol. weight	Equiv. weight	g/L
$NH_4H_2PO_4$	acid	115.04	115.04	0.345
NH_4NO_3	acid	80.05	80.05	0.240
$Ca(NO_3)_2\ 4H_2O$	basic	236.16	118.1	0.354
$CaSO_4\ 2H_2O$	neutral	172.18	86.09	0.344
KH_2PO_4	basic	120.1	120.1	0.480
NaOH	very basic	40	40	0.080
$MgSO_4\ 7H_2O$	neutral	246.5	123.25	0.740

Table 25. Chemicals used to prepare a nutrient solution containing 25 % NH_4^+ + 75 % NO_3^- used in the tissue nitrate experiment.

Chemical	Effect on acidity	Mol. weight	Equiv. weight	g/L
NH_4NO_3	acid	80.05	80.05	0.240
$Ca(NO_3)_2\ 4H_2O$	basic	236.16	118.1	0.118
$CaSO_4\ 2H_2O$	neutral	172.18	86.09	0.172
KH_2PO_4	basic	120.1	120.1	0.360
$CaH_4(PO_4)_2$	neutral	246.5	123.25	0.493
$Mg(NO_3)_2\ 6H_2O$	neutral	256.4	128.2	0.513
$NaNO_3$	basic	85	85	0.085
NaOH	very basic	40	40	0.160
K_2SO_4	neutral	173.26	87.13	0.349

Table 26. Chemicals used to prepare a nutrient solution containing 0 % NH_4^+ + 100 % NO_3^- used in the tissue nitrate experiment.

Chemical	Effect on acidity	Mol. weight	Equiv. weight	g/L
KNO_3	basic	101.1	101.1	0.303
$Ca(NO_3)_2\ 4H_2O$	basic	236.16	118.1	0.354
$Mg(NO_3)_2\ 6H_2O$	neutral	256.4	128.2	0.769
KH_2PO_4	basic	120.1	120.1	0.360
$CaH_4(PO_4)_2$	neutral	246.5	123.25	0.370
$CaSO_4\ 2H_2O$	neutral	172.18	86.09	0.172
NaOH	very basic	40	40	0.080
K_2SO_4	neutral	173.26	87.13	0.087

5. Influence of boron (B) on physiological disorders of lettuce

Experiment 1

1) Micronutrient stock solution lacking boron

Table 27. Amount of micronutrient fertilizers use to prepare a 1000x stock solution.

Element	Final conc.	Chemical formula	Formula weight	Amount in final solution	Amount in stock solution (1000 X)	Note
Cu	0.5 µM	$CuSO_4 \cdot H_2O$	177.61	0.089 mg/l	0.089 g/l	
Fe	10 µM	$FeSO4 \cdot H_2O$	169.62	1.69 mg/l	1.69 g/l	
Mn	10 µM	$MnSO_4 \cdot 3H_2O$	205.0	2.05 mg/l	2.05 g/l	
Mo	0.5 µM	$Na_2MoO_4 \cdot 2H_2O$	241.91	0.12 mg/l	0.12 g/l	
Zn	4 µM	$ZnSO_4 \cdot H_2O$	179.47	0.716 mg/l	0.72 g/l	

Use 1 ml of stock solution to make 1 liter of final fertilizer solution.

2) Amount of born (B) used to induce deficiency and toxicity symptoms

Table 28. Amount of micronutrient fertilizers use to prepare a 1000x stock solution.

No.	mM B	B (mg/liter)	H_3BO_3 (mg/liter)	H_3BO_3 (g/100 liter)	H_3BO_3 (mg/gal)	H_3BO_3 (g/50 gal)	Note
1	Control	0	0	0	0	0	RO water
2	0.02	0.216	0.566	0.0566	2.15	0.1075	Control (1x)
3	0.2	2.164	5.660	0.566	21.5	1.075	10 x
4	2.0	21.64	56.6	5.66	215.1	10.75	100

a. Experimental results

When plants were cultured with nutrient solutions that lack boron or have elevated levels of boron in the nutrient solution, little difference on plant was noticed. No detectable symptom of boron deficiency occurred on plants grown with nutrient solutions containing no boron. When B concentration in the nutrient solution was elevated to 0.2 mM and 2.0 mM, many plants showed necrotic spots on the leaf (Figs. 12 C and D).

The leaf disorder symptoms caused by elevated boron, however, were different from those leaf margin burns commonly observed (Fig. 13, Fig 14). Plans grown with different levels of boron concentrations are being examined for their tissue B contents. Further testing is needed to clarify if boron is implicated with the common occurrence of leaf margin burns in lettuce.

Fig. 30. Development of leaf edge burns 3SX821 lettuce grown with four different concentrations (0, 0.02, 0.2, 2.0 mM) of boron (B) in the nutrient solution.

Ansar	Baby Romaine
Bibb	Gem

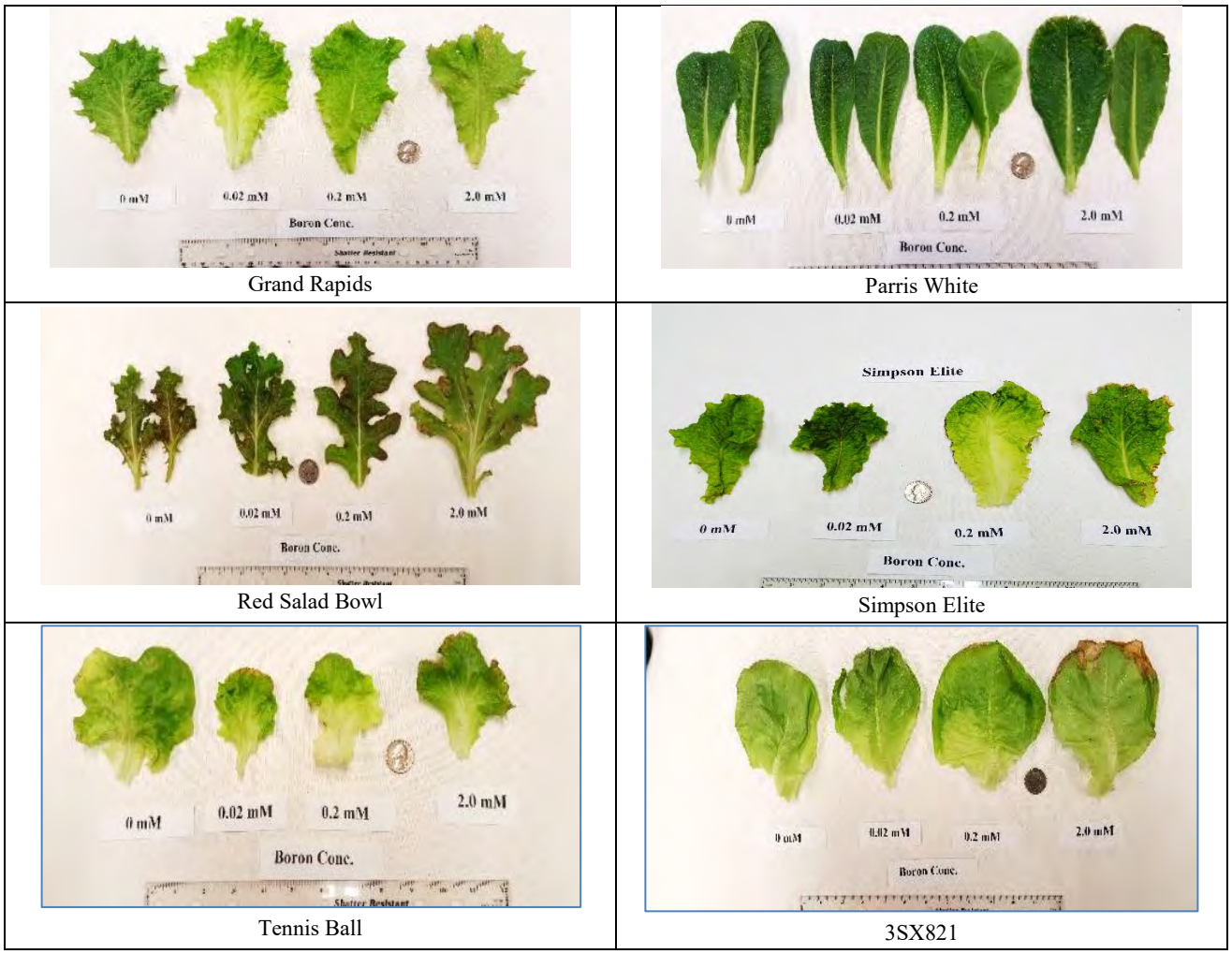

Fig. 31. Close-up pictures of lettuce leaves showing abnormalities caused by absence or elevated levels of boron in the nutrient solution.

Experiment 2

a. Greenhouse environmental condition
   Light intensity: The average light intensity measured during the day time was 249.5 $\mu mol \cdot m^{-2} \cdot s^{-}$ or 19,860 lux.
   Air and leaf temperature:
   The average ambient and leaf temperatures were: 23.8 ºC and 22.3 °C

b. Lettuce cultivars: Black Seeded Simpson, Rex RZ, and Parris Island.

c. Nutrient solutions:

   The nutrient solution used for this experiment contained the standard concentrations of macronutrients (meq/L: 5 Ca^{2+}, 2 Mg^{2+}, 6 K^{+}, 2 NH_4^{+}, 11 NO_3^{-}, 2 SO_4^{2-}, 2 $H_2SO_4^{-}$) and micronutrients (µM: 0.5 Cu, 20 Fe, 10 Mn, 0.5 Mo, 4 Zn) except boron. Four different concentrations of boron (0, 0.2 mM) were used to test their influence on physiological disorders (Table 11, 12 and 13).

Table 29. Amount of micronutrient fertilizers use to prepare a 1000x stock solution.

Elem.	Final conc.	Chemical formula	Formula weight	Amount in final solution	Amount in stock solution (1000 X)	Note
B	20 µM	$Na_2B_4O_7 \cdot 10H_2O$	381.37	7.63 mg/l	7.63 g/l	Boil to dissolve
Cl	-	$MgCl_2 \cdot 7H_2O$	221.24	-	-	Not used
Cu	0.5 µM	$CuSO_4 \cdot H_2O$	177.61	0.089 mg/l	0.089 g/l	
Fe	10 µM	$FeSO4 \cdot H_2O$	169.62	1.69 mg/l	1.69 g/l	
Mn	10 µM	$MnSO_4 \cdot 3H_2O$	205.0	2.05 mg/l	2.05 g/l	
Mo	0.5 µM	$Na_2MoO_4 \cdot 2H_2O$	241.91	0.12 mg/l	0.12 g/l	
Zn	4 µM	$ZnSO_4 \cdot H_2O$	179.47	0.716 mg/l	0.73 g/l	

Use 1 ml of stock solution to make 1 liter of final fertilizer solution.

Table 30. Amount of micronutrient fertilizers use to prepare a 1000x stock solution.

Element	Final conc.	Chemical formula	Formula weight	Amount in final solution	Amount in stock solution (1000 X)	Note
Cu	0.5 µM	$CuSO_4 \cdot H_2O$	177.61	0.089 mg/l	0.089 g/l	
Fe	10 µM	$FeSO4 \cdot H_2O$	169.62	1.69 mg/l	1.69 g/l	
Mn	10 µM	$MnSO_4 \cdot 3H_2O$	205.0	2.05 mg/l	2.05 g/l	
Mo	0.5 µM	$Na_2MoO_4 \cdot 2H_2O$	241.91	0.12 mg/l	0.12 g/l	
Zn	4 µM	$ZnSO_4 \cdot H_2O$	179.47	0.716 mg/l	0.74 g/l	

Use 1 ml of stock solution to make 1 liter of final fertilizer solution.

Table 31. Amount of micronutrient fertilizers use to prepare a 1000x stock solution.

No.	mM B	B (mg/liter)	H_3BO_3 (mg/liter)	H_3BO_3 (g/100 liter)	H_3BO_3 (mg/gal)	H_3BO_3 (g/50 gal)	Note
1	Control	0	0	0	0	0	RO water
2	0.2	2.164	5.660	0.566	21.5	1.075	10

d. Procedures and analysis

It has been known that the deficiency and excess level of boron (B) causes necrosis of leaf tissues in many plants. This experiment was conducted to determine if the lack or excessive levels of B can cause leaf edge burns on lettuce grown hydroponically.

Three cultivars of lettuce were used for hydroponic tub culture. Seeds were germinated on Rockwool cubes and seedlings were transplanted onto the Styrofoam board which was floated inside the tub filled with nutrient solution. Appearance of leaf edge burns and other symptoms were monitored.

e. Experimental results

When plants were cultured with nutrient solutions that lack boron or have elevated levels of boron in the nutrient solution, little difference on plant was noticed. When B concentration in the

nutrient solution was elevated to 2.0 mM, many plants showed necrotic spots in the previous experiment.

In this experiment, the leaf disorder symptoms occurred in all cultivars examined when the nutrient solution lacked boron. No boron-related abnormalities were observed when the nutrient solution contained 0.2 mM B. Boron deficiency symptoms were manifested by browning and death of newly formed leaves as well as blackening of roots in Black Seeded Simpson, interveinal chlorosis and browning in younger leaves in Rex RZ, and browning, blackening and distorted growth of newly formed leaves in Parris Island (Figs. 27, 28). The threshold leaf tissue concentrations of boron that cause deficiency symptom have yet to be determined by an ICP analyzer using the dried samples of lettuce grown in this experiment.

Table 32. Influence of boron (B) on the growth of lettuce cultured hydroponically for 4 weeks.

Cultivar and boron treatment	Height (cm)	Width (cm)	pH	Brix (%)	Chloro-phyll (SPAD)	Shoot fresh weight (g)	Shoot dry weight (g)	Bolt (1-5)	Leaf margin burns[z] (1-5)	Leaf mold (1-5)	Overall rating (1-5)
Black Seeded Simpson, 0.2 mM	26.7	11.7	6.5	3.8	29.1	41.0	5.2	5.0	5.0	5.0	5.0
Parris Island, 0.2 mM	21.3	15.7	6.4	5.0	42.9	105.0	7.5	5.0	5.0	5.0	5.0
Rex RZ, 0.2 mM	13.7	20.7	6.6	3.1	28.6	66.3	4.2	5.0	5.0	5.0	4.0
Black Seeded Simpson, 0 mM	12.7	11.0	6.6	8.3	35.0	21.3	4.1	2.0	3.0	5.0	2.0
Parris Island, 0 mM	12.0	15.0	6.4	9.4	45.2	31.3	5.8	1.0	3.0	5.0	2.3
Rex Rz, 0 mM	6.3	12.0	6.4	7.1	30.2	22.7	2.7	3.0	3.0	5.0	3.0

[z]Leaf margin burns was scored from 1 to 5, with 1 showing the highest leaf edge burn and 5 showing the lowest leaf edge burn.

E F

Fig. 32. Lettuce plants cultured hydroponically in tubs with and without boron in the nutrient solution: uce to induce boron deficiency and toxicity symptom: A-roots of lettuce grown without boron (left) and with boron (right), B-close-up picture of Black Seeded Simpson leaves that show deficiency symptom at the time of harvest, C-leaves of Rex RZ that show mild deficiency symptoms when grown without boron, D-normal leaves of Rex RZ grown with boron, E-leaves of Parris Island lettuce showing deficiency symptoms when grown without boron, F-leaves of Parris Island leaves that show no deficiency symptom when grown with boron in the nutrient solution. Pictures were taken after 4 weeks of hydroponic culture.

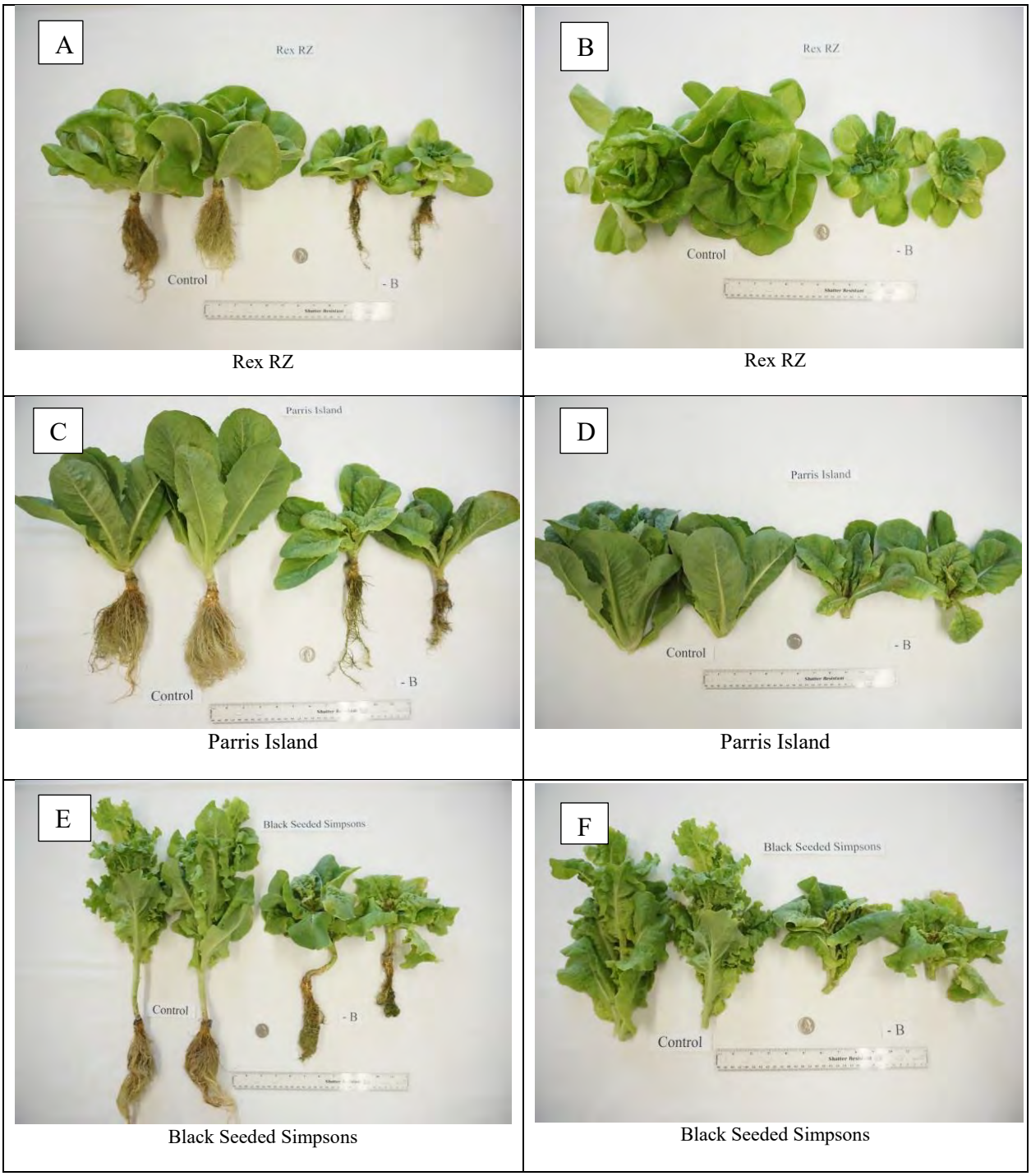

Fig. 33. Plants of three different lettuce cultivars harvested after four weeks of hydroponic culture with 0.2 mM boron (control) and no boron (-B) in the nutrient solution.

Experiment 3

Two cultivars (Rex RZ and Black Seeded Simpson) of lettuce are grown in tub culture using nutrients containing 0, 20, 40, 100 µM boron. The growth and abnormality symptom development were monitored for 20 days.

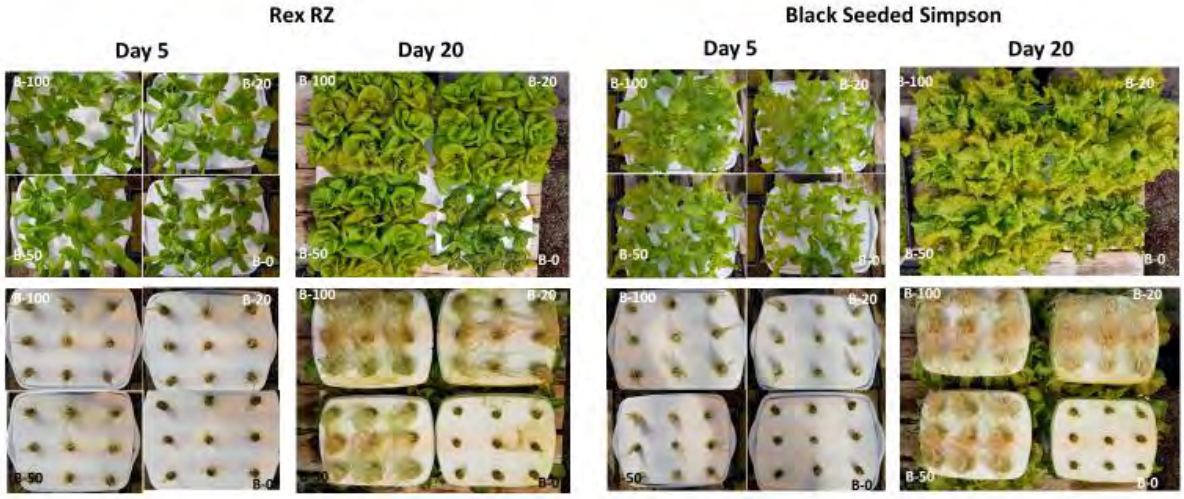

Fig. 34. Lettuce plants cultured hydroponically in tubs with 4 different boron levels (0, 20, 50, and 100 µM, shown in pictures as B-0, B-20, B-50, and B-100, respectively) in the nutrient solution to investigate boron deficiency (or toxicity) symptom and the early effects of B deficiency on photosynthesis

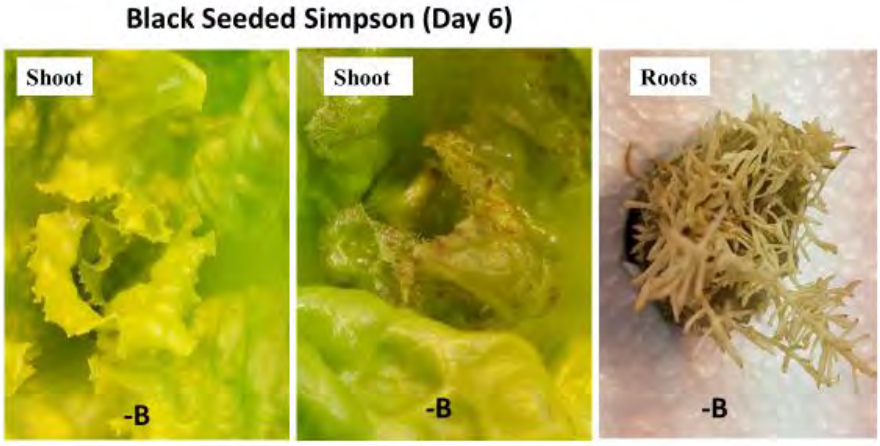

Fig. 35. The earliest symptoms in lettuce grown under nutrition solution without boron were 1) failure of roots to elongate and necrotic spots on shoot at day 6.

Experimental results

Plants grown for 5 days and 20 days at different levels of boron are shown in Fig. 34. As expected, new shoot and root growths were severely affected when grown in nutrient solusion containing no boron (-B, Fig. 35). We are currently investigating whether phothosynthetic potentials decreases with B deficiency before visible foliar symptoms appear as measured by the chlorophyll a fluorescence as an indicator in the leaves of lettuce. The physiological disorders caused by lack of boron were highly visible with reduced leaf size, browning of tissues, and necrosis.

6. **Performance of grafted plants for greenhouse production**

a. Objectives

1) To determine if grafted tomato plants perform better than non-grafted plants when cultured in the greenhouse
2) To compare the yield and quality of fruits produced by grafted plants compared to the non-grafted plants

b. Plant materials

1) Scion cultivars: Torero (Beef steak tomato, indeterminate)
Cannonball (NDSU release, determinate)

2) Rootstock cultivars: Maxifort (Johnny's Selected Seeds)
Blocking (rootstock tomato from Korea)

c. Grafting

Seedling grafts were made using the scion/rootstock combinations using the cleft grafting.

d. Plant culture

Grafted plants are grown in 7-inch pots filled with the commercial peat-light mix. Plants are grown in the Exolite covered greenhouse with 70 ^{o}F day/60 ^{o}F night temperatures. Plants are now in flowering stage. Fruit harvest will be made for 3-4 months. Fruit yield and quality will be compared between the grafted and non-grafted plants.

7. **Dissemination of information**

Publications in 2016.

1. Kim, C., L.J. Cihacek, and C.W. Lee. 2016. Changes in nitrate contents of hydroponically grown leaf lettuce as influenced by the ratio of ammonium and nitrate forms of nitrogen in nutrient solution. HortScience 51(9): S197.
2. Choi, S., C. Kim, Z. Wu, and C.W. Lee. 2016. Influence of artificial lights on nitrate and sugar contents of leaf lettuce. HortScience 51(9): S287.
3. McCollum, C., C. Kim, and C.W. Lee. 2016. Evaluation of lettuce cultivars for hydroponic culture. HortScience 51(9): S385.

Appendix I

Presentations at 2016 Annual Conference of ASHS

Influence of Artificial Lights on Nitrate and Sugar Contents of Leaf Lettuce

NDSU PLANT SCIENCES

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Introduction

High nitrate levels of leafy vegetables in diet may be linked to higher incidences of gastrointestinal cancer. This experiment was conducted to determine if different artificial lights influence the tissue nitrate and sugar concentration of leaf lettuce grown hydroponically as well as in pots under light emitting diode (LED), light emitting plasma (LEP) and high pressure sodium (HPS) lamps. The artificial lights were installed in individual cubicles (1 m x 1 m x 1.5 m) lined with fabrics that transmitted less than 3% natural light inside a greenhouse. The photosynthetic photon flux densities ($\mu mol \cdot m^{-2} s^{-1}$) inside the light boxes were: 89, 389, 400, and 142 $\mu mol \cdot m^{-2} s^{-1}$, respectively, for LED (2:1 mixture of red and blue lights), LEP, HPS, and natural light. Seedlings of 3 different cultivars (Parris Island, Black Seeded Simpson, Rex Rz) of lettuce were anchored on Styrofoam boards floated inside a tub containing 200 ppm N prepared with 20-20-20 commercial analysis fertilizer.

Materials and Methods

Artificial lighting was done inside three different chambers constructed with cotton fabrics that transmitted less than 5% of natural light. Chamber dimensions were 1m x 1m x 1.5 m (Fig. 1). Plants were grown hydroponically as well as in pots (containing BM mix). The distance from the lamp to plant level was 0.8 m, 0.6 m, and 0.9 meter for LEP, LED, HPS lights (Table. 1). LED light was composed of blue and red spectra. Plants were grown for 3 weeks using a nutrient solution containing 200 ppm N prepared with a commercial 20-20-20 fertilizer. Plants were also grown outside the lighting chamber under natural light conditions for growth comparisons. The high pressure sodium lamps (HPS) provided highest light intensity as well as highest temperature (Table 1). Light intensity under HPS and LEP was similar at the distance specified. LED lights provided the lowest light intensity (89 $\mu mol \cdot m^{-2} \cdot s^{-1}$) as compared to LEP (389 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and HPS (505 $\mu mol \cdot m^{-2} \cdot s^{-1}$).

Table 1. Environmental conditions used for artificial lighting experiments for growing lettuce.

Light source	Lux	ppfd[z] ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Air temperature (°C)	Leaf temperature (°C)
LED (light emitting diode)	5,040	89	31.4	32.1
Natural light	8,460	142	20.7	23
Supplementary light by LEP	2,000	352.3	20.1	29
Supplementary light by HPS	33,200	400	22.4	28.5
LEP (light emitting plasma)	25,700	389	33.7	35.5
HPS (high pressure sodium)	42,900	505	36	40.3

[z]Light measurement by photosynthetic photon flux density (ppfd).

Fig. 1. Light cages used for artificial lights LEP, LED, and HPS lamps for growing lettuce hydroponically.

Results

While the fresh weight yield of lettuce was highest under HPS lamps (Table 2), all plants had leaf margin burns when grown under HPS lamps, perhaps due to the combination of high temperature and high light intensity. Since lettuce require not more than 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the distance between the lamp and plants must be increased. Biomass yield under LED was the lowest.

Table 2. Influence of various artificial lights on growth of Parris Island transplanted in BM root media.

Treatment[z]	Shoot height (cm)	Shoot width (cm)	pH	Nitrate (mg/L)	Brix (%)	Chlorophyll content (SPAD)	Shoot Fresh Weight (g)	Shoot dry Weight (g)
Natural Light	20.2 b	8.3 c	5.6 c	6566 ab	3.3 ab	32.8 b	26.3 d	2.8 c
Supplemental light HPS	13.7 c	24.7 a	5.2 d	8400 a	4.2 a	55.8 a	114.0 bc	17.1 b
Supplemental light LEP	15.7 c	23.7 a	5.5 cd	8033 a	2.0 bc	37.8 ab	140.0 b	18.0 b
LEP	22.0 ab	18.5 b	6.4 b	4900 bc	2.3 bc	37.0 b	107.0 3	14.5 b
LED	23.8 a	7.2 c	7.1 a	5300 bc	1.5 c	33.8 b	51.7 d	2.1 c
HPS	14.5 c	23.0 ab	7.0 a	3700 c	2.9 abc	47.8 ab	187.0 a	30.2 a
Significant	***	***	***	***	*	ns	***	***
P-value	0.0000	0.0000	0.0000	0.0009	0.0205	0.0843	0.0000	0.0000

[z]LSD multiple range test significance * and *** at P≤0.05 and 0.0005, respectively.

Fig. 2. Parris Island lettuce grown under artificial lights (LEP, LED, HPS) in comparison to that grown under natural sunlight in the greenhouse.

Fig. 3. Leaves of Parris Island lettuce grown under artificial lights (LEP, LED, HPS) in comparison to that grown under natural sunlight in the greenhouse.

The shape, color, and general appearance of lettuce grown under LEP were much more desirable than that grown under either LED or HPS lamps (Figs. 2, 3, 4). Tissue nitrate concentration was highest when plants were grown under LEP (Table 2).

Fig. 4. Parris Island lettuce grown under artificial lights (LEP, LED, HPS) in comparison to that grown under natural sunlight in the greenhouse.

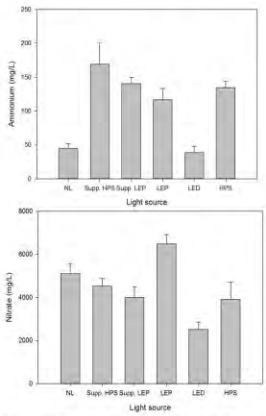

Fig. 5. NO_3^- and NH_4^+ concentrations calculated by multiplying and converting with fresh growth rate and dried sample value measured by Kjeldahl method (Abbreviations : NL : Natural light, Supp. HPS : Supplemental light HPS, Supp. LEP : Supplemental light LEP, LEP : Light emitting plasma, LED : light emitting diode, HPS : High pressure sodium lamps).

Conclusions

In both lettuce cultivars, the nitrate level was lowest when plants were grown under LED compared to other lights. Sugar content determined by a refractometer (Brix) was also lowest under LED, while both LEP and HPS provided highest Brix readings. When plants were grown in a peat-light mix (BM Mix)) in pots, tissue nitrate levels were about half of those obtained from hydroponic culture using the same nutrient solution. The pattern of nitrate and sugar accumulation also varied by cultivars under the same artificial lights.

Evaluation of Lettuce Cultivars for Hydroponic Culture

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Abstract

Sixty cultivars of lettuce were evaluated for their performance in hydroponic culture. Seeds were germinated in Rockwool slabs and seedlings at the two true-leaf stage were anchored 6 inch apart on an NFT (nutrient film techniques) system. Plants were grown for 4 weeks with a nutrient solution containing 200 ppm N (prepared using 20-20-20 commercial analysis fertilizer). Plant growth and quality were evaluated by fresh and dry weights, plant size and shape, chlorophyll content (SPAD), soluble solids content (Brix), pH, tissue nitrate (NO_3^-), bolting, and the presence of mold and leaf margin burns. The highest fresh weight yield was obtained by Giant Caesar, followed Ansar, Coastal Star, Vivian, and Rex Rz. The total soluble solids content as measured by a refractometer was highest in 11C3487, followed by Caesar's Favorite, Outredgeous, Balfour, and 3SX822. Tissue NO_3^- contents, which ranged from 4,000 ppm to 30,000 ppm, were lowest in Baby Romaine and Aroyo, followed by Aerostar and Encino. Refugio, Red Oak, and Four Seasons were the three cultivars that had the highest tissue nitrate contents. Cultivars with high propensity for bolting included Black Seeded Simpson, Looseleaf Blend, Arctic Crisp, Simpson Elite, and Tango. Cultivars with highest overall ratings were 11C3487, 3SX820, Vivian, Parris Island, Rex Rz, and Pomegranate Crunch.

Materials and Methods

Seeds of sixty cultivars of lettuce were planted on Rockwool cubes and seedlings were anchored on the channel of hydroponic NFT system. During the experiment, plants were grown with a nutrient solution containing 200 ppm N using the 20-20-20 commercial analysis fertilizer. Each unit contained four 6-foot long troughs which were slightly slanted to allow nutrient solution to flow into the sump bucket (5 gallon size). The nutrient solution was continuously applied to the plants (3 plants per cultivar) and collected into the bucket for recirculation. To compensate the loss of nutrient solution by plant absorption and transpiration, each sump bucket was replenished with new solutions as needed (Fig. 1).

A

B

Fig. 1. An NFT system used for growing 60 cultivars of lettuce: one week after seedling placement (A), and three weeks after transplanting (B).

Results and Discussion

When evaluated after 4 weeks of culture in March-April, plants of eight different cultivars showed bolting problems. The cultivars that formed flower stalks were: Arctic Crisp, Black Seeded Simpson, Emerald Oak, Grand Rapids, Looseleaf Blend, Simpson Elite, Tango, and Tennis Ball (see Table 1). A number of cultivars showed some signs of stem elongation that could eventually lead to flower stalk formation (cultivars scored less than 5.0 in Table 1). The incidents of developing bolting problem were higher in this experiment compared to previous experiments using the same cultivars. Usually bolting occurs when plants are exposed to high temperature under high light intensity. The greenhouse compartment was lighted with high pressure sodium lights from 8 a.m. to 10 p.m. during the plant growing period. During the day time, the average light intensity, air temperature, and leaf temperature in the greenhouse was 232.1 μmol $m^{-2}s^{-1}$, 23.5 °C, and 27 °C, respectively.

Table 1. Plant quality parameters of the sixty lettuce cultivars grown for 4 weeks with NFT system.

	Cultivar	Length (cm)	Width (cm)	Plant color[z]	Plant shape[y]	pH	Brix (%)[x]	Chlorophyll (SPAD)[w]	Plant fresh weight (g)	Plant dry weight (g)	Nitrate (ppm)[v]	Bolt (1-5)	Leaf margin burns (1-5)[u]	Leaf mold (1-5)	Overall rating (1-5)
1	Aerostar	23.5	25.7	DG	U	6.2	5.4	59.6	159.3	14.8	4,700	5.0	3.5	5.0	3.5
2	Ansar	26.3	24.7	DG	U	6.1	4.4	56.2	158.7	13.5	6,000	5.0	3.3	5.0	3.5
3	Arctic Crisp	77.7	19.9	DG	U	6.1	4.6	38.9	80.0	10.8	5,700	1.0	1.0	5.0	1.0
4	Aroyo	28.0	24.3	DG	U	6.1	3.9	66.8	141.0	14.3	4,000	4.3	4.2	5.0	4.0
5	Baby Romaine	21.8	16.3	G	U	6.2	5.4	51.6	95.0	9.9	4,000	5.0	3.7	5.0	3.8
6	Balfour	12.5	28.0	G	H	6.1	7.1	35.2	68.5	9.7	11,000	5.0	5.0	5.0	5.0
7	BBG	22.8	23.7	DG	U	6.1	3.4	47.9	109.3	10.8	14,000	5.0	2.0	5.0	2.6
8	Black Seeded Simpson	35.3	24.3	LG	U	5.5	4.8	27.5	170.3	11.6	14,000	1.0	3.0	5.0	2.0
9	Bolder	15.5	25.0	G	H	6.8	4.8	38.4	103.3	9.8	11,000	5.0	4.7	5.0	4.8
10	Buttercrunch	24.5	22.7	G	U	6.9	6.1	45.6	155.3	15.7	12,000	5.0	4.2	5.0	3.5
11	Butterhead	16.5	23.3	LG	LM	6.4	6.6	36.9	132.0	12.4	20,000	5.0	4.0	5.0	4.5
12	Caesar's Favorite	21.7	23.3	DG	U	6.2	7.4	62.8	106.7	10.8	16,000	5.0	3.3	5.0	3.3
13	Coastal Star	29.6	28.3	DG	U	6.6	6.1	59.2	167.0	15.1	22,000	4.7	4.0	5.0	4.0
14	Emerald Oak	28.7	21.0	G	U	5.5	5.3	47.4	130.3	12.3	20,000	1.0	2.0	5.0	2.0
15	Encino	19.3	30.0	LG	M	6.3	5.1	33.9	154.7	13.3	5,200	4.5	4.5	5.0	4.0
16	Four Seasons	13.3	25.0	GR	M	6.5	8.0	38.6	61.7	6.2	26,000	5.0	2.7	5.0	2.3
17	Gabriela	21.9	27.0	R	M	6.4	5.9	37.6	77.0	8.2	17,000	5.0	4.5	5.0	4.6
18	Giant Caesar	28.7	28.3	G	U	4.7	4.8	47.4	188.8	19.7	22,000	5.0	3.7	5.0	3.5
19	Grand Rapids	24.5	20.0	LG	U	5.9	5.7	35.7	98.5	9.4	19,000	1.6	4.0	5.0	1.6
20	Green salad bowl	12.8	27.3	G	LM	5.2	7.7	44.1	44.7	4.4	18,000	5.0	4.0	5.0	3.8
21	Green Ice	36.6	30.0	DG	M	4.3	5.2	37.4	112.3	11.2	26,000	5.0	4.0	5.0	4.6
22	Green Star	26.3	31.7	DG	U	6.1	10.3	42.9	154.0	14.7	32,000	4.0	4.2	5.0	3.5
23	Green Towers	25.3	19.7	DG	U	5.9	6.7	3.8	83.0	7.3	13,000	3.0	3.0	3.0	3.5
24	Iceberg A	23.7	24.3	LG	H	6.0	6.1	41.0	97.3	9.9	7,100	4.0	3.3	5.0	3.8
25	Igloo	20.3	27.3	DG	M	5.8	5.0	65.0	122.3	11.8	6,000	5.0	5.0	5.0	5.0
26	Jericho	19.8	29.0	G	U	5.7	3.8	43.1	130.7	15.8	11,000	5.0	3.0	5.0	2.3
27	Lettony	24.8	26.7	DG	U	5.8	3.3	60.1	145.3	15.4	6,200	4.3	4.0	5.0	4.8
28	Little Caesar	18.3	19.3	DG	U	6.9	4.3	44.3	135.7	12.7	12,000	5.0	3.7	5.0	3.3
29	Lollo	16.3	20.7	G	M	5.6	5.9	38.5	72.7	6.0	23,000	4.3	5.0	5.0	4.8
30	Looseleaf Blend	53.3	20.0	LG	U	5.8	3.9	27.8	137.6	13.5	19,000	1.0	2.7	5.0	1.3
31	Nevada	37.7	18.3	G	U	5.9	3.9	28.4	111.8	11.3	14,000	5.0	2.3	5.0	3.5
32	Oakleaf	17.0	24.3	LG	M	6.1	6.5	33.2	102.3	10.4	22,000	5.0	3.7	5.0	3.5
33	Outredgeous	18.8	33.0	RG	M	6.4	7.3	10.0	133.0	13.0	24,000	4.7	3.0	3.0	3.8
34	Parris Island	23.3	19.7	DG	U	6.8	4.6	47.1	143.3	15.3	14,000	5.0	3.7	5.0	3.7
35	Parris Island Cos	23.0	18.7	DG	U	6.6	5.9	48.3	129.7	12.3	14,000	5.0	3.7	5.0	3.6
36	Paris White	22.3	33.3	DG	U	5.8	5.3	48.1	111.7	12.8	20,000	5.0	4.0	5.0	4.2
37	Pomegranate Crunch	20.7	21.6	RG	U	5.6	5.3	45.8	79.0	7.6	18,000	5.0	5.0	5.0	5.0
38	Prizehead	18.7	23.3	LGR	M	4.1	9.2	25.6	87.3	8.7	39,000	5.0	4.0	5.0	3.3
39	Red Oak	17.0	28.3	RG	M	6.3	5.3	33.7	81.5	8.2	30,000	5.0	4.5	5.0	4.6
40	Red Romaine	28.8	30.3	RG	M	6.1	6.3	52.4	46.7	5.0	16,000	4.3	4.2	5.0	3.5
41	Refugio	29.7	28.3	R	M	5.5	6.3	39.5	40.7	3.8	31,000	5.0	4.7	5.0	3.5
42	Rex Rz	17.3	24.7	G	LM	6.3	5.2	40.2	154.0	14.6	27,000	5.0	4.5	5.0	4.5
43	Rhazes	20.8	28.3	RG	U	5.5	6.4	47.5	75.7	6.8	17,000	5.0	4.5	5.0	4.6
44	Romaine	29.7	28.0	DG	U	6.2	7.5	61.2	179.8	18.2	12,000	5.0	4.5	5.0	4.5
45	Rosalita	27.8	28.7	GR	U	6.5	7.4	58.8	194.3	18.8	12,000	4.0	4.5	5.0	3.5
46	Rouge d'Hiver	25.7	25.0	RG	U	6.3	6.6	47.7	87.7	9.2	12,000	4.0	1.8	3.0	1.5
47	Rouge Red	19.8	28.0	GR	M	6.5	3.3	72.6	88.0	10.1	9,900	5.0	3.5	5.0	4.0
48	Salad Bowl	22.0	15.3	LG	M	4.7	3.5	28.4	152.8	14.4	6,000	4.7	4.0	5.0	4.0
49	Silvia	29.8	27.3	GR	U	4.5	5.3	79.1	89.0	8.8	13,000	4.0	4.0	5.0	3.8
50	Simpson Elite	40.7	18.3	LG	U	6.1	4.1	72.3	144.3	13.3	16,000	1.0	4.0	5.0	1.0
51	Skibolt	30.3	38.7	LG	M	3.5	3.8	23.7	238.7	21.8	18,000	5.0	4.3	5.0	3.7
52	Summer Crisp	39.3	22.7	LG	U	5.6	5.1	35.5	132.8	13.5	14,000	5.0	3.0	5.0	3.5
53	Tango	66.3	19.7	G	U	5.3	8.9	46.3	87.5	8.6	19,000	1.0	1.0	5.0	1.0
54	Tennis Ball	24.7	18.3	LG	U	5.2	4.9	27.1	75.3	8.2	31,000	3.0	2.7	5.0	2.8
55	Vivian	28.7	28.0	DG	U	6.9	4.6	47.9	198.7	17.8	20,000	5.0	3.7	5.0	5.0
56	Winter Density	19.5	17.0	DG	U	6.0	6.3	35.4	75.3	7.5	6,000	4.7	4.0	5.0	5.0
57	11C3487	16.7	25.0	LG	LM	6.1	8.0	35.3	108.3	11.8	23,000	5.0	4.2	5.0	5.0
58	3SX820	10.0	24.7	LG	LM	6.1	5.8	35.3	109.7	11.1	22,000	5.0	5.0	5.0	5.0
59	3SX821	13.0	22.7	LG	LM	6.1	8.2	43.1	109.8	10.2	16,000	5.0	5.0	5.0	5.0
60	3SX822	15.7	24.7	LG	LM	6.2	8.9	32.6	125.7	13.8	21,000	5.0	4.0	5.0	5.0

[z]Plant color: DG-dark green, LMG-lime green, LG-light green, G-green, G-R-green-red, DR-dark red, G-green, R-G-red-green.
[y]Plant shape: LM-low mounded, U-upright, P-pyramidal, LV-low vase shape, M-mounded, V-vase, H-head.
[x]Percent sugar was measured by a refractometer.
[w]Measured by a SPAD meter.
[v]Measured by Horiba nitrate meter.
[u]Leaf edge burn was scored from 1 to 5, with 1 showing the highest leaf edge burn and 5 showing the lowest leaf edge burn.

Most plants developed leaf edge burns with a different degree of symptom expression (Table 1). The appearance of leaf margin burns perhaps was due to the bouts of temperature surge and low humidity caused by the failure of automatic temperature control system which occurred one weekend in March. Giant Caesar, Ansar, Coastal Star, Vivian, and Rex Rz produced highest fresh weights. The cultivars that scored the highest overall ratings were 11C3487, 3SX820, Vivian, Parris Island, Rex Rz, and Pomegranate Crunch. The general appearance of lettuce at the time of harvest is shown in Fig. 2.

Fig. 2. Plants of sixty different lettuce cultivars harvested after 6 weeks of culture on NFT system.

Acknowledgement

Support for this study was made available by a research grant fund received from the Specialty Crop Block Grant Program administered through the ND Department of Agriculture.

Changes in Nitrate Contents of Hydroponically Grown Leaf Lettuce as Influenced by the Ratio of Ammonium and Nitrate Forms of Nitrogen in Nutrient Solution

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Nitrates and Health

1. High nitrate concentration in drinking water causes creates the condition known as methemoglobinemia (the blue baby syndrome), in which blood lacks the ability to carry sufficient oxygen to the individual body cells causing the veins and skin to appear blue.
2. Nitrate in foods is converted into nitrite and nitroso-amines that could serve as carcinogen.

Leafy vegetables
↓
Nitrates
↓
Nitrite
↓
Nitrosamine
(carcinogen)

Nitrates in Water and Food

1. Drinking water
   EPA (1962) mandate: 10 ppm NO_3^--N
   (44.3 ppm NO_3^-)
2. Leafy vegetables
   European Union: about 5,000 ppm NO_3^-
   Concentration varies by crop and season.
   United States
   No regulation on nitrate content of vegetables.

Objectives

1. To determine the influence of nutrient solution N composition on leaf tissue nitrate concentration.
2. To characterize the growth and quality of lettuce grown with different combinations of NH_4^+ and NO_3^- in the nutrient solution.

Nutrient Solutions

1. Modified Hoagland solution.
2. Total nitrogen (N) concentration: 12 meq/liter.
3. Nitrogen compositions:
   Solution 1: 100% NH_4^+ + 0% NO_3^-
   Solution 2: 75% NH_4^+ + 25% NO_3^-
   Solution 3: 50% NH_4^+ + 50% NO_3^-
   Solution 4: 25% NH_4^+ + 75% NO_3^-
   Solution 5: 0% NH_4^+ + 100% NO_3^-

Solution 1: 100 % NH_4^+ + 0% NO_3^-

Nutrient	Ion concentration (meq/liter)												
	H^+	Ca^{2+}	Mg^{2+}	K^+	NH_4^+	Na^+	NO_3^-	SO_4^{2-}	$H_2PO_4^-$	Cl^-	HCO_3^-	OH^-	Total
Solution 1	0	4	3	4	12	0	0	6	11	2	4	0	46
1. $(NH_4)_2SO_4$					3			3					3
2. $NH_4H_2PO_4$					7				7				7
3. NH_4Cl					2					2			2
4. $MgSO_4 \cdot 7H_2O$			3					3					3
5. KH_2PO_4				4					4				4
6. $CaCO_3$		4									4		4
Total	0	4	3	4	12	0	0	6	11	2	4	0	46

Solution 2: 75 % NH_4^+ + 25 % NO_3^-

Nutrient	Ion concentration (meq/liter)												
	H^+	Ca^{2+}	Mg^{2+}	K^+	NH_4^+	Na^+	NO_3^-	SO_4^{2-}	$H_2PO_4^-$	Cl^-	HCO_3^-	OH^-	Total
Solution 2	0	6	4	5	9	0	3	7	9	1	4	0	48
$NH_4H_2PO_4$					7				7				7
NH_4NO_3					1		1						1
NH_4Cl					1					1			1
$CaNO_3 \cdot 4H_2O$		2					2						2
KH_2PO_4				2					2				2
$MgSO_4 \cdot 7H_2O$			4					4					8
K_2SO_4				3				3					3
$CaCO_3$		4									4		4
Total	0	6	4	5	9	0	3	7	9	1	4	0	48

Solution 3: 50 % NH_4^+ + 50 % NO_3^-

Nutrient	Ion concentration (meq/liter)												
	H^+	Ca^{2+}	Mg^{2+}	K^+	NH_4^+	Na^+	NO_3^-	SO_4^{2-}	$H_2PO_4^-$	Cl^-	HCO_3^-	OH^-	Total
Solution 3		7	6	4	6	2	6	10	7	0	0	2	50
$NH_4H_2PO_4$					3				3				3
NH_4NO_3					3		3						3
$CaNO_3 \cdot 4H_2O$		3					3						3
$CaSO_4 \cdot 2H_2O$		4						4					4
KH_2PO_4				4					4				4
NaOH						2						2	2
$MgSO_4 \cdot 7H_2O$			6					6					6
Total	0	7	6	4	6	2	6	10	7	0	0	2	50

Solution 4: 25 % NH_4^+ + 75 % NO_3^-

Nutrient	Ion concentration (meq/liter)												
	H^+	Ca^{2+}	Mg^{2+}	K^+	NH_4^+	Na^+	NO_3^-	SO_4^{2-}	$H_2PO_4^-$	Cl^-	HCO_3^-	OH^-	Total
Solution 4	0	7	4	7	3	5	9	6	7	0	0	4	52
NH_4NO_3					3		3						3
$CaNO_3 \cdot 4H_2O$		1					1						1
$CaSO_4 \cdot 2H_2O$		2						2					2
KH_2PO_4				3					3				3
$CaH_4(PO_4)_2$		4							4				4
$Mg(NO_3)_2 \cdot 6H_2O$			4				4						4
$NaNO_3$						1	1						1
NaOH						4						4	4
K_2SO_4				4				4					4
Total	0	7	4	7	3	5	9	6	7	0	0	4	52

Solution 5: 0 % NH_4^+ + 100 % NO_3^-

Nutrient	Ion concentration (meq/liter)												
	H^+	Ca^{2+}	Mg^{2+}	K^+	NH_4^+	Na^+	NO_3^-	SO_4^{2-}	$H_2PO_4^-$	Cl^-	HCO_3^-	OH^-	Total
Solution 5	0	8	6	7	0	2	12	3	6		0	2	46
KNO_3				3			3						3
$Ca(NO_3)_2 \cdot 4H_2O$		3					3						3
$Mg(NO_3)_2 \cdot 6H_2O$			6				6						6
KH_2PO_4				3					3				3
$CaH_4(PO_4)_2$		5							5				5
$CaSO_4 \cdot 2H_2O$		2						2					2
NaOH						2						2	2
K_2SO_4				1				1					1

A B

Fig. 1. The lettuce grown in the five different NH_4^+: NO_3^- ratio nutrient solutions in hydroponic tub cultures: A-seedlings anchored on Styrofoam boards, B-two weeks later.

Fig. 2. The lettuce grown in the five different NH_4^+: NO_3^- ratio nutrient solutions in hydroponic tub cultures.

Fig. 3. A close-up of lettuce plants grown for 4 weeks in five different nitrogen combinations in the nutrient solution.

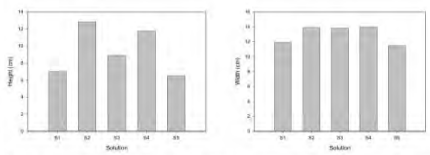


Fig. 4. Plant growth as influenced by five different combinations of NH_4^+ and NO_3^- forms of nitrogen: S1 (100% NH_4^+), S2 (75% NH_4^+, 25% NO_3^-), S3 (50% NH_4^+, 50% NO_3^-), S4 (25% NH_4^+, 75% NO_3^-), S5 (100% NO_3^-).

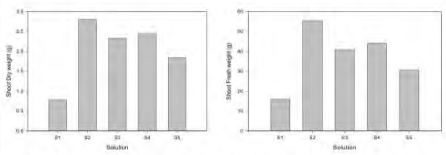


Fig. 5. Plant weight as influenced by five different combinations of NH_4^+ and NO_3^- forms of nitrogen: S1 (100% NH_4^+), S2 (75% NH_4^+, 25% NO_3^-), S3 (50% NH_4^+, 50% NO_3^-), S4 (25% NH_4^+, 75% NO_3^-), S5 (100% NO_3^-).

Fig. 6. Plant weight as influenced by five different combinations of NH_4^+ and NO_3^- forms of nitrogen: S1 (100% NH_4^+), S2 (75% NH_4^+, 25% NO_3^-), S3 (50% NH_4^+, 50% NO_3^-), S4 (25% NH_4^+, 75% NO_3^-), S5 (100% NO_3^-).

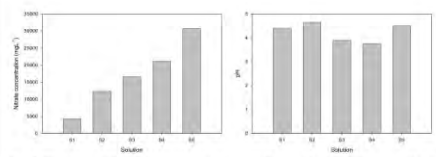


Fig. 7. Plant tissue pH and nitrate concentration as influenced by five different combinations of NH_4^+ and NO_3^- forms of nitrogen: S1 (100% NH_4^+), S2 (75% NH_4^+, 25% NO_3^-), S3 (50% NH_4^+, 50% NO_3^-), S4 (25% NH_4^+, 75% NO_3^-), S5 (100% NO_3^-).

Table 1. Influence of five different nutrient solutions on growth of Rex RZ lettuce for four weeks cultivation.

Treatment[x]	Plant height (cm)	Plant width (cm)	pH	Brix (%)	Chlorophyll content (SPAD)	Fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight(g)
S 1[y]	7.0 c	11.9 ab	4.4 abc	3.6 ab	26.8 a	16.0 c	0.8 c	10.3 c	1.67 b
S 2	12.8 a	13.9 a	4.7 a	3.6 ab	29.4 a	55.5 a	2.8 a	15.7 ab	2.07 ab
S 3	8.9 bc	13.8 a	3.9 bc	4.1 a	26.6 a	40.8 b	2.3 ab	12.7 bc	1.95 ab
S 4	11.8 ab	14.0 a	3.8 c	2.8 b	21.0 a	44.2 ab	2.4 ab	15.3 ab	1.89 ab
S 5	6.5 c	11.5 b	4.5 ab	4.6 a	23.9 a	30.7 b	1.8 b	17.8 a	2.28 a
Significance	***	*	*	ns	ns	***	***	**	ns
P-value	0.0004	0.0339	0.292	0.0521	0.3092	0	0	0.0043	0.1053

[x]Abbreviations in nutrient solution: S1 : NH_4^+ 100% + NO_3^- 0%, S2 : NH_4^+ 25% + NO_3^- 75%, S3 : NH_4^+ 50% + NO_3^- 50%, S4 : NH_4^+ 25% + NO_3^- 75%, S5 : NO_3^- 100%.
[y]Duncan's multiple range test significance: *, ** and *** at P≥0.05, 0.001, and 0.0005, respectively.

Fig. 8. Rex Rz lettuce plants grown hydroponically in solutions containing five different combinations of NH_4^+ and NO_3^- forms of nitrogen for 4 weeks.

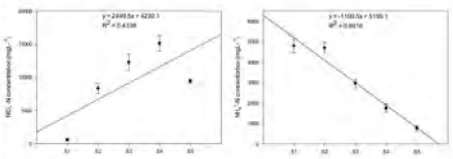


Fig. 9. NO_3^--N and NH_4^+-N concentrations in dried Rex RZ lettuce samples measured by Kjeldahl method: S1 = NH_4^+ 100% + NO_3^- 0%, S2 = NH_4^+ 25% + NO_3^- 75%, S3 = NH_4^+ 50% + NO_3^- 50%, S4 = NH_4^+ 25% + NO_3^- 75%, S5 = NO_3^- 100%).

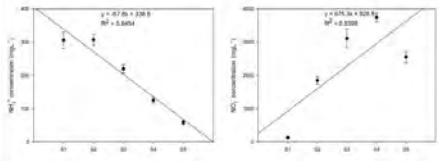


Fig. 10. NO_3^- and NH_4^+ concentrations calculated by multiplying and converting with fresh growth rate and dried sample value measured by Kjeldahl method: S1 = NH_4^+ 100% + NO_3^- 0%, S2 = NH_4^+ 25% + NO_3^- 75%, S3 = NH_4^+ 50% + NO_3^- 50%, S4 = NH_4^+ 25% + NO_3^- 75%, S5 = NO_3^- 100%).

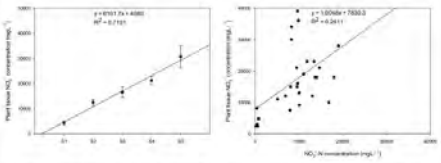


Fig. 11. Correlation between plant tissue NO_3^- (measured by using portable nitrate meter during harvesting) and NO_3^--N concentrations calculated measured by Kjeldahl method.

Fig. 12. Close-up of lettuce leave from different treatments.

Fig. 13. Close-up of lettuce grown with nutrient solution containing 100% NO_3^- form of nitrogen.

Fig. 14. Close-up of lettuce grown with nutrient solution containing 1:1 ratio of NH_4^+ and NO_3^- forms of nitrogen.

Tissue Nitrate Concentration Measurement

1. The Kjeldahl method
2. Portable nitrate meters

Horiba Nitrate Meter

Summary

1. The growth of the plants was the greatest when it was grown with 1:1 mixture of NH_4^+ and NO_3^- (solution 3). Tissue nitrate content in this treatment was about 4,000 ppm higher compared to solution 2.
2. Difference in biomass yield between the solution 3 and solution 2 was statistically not significant.
3. As tissue NO_3^- levels increased, plant sap pH values increased and the leaf chlorophyll index became lower. It appears that a hydroponic solution containing 75% NH_4^++25% NO_3^- can effectively be used for growing lettuce to lower leaf nitrate levels without sacrificing yield and produce quality.

Acknowledgements

Specialty Crop Block Grant administered through North Dakota Department of Agriculture.

Thank You!

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

Final Report

Partner Organization: North Dakota State University

Project Summary

Seed potato production in North Dakota and North America has been significantly impacted by Potato Virus Y (PVY). PVY is easily transmitted, primarily by aphid vectors, in a non-persistent manner. Significant yield losses of commercial production may result, and seed potato fields may fail certification due to low virus levels. Several potato cultivars are asymptomatic for visual symptoms, while new and recombinant PVY strains generally cause mild or no foliar symptoms. Visual assessment techniques and management tools are often unreliable, making a rapid, accurate assessment method for PVY infection in certified seed fields an important advancement. PVY infection generally decreases chlorophyll content, causing a decline in reflected light energy, particularly in the Near Infrared (NIR) wavelengths. Ten genotypes were evaluated using a Flame Miniature Spectroradiometer, utilizing OceanView v1.5 software, with a pole mounted sensor. Reflectance curves were generated for $PVY^{N:O}$ infected plants, compared to clean (uninfected) plants. A handheld SPAD 502 Plus Chlorophyll Meter was used to estimate chlorophyll content for plants from both treatments. Greenhouse study results indicate genotypes differ for reflectance and estimated chlorophyll content. Reflectance curves for infected plants varied from uninfected plants 4 weeks following emergence. Conversely, estimated chlorophyll content differed at 8 weeks post emergence.

The purpose of this project was to assess the potential for remotely sensing Potato Virus Y (PVY) in seed potato fields as an accurate, rapid management tool. Spectral reflectance and chlorophyll content (estimated) of infected and uninfected potato genotypes and newly released cultivars from the NDSU potato breeding program were obtained in field and greenhouse trials. Results will be validated in certified seed potato fields in the future.

The project is important and timely because the ND and MN seed potato industries have suffered significant declines since 1995, primarily due to PVY, an aphid vectored, viral disease. New PVY strains, with varying levels of symptom expression, along with new vector species (including soybean aphid) have resulted in an explosion of decertification of seed lots due to viral issues across North America, including ND and MN. The ordinary strain of PVY (PVY^{O}) causes mild to severe mosaic (depending upon cultivar), leaf drop, and leaf and stem necrosis. New strains including PVY^{N} and PVY^{NTN} symptoms are generally very mild or asymptomatic, and are often indistinguishable by the human eye. Additionally, PVY^{NTN} causes potato tuber necrotic ringspot disease (PTNRD), rendering tubers unmarketable for fresh consumption and processing.

This project does not build on any previously funded projects with the SCBGP.

Project Approach

A rapid, in-field diagnostic tool for assessment and identification of infected plants would be extremely valuable. The following 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.

In 2015, a field experiment was conducted at Grand Forks, ND, evaluating 10 genotypes (Table 1), inoculated with PVY^{O} and PVY^{N}, compared to the uninoculated controls. The canopies of each plot (clone, treatment, replicate) were analyzed for reflectance using a Flame Miniature Spectroradiometer (Ocean Optics, Dunedin, FL), utilizing OceanView v1.5 software (Ocean optics, Dunedin, FL), with a pole-mounted sensor. Irradiance was measured between 400 and 950 nm. A handheld SPAD 502 Plus Chlorophyll Meter (Spectrum Technologies, Inc., Plainfield, IL) was used to estimate the chlorophyll content in the optical density range of 650 to 940 nm. An enzyme-linked immunosorbent assay (ELISA) was used to confirm viral presence/absence in treatments. Spectral data was collected 3 times at 3 week intervals. Field trial results indicated that nearly all plots were infected with the recombinant strain $PVY^{N:O}$. Reflectance curves were generated; however, due to lack of virus-free controls, data is not presented. Results indicated PVY spread in the field; we did not use horticultural oil as a means to suppress aphid transmission.

Table 1. Genotypes utilized in 2015 and 2016 field and greenhouse trials.

2015 Genotypes	2016 Genotypes
Dakota Pearl * #	Chieftain
Dakota Ruby * #	Dakota Pearl
Dakota Trailblazer	Dakota Russet
ND7132-1R * #	Ivory Crisp
ND8305-1 * #	NorValley
Red LaSoda * #	Red LaSoda
Red LaSoda NY-20 #	Red Norland
Red Norland * #	Red Pontiac
Russet Burbank * #	Russet Burbank
Satina * #	Russet Norkotah
	Shepody
	Umatilla Russet

Two replicated greenhouse experiments (3 replicates) were also conducted in 2015, using 8 and 9 of the clones used in the field trial (denoted by a * and a #, respectively, in Table 1), and two treatments (virus-free and $PVY^{N:O}$ infected minitubers) planted in a randomized complete block design. PVY did not spread in the greenhouse based on ELISA results. Reflectance and chlorophyll levels were determined using the same instruments and technique. In the initial

experiment, reflectance data was collected 3 times at 3 week intervals, while in the second experiment, data were collected 3 times at 2 week intervals, all beginning 4 weeks post emergence. In the second experiment, the same stem was used throughout the assessments, while in the initial experiment different stems may have been used. Data is not presented for the initial trial due to slow emergence. Figures 1, 2 and 3, provide least squares means for the 9 clones evaluated, comparing the reflectance for virus-free and PVY infected minitubers.

Chlorophyll content was lower in the greenhouse (and field) for PVY infected clones. Compared to reflectance curves for the second greenhouse study, least square means comparing infected and virus-free plants, statistical differences were not found at 4 (Figures 4) and 6 weeks post emergence, but were statistically different at 8 weeks (Figure 5).

A similar field experiment was planted at Grand Forks, ND, in 2016, using PVY^{o} and $PVY^{n:o}$ (the most prevalent strain in ND and MN). Plants grown from disease-free minitubers (time did not allow production of PVY infected materials for planting in the field that would not be dormant) were inoculated in the field using infected foliar tissue and carborundum sprayed on with a paint gun. Despite this being a typical protocol, plant damage must not have been severe enough to provide wounds for virus entrance, as ELISA testing (done two times during the summer approximately 3 and 6 weeks post inoculation) results were negative for PVY infection. A new protocol for foliar inoculation has been garnered from A. Karosev (personal communication). Due to the PVY spread in the field in 2015, Aphoil® was applied twice weekly through the growing season. This mitigated PVY spread by aphid vectors. However, SPAD meter and reflectance readings were not taken due to the lack of PVY infection.

A greenhouse experiment was also conducted during the summer/fall of 2016 using Aphoil®. Minitubers of 3 cultivars (Dakota Pearl, Red LaSoda NY-20, and Russet Burbank) were planted in a randomized complete block design , with 3 replicates and 2 treatments (Aphoil® and untreated). Plots were sprayed with Aphoil® on Sept 29, at a concentration of 4% at a rate of 87 mL/m^2. Plots were evaluated for chlorophyll content using the SPAD meter and reflectance readings were taken 1, 4, and 8 days after treatment to determine if the Aphoil® will impact either. Based on reflectance data, the greatest differences in reflectance comparing treated and untreated plant materials existed between 520 and 670nm and between 730 and 950 nm, in the visible light (VIS) and near-infrared (NIR) areas of the electromagnetic spectrum respectively. Mineral oils, such as Aphoil® are an essential tool in certified seed potato grower's management toolbox for minimizing PVY infection by aphid vectors. Thus, it is crucial to understand how mineral oils affect reflectance when using spectral analysis as a potential PVY management tool. Our research continues in this area with a field trial in 2017.

Based on our results, one could conclude that remote sensing may have a place in certification, production and crop consulting. Growers, industry personnel and researchers need to be aware that reflectance varies by genotype and that virus-free and PVY infected plants of the same genotype will also have different reflectance patterns. This is an exciting area with much potential; however, continued and additional work is warranted.

Figure 1. Least squares means for reflectance curves determined from readings on December 22, 2015, 4 weeks post emergence, for virus-free (clean) and PVYN:O infected minitubers . Differences were significant at p = 0.01 based on analysis of variance (ANOVA).

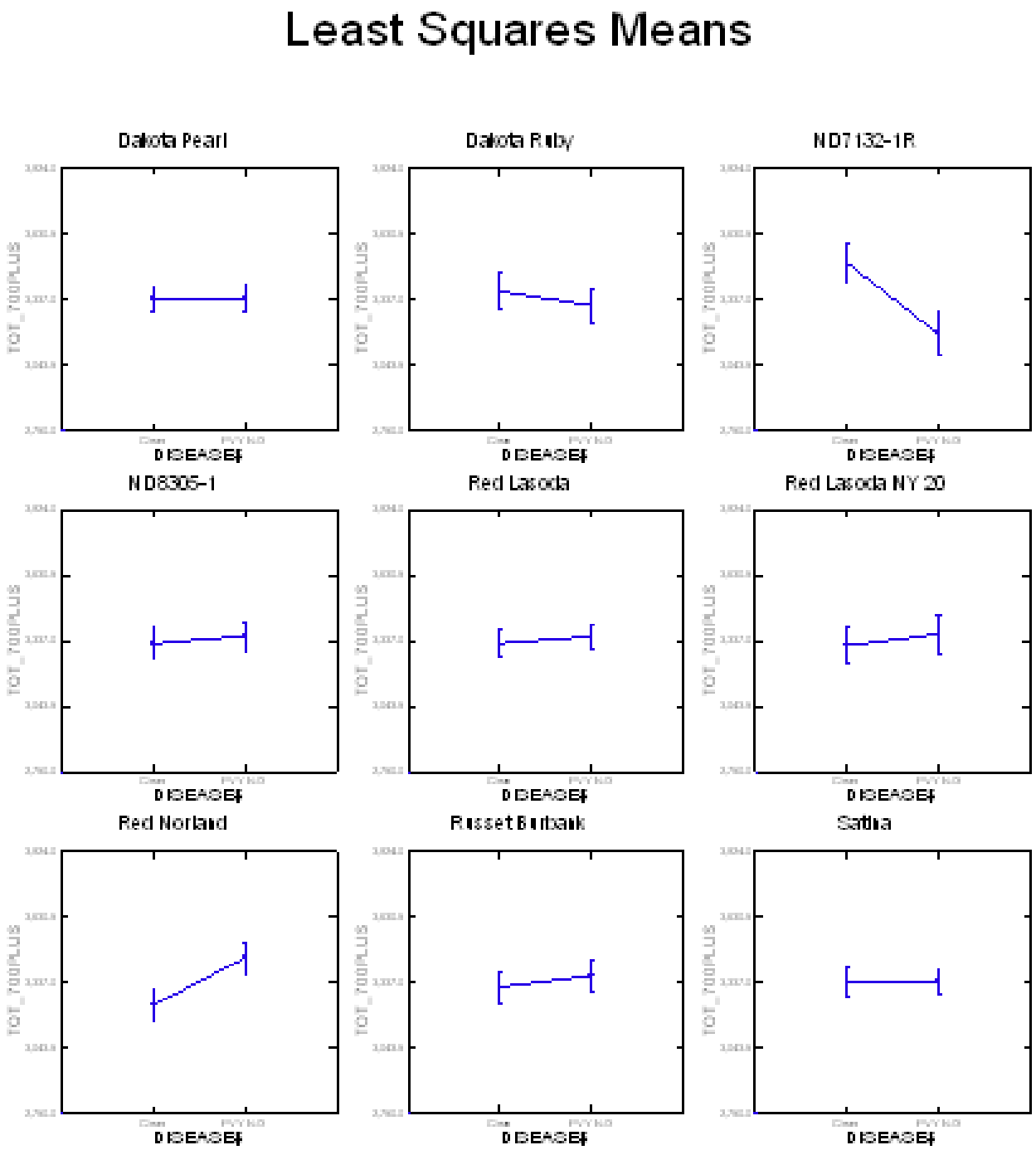


Figure 2. Least squares means for reflectance curves determined from readings on January 6, 2016, 6 weeks post emergence, for virus-free (clean) and PVYN:O infected minitubers. Differences were not statistically different.

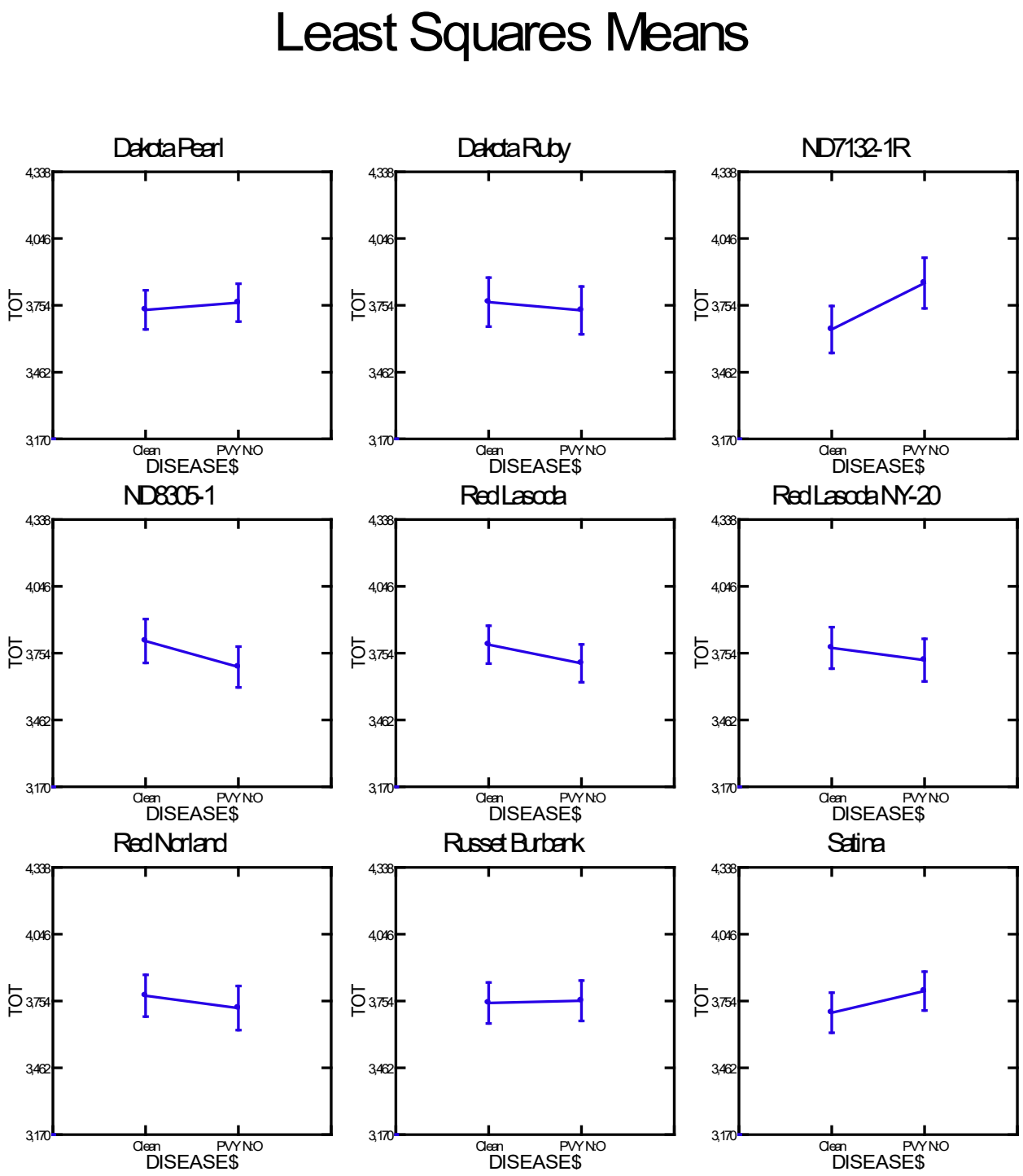


Figure 3. Least squares means for reflectance curves determined from readings on January 22, 2016, 8 weeks post emergence, for virus-free (clean) and PVYN:O infected minitubers. Differences were not statistically different.

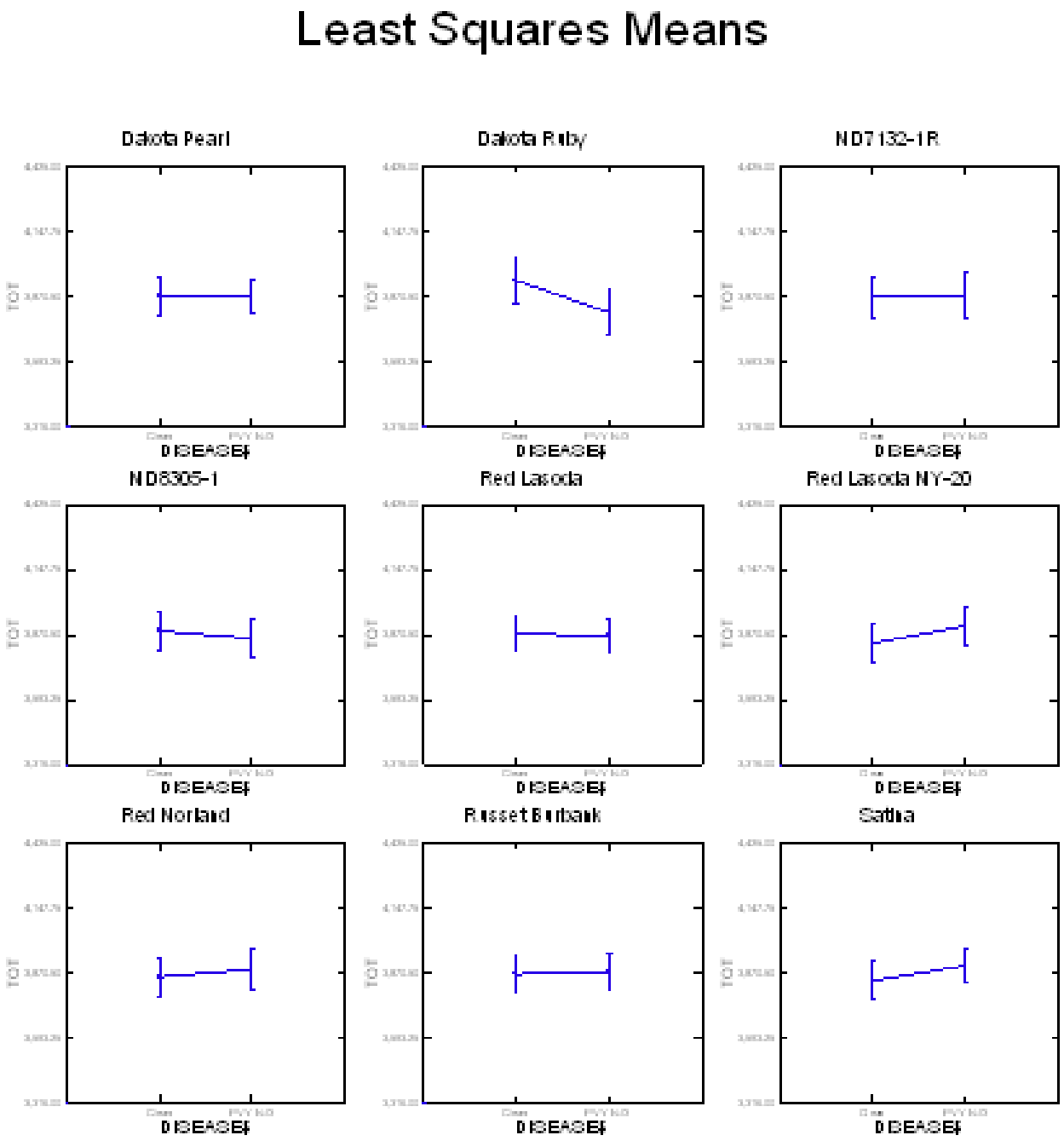


Figure 4. Least squares means for SPAD meter readings on December 22, 2015, 4 weeks post emergence, for virus-free (clean) and PVYN:O infected minitubers. Differences were not statistically different.

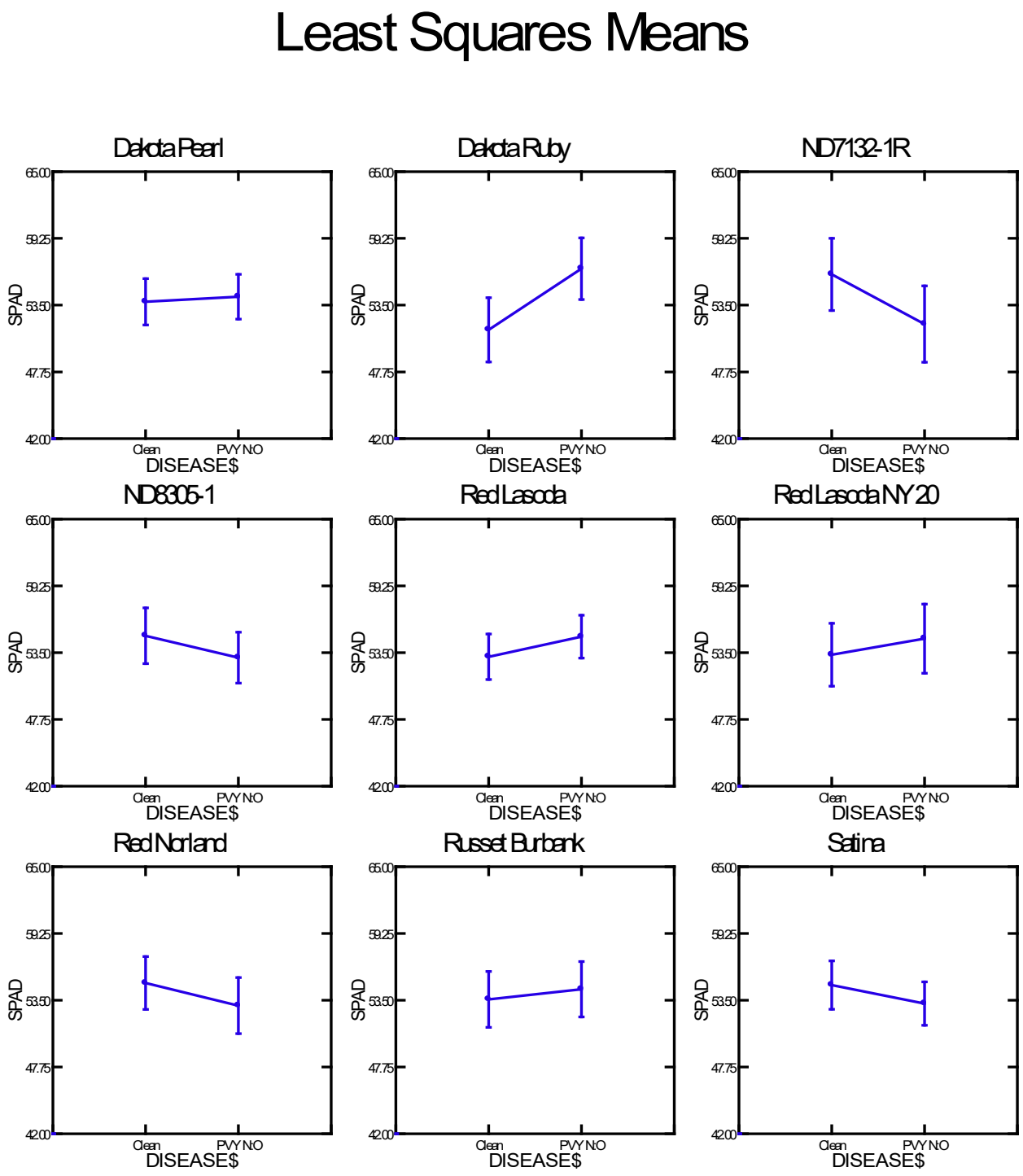
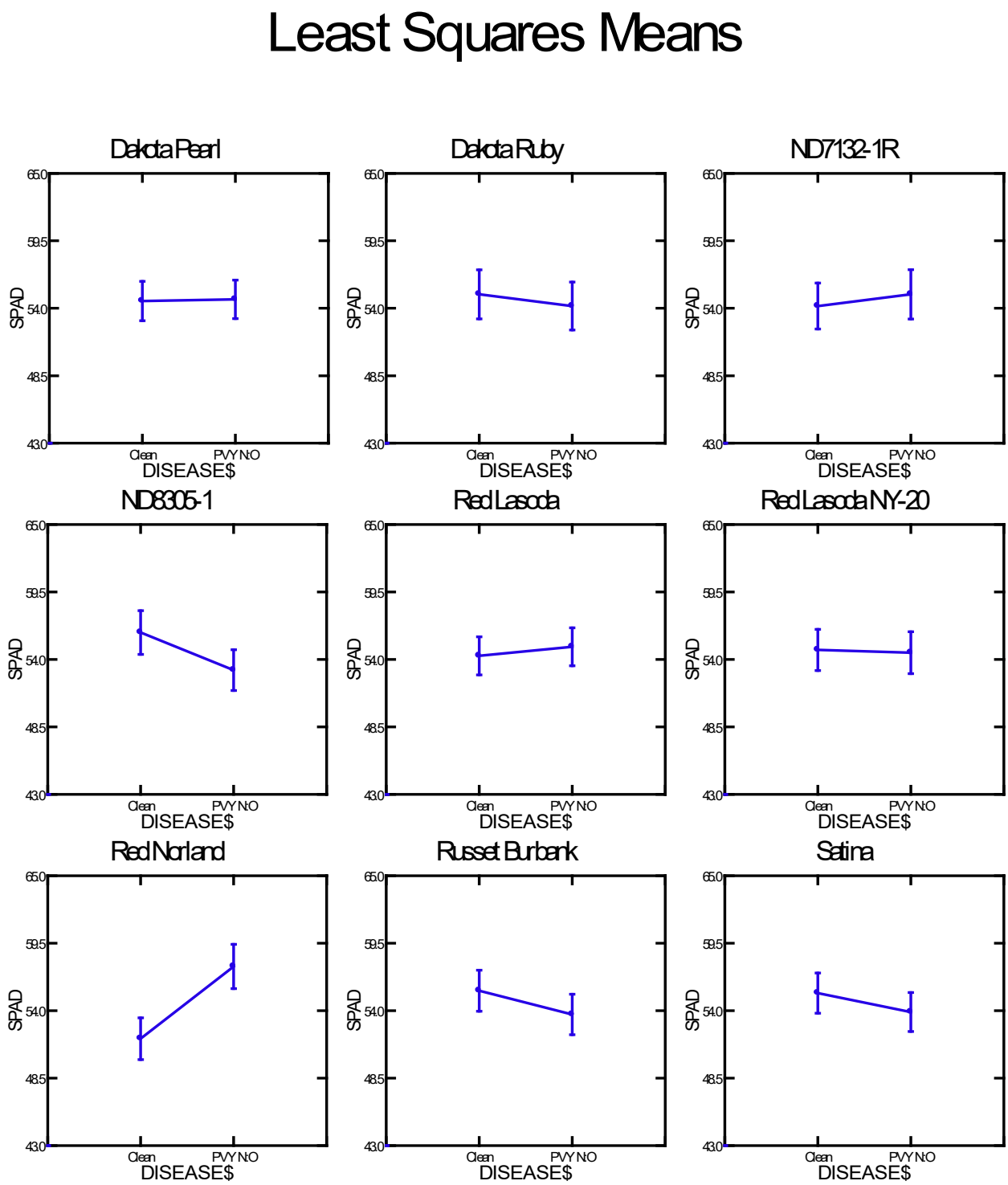


Figure 5. Least squares means for SPAD meter readings on January 22, 2015, 4 weeks post emergence, for virus-free (clean) and PVYN:O infected minitubers. Differences were statistically different at p = 0.01.



The graduate student participated in the Potato Association of America Annual Meeting in Grand Rapids, MI, in July 2016. His presentation entitled 'Remote Sensing of Potato Virus Y' was part of the graduate student oral paper competition. He will be participating at the annual meeting in Fargo, ND, July 23-27, 2017. The student also presented an oral update of his work at the Northern Plains Potato Growers Association Annual Field Day on August 25, 2016, to about 125 producers, industry personnel and other attendees at the Inkster, ND, field site, and participated in a graduate student symposium (oral paper presentation) in Saskatoon in March 2017.

Goals and Outcomes Achieved

GOAL:
This project was initiated to determine the wavelengths of reflected light associated with PVY infection of seed potato plants.
PERFORMANCE MEASURE:
Multi- and hyper-spectral data responses to PVY infection were measured and spectral reflectance curves constructed.
BENCHMARK:
Our benchmark was the comparison between PVY infected plants and those that were virus-free of the same cultivar, from greenhouse and field grown materials.
TARGET/PERFORMANCE MONITORING PLAN:
A technique based on spectral reflectance will provide a rapid, economical, and reliable method for determining in-field infection. Irradiance between 400 and 950 nm was determined to be satisfactory for recognizing PVY infected plants from virus-free plants.
Findings and management strategies have been and updates continue to be presented to potato producers and certification personnel at field days, research reporting conferences, and in the Valley Potato Grower magazine.

GOAL:
Our project will determine if these wavelengths (reflectance) are discernible in the greenhouse and field for new NDSU cultivar releases, advanced selections, and commonly grown cultivars by ND certified seed potato producers.
PERFORMANCE MEASURE
Multi- and hyper-spectral data responses to PVY infection were measured and spectral reflectance curves constructed for common cultivars produced in ND, new NDSU cultivar releases, and potential new releases (Table 1).
BENCHMARK:
Our benchmark was the comparison between virus-free and PVY infected plants of the same genotye, from greenhouse and field grown materials.
TARGET/PERFORMANCE MONITORING PLAN:
A technique based on spectral reflectance can provide a rapid, economical, and reliable method for determining in-field infection. Field results were difficult to obtain due to rampant virus spread in the plots in 2015 and lack of virus due to poor inoculation technique in 2016. We will continue to evaluate this in subsequent projects. Greenhouse results indicated differences 4 weeks post emergence of virus-free and PVY infected minitubers.

Our results and management strategies based upon them have been, and updates continue to be presented to seed and commercial potato producers, certification personnel and researchers at field days, research reporting conferences, and the Valley Potato Grower magazine, and will potentially be an important inclusion of cultivar releases published in the American Journal of Potato Research.

GOAL:
The proposed research will determine if PVY strains are discernible from one another in the greenhouse and field using spectral data.
PERFORMANCE MEASURE
Multi- and hyper-spectral data responses to PVY infection were measured, and spectral reflectance curves constructed for virus-free potato plants and those inoculated with different PVY strains (PVY^{O}, PVY^{N}).
BENCHMARK:
Our benchmark was the comparison between potato plants inoculated with different PVY strains, including those that are virus free of the same cultivar, from greenhouse and field grown materials.
TARGET/PERFORMANCE MONITORING PLAN:
Spectral reflectance provides a rapid, economical, and reliable method for determining status of in-field plants. Field trial results were confounded by viral strains recombining and thus our results are not publishable. We have not repeated in the greenhouse because more than 95% of PVY present in MN and ND have been identified as $PVY^{N:O}$ recombinants (N.C. Gudmestad, personal communication).
Results and management approaches will be presented to potato producers (seed and commercial) and certification personnel at field days, research reporting conferences, and via the Valley Potato Grower magazine. Additionally, results will be discussed at the NCCC215 regional potato breeding and genetics meeting, the Potato Association of America annual meeting, and will be submitted for publication in the American Journal of Potato Research.

GOAL:
Our proposed research will determine if PVY infection can be differentiated from nutrient deficiency (specifically nitrogen) and PVS (a common virus infecting commercial and seed potatoes, causing similar but again very mild symptoms) in the greenhouse and field using spectral data.
PERFORMANCE MEASURE
Multi- and hyper-spectral data responses to PVY infection versus nitrogen deficiency and PVS infection will be measured, vegetative index values determined and spectral reflectance curves constructed.
BENCHMARK:
Our benchmark will be the comparison between potato plants inoculated with PVY strains, compared to nitrogen stressed plants, those infected with PVS, and those that are virus free of the same cultivar, from greenhouse and field grown materials.
TARGET/PERFORMANCE MONITORING PLAN:
Spectral reflectance provides a rapid, economical, and reliable method for determining status of in-field plants. Being able to ascertain abiotic and biotic stresses from one another will be key in adoption by producers, certification agencies, and agri-consultants. Based on the size of data

sets for the field and greenhouse trials presented above, it was determined that this goal would have to become the focus of Specialty Crop Block Grant proposal submissions made in 2015 and 2016. It was beyond the scope of one master's level student to complete in 2-3 years. A second student is on board and additional funding was secured. Conclusions and management approaches will be presented to seed and commercial potato producers, certification personnel, and industry representatives at field days, research reporting conferences, via trade publications such as the Valley Potato Grower magazine, and be submitted for publication in the American Journal of Potato Research.

Beneficiaries

The beneficiaries of this project are the certified seed potato growers, approximately 63 in ND and MN. Commercial producers in this region (approximately 250) rely on these producers for seed; thus, protecting the ND/MN seed industries would have a positive impact by safeguarding the +$408M generated by the regional potato industry in 2016. Finally, our technology/technique will have global potential, as potatoes are the fourth most grown food crop worldwide, following wheat, corn and rice.

Lessons Learned

Per quarterly and annual reports, some problems did arise. In 2015, we experienced virus spread in the field, so at the end of the experiment we had $PVY^{n:o}$ present. In order to mitigate, Aphoil® was used in 2016. We successful prevented PVY spread by aphids using this mineral oil. However, this brought a new challenge, as we were unsure how this mineral oil would impact chlorophyll levels and spectral imaging.

Inoculation of the field experiment in 2016 using a paint spray gun and carborundum for wounding the potato leaf tissue and allowing for penetration of the virus did not work. Thus, SPAD meter readings and reflectance data for PVY infection could not be assessed. However, we have garnered a new protocol (successfully developed and employed by Dr. Alex Karasev, University of Idaho) to utilize in our current field and greenhouse trials.

We learned that it was better to do reflectance and SPAD meter readings more frequently (every 2 versus 3 weeks). And, in order to minimize error from a statistical stand point, we found that identifying the same stem for 4, 6 and 8-week post emergence readings was more accurate for the greenhouse and field trials, and similarly for the mineral oil experiment to use the same stem on days 1, 4 and 8 post treatment.
It was determined that objective 4 was beyond the scope of one student when working on objectives 1-3. A new graduate student started in January 2017, to focus on objective 4. Had hoped she would have been on board late August 2016; however, her paperwork was delayed.

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Specialty Crop International Expansion

Final Report

Partner Organization: North Dakota Department of Agriculture

Project Summary

This project focused on the international promotion of North Dakota's specialty crops available for export. To achieve this, the North Dakota Department of Agriculture (NDDA) focused on engaging specialty crop export companies, producers and commodity groups in various trade/buyers missions sponsored by the Food Export Association. By attending these events, North Dakota specialty crop groups were able to make face-to-face contact with potential buyers, participate in market research, and learn the details of exporting all while promoting/educating other parts of the world on the health benefits of specialty crops. This, in turn, has the potential to increase sales as well as to provide a sustainable future for the industry. As the world's population continues to grow, it is very important to keep an eye on world markets and to do our part in keeping our population healthy.

The major issue at hand is that the world's population is currently at 7.5 billion, and it's expected to reach 9.8 billion by 2050, according to forecasts by the United Nations. Ninety-five percent of the world's population currently lives outside of the United States and holds two-thirds of the world's purchasing power. This highlights the opportunity for the international promotion of North Dakota's specialty crops. Increased interest and demand can be created internationally by taking part in the various market promotion programs offered through NDDA in conjunction with Food Export Midwest.

There were three major objectives for this project. First, to connect North Dakota specialty crop producers with interested buyers overseas. This was accomplished by engaging them in Food Export's Specialty Food Buyers Missions or Focused Trade missions for specialty and natural products. Secondly, the project provided a great opportunity for the specialty crop producers who are interested in exporting but don't necessarily have the capital to spend on market research. Grant funds awarded allowed those producers to make contacts in various countries and find outlets/wholesale markets for their products. Finally, our exporters were able to educate the consumers in foreign countries on the health benefits of consuming various specialty crops, which, in turn, should create more demand in the marketplace.

NDDA currently has no state budget for any international activities involving expansion of specialty crops, so this project did not supplant state funds. As the world's population continues to increase, it is very important that we try our best to provide as many people with a healthy/reliable food chain, like pulse crops and dry edible beans. By ensuring that North Dakota pulse and dry edible bean producers, exporters, and commodity groups increase contacts in viable markets, while promoting the health benefits of these specialty crops, we should be able to provide a sustainable future for the industry.

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

Project Approach

This has been a slow but successful program for our Specialty Crop International Expansion Grant. Two companies, CalDak International and JM Grain, applied and were approved for grants to participate in international trade missions/shows with Food Export Midwest.

CalDak International of Fargo, ND, requested funds to take part in Food Export Midwest's Food Show Plus! program at SIAL China in Shanghai. The awarded funds were used to reimburse two individuals from the Fargo office for airfare, booth fee, registration fees, and meal expenses while traveling, as it was written into the grant. The purpose of their project was to promote North Dakota dry edible beans and confection sunflower seeds to qualified buyers in the regions of China, Hong Kong and Taiwan. As food safety has been a main issue in these markets, more consumers are looking for higher-quality U.S. food products.

CalDak International chose to participate in SIAL China because it's Asia's largest international food tradeshow that targets importers/distributers in Shanghai, China. Attendance at the show was 2,734 exhibitors and more than 55,000 attendees. Their booth was located in the USA pavilion, which generated a lot of attention due to its close proximity to the exhibit entrance. CalDak reported they had 15 individual meetings with qualified buyers, made contact with over 60 purchasers/traders, and handed out more than 150 brochures on North Dakota's dry edible beans and confection sunflowers.

JM Grain of Garrison, ND, requested funds to take part in Food Export Midwest's Focused Trade Missions to Singapore, the Philippines, and Cambodia in Shanghai. JM Grain promotes and markets pulse crops to international markets. The trade missions allowed them to expand markets for our specialty crops into countries that import and need specialty crops. Market expansion is necessary to give the farmer an outlet for his products that would not exist without exporting. JM Grain has not previously exported into Singapore or Cambodia and they believe the Philippines has an expanded need that the United States/North Dakota can fill if they can form the relationships necessary to develop and meet those needs. The costs as well as research would be prohibitive for their company alone; thus, the grant supports the project and allows it to make possible expansion of markets. Funds were used to reimburse travelers for airfare, registration fees, meals and hotel expenses as written into the grant application.

JM Grain representatives met with buyers and merchandisers in Singapore, the Philippines, and Cambodia during a trade mission to southeast Asia in October 2015. Opportunities for exporting specialty crops of peas, lentils and chickpeas into all three markets were explored. Singapore is almost entirely dependent on imports for its food requirements as well as being a major distribution point into other southeast Asia counties. They met with nine potential customers ranging from wholesale and redistribution companies to the retail market sector. Singapore is most interested in whole peas for snack foods. In the Philippines, they found the market to be very positive for the specialty crops of peas, chickpeas and, to a lesser extent, lentils. They discussed business with eight canneries, snack food and marketing companies. Cambodia is an emerging market for specialty crops. The retail sector imports peas and chickpeas and repackages them for the higher-end grocery stores. They met with seven companies, primarily from the retail sector, as well as a company that is interested in bulk product for re-exporting.

Specialty crop funds were only used for allowable activities, no unallowable activities such as animal feed were associated with this trip.

JM Grain participated in the Food Export Midwest Trade Missions to Singapore, the Philippines, and Cambodia during October (Oct. 17-19 in Singapore; Oct. 20-21 in the Philippines; and October 22-23 in Cambodia), meeting with potential buyers, visiting retail markets in each country and learning about trade barriers as well as trade agreements. During this time they met with a total of 24 contacts beginning the relationships necessary to conduct business in each location. Their understanding of market trends, demands and opportunities for each country, through the conversations, presentations, statistics, market research and embassy support is invaluable as they venture into potential contracts. In Singapore, they visited with a large company's representative that could result in contracts. The Philippines experience allowed them to explain and offer how our specialty crops can be used for human consumption. The result was JM Grain sending peas for a soak test to one of their largest canneries. At this time, they haven't sold any peas yet but are keeping in contact with some of the big players for the cannery business and snack food industry in the Philippines. Cambodia is at the developing stages and the visit puts JM Grain on the ground floor for sending product into their retail stores. This was a first-time trade mission into this country and they were able to participate through the grant.

Marketing materials were also developed and distributed to promote North Dakota specialty crops. The brochures highlighted dry edible beans, dry edible peas, lentils, honey, confection sunflower and potatoes. The brochures include stats for each commodity and contact information for the associated commodity group.

Goals and Outcomes Achieved

Expected Measurable Outcomes:
Goal: To increase foreign market interest/awareness of North Dakota pea/lentil, dry bean and commodity groups.

Performance Measure: Quarterly surveys/questionnaire submissions will be required.
Benchmark: Our benchmark is zero, because this was a new program.
Target: To attract five to eight export companies with foreign export market awareness.

Progress was monitored by tracking the amount of contacts made in various countries, as well as monitoring sales. After exporters returned from their trips, NDDA required them to fill out a short questionnaire, type a written report on the market they visited and report any increases in sales. The surveys and reports were required to the department quarterly. NDDA compiled the data in a 2015 report to send out to all specialty crop groups/producers to show the impact that this program had on exports throughout the year.

Two companies, CalDak International and JM Grain, applied and were approved for grants to participate in international trade missions/shows with Food Export Midwest. CalDak exceeded expectations by meeting with 15 different qualified buyers, and making contact with 60 purchasers/traders. Their goal after they returned was to follow up with these individuals and to start making offers on confection sunflowers and dry edible beans. Currently prices are not

competitive and the international buyers are sourcing from other countries but they keep in contact in anticipation of the markets changing. JM Grain exceeded expectations by meeting with at least 24 different qualified buyers while traveling to all three countries. JM Grain plans to solidify contracts both in Singapore and the Philippines with ongoing conversation with Cambodia. They met with a buyer from the Philippines earlier this year in the U.S. and have continued contact through email and phone.

The marketing materials developed to promote North Dakota specialty crops were distributed for use by companies on specialty crop missions. The brochures are an easy reference for interested buyers to be able to get into contact with specialty crop commodity groups.

Beneficiaries

North Dakota pulse, dry edible bean and confection sunflower producers, exporters and commodity groups were able to increase contacts in viable markets while promoting the health benefits of these specialty crops. The relationship-building that took place with buyers in China, Hong Kong, Taiwan, Singapore, Cambodia and the Philippines will benefit North Dakota's reputation for quality food products.

Overall, the beneficiaries of the project were the two exporters and the 110 producers that supply them, the 835 confection sunflower growers in North Dakota, the 1,700 dry bean growers in North Dakota, the 1,111 pulse growers in North Dakota and the reputation of the state. *Note: Sunflower and dry bean numbers based on reports from North Dakota commodity groups. Pulse number based on latest available census of agriculture (2012).*

They were able to increase contacts in viable markets while promoting the health benefits of these specialty crops. North Dakota's reputation will benefit due to the relationships built with buyers in China, Hong Kong, Taiwan, Singapore, Cambodia and the Philippines.

Lessons Learned

The goal was to increase foreign market interest and awareness of North Dakota pea/lentil, dry bean and commodity groups. The target was to attract five to eight export companies with foreign export market awareness. Foreign market interest and awareness was definitely increased through the project. CalDak International had 15 individual meetings with qualified buyers, made contact with over 60 purchasers/traders and handed out more than 150 brochures on North Dakota's dry edible beans and confection sunflowers. JM Grain exceeded expectations by meeting with at least 24 different qualified buyers while traveling to Singapore, the Philippines and Cambodia. As relationships take time to build, these companies are continuing to follow up with buyers from these companies. The target of five to eight export companies was not reached. In the end, two companies were able to take part in the program. This was due to several reasons. One of the difficulties was that the Food Export deadlines to register for activities closed very early in the program year. In some cases, it was hard for companies to make a commitment that far in the future. Another factor was the availability of competing financing for similar projects that had fewer reporting requirements.

For the companies that were able to work within the timelines and guidelines, this was a very positive experience for their companies, North Dakota producers and commodity groups. The connections made provide a solid foundation for the expansion of North Dakota specialty crop exports.

Contact Information

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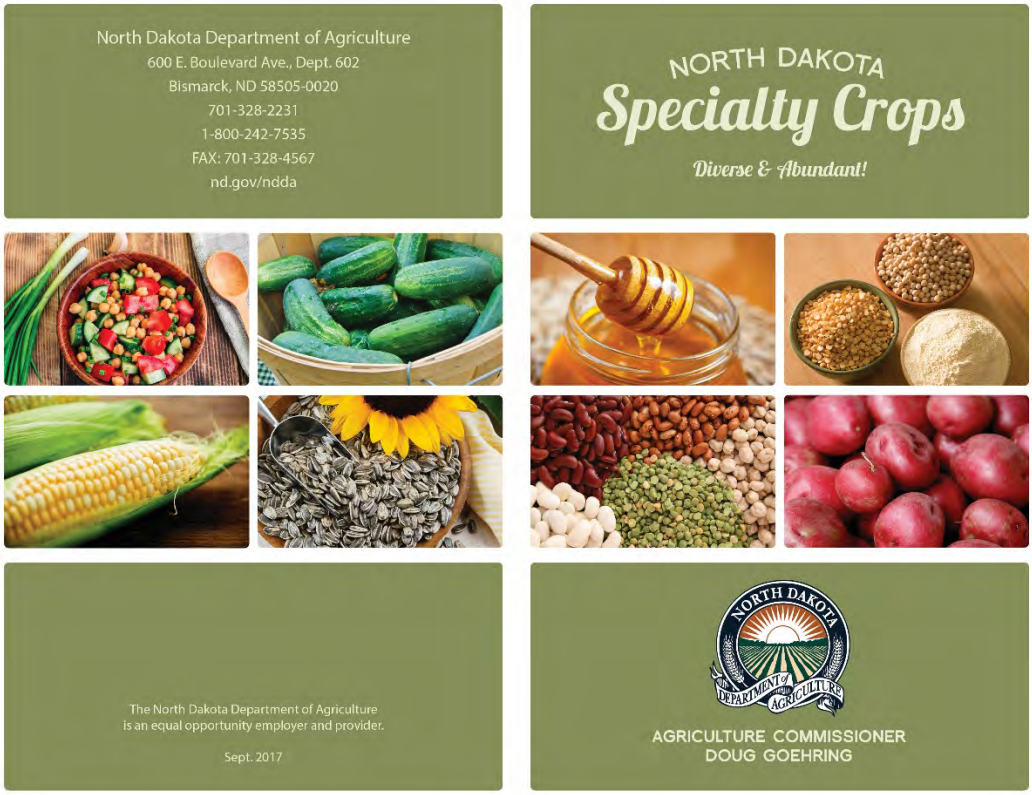
Additional Information

The marketing piece developed to promote North Dakota specialty crops (outside and inside).



North Dakota is home to a variety of specialty crops and is a leader in the production of dry edible beans, dry edible peas, lentils, honey, confection sunflower and potatoes.

Dry Edible Beans

North Dakota is the No. 1 producer of dry edible beans in the U.S., producing nearly 30 percent of the U.S. total. Some of the top varieties include pinto beans, black beans and navy beans. Dark red kidney beans, great northern beans, pink beans and small red beans are also grown.

Contact
Northarvest Bean Growers Assoc.
50072 East Lake Seven Road
Frazee, MN 56544
Ph: 218-334-6351
Email: nhbean@loretel.net
Web: www.northarvestbean.org

Dry Edible Peas

North Dakota is the No. 1 producer of dry edible peas in the U.S., producing more than 44 percent of the U.S. total. In 2016, North Dakota farmers produced more than 1.3 billion pounds of dry edible peas valued at $131.2 million.

Contact
Northern Pulse Growers Assoc.
1710 Burnt Boat Drive Suite 4
Bismarck, ND 58501
Ph: 701-222-0128
Email: info@northernpulse.com
Web: www.northernpulse.com

Lentils

North Dakota is the No. 2 producer of lentils in the U.S., producing more than 40 percent of the U.S. total. In 2016, North Dakota farmers produced more than 422 million pounds of lentils valued at $104.8 million.

Contact
Northern Pulse Growers Assoc.
1710 Burnt Boat Drive Suite 4
Bismarck, ND 58501
Ph: 701-222-0128
Email: info@northernpulse.com
Web: www.northernpulse.com

Honey

North Dakota is the No. 1 producer of honey in the U.S., producing more than 23 percent of the U.S. total. In 2016, North Dakota bees produced more than 37 million pounds of honey valued at $65.4 million.

Contact
North Dakota Beekeepers Assoc.
Will Nissen, President
900 38th St.
Minot, ND 58701
Ph: 701-838-4726
Email: nissen@minot.com
facebook.com/NDBeeAssociation/

Confection Sunflower

North Dakota is the No. 2 producer of non-oil, or confection, sunflower in the U.S., producing nearly 30 percent of the U.S. total. In 2016, North Dakota farmers produced more than 85 million pounds of confection sunflower valued at $22.3 million.

Contact
National Sunflower Association
John Sandbakken, Executive Director
2401 46th Ave. SE, Suite 206
Mandan, ND 58554
Ph: 701-328-5100
Web: www.sunflowernsa.com

Potatoes

North Dakota is the No. 4 producer of potatoes in the U.S. In 2016, North Dakota farmers produced more than 2.3 billion pounds of potatoes valued at $210 million.

Contact
Northern Plains Potato Growers Assoc.
Chuck Gunnerson, President
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420 Business Hwy. 2
East Grand Forks, MN 56721
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Web: www.nppga.org

Studies of Cold Acclimation of Winter Legumes

Final Report

Partner Organization: North Dakota State University

Project Summary

Research was conducted at North Dakota State University to identify QTL related to winter hardiness that can be used in breeding more winter hardy winter pea varieties for production in North Dakota. There are relatively few pea varieties that can be reliably produced in the region. A greenhouse methodology developed to identify consistent differences in freeze injury among lines was validated. The method was used to identify QTL using two mapping populations. Data were collected in the greenhouse using our new method and from the field on winter survival. 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. Future work on this project includes collecting field survival data on the second year of the field studies. Results from this study will allow for use of molecular marker-assisted selection to develop pea varieties with improved winter hardiness.

The ultimate 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. The specific objectives can be summarized as characterizing the agronomic and genetic components of winter pea production such that optimum agronomic practices and superior varieties can be developed. Significant delays in planting in the spring can be experienced which reduce seed yield and may result in situations where planting is not possible that season. The ability to establish a pulse crop in the fall eliminating the need for spring planting could alleviate difficulty in establishing a spring legume crop and maintain this agronomically valuable crop in rotations. 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. This project will provide growers valuable insight into production of fall-sown peas, new high yielding varieties and genetic characterization of winter hardiness and development of genetic markers will aid plant breeders in variety development.

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

Project Approach

The winter pea crop is sown in early fall allowing the plants to achieve a small rosette morphology with several branches prior to the onset of dormancy as temperatures decrease and snow cover occurs. Plants often have 2-5 branches achieving vine lengths of 7-15 cm and have 4-8 short internodes. It is typical that most if not all aboveground vegetative material is killed during the winter period and that plant growth and harvested seed is ultimately derived from axillary and nodal meristems at or below the soil surface. It is important to protect the nodes near the soil surface and the below ground epicotyl for successful winter pea survival (Dowker 1969).

In early spring as temperatures rise and snow melts the plants resume vegetative growth and it is at this stage that they are most vulnerable to cold and freezing injury since they have lost cold acclimation. Surviving plants continue to grow, flower, and are typically harvested 2-3 weeks prior to spring-sown varieties.

Current winter pea cultivars including Windham, Specter and others developed in Europe, despite being selected under relatively mild winter temperatures, have shown excellent winter survival and produced seed yield at Minot and Fargo, ND, in 2010 under harsh environments characteristic of the US Midwest. Winter survival at these two locations again in 2011 add further evidence that levels of winter hardiness currently available in the germplasm is adequate for production, but it is expected that additional breeding and selection in the harsh Midwest environments will allow genotypes with increased levels of hardiness to be identified. Establishment and demonstration of appropriate production methods for winter peas is necessary to transfer this technology to producers in the region and ensure successful crop production.

Winter legumes promise many advantages to producers and production across the US is expected to expand with the introduction of improved cultivars. Fall-sown legumes 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. Classic examples of this include the Camas Prairie region of northern Idaho (Muehlbauer 1996). Many fields in the Midwest, especially ND, are also good examples where fall-sowing would be a benefit. Fall-sown winter peas use available moisture more efficiently and typically flower during a cooler period of the summer minimizing floral abortion.

Successful field trials in ND in 2009 and 2010 indicate that production 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. Adoption of fall-sown pea production will require establishment of agronomic practices and characterization of genetic factors contributing to winter pea production.

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. In addition, the winter pea germplasm will be characterized for nutritional quality which is expected to provide growers and their seed processors with added marketing power when selling peas in the marketplace. 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.

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 were used where polymorphism can 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.

The NDSU pulse crop breeding program has several populations and a collection of germplasm with purported winter adaptation. These genotypes and populations were screened with markers identified with linkage to traits (QTL) associated with winter hardiness. Utility of the markers was validated across multiple populations and the degree of polymorphism and marker phase will be evaluated across the multiple winter hardy genotypes. Individual selections made based on marker genotype were verified and validated in the field.

- Genotyped the Shawnee'/'Melrose' and 'Medora'/Melrose' mapping populations, which allowed us to address objective 1.
- Grew the Shawnee'/'Melrose' and 'Medora'/Melrose' mapping populations in the field and greenhouse so data on winter survival and area under the injury curve could be collected, which allowed us to address objective 2.
- This project was specific to pea and will not be applicable to other crops.

1) The two mapping populations used in this study were increased in the field at Prosper, ND in 2015 and 2016. Adequate quantities of quality seed were obtained to complete the GH screening and evaluation for freezing tolerance.

2) DNA samples were collected from single plants of each recombinant inbred line and corresponding parents grown in the greenhouse. These samples were submitted for genotype by sequencing and analysis for identification of single nucleotide polymorphisms.
3) Field trials were established in the fall of 2015 and 2016 to evaluate winter survival of each genetic population. Stand counts were taken in the fall of each year, however, survival was only sufficient for the 2015 sowing to allow differential survival ratings to be taken. The 2016 sowing was evaluated on April 12, 2017 and complete death of the trial was observed due to low snow cover and severe cold conditions.
4) Greenhouse evaluation of the genetic populations has been completed and the data analyzed for distribution of survival ratings and combined with the genetic maps to identify quantitative trait loci.
5) The graduate student, Ms. Courtney Holdt, and the PI, Kevin McPhee, worked closely to ensure the project progressed and objectives were met.
6) The graduate student and the field crew made numerous trips to the field to establish the trials, maintain weed control, monitor for insect infestations, collect data and harvest. These activities occurred during the 2015 and 2016 summer field seasons.
7) Courtney Holdt attended the CSSA meetings in Arizona and attended two overseas conferences where she presented the progress/results of her project.
8) RNA extraction and RNAseq analysis were conducted on a time course set of samples for plants subjected to acclimation conditions. These samples were sequenced and the resulting files are currently being analyzed.
9) High density genetic maps were developed for both genetic populations. These genetic maps were used to identify quantitative trait loci contributing to freezing tolerance. The three QTL identified correspond to previously identified QTL lending confidence to the results.

Goals and Outcomes Achieved

Hartley's test for homogeneity proved that analysis of 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 (data not presented). Variation due to genotype was significant for both mapping populations.

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.

Quantitative trait analysis detected three significant QTL using AUIC values. When field survival was analyzed in the same manor, two significant QTL were found in close proximity to those found by the greenhouse protocol (Figure 1).

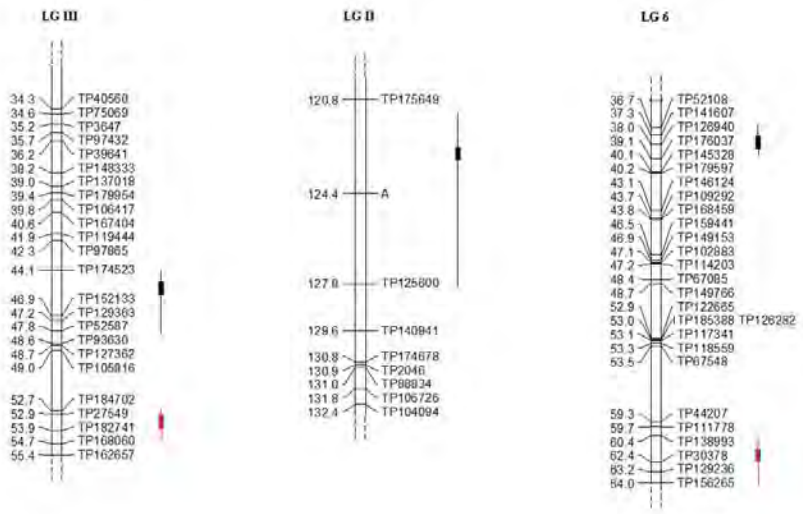

Figure 1. QTL for winter hardiness (black) and field survival (red) identified for the 'Shawnee'/'Melrose' population with Qgene and supported by QTL IciMapping on LG1, LG4 and LG6.

Our goal of two F7-derived mapping populations being developed was successful and WTLs for winter hardiness were identified and aligned with previously identified WTL from unrelated populations. We developed two genetic maps based on single nucleotide polymorphism markers and recorded the phenotypic data documenting the response of pea genotypes to cold and/or freezing conditions. This data from a freeze chamber was used to identify the QTL.
Our benchmark did not have genetic maps existing and the QTL based on Midwest environmental conditions had not been identified due to poor stand establishment or complete winter kill of the nursery. We completed our target of developing two genetic maps for winter x spring pea crosses with high market density. We also completed our target for QTL. QTL and their associated markers were identified based on artificial conditions in freezing chambers, however, inadequate field data prevented identification of WTLs based on natural field environmental conditions.

Beneficiaries

- Results from this research will benefit researchers that devlop winter pea varieties adapted to the northern Great Plains. Data will be used to more efficiently develop improved pea varieties using marker-assisted selection.
- The pulse breeding program at NDSU developed a new method of greenhouse screening for identification of winter hardy pea varieties.

I estimate the number of researchers that will benefit to be in the range of 200 or more worldwide. The number of people in the breeding program was 9.
Presentations:

- CSSA – 50 attendees
- International Legume meeting, Zambia, Africa - 150
- ILS, Portugal – 250
- Field Days – 150

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

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. The title of the paper she presented was *Evaluation of Winter Hardiness in Pea (Pisum sativum L.).* The abstracts and related citations are below.

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.

Enhance Potato Production and Marketing in Western North Dakota

Final Report

Partner Organization: WADF03, INC. d.b.a Williston Ag Diversification Group

Project Summary

This project enhanced the competitiveness of specialty potato variety MonDak Gold with limited commercial grower production in 2015, 2016, and 2017 for both specialty potato products and fresh pack markets. MonDak Gold is a specialty variety with superior culinary attributes, low levels of acrylamides, yellow flesh, resistance to sugar sweetening in storage, and suited to processing for superiority in making processed potato products from long term storage. Low levels of Acrylamide are an attribute in quality and marketability over Russet Burbank and other currently grown potato varieties. This project has helped attract potential new potato processors and fresh pack companies to the MonDak region. Research was conducted identified other novel varieties for adaptation to North Dakota with potential for specialty markets. The project served as a catalyst for innovative potato products to help attract investors to consider locating a potato processor in western North Dakota, and delivered a positive nutritional messages about potatoes to consumers about more nutritious potato products.

Project Approach

This project conducted research on multi-purpose specialty potato varieties/breeding lines that exhibited both excellent processing and fresh market attributes. The project provided a seed supply of MonDak Gold potatoes, a multi-purpose, red skin, yellow flesh potato variety with excellent culinary attributes such as taste, texture and color for commercial potato growers to supply MonDak Gold potatoes for introducing the MonDak Gold potato variety to specialty markets.

The project tested and evaluated newly breed advanced specialty breeding lines/cultivars from North Dakota State University, University of Minnesota and the Tri-State Potato Breeding Program in Idaho, Washington and Oregon, to serve as a catalyst for innovative potato products and use, deliver positive nutritional messages about potatoes to the trade as well as consumers and to create interest to attract a potato processor to our area. Western North Dakota was one of 2 possible locations considered by a group of investors for a specialty potato processing facility, with the other possible location in Southern Idaho.

The project built off of previously funded by SCBGP by producing new mini-tuber, Generation 1 and Generation 2 MonDak Gold seed potatoes for limited commercial grower production and marketing in 2015, 2016, and 2017. The growers participating conducted hands on test marketing of the MonDak Gold potatoes in their specialty potato products and fresh pack markets. The grant project provided evaluations of “niche” market potato lines and strip trials production and testing of advanced “niche” market multi-purpose potato lines for yield, disease resistance, desired agronomic characteristics and fry, roasted and fresh market qualities.

The objectives accomplished included the:

1) Evaluation of "niche" market potato breeding lines/cultivars grown in the irrigated Nesson Valley Research and Development (NVRD), Ray, North Dakota for yield, agronomic characteristics, disease resistance, storability, grading, fries, roasted and fresh market quality factors, in the fall and winter of 2014-2015, 2015-2016, and 2016-2017.
2) Evaluation of strip trials for advanced "niche" market multi-purpose potato breeding lines/cultivars grown at the NVRD station for advance French fry, chip, roasted and fresh market quality assessment in the fall and winter of 2014-2015. Strip trials allowed for the commercial handling of newly bred lines using commercial size equipment for determining and identifying varietal quality in a real-world growing environment.
3) Produced mini-tubers of MonDak Gold potato variety by Valley Tissue Culture in Halstad, MN in 2015 and 2016.
4) Produced Generation 0 class seed of MonDak Gold in 2015 at the Enander Seed farm in Grenora, North Dakota and Generation 1 seed production in 2016.
5) Produced a limited supply of MonDak Gold in 2015 at NVIRP, the Bill Sheldon Potato Farm, Ray, ND, and the Jonk Seed Farm, Forest River, ND for test marketing in both fresh and processing industries for 2015-2016 and 2016-2017.

Goals and Outcomes Achieved

Chuck Stadick, Spud Viking Potato Consulting, Dr. Susie Thompson, NDSU Potato Breeder, Spencer Bariball, University of Minnesota Potato Researcher, Dr. Sanjay Gupta, University of Minnesota Potato Nutritionist, Keith Olson, Regional Director of the Small Business Development Center, and Jerald Bergman, NDSU Williston Research Extension Center Director and Project Coordinator, attended the National Potato Council Annual Meeting and Expo, January 7-9, 2015 at Orlando, Florida. The Williston Ag Diversification Group hosted a trade show booth to promote the MonDak Gold potato variety for the fresh pack and processing industries and to promote the MonDak region (western North Dakota and eastern Montana) for potato production and processing plants. The MonDak Gold potato variety was displayed at our trade show exhibit booth and created a lot of interest due to its attributes of high fry color quality, low acrylamides, and long storage life.

The test results of the 2014 Potato Breeding Lines/Cultivars and Advanced Strip Trials grown and evaluated at the NDSU Williston Research Extension Center Irrigated Nesson Valley site were completed (see attachment 1 and 2). The Williston, ND test results from NDSU, University of Minnesota, USDA Potato Testing Laboratory, East Grand Forks, MN, and Ag World Support Systems, Moses Lake, WA were presented at the Northern Plains Potato Growers Association Annual Meeting, February 17, 2015 at the Alerus Center, Grand Forks.

Mini-tubers of the MonDak Gold potato variety were produced from tissue culture by Valley Tissue Culture, Halstad, MN and delivered to the Enander Seed Farm, Grenora, ND for 2015 seed potato generation increase of the MonDak Gold potato variety. MonDak Gold potatoes produced in 2014 were distributed to end users for product testing and evaluations in April-June, 2015. Granrud's Lefse Shack, Opheim, MT reported that the MonDak Gold potato produced an excellent lefse product and is now their preferred red potato variety for lefse making. Spud Viking Potato Consultant, Chuck Stadick, reported the MonDak Gold potato variety can compete with the benchmark yellow flesh variety Yukon Gold. Organic growers in Minnesota who

expressed a keen interest in producing and test marketing the MonDak Gold potato variety in 2015 received MonDak Gold potatoes for planting, performance testing, and test marketing to organic consumers.

Valley Tissue Culture, Halstad, Minnesota produced 583 pounds of mini-tubers in 2015 and 227.5 pounds of mini-tubers in 2016-17 for grow out at the Enander Seed Farm in Grenora, ND.

The 2015 potato trials at the NDSU Williston Research Extension Center Nesson Valley Irrigated site included newly bred advanced specialty breeding lines/cultivars from NDSU to identify and introduce new, improved potato varieties, specialty potato products, and more nutritious potatoes to consumers (see attachment 3). The MonDak Gold potato variety planted on 4-5 irrigated acres at the Nesson Valley Irrigated site provided MonDak Gold potatoes for test marketing by interested end users in the fall of 2015. Mini-tubers produced by Valley Tissue Culture, Halstad, MN, generation increased at the Enander Seed Farm, Grenora, ND to provide Generation 0 seed potatoes for 2016.

Distribution and test marketing of the 2014 MonDak Gold potato crop coming out of long term storage occurred during the April-June, 2015 period. Over 100,000 pounds of MonDak gold potatoes were distributed to 72 businesses and over 400 individuals. The end users utilized the MonDak Gold potatoes in their potato products as well as for use as seed potatoes for 2015 planting and production purposes. Feedback was very positive by all the consumers that received the MonDak Gold potatoes.

Our MonDak Ag Showcase and the Nesson Valley Irrigated field day events were held on July 9-10, 2015. Special presentations were made during these events on potato research, varieties, and market development by Jerald Bergman, WREC Director.

The MonDak Gold potato commercial plantings at the Williston Research Extension Center and The Bill Sheldon Farm in Nesson Valley were harvested and utilized in our test marketing efforts.

The Nesson Valley potato variety trials were harvested on September 30, 2015 by Dr. Susie Thompson, NDSU Potato Breeder and WREC staff. The harvested plot samples were delivered to NDSU, the USDA-ARS Potato Laboratory in east Grand Forks, MN, and the University of Minnesota for yield and quality determinations, storability tests, etc. Three hundred pounds of MonDak Gold potatoes harvested from a commercial field at Williston Research Extension Center were also delivered to the USDA-ARS Potato Testing Laboratory for long term storability and quality testing.

MonDak Gold variety of potatoes from the 2015 production were distributed to end users for product testing and evaluations. Mini-tubers of MonDak Gold from tissue culture produced at Valley Tissue Culture, Halstad, MN were generation increased at the Enander Certified Seed Farm, Grenora, ND in 2015.

MonDak Gold test results were obtained from the National Fry Process Trials. The National Fry Process Trials were grown in North Dakota, Wisconsin, Maine, Idaho, and Washington. Data

includes yield specific gravity, sugars, tuber descriptions (length, shape, size, and distribution), acrylamide, and asparagine. The MonDak Gold potato variety was considerably lower in acrylamides than the commercially grown varieties Russet Burbank and Ranger Russet. The MonDak Gold variety was also considerably lower in glucose sugars than Russet Burbank (attachment 4).

Chris Blackwell, Global Grid Advisors/Viall Farms Consulting, Dr. Susie Thompson, NDSU Potato Breeder, Spencer Bariball, University of Minnesota Potato Researcher, and Dr. Jerald Bergman, NDSU Williston Research Extension Center Director and Project Coordinator, attended the National Potato Council Annual Meeting and Expo, January 12-14, 2016 at Las Vegas, Nevada. The Williston Ag Diversification Group hosted a trade show booth to promote the MonDak Gold potato variety for the fresh pack and processing industries and to promote the MonDak region (western North Dakota and eastern Montana) for potato production and processing plants.

Our team answered inquiries from attendees regarding the potential of MonDak Gold potato variety for processing and fresh pack and the potential for potato production and processing in western North Dakota including available irrigated land for production and infrastructure available for potato processing facilities. The MonDak Gold potato variety was displayed at our trade show exhibit booth.

Chris Blackwell, Dr. Susie Thompson, and Dr. Jerald Bergman also attended and participated in the Northern Plains Potato Growers Association and Annual Meeting/Research Reporting Conference, February, 12, 2016 at the Alerus Center, Grand Forks, ND.

Granrud's Lefse Shack, Opheim, MT reported that the MonDak Gold potato again produced an excellent lefse product and continues to be their preferred red potato variety for lefse making. Organic growers in Minnesota expressed a keen interest in producing and test marketing the MonDak Gold potato variety again in 2016. An endorsement for MonDak Gold potatoes by Riverbend Farms that conducted test marketing of MonDak Gold potatoes in 2015 is included in an attachment (see attachment 5).

2016 potato variety trials were planted and harvested at the NDSU Williston Research Extension Center Nesson Valley Irrigated site and included MonDak Gold potatoes and newly bred advanced specialty breeding lines/cultivars from NDSU (see attachment 6). The MonDak Gold potato variety was grown at Nesson Valley in May 2016 to provide MonDak Gold potatoes for test marketing by end users in the fall and winter of 2016-17. Generation 0 MonDak Gold potatoes produced at the Enander Seed Farm in 2016 for seed multiplication and advanced to Generation 1. The Jonk Potato Farm, Forest River, ND produced MonDak Gold potatoes in 2016 for organic production and test marketing as a specialty variety in the health and diabetic markets in 2016-2017(see testimony letter in attachment 7). Valley Tissue Culture, Halstad, MN produced mini-tubers in 2016 for 2017 generation increase at the Enanders Seed Farm, Grenora, ND. The MonDak Ag Showcase and NDSU Williston Research Extension Center, and the Irrigated Nesson Valley Field Day event held in July 2016 showcased potato production, the MonDak Gold potato variety, and other the specialty potato varieties for specialty markets. The Nesson Valley potato variety trial, the MonDak Gold commercial potatoes and the early blight

disease management in MonDak Gold potatoes results were published (see attachment 8). The MonDak Gold potato variety seed potatoes at the Enander Certified Seed Farm, Grenora, ND and the Jonk Potato Farm, Forest River, ND were utilized for seed potatoes and for test marketing in 2017.

These activities achieved the performance goals in evaluating promising newly breed "niche" market potato breeding lines and producing MonDak Gold seed potatoes for limited commercial production and test marketing.

In 2016, eighteen advanced fresh market and dual-purpose russet selections and commercially acceptable check cultivars were included in the trial planted at the Nesson Valley Research Extension Center, east of Williston, ND. Agronomic, yield and grade, and quality evaluations for the entries are summarized in Tables 1-7 (attached) in comparison to industry standards. The trial identified 4 promising specialty market varieties for fresh market; ND6002-1R (Red), ND7132-1R (Red), ND7818-1 (Yellow), and ND7982-1R (Red) and 5 dual-purpose russet selections; AND97279-5, AND00618, ND78825-7, ND8068-5, and ND039194AB-1. The most advanced dual-purpose russet selection is ND8068-5Russ, a very early maturing selection, seven to ten days earlier than standard Russet Norkotah. AND00618-2RussY is notable for its attractive tubers and soft free tubers. Dual-purpose indicates that the clones are suitable both for processing into frozen products such as French fries, and that they are also suitable for tablestock (baking for example).

A commercial supply of MonDak Gold potatoes was produced in 2015 and 2016 at Nesson Valley for test marketing in 2016 and 2017. The Jonk Potato Farm Forest River, ND also produced commercial MonDak Gold potatoes for organic production and test marketing in 2016-2017. MonDak Gold variety potatoes were distributed to over 72 businesses and over 400 individuals in the MonDak region to promote the MonDak Gold potato variety and deliver a positive nutritional message about potatoes to end users in the high end restaurants, grocery stores, organic market outlets, lefse product manufacturers, and consumers.

Diabetic consumers continue to report to us that the MonDak Gold potato variety is lower in sugar. This was supported by the National Fry Process Trials results were the MonDak Gold potato variety was considerably lower in glucose sugar than Russet Burbank. However, the University of Minnesota laboratory analyses of MonDak Gold potatoes for resistant starch and glycemic index did not verify the diabetic consumer reports (see attachment 9).

Beneficiaries

This project served as a catalyst for market development of innovative potato varieties, potato products, and uses to deliver positive nutritional message about potatoes to the trade and consumers and to interest and attract potato processors and fresh pack operations to the MonDak region. This project created a nucleus of MonDak Gold virus free mini-tubers, generation 0 and generation 1 seed to provide test marketing and seed to commercial growers. Limited specialty markets have been established in the high-end restaurants, organic market, fresh pack, and in lefse products.

MonDak Gold, a multi-purpose red skin, yellow flesh potato variety was demonstrated to have excellent culinary attributes and suited for processing with superiority over the industry potato standard Russet Burbank potatoes in both traditional and specialty potato products.

This project served as a catalyst for innovative potato products and other newly bred potato varieties from the University of Minnesota and North Dakota State University Potato Breeding and Research programs for the fresh pack, French fry, roasting chips, niche and traditional markets and delivered a positive nutritional message about potatoes to trade and consumers. The potential production and irrigation development in the MonDak region has the potential to create new economic wealth and jobs in the MonDak region and northern Great Plains.

The impact of "niche" markets will be realized by how fast and what quantities the MonDak Gold and other specialty potatoes breeding lines replace existing standard varieties because of their superior fry, roasting, fresh market, and specialty potato qualities. "Niche" processing and fresh market opportunities of specialty potato products will help diversity the MonDak agricultural region into potato production and processing.

Variety development is long-term, and the collaborative effort between the North Dakota State University Potato Breeding program and the North Dakota State Williston Research Extension center will continue working together with the Williston Regional Economic Development Group to develop and promote these newly developed (developing) cultivars for specialty markets and adaptation to the MonDak and Northern Plains region.

MonDak Gold variety potatoes were distributed to over 72 businesses and over 400 individuals in the MonDak region to promote the MonDak Gold potato variety and deliver a positive nutritional message about potatoes to end users in the high-end restaurants, grocery stores, organic market outlets, lefse product manufacturers, and consumers.

MonDak Gold potato variety was showcased at several tradeshows and expos. Around 1,900 people attended the National Potato Council Annual Meeting and Expo in January 7-9, 2015 in Orlando, Florida and around 2,100 attended the same event January 12-14, 2016 in Las Vegas, Nevada. An estimated 250 people attended the Northern Plains Potato Growers Association Annual Meetings in both February 2015 and February 2016 at the Alerus Center in Grand Forks, North Dakota. Our MonDak Ag Showcase and the Nesson Valley Irrigated field day events that were held in July 2015 was attended by 500 guests and the MonDak Ag Showcase and the Nesson Valley Irrigated field day events held in July 2016 was attended by 600 guests.

Lessons Learned

This project was an extensive collaborative effort of the potato research programs at the University of Minnesota and North Dakota State University Breeding Programs with North Dakota State University Williston Research Extension Center, the Williston Area Economic Diversification Group, and Chuck Stadick, Spud Viking Potato Consulting. The Northern Plains Potato Growers Association provided much valuable assistance. All these organizations and individuals worked together to conduct potato research and varietal test marketing and promotion of MonDak Gold and other advanced specialty potato breeding lines nationwide.

Dr. Christian Thill, University of Minnesota Potato Breeder, passed away unexpectedly on August 7, 2014. His passion, enthusiasm, knowledge, and collaboration efforts for the test marketing was greatly missed, especially in the organic and fresh pack markets. Due to the unfortunate passing of Dr. Thill, the testing and evaluation of the University of Minnesota potato breeding lines/cultivars were discontinued in 2105 by the University of Minnesota administration except for this project's continued production and test marketing of the MonDak Gold potato variety. The testing and evaluation of the NDSU potato breeding lines/cultivars were continued at the NDSU Williston Research Extension Center Nesson Valley Irrigated site under the direction of Dr. Susie Thompson, NDSU Potato Breeder, Fargo, ND and included the University of Minnesota MonDak Gold potato variety.

This project has helped develop and promote newly developed (developing) cultivars for specialty markets and adaptation to the MonDak and Northern Plains region. Valley Tissue Culture, Halsted, MN and Enander Seed Farm, Grenora, ND play a major role in the positive outcomes of a supply of MonDak Gold certified seed for commercial production and test marketing for processing into fries, chips, fresh market, baking, mashing, roasting, lefse, and microwave cooking products.

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Additional Information

See attachments.

Processing Potato Trial
Williston, North Dakota
2014

Asunta (Susie) Thompson, Jerald Bergman, and Tyler Tjelde

In order to meet the objectives of the potato improvement team, yield and evaluation trials were grown at eight locations in North Dakota and Minnesota in 2014, five irrigated (Larimore, Oakes, Inkster, Williston, and Park Rapids) and three non-irrigated locations (Hoople, Crystal and Grand Forks). Twenty-two advancing dual-purpose russet selections and commercially acceptable cultivars were grown at the Nesson Research Extension Center, near Williston, ND. The trial was planted on May 6, in a randomized complete block, with four replicates. Rows were 36 inches apart, and the within-row spacing was 12 inches. Cultural practices typical of the growing area were used during the growing season, including sprinkler irrigation (linear). Vine notes were not taken in 2014 prior to vinekill/senescence. As in 2013, early blight was prevalent in some clones by early July. The plot was harvested on September 24; days to harvest was 140. A dinner was held showcasing potato dishes and potato displays.

Yield and grade, quality evaluations, and French fry quality for the Williston processing trial entries are summarized Tables 1, 2 and 3, respectively. The NDSU potato breeding program attempts to focus on high yielding, earlier maturing clones because of our often short growing season. Total yield ranged from 164 cwt./acre to 540 cwt./acre for ND081476B-9Russ and Shepody, respectively; the mean was 371 cwt./acre. Similarly, Shepody produced 472 cwt./acre US. No. 1 yield, and ND081746B-9Russ just 136 cwt./acre. Percentage US No. 1 yield ranged from 75% (Umatilla Russet) to 93% (Dakota Trailblazer), with a mean of 85%. Relative to 2013, the tuber size profile for all clones tended to be larger. The mean percentage of tubers 6-12 ounces was 49%. Dakota Trailblazer produced 61% in this category, while Shepody produced 34%. Several selections and check cultivars had high percentages of oversized (>12 ounces) tubers, including AND00618-2RussY, ND071081-4Russ, ND081476B-3Russ, Ranger Russet, Shepody and Teton Russet; all exceeded 25%. Few US No. 2 grade tubers were produced and only ND081563C-2Russ and Shepody produced many culls (10%). Umatilla Russet was small and somewhat spindly in all of our trials in 2014. Alpine Russet tends to be somewhat triangular due to a pointed apical end.

Mean specific gravity was 1.0946, with a range of 1.0820 to 1.1119, for ND081476B-9Russ and Dakota Trailblazer, respectively (Table 2). Specific gravity levels for many selections and cultivars were very high and out of processor specifications for this quality attribute. Percentage hollow heart and brown center ranged from 0 to 6%, with a mean of 1%. All trial entries were evaluated for blackspot and shatter bruise potential. Blackspot bruise potential is assessed using the abrasive peel method of Pavek and Corsini. On a scale of 1 (none) to 5 (severe), clones ranged from 1.4 for ND081586B-3Russ, to 4.8 for ND071081-2Russ. Shatter bruise potential is assessed using a bruising chamber with digger chain link baffles, and rated on a scale of 1 (none) to 5 (many and severe). The mean across trial entries was 1.9, with a range of 1.4 to 2.4.

Our research is designed to identify processing (both chip and frozen) germplasm that will reliably and consistently process from long-term cold (38F and/or 42F, 3.3C and/or 5.5C,

respectively) storage. A field (zero time) sample was collected during grading for immediate French fry processing, and after eight weeks storage from 45F (7.2C) for fry color, stem end fry color, sugar ends, and other defects. Several advancing selections and industry standards had outstanding French fry color when fried at harvest and after storage at 45F for 8 weeks (Table 3). Many demonstrate sugar end resistance. All clones had acceptable French fry color from the field, and had leachable sugar ends with the exception of ND081563C-2Russ, ND092019C-4Russ, Russet Norkotah, and Shepody. Percentage sugar ends ranged from 8% to 92%, for Dakota Trailblazer and ND102677B-3Russ, respectively. Our program assesses a sugar end as any color deviation from the main fry. This is more stringent than the processing industry that requires a score of a 3 or 4 on the color chart to be called a sugar end. Clones with stem end colors 2 or less can often be managed during processing/manufacturing. Following 8 weeks of storage at 45F, Russet Norkotah (used as a poor processing control) produced the darkest French fry colors. Stem end color ranged from 0.7 to 2.9. Russet Burbank and Teton Russet, amongst others, exhibited classic sugar ends from storage. French fry/frozen processing selections will also evaluated from 45F storage in June.

Promising advancing dual-purpose (frozen processing and tablestock) russet selections include ND8068-5Russ, despite its yield performance in this trial. ND8068-5Russ has early maturity, seven to ten days earlier than Russet Norkotah. Unlike Russet Norkotah, it processes from the field and 45F storage. It does not compete with Russet Norkotah or Shepody when grown full season, but instead would be very suitable for early harvest and to open processing plants. AND00618-2RussY has received some interest as a dual-purpose specialty due to its beautiful yellow flesh; hollow heart has been noted. This trial was managed as for Russet Burbank. Perhaps managing nitrogen and water more specifically for this selection would minimize that internal defect. Dakota Trailblazer continues to be a top performer, although yield potential was reduced.

We wish to express our gratitude to the North Dakota Department of Agriculture for Specialty Crops Block Grant Funding, the Northern Plains Potato Growers Association and MN Area II Potato Research and Promotion Council, R.D. Offutt Co., Hoverson Farms, Enander Seed Farm, and Jorde Certified Seed for research funding and certified seed potatoes in support of the processing trial at the Nesson Research Extension Center. Additionally, we are grateful for the support of the Williston Area Economic Development Group and Chuck Staddick. Finally, a sincere thank you to the Nesson staff and Richard Nilles, in maintaining and harvesting the research plot at Nesson.

Table 1. Yield and grade for advanced processing selections and cultivars, full season, Nesson Valley near Williston, ND, 2014.

Clone	Total Yield Cwt./A	US No. 1 Cwt./A	US No. 1 %	0-4 oz. %	4-6 oz. %	6-12 oz. %	>12 oz. %	US No. 2 %	Culls %
1. AND00618-2RussY	352	318	90	5	13	47	30	1	4
2. ND8068-5Russ	273	215	79	21	35	44	0	0	0
3. ND8339C-1Russ	442	394	89	10	24	52	13	0	1
4. ND071081-2Russ	378	332	88	12	24	52	12	0	0
5. ND071081-4Russ	412	384	93	5	7	44	42	0	2
6. ND081476B-3Russ	234	211	90	5	12	41	37	1	4
7. ND081476B-9Russ	164	136	82	9	21	49	12	3	6
8. ND081478B-2RussY	303	248	82	14	21	51	10	2	3
9. ND081563C-2Russ	490	385	79	10	18	49	12	1	10
10. ND081586B-3Russ	384	343	89	11	21	53	16	0	0
11. ND092019C-4Russ	316	237	75	20	29	38	8	0	4
12. ND102676B-1Russ	375	312	83	15	12	52	10	0	1
13. ND102677B-3Russ	228	193	82	18	21	53	8	0	0
14. Alpine Russet	473	415	88	8	15	50	22	1	4
15. Bannock Russet	385	316	82	17	19	47	17	1	1
16. Dakota Trailblazer	349	325	93	7	12	61	20	1	0
17. Ranger Russet	395	350	88	6	13	51	25	1	5
18. Russet Burbank	447	381	84	8	17	46	21	1	7
19. Russet Norkotah	392	328	84	16	26	55	4	0	0
20. Shepody	540	472	88	2	5	34	49	0	10
21. Teton Russet	409	360	88	4	7	52	29	2	6
22. Umatilla Russet	422	327	77	19	22	47	8	0	4
Mean	371	317	85	11	18	49	18	1	3
LSD ($\propto$=0.05)	76	73	8	6	5	8	10	2	4

Table 2. Quality attribute assessments for advanced processing selections and cultivars, full season, Nesson Valley near Williston, ND, 2014.

Clone	Specific Gravity[1]	% Hollow Heart[2]	Black-spot Bruise[3]	Shatter Bruise[4]
1. AND00618-2RussY	1.0921	0	1.9	2.0
2. ND8068-5Russ	1.0933	0	3.5	2.0
3. ND8339C-1Russ	1.0938	1	2.6	2.3
4. ND071081-2Russ	1.0934	0	4.8	1.9
5. ND071081-4Russ	1.1020	0	5.0	1.8
6. ND081476B-3Russ	1.0930	1	2.3	1.8
7. ND081476B-9Russ	1.0820	0	2.0	1.4
8. ND081478B-2RussY	1.0904	0	1.8	2.1
9. ND081563C-2Russ	1.1050	0	4.0	2.4
10. ND081586B-3Russ	1.0916	0	1.4	2.4
11. ND092019C-4Russ	1.1036	0	2.8	2.0
12. ND102676B-1Russ	1.1042	6	3.7	1.5
13. ND102677B-3Russ	1.0973	0	2.8	1.4
14. Alpine Russet	1.0928	0	3.6	2.0
15. Bannock Russet	1.0942	1	2.3	2.0
16. Dakota Trailblazer	1.1119	1	2.6	1.4
17. Ranger Russet	1.1011	0	3.0	1.5
18. Russet Burbank	1.0913	1	2.3	2.2
19. Russet Norkotah	1.0838	1	2.4	1.5
20. Shepody	1.0845	0	2.7	2.1
21. Teton Russet	1.0882	0	3.8	2.1
22. Umatilla Russet	1.0927	1	3.2	2.0
Mean	1.0946	1	2.9	1.9
LSD ($\propto$=0.05)	0.0050	3	1.4	1.4

[1] Determined using weight-in-air, weight-in-water method.
[2] Hollow heart includes brown center.
[3] Blackspot bruise determined by the abrasive peel method, scale 1-5, 1=none, 5=severe.

Table 3. French fry evaluations following harvest and after 8 weeks storage at 45F, full season trial, Nesson Valley near Williston, ND, 2014.

Clone	Fry Color[1]	Stem-end Color	% Sugar End[2]	Fry Color[1]	Stem-end Color	% Sugar End[2]
	Field Fry			Following 8 wks. at 45F		
1. AND00618-2RussY	1.5	2.1	33	1.2	1.6	59
2. ND8068-5Russ	0.9	1.2	50	0.5	0.7	50
3. ND8339C-1Russ	1.2	1.9	54	0.9	1.4	58
4. ND071081-2Russ	1.4	1.6	25	1.0	1.2	17
5. ND071081-4Russ	0.8	1.4	50	0.7	1.1	58
6. ND081476B-3Russ	0.9	1.7	59	1.0	1.8	59
7. ND081476B-9Russ	0.9	1.8	75	0.6	1.7	75
8. ND081478B-2RussY	1.9	2.1	25	1.3	1.7	50
9. ND081563C-2Russ	2.4	2.6	25	1.2	1.5	33
10. ND081586B-3Russ	0.5	1.5	83	0.6	1.5	67
11. ND092019C-4Russ	2.1	3.3	42	2.1	2.9	42
12. ND102676B-1Russ	0.9	1.3	42	1.0	1.5	59
13. ND102677B-3Russ	0.6	2.3	92	0.6	2.4	100
14. Alpine Russet	0.5	0.5	8	0.4	0.5	25
15. Bannock Russet	0.8	2.1	83	0.6	1.7	92
16. Dakota Trailblazer	0.5	0.5	8	0.4	0.9	25
17. Ranger Russet	1.6	2.1	33	0.8	2.1	75
18. Russet Burbank	1.0	2.6	75	1.0	2.5	67
19. Russet Norkotah	2.5	3.0	33	2.3	2.3	8
20. Shepody	2.8	3.3	42	0.8	2.4	84
21. Teton Russet	1.1	2.5	75	1.2	2.6	92
22. Umatilla Russet	1.1	2.0	42	1.0	1.3	50
Mean	1.2	2.0	50	0.9	1.7	56
LSD ($\propto$=0.05)	0.9	0.9	54	0.7	1.0	50

[1] Fry color scores: 0.1 corresponds to 000, 0.3 corresponds to 00, 0.5 corresponds to 0, 1.0 equals 1.0 and subsequent numbers follow French fry rating scale 000 to 4.0.

[2] In our evaluation, any stem end darker than the main fry is considered a sugar end, the worst case scenario. The processing industry defines a sugar end as a 3.0 or darker.

2014 POTATO BREEDING UNIVERSITY OF MINNESOTA RESEARCH SUMMARY

Project Title: **Potato Breeding and Genetics University of Minnesota**

Project Leader: Dr. Emily Hoover (as of August 2014), University of Minnesota, 1970 Folwell Avenue, St. Paul, Minnesota 55108. 612-624-6220. hoover@umn.edu

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GOALS OF THIS RESEARCH

The objective of this research is to develop and release potato varieties adapted to Minnesota and North Dakota. Selection will emphasize lines having superior yield, quality, and host plant resistance to biotic and abiotic stress.

2014 RESEARCH OBJECTIVES

OBJECTIVE 1	BREEDING, EVALUATION, AND SELECTION FOCUSED ON FRY AND CHIP PROCESSING AND FRESH MARKET RUSSET, RED AND YELLOW VARIETIES, GROWER FIELD TRIALS (Fry & Chip processing, Fresh russet, Fresh Red, and Fresh Yellow)
OBJECTIVE 2	TISSUE CULTURE BANK MANAGEMENT, VIRUS CLEAN-UP RESEARCH
OBJECTIVE 3	NATIONAL TRIALS (NFPT & NCPT); REGIONAL TRIAL (NCRPVT)
OBJECTIVE 4	OUTREACH

SUMMARY

Research emphasized the development, evaluation and release of potato varieties with improved yield, quality, and resistance to biotic and abiotic stress.

Objective 1

SELECTION AND CLONAL ADVANCEMENT:

Breeding lines advance through the UM program in generations. Early generations are Single-hills, and Generation 1 (G1); Mid-generations are G2, G3, and G4; Late-generations are G5 and G6. By the time a selected clone moves to G2 and beyond, sufficient breeder's seed is available for multi-location evaluations.

Single-hills: Represent selected clones from new hybrid crosses. After a cross and sowing of new hybrid seed, seedlings, are first grown in the greenhouse to produce mini-tubers. These minitubers are planted to the field as *single-hills*. In 2014 we had 101 families (crosses). Single hills were grown in Gully, MN, Williston, ND, and Sikeston, MO.

Generation1: Single-hills selected from the previous year are planted for the first time in the field using normal plant spacing and production practices as *G1*. Typically, only 4 to 8-hills of each clone are available for planting. 39 families consisting of 1200 clones were evaluated and screened for cold induced sweetening.

Early Generations Planted in 2014

Market	Gully SH	Gully G1	Williston SH	Williston G1	Sikeston SH
Reds	131	10	10	3	-
Yellows	53	3	1	0	-
Russets	178	15	14	13	-
Chip	1163	25	0	4	66
Total	1525	53	25	20	66

Generation 2-6: Selected *G1* clones are moved to the next year as *G2* selections. Typically, sufficient seed is available to evaluate the clones from multiple locations using replicated plots. Additionally, the clones are segregated into market-type and planted as *Fresh, Processing, or Chipping Trials.* Selected *G2 clones and beyond* are evaluated at multiple locations using replicated plots, and more comprehensive data is collected including yield, size and grade, internal and external physiological defects, specific gravity and processing quality. In 2014 the *G3's and beyond* were planted at Becker Early harvest, Becker Late harvest, Williston, Crystal, and Grand Forks.

Locations	2014 Number of Clones Tested by Generation*								
	Total	G2	G3	G4	G5	G6	G7	G12	G16
Fresh Market									
Becker Early	36	5	5	18	-	1	2	3	2
Becker Late	38	7	5	18	-	1	2	3	2
Williston	36	7	5	18	-	1	2	-	3
Crystal	9	-	-	9	-	-	-	-	-
Grand Forks	37	5	5	18	-	1	2	3	3
Processing									
Becker Late	57	20	13	12	5	-	4	1	2
Williston	57	20	13	12	5	-	4	1	2
Grand Forks	51	15	12	12	5	-	4	1	2
Chippers									
Becker Late	29	6	7	2	2	3	8	1	-
Williston	29	6	6	2	2	3	8	1	1
Grand Forks	42	19	6	2	2	3	8	1	1

***Totals include dual purpose clones**

G3 clones and above were also evaluated in *Grower Field Trials at Peterson Farms* (24), *Dechene Farms* (24), *Hammer Farms* (12), *Five Star Produce* (3), *and Goener Farms* (12).

Generation 7 and beyond - Advanced, Seed Spacing, and Advanced Processing Trials:
After *G6,* several of our clones are evaluated in *seed spacing*, and *Processing Trials.*

At Williston, ND 11 advanced clones were evaluated in strip trials. Trials are planted in 100 to 400 hill strips. Tubers were harvested and evaluated by Ag World, Grand Forks, ND for processing quality.

Attachment 2

2014 University of Minnesota
Potato Breeding and Genetics

Williston, ND
Preliminary Yield Trials

					Size Distribution																	
					<2oz		2-4oz		4-6oz		6-8oz		8-10oz		10-12oz		12-14oz		>14	
Loc	Clone	Trial	Mkt	Skin	*cnt	Cwt	cnt	Cwt	cnt	Cwt	cnt	Cwt	cnt	Cwt	cnt	Cwt	cnt	Cwt	cnt	Cwt
W	Atlantic	Chk	C	W	3	2.054	18	23.107	20	39.653	23	63.773	12	42.278	10	43.932	4	20.911	9	61.762
W	MN02467Rus/Y	G12	FF/FM	Rus	9	5.727	29	36.501	23	46.157	25	70.356	15.5	56.441	14	61.455	4	20.618	5.5	33.962
W	MN04844-07	G10	FM/C	W	51	31.865	97.5	113.097	17	31.758	3	8.059	3	11.254	1	4.236	0	0	0	0
W	MN07014GFB-01LW	G6	FF	LW	22	14.249	58.5	69.878	35	68.487	16	44.896	10	35.574	3.5	15.94	4	21.096	5	35.174
W	MN07023BB-01Rus	G6	FF	Rus	10	6.49	29	35.745	46	90.86	23	63.702	18	66.183	12	52.205	7	36.886	6	41.079
W	MN07051BB-01Rus	G6	FF	Rus	16	9.913	46	57.982	42	83.899	15	42.178	8	27.971	2	8.958	2	10.412	0	0
W	MN07106GFB-01	G6	C	W	33	19.983	68	83.685	40	79.506	12	33.662	2	7.217	0	0	0	0	1	6.447
W	MN07112WB-01W/P	G6	FM	W/Purple	146	83.1	161	183.859	79	153.035	37	101.372	20	71.561	13	58.424	8	42.377	3	21.681
W	MN07257BB-01Rus	G6	FF	Rus	12	7.332	47	57.895	44	88.278	33	92.101	21	74.542	9	39.895	6	31.851	1	6.618
W	MN07286GFB-01R/Y	G6	FM	Red	47	29.797	70	82.572	40	79.135	16	45.629	11	39.082	4	17.673	1	5.434	0	0
W	MN07269BB-01	G6	C	W	68	41.172	118.5	136.632	21.5	39.325	4	11.126	0	0	0	0	0	0	0	0
W	MN07312BB-01	G6	C	W	27	17.915	81	97.706	52	100.416	12	33.135	3	10.94	1	4.122	0	0	0	0
W	MN07330BB-01	G6	C	W/Red splash	28	17.715	59	70.848	54	110.258	24	67.567	8	28.556	4	17.416	2	10.084	0	0
W	MN08001BB-01R	G6	FM	Red	14	8.216	42	53.432	44	85.183	27	77.495	14	50.303	11	49.837	3	15.234	3	18.457
W	MN08025BW-01	G6	C	W	66	39.368	88	106.678	16	30.767	7	18.4	1	3.537	1	4.136	0	0	0	0
W	MN08102BW-01	G6	C	W	31	18.643	63	79.52	43	86.481	21	58.167	14	49.638	1	4.208	0	0	2	15.419
W	MN09059BB-01	G5	C	W	33	20.04	118	141.025	52	98.048	7	18.457	2	6.732	0	0	1	5.434	0	0
W	MN09075BW-01Rus	G5	FF	Rus	5.5	3.295	26	32.757	26.5	55.122	17.5	48.646	17.5	63.055	11	48.076	3.5	18.429	5.5	37.093
W	MN09107BB-01Rus	G5	FF	Rus	24	14.57	62.5	76.254	38.5	75.376	32	89.176	11	39.696	6.5	28.656	3	15.319	2.5	14.977
W	MN09135BW-01Rus	G5	FF	Rus	14.5	9.435	38.5	47.969	37.5	75.961	16.5	46.1	7	24.655	4	17.238	3	16.075	1	6.504
W	MN09151BW-01Rus	G5	FF	Rus	10	6.69	24	31.608	16	31.979	26	69.692	5	16.96	12	52.833	2	10.969	2	12.381
W	MN09152BW-01Rus	G5	FF	Rus	5.5	3.516	16.5	21.039	26.5	53.161	20	56.039	16	57.839	21.5	94.162	7	36.508	17	120.692
W	MN10001PLWR-03LW	G4	FF/FM	LW	3	1.954	8.5	10.191	15	29.854	12.5	34.646	18	64.757	11	48.554	10.5	53.888	10.5	67.738
W	MN10001PLWR-14R	G4	FM	Red	4.5	2.746	12.5	15.169	19	39.311	16.5	47.576	18	65.52	12	53.032	7	36.843	2.5	15.726
W	MN10003PLWR-02R	G4/NCR	FM	Red	25	15.112	79	96.758	58.5	116.891	25.5	70.37	7.5	26.923	6	25.546	2	10.099	0	0
W	MN10003PLWR-03R	G4/NCR	FM	Red	20	11.354	57	69.45	47	93.655	30	86.381	5	17.473	1	4.179	2	10.127	0	0
W	MN10003PLWR-07R	G4/NCR	FM	Red	13	7.118	27	30.268	17	33.876	7	19.698	10	36.215	3	13.451	0	0	1	6.889
W	MN10008PLWR-06R	G4	FM	Red	30	17.744	101	123.908	45	86.481	8	22.223	4	13.836	1	4.736	0	0	0	0
W	MN10010WW-06Rus	G4	FF	Rus	4	2.667	26	32.735	34	68.109	32	89.348	22	78.179	15	66.854	8	41.051	4	25.532
W	MN10013PLWR-04	G4/NCR	C/FM	W	19	11.197	91	113.425	74	146.474	26	69.307	9	31.651	2	8.943	1	4.964	0	0
W	MN10020PLWR-04R	G4/NCR	FM	Red	7	4.336	38	46.257	26	51.092	19	52.747	8	29.326	3	13.165	3	15.947	0	0
W	MN10023BB-01Rus	G4	FF	Rus	4.5	3.031	13	16.753	26	51.827	17	48.154	8	28.263	4.5	20.04	3	15.84	1	6.347
W	MN10023BW-01Rus	G4	FF	Rus	2.5	1.555	16.5	20.447	26.5	54.081	21	59.843	11	39.938	7	30.938	4	21.303	4	28.77
W	MN10024PLWR-09R	G4	FM	Red	15	8.33	29	33.02	13	24.534	3	7.503	1	3.908	3	13.864	1	5.563	1	5.834
W	MN10024PLWR-11R	G4	FM	Red	14	8.658	23	27.657	23	47.855	19	53.86	9	31.266	4	17.658	1	5.135	2	11.639
W	MN10025PLWR-07R	G4/NCR	FM	Red	5	2.724	13	17.587	11	22.965	8	22.308	15	53.76	3	13.579	9	47.441	1	7.246
W	MN10025PLWR-20R	G4	FM	Red	26	15.276	75	89.661	35	69.778	10	26.901	1	3.338	0	0	0	0	0	0
W	MN10030WB-04Rus	G4	FF	Rus	8	5.007	43.5	52.505	50.5	101.094	21.5	59.503	10	35.602	4	18.093	1	5.263	1.5	10.37
W	MN10053BW-01Rus	G4	FF	Rus	7	4.586	37.5	46.179	40	79.548	21.5	61.348	15	54.017	8	35.438	5	25.931	5	34.818
W	MN10054BW-01Rus	G4	FF	Rus	0	0	8	10.177	15.5	31.223	15.5	43.354	8	29.162	9.5	42.598	6.5	34.154	14.5	97.87
W	MN10056WB-10Rus	G4	FF	Rus	19	11.518	43.5	53.781	24	46.842	8.5	23.72	4.5	15.961	1.5	6.854	0	0	2	13.172
W	MN10056WW-05Rus	G4	FF	Rus	12.5	8.301	40.5	49.124	26	49.395	6	16.624	2	6.932	0	0	0	0	0	0
W	MN10056WW-10Rus	G4	FF	Rus	5	2.96	21.5	26.174	35	70.134	22	60.478	14.5	52.64	2.5	10.655	2	10.163	0	0
W	MN100648W-01Rus	G4	FF	LW	33.5	21.823	58.5	72.602	49.5	97.592	29	81.702	12	43.461	7.5	33.363	4	20.896	3	18.429
W	MN11026WB-07Rus	G3	FF	Rus	17	10.47	44	55.015	34	68.252	21	59.323	27	96.052	8	35.417	5	27.144	5	35.902
W	MN11027WW-06Rus	G3	FF	Rus	2	1.134	8.5	10.84	14.5	29.048	18.5	51.848	14	51.363	7	30.688	7.5	39.724	13.5	95.631
W	MN11031WW-01Rus	G3	FF	Rus	6.5	3.737	22	25.418	23	45.473	20	57.704	14	49.944	12	53.567	3.5	18.044	9	59.287
W	MN11035WB-06LW	G3	FF	LW	4.5	3.003	14	17.601	19.5	40.316	14.5	39.881	14	51.585	11.5	51.15	9.5	50.436	10	71.026
W	MN11037PLWRGR-04R	G3	FM	Red	30	17.787	66	78.521	39	75.084	6	17.473	0	0	0	0	0	0	0	0
W	MN11040WB-04Rus	G3	FF	Rus	27	16.453	48.5	57.14	25.5	50.023	6.5	17.737	4	13.608	2	8.523	2	9.985	0	0
W	MN11040WB-07RusCT	G3	FF	Rus	6	3.366	18	21.966	21	44.369	18	50.565	9	31.993	4	17.088	3	15.576	2	12.524
W	MN11040WB-12Rus	G3	FF	Rus	9	5.078	32	40.58	43	85.639	20	55.058	5	17.245	2	8.444	1	5.406	0	0
W	MN11042PLWRGR-03R	G3	FM	Red	21.5	13.272	58.5	72.495	39	79.156	13	34.718	5.5	19.869	2	8.701	1	4.992	1	5.976
W	MN11048WW-04Rus	G3	FF	Rus	9	5.42	43.5	54.737	52	105.138	23.5	65.313	10.5	37.221	2.5	10.869	2	10.598	0	0
W	MN11057WB-03Rus	G3	FF	Rus	12	8.066	44.5	55.543	40.5	79.456	16.5	45.587	9.5	33.192	1.5	6.469	0	0	0	0
W	MN11057WB-04Rus	G3	FF	Rus	7.5	5.342	32	40.166	26.5	56.177	28	78.265	14	50.451	2	8.843	1	5.092	1.5	9.749
W	MN11057WW-04Rus	G3	FF	Rus	18	11.311	47	59.622	50	100.816	24	66.141	6	21.695	3	13.18	0	0	2	15.547
W	MN11059PLWRGR-07R	G3	FM	Red	52	31.209	97	112.526	43	82.843	10	26.188	4	14.649	1	4.493	2	10.355	1	7.602
W	MN11124PLWRGR-01Rus	G3	FF	Rus	1	0.442	9	11.397	8	15.776	10	28.442	10	36.273	11	49.281	6	31.694	21	152.678
W	MN11136PLWRGR-10	G3	C	W	4	2.354	19	23.521	20	38.811	16	46.143	16	57.197	15	65.813	9	47.726	8	57.597
W	MN11136PLWRGR-11	G3	C	W	26	16.332	104	123.566	54	106.521	16	42.563	3	10.427	1	4.678	2	10.512	0	0
W	MN11142PLWRGR-01	G3	C	W	18	10.455	32	40.152	37	75.74	22	60.421	8	27.629	0	0	0	0	0	0
W	MN11153PLWRGR-03	G3	C	W	24	14.92	63	77.138	40	79.663	15	42.306	6	20.739	7	31.623	0	0	0	0
W	MN11189PLWRGR-02	G3	C	W	50	29.996	81	95.082	60	121.455	19	53.047	7	25.133	6	27.015	1	5.092	0	0
W	MN12004WB-01R	G2	FM	Red	13	8.159	19	22.993	20	38.94	10	28.527	8	29.012	3	13.978	2	10.598	2	15.405
W	MN12004WW-01R	G2	FM	Red	15	9.072	28	35.745	23	48.268	15	42.163	4	13.864	1	4.607	1	5.035	3	20.739
W	MN12028WB-01Rus	G2	FF	Rus	6	3.837	25	31.594	22	42.934	32	88.606	14	51.62	10	44.859	5	26.559	8	58.253
W	MN12088WW-01Rus	G2	FF	Rus	7	4.165	18	22.365	19	39.339	21	59.565	17	62.09	8	36.358	8	42.477	22	164.104
W	MN12113WW-01Rus	G2	FF	Rus	10	5.663	20	24.448	29	58.666	23	63.973	22	79.934	9	40.594	8	41.222	1	5.962
W	MN12114WB-02Rus	G2	FF	LW	16	9.913	35	42.306	26	49.709	19	53.646	6	20.996	1	4.094	0	0	1	5.877
W	MN12114WW-02LW	G2	FF	LW	6	3.766	41	53.746	26	50.693	18	49.252	17	60.278	7	30.068	2	10.227	0	0
W	MN12124WB-03Rus	G2	FF	Rus	1	0.799	8	10.07	23	47.384	14	39.439	17	62.175	11	48.154	6	31.266	14	98.12
W	MN12124WW-01Rus	G2	FF	Rus	2	1.255	15	19.769	27	54.53	23	64.572	19	69.05	14	61.933	9	46.057	16	109.274
W	MN12138WW-01	G2	C	W	10	6.076	25	30.481	21	42.653	21	59.422	13	46.771	5	22.565	4	20.896	1	5.82
W	MN18747	G16	FF/FM	LW	2	1.155	3	3.152	6	13.123	10	29.868	10	36.13	16	70.12	5	26.787	18	131.825
W	MonDak Gold	G16	FF/FM	Red	9	5.834	26	33.377	29	61.205	41	115.664	28	102.442	22	97.35	6	31.323	10	65
W	NDMN120013WB-01Rus	G2	FF	Rus	8	4.564	36	44.56	53	112.555	25	69.307	20	71.404	9	39.04	5	26.459	2	14.663
W	NDMN120013WW-01Rus	G2	FF	Rus	0	0	14	17.644	24	47.027	31	88.135	21	75.868	15	66.298	11	58.139	2	12.338
W	NDMN120015WB-01Rus	G2	FF	Rus	18	10.897	64	80.946	31	59.323	9	24.819	3	10.698	1	4.436	0	0	0	0
W	NDMN120015WW-02Rus	G2	FF	Rus	7	4.194	35	43.319	29	56.256	24	63.851	6	21.567	5	22.037	1	5.206	1	5.877
W	NDMN120020WW-01Rus	G2	FF	Rus	32	19.655	80	93.37	11	20.04	1	3.052	1	3.951	0	0	0	0	0	0
W	NDMN120022WB-01Rus	G2	FF	Rus	27	17.302	61	72.73	37	72.688	7	18.5	3	10.07	2	9.243	0	0	0	0
W	NDMN120022WW-01Rus	G2	FF	Rus	17	10.912	58	68.822	55	111.442	27	76.838	15	54.402	0	0	2	9.956	2	11.768
W	NDMN120024WW-02Rus	G2	FF	Rus	5	3.124	43	53.917	35	69.179	27	74.342	13	45.843	7	31.708	3	15.562	1	5.934
W	NDMN120029WW-01Rus	G2	FF	Rus	6	3.281	15	19.498	18	37.314	17	49.267	6	22.209	8	35.93	6	32.165	19	139.028
W	NDMN120048WW-01Rus	G2	FF	Rus	12	7.118	22	28.427	24	47.256	21	58.852	11	38.726	6	25.846	0	0	1	5.763
W	NDMN120053WB-02Rus	G2	FF	Rus	20	11.696	37	42.035	18	35.231	4	10.641	3	10.598	0	0	0	0	0	0
W	NDMN120053WW-01Rus	G2	FF	Rus	73	43.747	101	116.976	19	37.528	2	5.905	0	0	0	0	0	0	0	0
W	NDMN120058WW-01Rus	G2	FF	Rus	7	4.436	24	31.166	32	65	28	77.01	13	47.37	5	22.066	1	5.278	6	38.055
W	NDMN120063WW-01Rus	G2	FF	Rus	8	5.192	51	62.09	42	86.609	23	62.204	1	3.38	4	17.288	0	0	0	0
W	NorValley	Chk	C	W	14	7.988	29	35.017	46	92.671	36	100.388	27	97.193	12	52.333	8	41.45	7	44.36
W	Red Lasoda	Chk	FM	Red	9.5	5.677	18.5	23.014	18	36.094	27	76.482	19	68.894	11.5	50.294	10	52.982	15	106.999
W	Red Norland	Chk	FM	Red	9	5.206	8	10.798	7	13.978	16	45.316	15	56.641	10	44.66	4	21.167	14	97.82
W	Russet Burbank	Chk	FF	Rus	11	6.668	31.5	38.405	48.5	97.549	37.5	105.302	27.5	99.211	24.5	107.149	17	89.305	14.5	100.994
W	Shepody	Chk	FF	LW	2.5	1.533	4	4.671	8.5	16.96	6.5	18.621	10	36.593	8	34.525	6.5	34.825	23.5	174.316
W	Yukon Gold	Chk	FM	W	4	1.897	7	7.959	10	20.44	5	13.75	7	25.261	8	35.716	6	30.752	8	49.267

*cnt= count (# tubers)

Attachment 2

	<2oz		2-4 oz		4-6 oz		6-8 oz		8-10 oz		10-12 oz		12-14 oz		>14 oz		4-6 oz			6-10 oz			>10 oz			total	
	cnt	Cwtyld	cnt	Cwtyld	cnt	Cwtyld	cnt	Cwtyld	cnt	Cwtyld	cnt	Cwtyld	cnt	Cwtyld	cnt	Cwtyld	cnt	cwtyld	%	cnt	cwtyld	%	cnt	cwtyld	%	cnt	cwtyld
MonDak Gold	173	7.943	279	25.077	190	29.703	173	37.644	193	54.314	194	66.585	139	56.032	290	161.366	150.0	29.7	11.6	366.0	92.0	22.4	623.0	284.0	38.2	1631.0	438.7
Culls					2	0.28			2	0.574			1	0.387	6	3.868	2.0	0.3	18.2	2.0	0.6	18.2	7.0	4.3	63.6	11.0	5.1

Attachment 2

Ag World Support Systems

Inspection Report

Grower: Williston Research Extension Center **Contract #** 100010 U of M Test Lots **Report #:** 33000438

Processor Name: Simplot Grand Forks

GROSS WEIGHT: 303 ***FEE:*** $60.00

Date Sampled: 01-Dec-14
Date Inspected: 01-Dec-14
Field Number: MN 1000/PLWR-
Storage Number:

	Total Exam	Washed Weight	Water Gain	Net Sample Weight	FM Dirt
Lbs.	303.0	290.0	2.9	287.1	15.9
%			1.0%		5.2%

	Graded Weight	Unus-able	Size Breakdown: nder Size	US No 2 for Processing: 4oz	6 oz	10 oz+	Unusables Breakdown: Soft Rot	Freeze Dmg	*Symptoms Scored	Non	Sun Burn	Insect Dmg	Other	Pitted Scab	Pink Eye
Lbs.	190.0	12.8	11.2	19.0	58.0	89.0	1.1	0.0	0.0	0.0	1.0	0.0	10.7	0.0	0.0
Internal		9.1		0.0	3.2	5.9									
Total:	190.0	21.9	11.2	19.0	54.8	83.1	1.1	0.0	0.0	0.0	1.0	0.0	10.7	0.0	0.0
%	100.0%	11.5%	5.9%	10.0%	28.8%	43.7%	0.6%	0.0%	0.0%	0.0%	0.5%	0.0%	5.6%	0.0%	0.0%

Size	Lbs Exm	I.B.S.	Brn Cen	H.H.	Net Nec	VRB	Other	Total	T.H.H.
4 oz +	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
6 oz +	20.0	0.0	0.0	1.1	0.0	0.0	0.0	1.1	0.0
		0.0%	0.0%	5.5%	0.0%	0.0%	0.0%	5.5%	0.0%
10 oz +	30.0	0.0	0.0	2.0	0.0	0.0	0.0	2.0	1.7
		0.0%	0.0%	6.7%	0.0%	0.0%	0.0%	6.7%	5.7%

	Lbs.	*%*
Bruise	44.7	51.7%
Bruise Free	41.8	48.3%
Total	86.5	100.0%

INTERNAL UNUSABLES BREAKDOWN

	I.D.	H.H.	Net	VRB	Other	T.H.H.
Lbs.	0.0	9.1	0.0	0.0	0.0	5.0
%	0.0%	4.8%	0.0%	0.0%	0.0%	2.7%

Fry Color Analysis

	0	*1*	*2*	*3*	*4*	*SE*	*HS*	*DE*	*Strips*	*Fry Points*
Strips	13	5	2	0	0	0	0	0	20	9
%	65%	25%	10%	0%	0%	0%	0%	0%		

Specific Gravity Measurements	*CPP*	*Avg Temp*
1.0774	**Water**	64
1.0850	**Tuber**	55
1.0890		

Uncorrected Average Specific Gravity: 1.0838 **Ticket #:**
Specific Gravity Correction: -0.0007
Corrected Average Specific Gravity: **1.0831**

Comments:

Sample Method: Submitted

***Symptoms = Characteristics of Late Blight/Pink Rot/ Pink Eye**
Nonscorable symptoms are not included in Unusables

I, the undersigned , on behalf of Ag World Support Systems, do hereby certify that at the request of the applicant and on the date indicated, samples of the herein described products were inspected and the quality and/or condition as shown by said samples were herein stated.

Signature:_____

Attachment 2

Ag World Support Systems

Inspection Report

Grower: Williston Research Extension Center **Contract #** 100010 U of M Test Lots **Report #:** 33000439

Processor Name: Simplot Grand Forks

GROSS WEIGHT: 362 ***FEE:*** $60.00

Date Sampled: 01-Dec-14
Date Inspected: 01-Dec-14
Field Number: MN 02467-Rus/7
Storage Number:

	Total Exam	Washed Weight	Water Gain	Net Sample Weight	FM Dirt
Lbs.	362.0	359.4	3.6	355.8	6.2
%			1.0%		1.7%

	Graded Weight	Unus-able	Size Breakdown: nder Size	US No 2 for Processing 4oz	6 oz	10 oz+	Unusables Breakdown: Soft Rot	Freeze Dmg	*Symptoms Scored	Non	Sun Burn	Insect Dmg	Other	Pitted Scab	Pink Eye
Lbs.	259.4	0.0	25.4	49.0	101.0	84.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Internal		2.0		0.0	2.0	0.0									
Total:	259.4	2.0	25.4	49.0	99.0	84.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
%	100.0%	0.8%	9.8%	18.9%	38.2%	32.4%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Size	Lbs Exm	I.B.S.	Brn Cen	H.H.	Net Nec	VRB	Other	Total	T.H.H.
4 oz +	19.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
6 oz +	30.0	0.0	0.0	0.6	0.0	0.0	0.0	0.6	0.0
		0.0%	0.0%	2.0%	0.0%	0.0%	0.0%	2.0%	0.0%
10 oz +	30.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

	Lbs.	*%*
Bruise	12.0	14.2%
Bruise Free	72.8	85.8%
Total	84.8	100.0%

INTERNAL UNUSABLES BREAKDOWN

	I.D.	H.H.	Net	VRB	Other	T.H.H.
Lbs.	0.0	2.0	0.0	0.0	0.0	0.0
%	0.0%	0.8%	0.0%	0.0%	0.0%	0.0%

Fry Color Analysis

	0	*1*	*2*	*3*	*4*	*SE*	*HS*	*DE*	*Strips*	*Fry Points*
Strips	60	0	0	0	0	0	0	0	60	0
%	100%	0%	0%	0%	0%	0%	0%	0%		

Specific Gravity Measurements	*CPP*	*Avg Temp*	
1.0927		**Water**	65
		Tuber	56

Uncorrected Average Specific Gravity: 1.0927 **Ticket #:**
Specific Gravity Correction: -0.0007
Corrected Average Specific Gravity: **1.0920**

Comments:

Sample Method: Submitted

***Symptoms = Characteristics of Late Blight/Pink Rot/ Pink Eye**
Nonscorable symptoms are not included in Unusables

I, the undersigned , on behalf of Ag World Support Systems, do hereby certify that at the request of the applicant and on the date indicated, samples of the herein described products were inspected and the quality and/or condition as shown by said samples were herein stated.

Signature:_____

Attachment 2

Ag World Support Systems

Inspection Report

Grower: Williston Research Extension Center **Contract #** 100010 U of M Test Lots **Report #:** 33000440

Processor Name: Simplot Grand Forks

GROSS WEIGHT: 364 ***FEE:*** $60.00

Date Sampled: 01-Dec-14
Date Inspected: 02-Dec-14
Field Number: MN10013PLWR-
Storage Number:

	Total Exam	Washed Weight	Water Gain	Net Sample Weight	FM Dirt
Lbs.	364.0	359.6	3.6	356.0	8.0
%			1.0%		2.2%

	Graded Weight	Unus-able	Size Breakdown: nder Size	US No 2 for Processing: 4oz	6 oz	10 oz+	Unusables Breakdown: Soft Rot	Freeze Dmg	*Symptoms Scored	Non	Sun Burn	Insect Dmg	Other	Pitted Scab	Pink Eye
Lbs.	259.6	1.0	6.6	15.0	65.0	172.0	0.0	0.0	0.0	0.0	0.5	0.0	0.5	0.0	0.0
Internal		0.0		0.0	0.0	0.0									
Total:	259.6	1.0	6.6	15.0	65.0	172.0	0.0	0.0	0.0	0.0	0.5	0.0	0.5	0.0	0.0
%	100.0%	0.4%	2.5%	5.8%	25.0%	66.3%	0.0%	0.0%	0.0%	0.0%	0.2%	0.0%	0.2%	0.0%	0.0%

Size	Lbs Exm	I.B.S.	Brn Cen	H.H.	Net Nec	VRB	Other	Total	T.H.H.
4 oz +	3.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
6 oz +	20.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
10 oz +	40.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

	Lbs.	*%*
Bruise	39.7	44.8%
Bruise Free	48.9	55.2%
Total	88.6	100.0%

INTERNAL UNUSABLES BREAKDOWN

	I.D.	H.H.	Net	VRB	Other	T.H.H.
Lbs.	0.0	0.0	0.0	0.0	0.0	0.0
%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Fry Color Analysis

	0	*1*	*2*	*3*	*4*	*SE*	*HS*	*DE*	*Strips*	*Fry Points*
Strips	51	9	0	0	0	15	0	0	60	5
%	85%	15%	0%	0%	0%	25%	0%	0%		

Specific Gravity Measurements ***CPP*** ***Avg Temp***

1.0745

Water 43

Tuber 55

Uncorrected Average Specific Gravity: 1.0745 **Ticket #:**
Specific Gravity Correction: 0.0004
Corrected Average Specific Gravity: **1.0749**

Comments:

Sample Method: Submitted

***Symptoms = Characteristics of Late Blight/Pink Rot/ Pink Eye**
Nonscorable symptoms are not included in Unusables

I, the undersigned , on behalf of Ag World Support Systems, do hereby certify that at the request of the applicant and on the date indicated, samples of the herein described products were inspected and the quality and/or condition as shown by said samples were herein stated.

Signature:_____

Attachment 2

Ag World Support Systems

Inspection Report

Grower: Williston Research Extension Center **Contract #** 100010 U of M Test Lots **Report #:** 33000441

Processor Name: Simplot Grand Forks

GROSS WEIGHT: 404 ***FEE:*** $60.00

Date Sampled: 01-Dec-14

Date Inspected: 02-Dec-14

Field Number: Mon Dak Gold

Storage Number:

	Total Exam	Washed Weight	Water Gain	Net Sample Weight	FM Dirt
Lbs.	404.0	391.4	3.9	387.5	16.5
%			1.0%		4.1%

	Graded Weight	Unus-able	Size Breakdown: nder Size	US No 2 for Processing: 4oz	6 oz	10 oz+	Unusables Breakdown: Soft Rot	Freeze Dmg	*Symptoms Scored	Non	Sun Burn	Insect Dmg	Other	Pitted Scab	Pink Eye
Lbs.	291.4	3.8	29.6	25.0	99.0	134.0	0.0	0.0	0.0	0.0	1.7	0.0	2.1	0.0	0.0
Internal		0.0		0.0	0.0	0.0									
Total:	291.4	3.8	29.6	25.0	99.0	134.0	0.0	0.0	0.0	0.0	1.7	0.0	2.1	0.0	0.0
%	100.0%	1.3%	10.2%	8.6%	34.0%	46.0%	0.0%	0.0%	0.0%	0.0%	0.6%	0.0%	0.7%	0.0%	0.0%

Size	Lbs Exm	I.B.S.	Brn Cen	H.H.	Net Nec	VRB	Other	Total	T.H.H.
4 oz +	5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
6 oz +	30.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
10 oz +	40.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

	Lbs.	*%*
Bruise	36.4	43.9%
Bruise Free	46.6	56.1%
Total	83.0	100.0%

INTERNAL UNUSABLES BREAKDOWN

	I.D.	H.H.	Net	VRB	Other	T.H.H.
Lbs.	0.0	0.0	0.0	0.0	0.0	0.0
%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Fry Color Analysis

	0	*1*	*2*	*3*	*4*	*SE*	*HS*	*DE*	*Strips*	*Fry Points*
Strips	60	0	0	0	0	0	0	0	60	0
%	100%	0%	0%	0%	0%	0%	0%	0%		

Specific Gravity Measurements	*CPP*	*Avg Temp*	
1.0886		**Water**	45
		Tuber	56

Uncorrected Average Specific Gravity: 1.0886 **Ticket #:**

Specific Gravity Correction: 0.0004

Corrected Average Specific Gravity: **1.0890**

Comments:

Sample Method: Submitted

***Symptoms = Characteristics of Late Blight/Pink Rot/ Pink Eye**
Nonscorable symptoms are not Included in Unusables

I, the undersigned , on behalf of Ag World Support Systems, do hereby certify that at the request of the applicant and on the date indicated, samples of the herein described products were inspected and the quality and/or condition as shown by said samples were herein stated.

Signature:_____

Attachment 3

Processing Potato Trial
Nesson Valley, North Dakota
2015

Asunta (Susie) Thompson, Jerald Bergman, Tyler Tjelde
North Dakota State University

Areas of North Dakota and Minnesota, both part of the northern plains potato production region, produce potatoes for all markets, including tablestock, seed, and processing. Approximately 60% of production in ND and MN is for processing (frozen including French fries, and chips). Russet Burbank accounts for about 35% and Prospect Russet about 15% of the acreage in North Dakota. Russet Burbank, the industry standard for frozen processing and russet fresh marketing, has several shortcomings. It is a long-season cultivar, requiring high amounts of inputs including fertilizer and water, and Russet Burbank is susceptible to many pests (pathogens, insects, nematodes), but more importantly to many stresses including temperature and moisture. Producers, processors, and consumers are interested in cultivars that are more environmentally and economically sustainable, and that possess excellent processing qualities including low sugar accumulation and high specific gravity.

In order to identify these outstanding genotypes, the North Dakota State University (NDSU) potato breeding program as part of the potato improvement team conducts crossing, selection, evaluation, and cultivar development activities across North Dakota and western Minnesota. Field research trials were grown at eight sites in 2015; five sites were irrigated (Larimore, Oakes, Inkster, Williston, and Park Rapids), while three were non-irrigated (Hoople, Crystal and Grand Forks) sites. These trials aid in identifying selections with high yield potential, disease and pest resistance, stress tolerance, adaptability, and consumer quality attributes for tablestock and processing genotypes. This report summarizes the results from the Nesson Valley Processing Trial. Many of the entries are russet selections considered to be dual-purpose, in that they produce acceptable frozen processed products including French fries, and are also suitable for tablestock.

Eighteen advancing dual-purpose russet selections and commercially acceptable cultivars were included in the trial planted on May 4, 2015. The field plot design was a randomized complete block with four replicates; cultural practices typical of the growing area were used during the growing season, including sprinkler irrigation. Rows were 36 inches apart, with a 12-inch within-row spacing. The trial was harvested on September 30; days to harvest totaled 149.

Agronomic and quality evaluations, yield and grade, and French fry quality are summarized in Tables 1, 2 and 3, respectively. Percentage stand ranged from 81 to 100% (Table 1). Ranger Russet and Shepody had the lowest percentage stand and were the only two statistically significant from the other entries. There was not a clear reason for low stand in these two processing industry standards.

Total yields were significantly different, ranging from 248 cwt./acre for ND113099-2Russ to 424 cwt./acre for Umatilla Russet (Table 2). Russet Burbank and Umatilla Russet total yields were significantly higher than for all other clones. ND8068-5Russ is an exceptionally early clone,

with maturity 7 to 10 days earlier than a standard Russet Norkotah. Total yields for this selection will not compete with those of later maturity in a full season trial such as the trial at Nesson Valley. Total yields and yield of US No. 1 tubers was less than in 2014. Yield of US No. 1s ranged from 152 for AND97279-5Russ and ND102647-3Russ, to 307 for Umatilla Russet. Similarly, percentage US No. 1s ranged from 52% for AND97279-5Russ and ND102647-3Russ, to 91% for Dakota Trailblazer. The tuber size profile was smaller than for entries in 2014. The percentage of 6 to 12 ounce size tubers for most clones was in the 40 to mid-50 percentage range. Several entries had more than 40% undersized tubers (tubers 0-4 ounces), an undesirable trait for processing genotypes. Similarly, many of these same entries, including AND97279-5Russ, ND8068-5Russ, ND102647-3RussND113099-2Russand ND113526CB-1Russ, produced 30% or more of tubers om the 4-6 ounce category. Tubers in the 6-12 ounce range, tend to be the most desirable for French fry processing. Industry standards led the way in terms of percentage of yield for this grade category, along with new selections ND113065CB-1Russ and ND113065CB-2Russ. ND7882b-7Russ, Bannock Russet and Dakota Trailblazer had the most propensity to produced oversized tubers (>12 ounces). All entries had few US No. 2 tubers and Culls, with the exception of Russet Burbank. Russet Burbank, the industry standard for French fry production and russet tablestock, is very sensitive to environmental conditions, particularly moisture stress. Uneven watering results in misshapen tubers. Tubers per plant ranged from 5.0 to 10.5 for Dakota Trailblazer and Ranger Russet, respectively; mean tuber number per plant was 7.7 (Table 1).

Several quality attributes are presented in Table 1. Mean specific gravity was 1.0910, with a range of 1.0825 for Russet Norkotah to 1.1196 for Dakota Trailblazer (Table 1). Hollow heart and brown center were not a significant problem for any entries, thought ND113526CB-1Russ should be monitored in future trials. Blackspot bruise potential is assessed using the abrasive peel method of Pavek and Corsini. Blackspot bruises result when polyphenol oxidase and tyrosine combine within damaged cells due to tubers bumping around during harvest and handling. Usually the skin is not broken and bruises are difficult to detect without peeling. Based on a scale of 1 (none) to 5 (severe), clones ranged from 1.8 for Bannock Russet to 4.6 for Ranger Russet (the susceptible check in this assessment). Our results indicate producers should use management practices to maximize the marketing of bruise-free tubers, including a pre-harvest irrigation if appropriate, maintain belts and conveyors full of tubers and soil as the potatoes move through the harvester, limit drops, and utilize padding on harvesters, in trucks, and on conveyors going into storage. Shatter bruise potential was assessed using a bruising chamber with digger chain link baffles. As for blackspot, tubers are shattered after pulp temperatures in storage reach 45F. Entries are rated on a scale of 1 (none) to 5 (many and severe). Clones ranged from 1.5 to 2.6 for ND11526CB-1Russ and Alpine Russet, respectively. Keeping tubers properly hydrated, using bruise-free management techniques, and minimizing damage limit shatters. Shatter bruises may serve as an entrance point for pathogens such as *Fusarium graminearum*, *Fusarium sambucinum* and *Fusarium coeruleum*. as well as *Alternaria solani*, the causal agent of foliar and tuber early blight.

All advancing selections and industry standards had acceptable French fry color when fried at harvest (Table 3). Many demonstrate sugar end resistance, and while most clones considered resistant to sugar ends had some, they were light enough to be manageable during processing.

ND8068-5Russ, ND113526CB-1Russ, Alpine Russet, Bannock Russet, Dakota Trailblazer and MonDak Gold would be considered resistant based on fry scores after harvest. A better assessment would be following 8 weeks storage at 45F. ND113174B-2 Russ, Russet Norkotah, and Russet Burbank had the most severe and the most sugar ends as defined by the industry. Our program assesses a sugar end as any color deviation from the main fry. This is more stringent than the processing industry that requires a score of a 3 or 4 on the color chart to be called a sugar end. Clones with stem end colors 2 or less from storage can usually be managed during processing. Our research efforts are designed to identify processing (both chip and frozen) germplasm that will reliably and consistently process from long-term cold 38F and 42F (3.3C and/or 5.5C) storage. As we grade, a field (zero time) sample is collected for immediate French fry processing. French fry/frozen processing selections are also evaluated from 45F (7.2C) storage after eight weeks, and again the following May or June for fry color, stem end fry color, sugar ends, and other defects. All clones with processing potential (chip and frozen) are chipped from 38F after 8 weeks of storage as we seek genotypes that will reliable process from that temperature. Entries from this trial were not French fried from long-term storage in June/July 2016.

Promising advancing dual-purpose (frozen processing and tablestock) russet selections included ND8068-5Russ, ND113065CB-1Russ and ND113065CB-2Russ. The most advanced is ND8068-5Russ. ND8068-5Russ has very early maturity, thus will not compete with late season cultivars for yield. However, ND8068-5Russ sizes early and has potential as an early russet for packing or may be used in place of Shepody or Ranger Russet as a clone for opening processing plants in mid-July. It will be considered for release in late 2016 or early 2017.

We wish to express our gratitude to Richard Nilles (research specialist potato breeding) and the staff at the Nesson Research Extension Center for assisting with planting, plot maintenance, and harvesting this trial. We are appreciative and grateful to our many grower, industry, and research cooperators in North Dakota, the North Dakota Specialty Crops Block Grant Program (NDDA), and to RD Offutt Company and Enander Seed for certified seed potatoes in support of this processing research.

Attachment 3

Table 1. Agronomic and quality evaluations for advanced processing selections and cultivars, full season, Nesson Valley, ND, 2015.

Clone	% Stand	Tubers Per Plant	Specific Gravity[1]	% Hollow Heart[2]	Black-spot Bruise[3]	Shatter Bruise[4]	General Rating
1. AND97279-5Russ	100	9.8	1.1008	0	3.7	2.4	2.9
2. ND7882b-7Russ	88	5.9	1.0874	0	4.2	1.6	3.4
3. ND8068-5Russ	98	7.4	1.0908	0	4.5	2.3	3.0
4. ND102647-3Russ	98	9.2	1.0832	0	2.5	2.0	2.6
5. ND113065CB-1Russ	99	8.4	1.0842	0	4.0	1.8	3.8
6. ND113065CB-2Russ	94	7.2	1.0824	0	3.6	1.8	4.0
7. ND113099-2Russ	96	6.0	1.0786	0	3.1	2.3	3.1
8. ND113174B-1Russ	98	9.8	1.1108	0	2.4	2.0	3.0
9. ND113526CB-1Russ	99	9.5	1.0914	3	3.5	1.5	2.3
10. Alpine Russet	96	6.7	1.0883	0	2.9	2.6	3.5
11. Bannock Russet	99	6.2	1.0934	0	1.8	2.5	3.8
12. Dakota Trailblazer	95	5.0	1.1196	0	2.1	2.4	4.4
13. MonDak Gold	93	7.4	1.0902	0	4.5	2.6	2.0
14. Ranger Russet	81	10.5	1.0940	0	4.6	1.9	3.1
15. Russet Burbank	95	8.5	1.0838	0	3.4	2.3	3.1
16. Russet Norkotah	94	6.1	1.0825	0	4.2	1.6	4.4
17. Shepody	83	6.6	1.0888	0	2.6	1.8	3.4
18. Umatilla Russet	99	9.3	1.0875	1	2.5	2.3	3.4
Mean	95	7.7	1.0910	0	3.6	2.1	3.3
LSD ($\propto$=0.05)	10	2.3	0.0049	2	0.8	0.7	0.6

[1] Determined using weight-in-air, weight-in-water method.
[2] Hollow heart includes brown center.
[3] Blackspot bruise determined by the abrasive peel method, scale 1-5, 1=none, 5=severe.
[4] Shatter bruise is evaluated using a bruising chamber with digger chain link baffles. Tubers are stored at 45F prior bruising. Shatter bruises are rated on a scale of 1-5, with 1 = none and 5 = many and severe.
[5] The general rating is a score given based on appearance, yield, tuber size distribution, external defects, similar to a breeder's merit score.

Attachment 3

Table 2. Yield and grade for advanced processing selections and cultivars, full season, Nesson Valley, ND, 2015.

Clone	Total Yield Cwt./A	US No. 1 Cwt./A	US No. 1 %	0-4 oz. %	4-6 oz. %	6-12 oz. %	>12 oz. %	US No. 2 %	Culls %
1. AND97279-5Russ	296	152	52	47	34	17	1	0	1
2. ND7882b-7Russ	282	223	79	15	19	39	22	1	4
3. ND8068-5Russ	255	154	61	35	33	27	1	0	4
4. ND102647-3Russ	291	152	52	47	33	18	1	1	0
5. ND113065CB-1Russ	338	248	73	26	29	42	2	0	0
6. ND113065CB-2Russ	271	196	73	27	27	41	5	0	0
7. ND113099-2Russ	248	183	74	24	34	33	7	1	2
8. ND113174B-1Russ	328	185	55	41	29	25	2	0	3
9. ND113526CB-1Russ	286	160	56	44	33	23	0	1	0
10. Alpine Russet	334	275	82	15	20	48	14	2	0
11. Bannock Russet	356	303	85	13	18	48	20	0	2
12. Dakota Trailblazer	305	279	91	9	18	56	18	0	0
13. MonDak Gold	284	213	75	25	29	43	3	0	0
14. Ranger Russet	374	298	80	18	20	45	14	1	2
15. Russet Burbank	420	294	69	17	20	40	10	2	11
16. Russet Norkotah	271	216	80	19	21	47	13	0	0
17. Shepody	266	207	77	19	24	41	12	1	2
18. Umatilla Russet	424	307	72	23	25	37	11	2	3
Mean	313	225	71	26	26	37	9	1	2
LSD ($\propto$=0.05)	57	61	11	11	9	11	12	1	2

Attachment 3

Table 3. French fry evaluations following harvest, full season trial, Nesson Valley, ND, 2015.

Clone	Fry Color[1]	Stem-end Color	% Sugar End[2]
	Field Fry		
1. AND97279-5Russ	0.6	1.3	42
2. ND7882b-7Russ	0.8	1.4	67
3. ND8068-5Russ	0.5	1.0	17
4. ND102647-3Russ	0.5	1.4	42
5. ND113065CB-1Russ	1.3	1.8	25
6. ND113065CB-2Russ	1.0	1.6	17
7. ND113099-2Russ	1.3	2.0	50
8. ND113174B-1Russ	0.6	2.0	75
9. ND113526CB-1Russ	0.5	1.0	25
10. Alpine Russet	1.0	1.1	8
11. Bannock Russet	0.8	1.1	50
12. Dakota Trailblazer	0.5	0.8	25
13. MonDak Gold	0.6	0.7	8
14. Ranger Russet	0.8	1.5	25
15. Russet Burbank	0.6	2.4	100
16. Russet Norkotah	1.9	2.9	33
17. Shepody	0.6	1.1	50
18. Umatilla Russet	0.8	1.4	50
Mean	0.8	1.5	39
LSD ($\propto$=0.05)	0.5	1.1	48

[1] Fry color scores: 0.1 corresponds to 000, 0.3 corresponds to 00, 0.5 corresponds to 0, 1.0 equals 1.0; subsequent numbers follow French fry rating scale 000 to 4.0. Scores of 3.0 and above are unacceptable because adequate sugars cannot be leached from the tuber flesh to make an acceptable fry of good texture.

[2] Any stem end darker than the main fry is considered a sugar end in these evaluations, thus mirroring the worst case scenario. The processing industry defines a sugar end as a 3.0 or darker.

MonDak Gold Quality Evaluations

NFPT SCRI data

http://hort-fms.cals.wisc.edu/fmi/webd#

Yield and specific gravity (MDG)

Trial	Year	State	Breeder Merit	Tuber shape, flesh color	Total yield (lbs) Clone	Total yield (lbs) Burbank	Total yield (lbs) Ranger	Marketable yield (%) Total	Marketable yield (%) >6 oz	Marketable yield (%) >10 oz	Specific gravity Harvest		Specific gravity Early season		Specific gravity Mid season	
NFPT	2012	ID	marginal	oblong, lt. yellow	102	57	77	90	82	54	9/20	1.087	10/25	1.088	2/20	1.079
NFPT	2011	ID	marginal	oblong, lt. yellow	60	73	90	91	73	44	9/22	1.086	10/19	1.089	1/12	1.083
NFPT	2012	ND	marginal	oblong, lt. yellow	76	79	75	84	62	26	9/20	1.103	11/1	1.105	2/26	1.099
NFPT	2011	ND	keep	oblong, yellow	58	89	80	75	50	7	10/7	1.083	10/20	1.089	1/11	1.080
NFPT	2012	WA	marginal	oval, white	158	138	211	93	88	69	9/25	1.081	10/24	1.084	2/20	1.080
NFPT	2011	WA	marginal	oval, yellow	104	128	125	85	79	65		1.081	10/11	1.081	1/12	1.079
NFPT SCRI	2012	WI	marginal	oval	129	159	98	56	32	6	9/24	1.072	11/1	1.077	2/26	1.076
SCRI Demo	2011	WI		oblong	107	86	62	54	22	0	9/8	1.078				
NFPT SCRI	2012	ME	keep	long, yellow	109	70	62	69	45	12	10/3	1.082	11/7	1.072	3/5	1.078
Average across site-years					100	98	93	78	59	32		1.084		1.086		1.082

Sugars (MDG)

Trial	Year	State	Sucrose (mg/g fresh weight) Early season		Mid season		Late season		Glucose (mg/g fresh weight) Early season		Mid season		Late season	
NFPT	2012	ID	11/26	1.765	2/20	1.590	7/25	1.485	11/26	0.162	2/20	0.714	7/25	1.673
NFPT	2011	ID	10/19	1.545	1/10	0.958	5/12	1.059	10/19	0.232	1/10	0.248	5/12	0.345
NFPT	2012	ND	11/26	0.870	2/26	1.623	7/25	3.071	11/26	0.335	2/26	0.154	7/25	1.778
NFPT	2011	ND	10/20	2.255	1/11	3.712	5/12	2.246	10/20	0.208	1/11	0.529	5/12	0.402
NFPT	2012	WA	11/26	2.076	2/20	2.136	7/25	1.934	11/26	0.150	2/20	0.270	7/25	0.649
NFPT	2011	WA	10/11	1.728	1/12	1.490	5/12	0.940	10/11	0.127	1/12	0.377	5/12	0.065
NFPT SCRI	2012	WI	11/27	2.053	2/26	3.002	7/25	3.749	11/27	0.351	2/26	0.957	7/25	4.799
SCRI Demo	2011	WI												
NFPT SCRI	2012	ME	11/29	1.971	3/5	1.002	7/25	1.531	11/29	0.257	3/5	0.712	7/25	0.574
Average across site-years				1.783		1.939		2.002		0.228		0.495		1.286

Field day (MDG)

Trial	Year	State	Tuber length	Tuber shape	Size distribution	Area uniformity	Variety merit	Overall rating	Rank at location
NFPT	2012	ID	1.46	1.92	1.91	2.57	2.48	2.07	51 / 88
NFPT	2012	ND	2.00	2.52	2.46	2.57	2.48	2.41	66 / 88
NFPT	2012	WA	1.91	2.31	2.25	2.57	2.48	2.30	64 / 88
NFPT SCRI	2012	WI	2.34	2.34	2.31	2.57	2.48	2.41	66 / 88
NFPT SCRI	2012	ME							/
NFPT	2011	ID							/
NFPT	2011	ND							/
NFPT	2011	WA							/
SCRI Demo	2011	WI							/
Average across site-years			1.93	2.27	2.23	2.57	2.48	2.30	

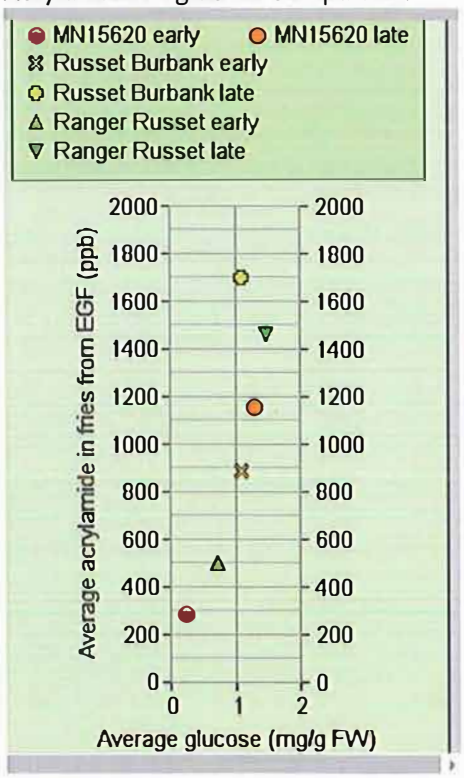
Acrylamide (MDG)

Trial	Year	State	Clone acrylamide, EGF process (ppb)						Acrylamide Russet Burbank (ppb)			Acrylamide Ranger Russet (ppb)		
			Early season		Mid season		Late season		Early season	Mid season	Late season	Early season	Mid season	Late season
NFPT	2012	ID	11/27		3/1	287	6/25	1330	674	1330	1540	382	1010	1960
NFPT	2011	ID	10/19	185	1/10	388	5/12	430	1388	1550	2735	568	1100	1445
NFPT	2012	ND	11/29	162	3/11	486	7/9	871	546	505	1050	417	549	408
NFPT	2011	ND	10/20	360	1/11	663	5/12	1278	723	878	1535	445	893	2638
NFPT	2012	WA	11/19	256	3/6	895	6/25	1940	821	4030	2350	527	753	1310
NFPT	2011	WA	10/11	120	1/12	258	5/12	1305	475	1243	2108	480	1330	2278
NFPT SCRI	2012	WI	12/4	674	3/12	1130	7/9	1550	1730	1010	1270	688	1600	1250
SCRI Demo	2011	WI				506		486		948	1570		617	1130
NFPT SCRI	2012	ME	12/11	218	3/18	527	7/9	1180	689	871	1110	453	495	700
Average across site-years				282		571		1152	881	1374	1696	495	927	1458

Asparagine (MDG)

Trial	Year	State	Asparagine (mg/g fresh weight)						Asparagine (molar percent of total amino acids)					
			Early season		Mid season		Late season		Early season		Mid season		Late season	
NFPT	2012	ID	11/27	2.9			6/25	2.7	11/27	30.7			6/25	28.2
NFPT	2011	ID	10/19	1.1	1/10		5/12	1.5	10/19		1/10		5/12	
NFPT	2012	ND	11/29	2.8			7/9	3.5	11/29	39.3			7/9	37.1
NFPT	2011	ND	10/20		1/11		5/12	1.9	10/20		1/11		5/12	
NFPT	2012	WA	11/29	3.1			6/25	3.0	11/29	31.6			6/25	29.2
NFPT	2011	WA	10/11	2.5	1/12		5/12	1.6	10/11		1/12		5/12	
NFPT SCRI	2012	WI	12/4	3.1			7/9	3.6	12/4	34.9			7/9	30.1
SCRI Demo	2011	WI												
NFPT SCRI	2012	ME	12/8	2.7			7/9	3.4	12/8	34.2			7/9	33.8

Acrylamide vs glucose comparison



Russet Burbank

Yield and specific gravity (RB)

Trial	Year	State	Breeder Merit	Tuber shape, flesh color	Total yield (lbs)			Marketable yield (%)			Specific gravity					
					Clone	Burbank	Ranger	Total	>6 oz	>10 oz	Harvest		Early season		Mid season	
NFPT	2014	ID	keep	long, white	127	113	108	75	70	21	9/17	1.082				
NFPT	2014	ID	drop	long, white	99	113	108	61	64	27	9/17	1.081				
NFPT	2013	ID	marginal	long, white	78	78	94	75	58	25	9/17	1.078	12/17	1.072	2/3	1.085
NFPT	2012	ID	drop	long, white	57	57	77	66	51	19	9/20	1.068	10/25	1.070	2/20	1.067
NFPT	2011	ID	marginal	long, white	73	73	90	88	71	35	9/22	1.080	10/19	1.070	1/12	1.073
NFPT	2014	ND	drop	long	116	116	148	22	87	43	9/29	1.087				
NFPT	2014	ND	marginal	long	116	116	148	22	87	43	9/29	1.088				
NFPT	2013	ND	marginal	long	142	142	136	76	52	18	10/3	1.091	12/23	1.090	2/17	1.083
NFPT	2012	ND	marginal	long, white	79	79	75	85	67	44	9/20	1.100	11/1	1.094	2/26	1.101
NFPT	2011	ND	marginal	long, white	89	89	80	68	38	7	10/8	1.079	10/20	1.087	1/11	1.087
NFPT	2014	WA	marginal	oblong	154	147	140	86	86	59	9/17	1.080				
NFPT	2014	WA	marginal	oblong	141	147	140	89	88	45	9/17	1.080				
NFPT	2013	WA	marginal	oblong	121	121	152	80	63	35	9/24	1.074	12/11	1.070	2/3	1.068
NFPT	2012	WA	drop	oval, white	138	138	211	87	77	50	9/25	1.071	10/24	1.081	2/20	1.081
NFPT	2011	WA	drop	oval, white	128	128	125	84	66	22		1.064	10/11	1.083	1/12	1.081
NFPT SCRI	2014	WI	keep	oblong	174	182	121	76	84	57	9/26	1.074				
NFPT SCRI	2014	WI	keep	oblong	190	182	121	83	85	49	9/26	1.077				
NFPT SCRI	2013	WI		oblong	148	148	188	87	80	36			1/13	1.081	2/24	1.075
NFPT SCRI	2012	WI	marginal	long	159	159	98	33	17	3	9/24	1.068	11/1	1.075	2/26	1.072
SCRI Demo	2011	WI		oblong	86	86	62	40	11	1	9/8	1.079				
NFPT SCRI	2014	ME	keep	long, white	140	91	97	69	70	24	9/30	1.084				
NFPT SCRI	2014	ME	keep	long, white	116	91	97	73	71	23	9/30	1.085				
NFPT SCRI	2013	ME	marginal	, white	188	188	131	63	48	28	9/27	1.077	1/21	1.080	3/3	1.079
NFPT SCRI	2012	ME	marginal	long, white	70	70	62	49	28	12	10/3	1.077	11/7	1.071	3/5	1.073
SCRI Demo	2014	MI	keep	long, white	51	52	54	61	33	27	9/12	1.071				
SCRI Demo	2013	MI														
Average across site-years					119	116	114	68	62	30		1.079		1.079		1.079

Field day evaluations (RB)

Trial	Year	State	Tuber length	Tuber shape	Size distribution	Area uniformity	Variety merit	Overall rating	Rank at location
NFPT	2014	ID	1.44	1.89	1.94	1.89	2.37	1.91	41 / 62
NFPT	2014	ID	1.67	2.39	2.11	2.33	2.47	2.19	62 / 62
NFPT	2014	ND	1.56	2.67	1.61	1.89	2.37	2.02	48 / 69
NFPT	2014	ND							/
NFPT	2014	WA	1.22	1.44	1.56	1.89	2.37	1.70	13 / 70
NFPT	2014	WA	1.89	2.33	1.94	2.33	2.47	2.19	60 / 70
NFPT SCRI	2014	WI	2.06	2.33	2.33	1.89	2.37	2.20	67 / 70
NFPT SCRI	2014	WI	1.78	2.11	2.50	2.33	2.47	2.24	70 / 70
NFPT SCRI	2014	ME	1.78	2.50	2.22	1.89	2.37	2.15	20 / 47
NFPT SCRI	2014	ME	2.28	2.44	2.72	2.33	2.47	2.45	43 / 47
SCRI Demo	2014	MI							/
NFPT	2013	ID	1.84	2.43	2.06	2.54	2.67	2.31	57 / 74
NFPT	2013	ND							/
NFPT	2013	WA	2.15	2.57	2.41	2.54	2.67	2.47	67 / 78
NFPT SCRI	2013	WI	1.89	2.09	2.02	2.54	2.67	2.24	52 / 78
NFPT SCRI	2013	ME	2.01	2.78	2.09	2.54	2.67	2.42	44 / 59
SCRI Demo	2013	MI							/
NFPT	2012	ID	1.82	2.41	2.47	2.51	2.57	2.36	71 / 88
NFPT	2012	ND	1.64	2.54	1.94	2.51	2.57	2.24	54 / 88
NFPT	2012	WA	2.47	2.32	2.14	2.51	2.57	2.40	71 / 88
NFPT SCRI	2012	WI	2.59	2.59	1.96	2.51	2.57	2.45	71 / 88
NFPT SCRI	2012	ME							/
NFPT	2011	ID							/
NFPT	2011	ND							/
NFPT	2011	WA							/
SCRI Demo	2011	WI							/
Average across site-years			1.89	2.34	2.12	2.29	2.51	2.23	

Acrylamide (RB)

Trial	Year	State	Clone acrylamide, EGF process (ppb)						Acrylamide Russet Burbank (ppb)			Acrylamide Ranger Russet (ppb)		
			Early season		Mid season		Late season		Early season	Mid season	Late season	Early season	Mid season	Late season
NFPT	2014	ID	12/16	1520			7/1	819	1935		721	845		1190
NFPT	2014	ID	12/16	2350			7/1	622	1935		721	845		1190
NFPT	2013	ID	12/17	1440	3/18	1200	7/8	626	1440	1200	626	1280	517	915
NFPT	2012	ID	11/27	674	3/1	1330	6/25	1540	674	1330	1540	382	1010	1960
NFPT	2011	ID	10/19	1388	1/10	1550	5/12	2735	1388	1550	2735	568	1100	1445
NFPT	2014	ND	12/16	1890			7/8	778	1820		747	1370		981
NFPT	2014	ND	12/16	1750			7/8	716	1820		747	1370		981
NFPT	2013	ND	12/23	2020	3/18	1960	7/8	1440	2020	1960	1440	1630	1400	1830
NFPT	2012	ND	11/29	546	3/11	505	7/9	1050	546	505	1050	417	549	408
NFPT	2011	ND	10/20	723	1/11	878	5/12	1535	723	878	1535	445	893	2638
NFPT	2014	WA	12/4	1430			7/1	1010	1400		1700	1290		1730
NFPT	2014	WA	12/4	1370			7/1	2390	1400		1700	1290		1730
NFPT	2013	WA	12/11	3010	3/18	1890	7/8	1390	3010	1890	1390	3240	927	886
NFPT	2012	WA	11/19	821	3/6	4030	6/25	2350	821	4030	2350	527	753	1310
NFPT	2011	WA	10/11	475	1/12	1243	5/12	2108	475	1243	2108	480	1330	2278
NFPT SCRI	2014	WI	12/24	1610			7/27	1245	1465		1013	748		2173
NFPT SCRI	2014	WI	12/24	1320			7/27	780	1465		1013	748		2173
NFPT SCRI	2013	WI	1/13	1100	3/27	340	7/8	523	1100	340	523	1358	578	930
NFPT SCRI	2012	WI	12/4	1730	3/12	1010	7/9	1270	1730	1010	1270	688	1600	1250
SCRI Demo	2011	WI				948		1570		948	1570		617	1130
NFPT SCRI	2014	ME	12/24	835			7/29	153	908		174	495		275
NFPT SCRI	2014	ME	12/24	980			7/29	195	908		174	495		275
NFPT SCRI	2013	ME	1/21	1413	4/3	1435	7/8	1420	1413	1435	1420	1093	898	1058
NFPT SCRI	2012	ME	12/11	689	3/18	871	7/9	1110	689	871	1110	453	495	700
SCRI Demo	2014	MI												
SCRI Demo	2013	MI												
Average across site-years				1351		1371		1224	1351	1371	1224	959	905	1310

Asparagine (RB)

Trial	Year	State	Asparagine (mg/g fresh weight)						Asparagine (molar percent of total amino acids)					
			Early season		Mid season		Late season		Early season		Mid season		Late season	
NFPT	2014	ID					7/8	3.0					7/8	
NFPT	2014	ID					7/8	3.5					7/8	
NFPT	2013	ID					7/8	3.1					7/8	
NFPT	2012	ID	11/27	4.1			6/25	4.1	11/27	36.6			6/25	36.0
NFPT	2011	ID	10/19	1.5	1/10		5/12	1.7	10/19		1/10		5/12	
NFPT	2014	ND												
NFPT	2014	ND												
NFPT	2013	ND												
NFPT	2012	ND	11/29	3.6			7/9	4.1	11/29	42.5			7/9	43.6
NFPT	2011	ND	10/20	1.3	1/11		5/12	1.5	10/20		1/11		5/12	
NFPT	2014	WA												
NFPT	2014	WA												
NFPT	2013	WA					7/8	4.1					7/8	
NFPT	2012	WA	11/29	3.0			6/25	3.2	11/29	31.9			6/25	36.1
NFPT	2011	WA	10/11	2.8	1/12		5/12	1.8	10/11		1/12		5/12	
NFPT SCRI	2014	WI					7/8	1.3					7/8	
NFPT SCRI	2014	WI					7/8	1.4					7/8	
NFPT SCRI	2013	WI												
NFPT SCRI	2012	WI	12/4	2.9			7/9	3.4	12/4	36.9			7/9	37.8
SCRI Demo	2011	WI												
NFPT SCRI	2014	ME												
NFPT SCRI	2014	ME												
NFPT SCRI	2013	ME												
NFPT SCRI	2012	ME	12/8	3.1			7/9	4.0	12/8	38.6			7/9	41.2
SCRI Demo	2014	MI												
SCRI Demo	2013	MI												

Sugars (RB)

Trial	Year	State	Sucrose (mg/g fresh weight) Early season		Mid season		Late season		Glucose (mg/g fresh weight) Early season		Mid season		Late season	
NFPT	2014	ID	9/29	0.613	3/4	1.083	6/29	0.637	9/29	0.493	3/4	0.938	6/29	1.040
NFPT	2014	ID	9/29	0.951	3/4	1.412	6/29	0.815	9/29	0.486	3/4	1.306	6/29	1.227
NFPT	2013	ID	12/17	0.758	4/7	0.929	7/9	0.417	12/17	1.244	4/7	1.503	7/9	0.704
NFPT	2012	ID	11/26	0.764	2/20	1.015	7/25	0.740	11/26	0.823	2/20	0.914	7/25	0.913
NFPT	2011	ID	10/19	1.248	1/10	0.550	5/12	0.426	10/19	1.600	1/10	1.179	5/12	1.227
NFPT	2014	ND	1/7	1.010	3/4	2.397	6/29	1.471	1/7	2.035	3/4	1.034	6/29	0.664
NFPT	2014	ND	1/7	1.020	3/4	3.506	6/29	0.907	1/7	2.273	3/4	0.749	6/29	1.164
NFPT	2013	ND	12/23	0.845	4/7	1.469	7/9	1.719	12/23	2.351	4/7	3.016	7/9	4.492
NFPT	2012	ND	11/26	1.175	2/26	1.143	7/25	0.759	11/26	0.494	2/26	0.217	7/25	0.170
NFPT	2011	ND	10/20	1.102	1/11	1.270	5/12	0.997	10/20	1.567	1/11	1.600	5/12	2.269
NFPT	2014	WA	9/29	0.746	3/4	1.655	6/29	0.654	9/29	0.980	3/4	1.051	6/29	0.303
NFPT	2014	WA	9/29	0.496	3/4	1.188	6/29	0.343	9/29	0.691	3/4	1.632	6/29	1.778
NFPT	2013	WA	12/11	0.842	4/7	1.004	7/9	0.115	12/11	2.255	4/7	2.823	7/9	1.751
NFPT	2012	WA	11/26	1.051	2/20	1.331	7/25	0.754	11/26	1.132	2/20	0.735	7/25	0.761
NFPT	2011	WA	10/11	1.359	1/12	0.697	5/12	0.862	10/11	0.719	1/12	1.229	5/12	0.995
NFPT SCRI	2014	WI	1/7	1.149	3/4	1.467	6/29	1.057	1/7	0.613	3/4	0.845	6/29	0.648
NFPT SCRI	2014	WI	1/7	0.850	3/4	0.969	6/29	0.701	1/7	0.673	3/4	0.742	6/29	0.810
NFPT SCRI	2013	WI	1/13	1.083	4/7	1.159	7/9	0.889	1/13	0.423	4/7	0.514	7/9	0.540
NFPT SCRI	2012	WI	11/27	1.632	2/26	1.467	7/25	0.730	11/27	1.645	2/26	1.435	7/25	1.930
SCRI Demo	2011	WI												
NFPT SCRI	2014	ME	1/7	1.490	3/4	0.929	6/29	0.946	1/7	0.796	3/4	0.083	6/29	0.157
NFPT SCRI	2014	ME	1/7	1.545	3/4	2.026	6/29	0.801	1/7	0.534	3/4	0.325	6/29	0.062
NFPT SCRI	2013	ME	1/21	0.103	4/7	0.389	7/9	0.131	1/21	1.939	4/7	1.186	7/9	2.475
NFPT SCRI	2012	ME	11/29	1.163	3/5	1.222	7/25	0.902	11/29	0.937	3/5	0.908	7/25	0.566
SCRI Demo	2014	MI												
SCRI Demo	2013	MI												
Average across site-years				1.000		1.316		0.773		1.161		1.129		1.158

Jerald Bergman

From: Greg Reynolds <greg.reynolds@frontier.com>
Sent: Monday, February 22, 2016 10:46 AM
To: Jerald Bergman
Subject: mondak gold endorsement

Hi Jerry,

We have been getting a few MonDak Gold seed potatoes for the past few years and selling them as fresh market potatoes. They have been popular across all our market segments. Most of our restaurant customers are using them for french fries and prefer them to anything else they have used. The school cafeterias like them because they are are smooth skinned and easy to wash, the kids eat them readily. The food co-ops also found them easy to sell.

For everyone growing them they are a productive potato with very few culls.

Greg Reynolds
Riverbend Farm
5405 Calder Ave SE
Delano, MN 55328
763-972-3295

Attachment 6

Processing Potato Trial
Nesson Valley, North Dakota
2015

Asunta (Susie) Thompson, Jerald Bergman, Tyler Tjelde
North Dakota State University

Areas of North Dakota and Minnesota, both part of the northern plains potato production region, produce potatoes for all markets, including tablestock, seed, and processing. Approximately 60% of production in ND and MN is for processing (frozen including French fries, and chips). Russet Burbank accounts for about 35% and Prospect Russet about 15% of the acreage in North Dakota. Russet Burbank, the industry standard for frozen processing and russet fresh marketing, has several shortcomings. It is a long-season cultivar, requiring high amounts of inputs including fertilizer and water, and Russet Burbank is susceptible to many pests (pathogens, insects, nematodes), but more importantly to many stresses including temperature and moisture. Producers, processors, and consumers are interested in cultivars that are more environmentally and economically sustainable, and that possess excellent processing qualities including low sugar accumulation and high specific gravity.

In order to identify these outstanding genotypes, the North Dakota State University (NDSU) potato breeding program as part of the potato improvement team conducts crossing, selection, evaluation, and cultivar development activities across North Dakota and western Minnesota. Field research trials were grown at eight sites in 2015; five sites were irrigated (Larimore, Oakes, Inkster, Williston, and Park Rapids), while three were non-irrigated (Hoople, Crystal and Grand Forks) sites. These trials aid in identifying selections with high yield potential, disease and pest resistance, stress tolerance, adaptability, and consumer quality attributes for tablestock and processing genotypes. This report summarizes the results from the Nesson Valley Processing Trial. Many of the entries are russet selections considered to be dual-purpose, in that they produce acceptable frozen processed products including French fries, and are also suitable for tablestock.

Eighteen advancing dual-purpose russet selections and commercially acceptable cultivars were included in the trial planted on May 4, 2015. The field plot design was a randomized complete block with four replicates; cultural practices typical of the growing area were used during the growing season, including sprinkler irrigation. Rows were 36 inches apart, with a 12-inch within-row spacing. The trial was harvested on September 30; days to harvest totaled 149.

Agronomic and quality evaluations, yield and grade, and French fry quality are summarized in Tables 1, 2 and 3, respectively. Percentage stand ranged from 81 to 100% (Table 1). Ranger Russet and Shepody had the lowest percentage stand and were the only two statistically significant from the other entries. There was not a clear reason for low stand in these two processing industry standards.

Total yields were significantly different, ranging from 248 cwt./acre for ND113099-2Russ to 424 cwt./acre for Umatilla Russet (Table 2). Russet Burbank and Umatilla Russet total yields were significantly higher than for all other clones. ND8068-5Russ is an exceptionally early clone,

with maturity 7 to 10 days earlier than a standard Russet Norkotah. Total yields for this selection will not compete with those of later maturity in a full season trial such as the trial at Nesson Valley. Total yields and yield of US No. 1 tubers was less than in 2014. Yield of US No. 1s ranged from 152 for AND97279-5Russ and ND102647-3Russ, to 307 for Umatilla Russet. Similarly, percentage US No. 1s ranged from 52% for AND97279-5Russ and ND102647-3Russ, to 91% for Dakota Trailblazer. The tuber size profile was smaller than for entries in 2014. The percentage of 6 to 12 ounce size tubers for most clones was in the 40 to mid-50 percentage range. Several entries had more than 40% undersized tubers (tubers 0-4 ounces), an undesirable trait for processing genotypes. Similarly, many of these same entries, including AND97279-5Russ, ND8068-5Russ, ND102647-3RussND113099-2Russand ND113526CB-1Russ, produced 30% or more of tubers om the 4-6 ounce category. Tubers in the 6-12 ounce range, tend to be the most desirable for French fry processing. Industry standards led the way in terms of percentage of yield for this grade category, along with new selections ND113065CB-1Russ and ND113065CB-2Russ. ND7882b-7Russ, Bannock Russet and Dakota Trailblazer had the most propensity to produced oversized tubers (>12 ounces). All entries had few US No. 2 tubers and Culls, with the exception of Russet Burbank. Russet Burbank, the industry standard for French fry production and russet tablestock, is very sensitive to environmental conditions, particularly moisture stress. Uneven watering results in misshapen tubers. Tubers per plant ranged from 5.0 to 10.5 for Dakota Trailblazer and Ranger Russet, respectively; mean tuber number per plant was 7.7 (Table 1).

Several quality attributes are presented in Table 1. Mean specific gravity was 1.0910, with a range of 1.0825 for Russet Norkotah to 1.1196 for Dakota Trailblazer (Table 1). Hollow heart and brown center were not a significant problem for any entries, thought ND113526CB-1Russ should be monitored in future trials. Blackspot bruise potential is assessed using the abrasive peel method of Pavek and Corsini. Blackspot bruises result when polyphenol oxidase and tyrosine combine within damaged cells due to tubers bumping around during harvest and handling. Usually the skin is not broken and bruises are difficult to detect without peeling. Based on a scale of 1 (none) to 5 (severe), clones ranged from 1.8 for Bannock Russet to 4.6 for Ranger Russet (the susceptible check in this assessment). Our results indicate producers should use management practices to maximize the marketing of bruise-free tubers, including a pre-harvest irrigation if appropriate, maintain belts and conveyors full of tubers and soil as the potatoes move through the harvester, limit drops, and utilize padding on harvesters, in trucks, and on conveyors going into storage. Shatter bruise potential was assessed using a bruising chamber with digger chain link baffles. As for blackspot, tubers are shattered after pulp temperatures in storage reach 45F. Entries are rated on a scale of 1 (none) to 5 (many and severe). Clones ranged from 1.5 to 2.6 for ND11526CB-1Russ and Alpine Russet, respectively. Keeping tubers properly hydrated, using bruise-free management techniques, and minimizing damage limit shatters. Shatter bruises may serve as an entrance point for pathogens such as *Fusarium graminearum*, *Fusarium sambucinum* and *Fusarium coeruleum*. as well as *Alternaria solani*, the causal agent of foliar and tuber early blight.

All advancing selections and industry standards had acceptable French fry color when fried at harvest (Table 3). Many demonstrate sugar end resistance, and while most clones considered resistant to sugar ends had some, they were light enough to be manageable during processing.

ND8068-5Russ, ND113526CB-1Russ, Alpine Russet, Bannock Russet, Dakota Trailblazer and MonDak Gold would be considered resistant based on fry scores after harvest. A better assessment would be following 8 weeks storage at 45F. ND113174B-2 Russ, Russet Norkotah, and Russet Burbank had the most severe and the most sugar ends as defined by the industry. Our program assesses a sugar end as any color deviation from the main fry. This is more stringent than the processing industry that requires a score of a 3 or 4 on the color chart to be called a sugar end. Clones with stem end colors 2 or less from storage can usually be managed during processing. Our research efforts are designed to identify processing (both chip and frozen) germplasm that will reliably and consistently process from long-term cold 38F and 42F (3.3C and/or 5.5C) storage. As we grade, a field (zero time) sample is collected for immediate French fry processing. French fry/frozen processing selections are also evaluated from 45F (7.2C) storage after eight weeks, and again the following May or June for fry color, stem end fry color, sugar ends, and other defects. All clones with processing potential (chip and frozen) are chipped from 38F after 8 weeks of storage as we seek genotypes that will reliable process from that temperature. Entries from this trial were not French fried from long-term storage in June/July 2016.

Promising advancing dual-purpose (frozen processing and tablestock) russet selections included ND8068-5Russ, ND113065CB-1Russ and ND113065CB-2Russ. The most advanced is ND8068-5Russ. ND8068-5Russ has very early maturity, thus will not compete with late season cultivars for yield. However, ND8068-5Russ sizes early and has potential as an early russet for packing or may be used in place of Shepody or Ranger Russet as a clone for opening processing plants in mid-July. It will be considered for release in late 2016 or early 2017.

We wish to express our gratitude to Richard Nilles (research specialist potato breeding) and the staff at the Nesson Research Extension Center for assisting with planting, plot maintenance, and harvesting this trial. We are appreciative and grateful to our many grower, industry, and research cooperators in North Dakota, the North Dakota Specialty Crops Block Grant Program (NDDA), and to RD Offutt Company and Enander Seed for certified seed potatoes in support of this processing research.

Attachment 6

Table 1. Agronomic and quality evaluations for advanced processing selections and cultivars, full season, Nesson Valley, ND, 2015.

Clone	% Stand	Tubers Per Plant	Specific Gravity[1]	% Hollow Heart[2]	Black-spot Bruise[3]	Shatter Bruise[4]	General Rating
1. AND97279-5Russ	100	9.8	1.1008	0	3.7	2.4	2.9
2. ND7882b-7Russ	88	5.9	1.0874	0	4.2	1.6	3.4
3. ND8068-5Russ	98	7.4	1.0908	0	4.5	2.3	3.0
4. ND102647-3Russ	98	9.2	1.0832	0	2.5	2.0	2.6
5. ND113065CB-1Russ	99	8.4	1.0842	0	4.0	1.8	3.8
6. ND113065CB-2Russ	94	7.2	1.0824	0	3.6	1.8	4.0
7. ND113099-2Russ	96	6.0	1.0786	0	3.1	2.3	3.1
8. ND113174B-1Russ	98	9.8	1.1108	0	2.4	2.0	3.0
9. ND113526CB-1Russ	99	9.5	1.0914	3	3.5	1.5	2.3
10. Alpine Russet	96	6.7	1.0883	0	2.9	2.6	3.5
11. Bannock Russet	99	6.2	1.0934	0	1.8	2.5	3.8
12. Dakota Trailblazer	95	5.0	1.1196	0	2.1	2.4	4.4
13. MonDak Gold	93	7.4	1.0902	0	4.5	2.6	2.0
14. Ranger Russet	81	10.5	1.0940	0	4.6	1.9	3.1
15. Russet Burbank	95	8.5	1.0838	0	3.4	2.3	3.1
16. Russet Norkotah	94	6.1	1.0825	0	4.2	1.6	4.4
17. Shepody	83	6.6	1.0888	0	2.6	1.8	3.4
18. Umatilla Russet	99	9.3	1.0875	1	2.5	2.3	3.4
Mean	95	7.7	1.0910	0	3.6	2.1	3.3
LSD ($\propto$=0.05)	10	2.3	0.0049	2	0.8	0.7	0.6

[1] Determined using weight-in-air, weight-in-water method.
[2] Hollow heart includes brown center.
[3] Blackspot bruise determined by the abrasive peel method, scale 1-5, 1=none, 5=severe.
[4] Shatter bruise is evaluated using a bruising chamber with digger chain link baffles. Tubers are stored at 45F prior bruising. Shatter bruises are rated on a scale of 1-5, with 1 = none and 5 = many and severe.
[5] The general rating is a score given based on appearance, yield, tuber size distribution, external defects, similar to a breeder's merit score.

Table 2. Yield and grade for advanced processing selections and cultivars, full season, Nesson Valley, ND, 2015.

Clone	Total Yield Cwt./A	US No. 1 Cwt./A	US No. 1 %	0-4 oz. %	4-6 oz. %	6-12 oz. %	>12 oz. %	US No. 2 %	Culls %
1. AND97279-5Russ	296	152	52	47	34	17	1	0	1
2. ND7882b-7Russ	282	223	79	15	19	39	22	1	4
3. ND8068-5Russ	255	154	61	35	33	27	1	0	4
4. ND102647-3Russ	291	152	52	47	33	18	1	1	0
5. ND113065CB-1Russ	338	248	73	26	29	42	2	0	0
6. ND113065CB-2Russ	271	196	73	27	27	41	5	0	0
7. ND113099-2Russ	248	183	74	24	34	33	7	1	2
8. ND113174B-1Russ	328	185	55	41	29	25	2	0	3
9. ND113526CB-1Russ	286	160	56	44	33	23	0	1	0
10. Alpine Russet	334	275	82	15	20	48	14	2	0
11. Bannock Russet	356	303	85	13	18	48	20	0	2
12. Dakota Trailblazer	305	279	91	9	18	56	18	0	0
13. MonDak Gold	284	213	75	25	29	43	3	0	0
14. Ranger Russet	374	298	80	18	20	45	14	1	2
15. Russet Burbank	420	294	69	17	20	40	10	2	11
16. Russet Norkotah	271	216	80	19	21	47	13	0	0
17. Shepody	266	207	77	19	24	41	12	1	2
18. Umatilla Russet	424	307	72	23	25	37	11	2	3
Mean	313	225	71	26	26	37	9	1	2
LSD ($\propto$=0.05)	57	61	11	11	9	11	12	1	2

Table 3. French fry evaluations following harvest, full season trial, Nesson Valley, ND, 2015.

Clone	Fry Color[1]	Stem-end Color	% Sugar End[2]
	Field Fry		
1. AND97279-5Russ	0.6	1.3	42
2. ND7882b-7Russ	0.8	1.4	67
3. ND8068-5Russ	0.5	1.0	17
4. ND102647-3Russ	0.5	1.4	42
5. ND113065CB-1Russ	1.3	1.8	25
6. ND113065CB-2Russ	1.0	1.6	17
7. ND113099-2Russ	1.3	2.0	50
8. ND113174B-1Russ	0.6	2.0	75
9. ND113526CB-1Russ	0.5	1.0	25
10. Alpine Russet	1.0	1.1	8
11. Bannock Russet	0.8	1.1	50
12. Dakota Trailblazer	0.5	0.8	25
13. MonDak Gold	0.6	0.7	8
14. Ranger Russet	0.8	1.5	25
15. Russet Burbank	0.6	2.4	100
16. Russet Norkotah	1.9	2.9	33
17. Shepody	0.6	1.1	50
18. Umatilla Russet	0.8	1.4	50
Mean	0.8	1.5	39
LSD ($\propto$=0.05)	0.5	1.1	48

[1] Fry color scores: 0.1 corresponds to 000, 0.3 corresponds to 00, 0.5 corresponds to 0, 1.0 equals 1.0; subsequent numbers follow French fry rating scale 000 to 4.0. Scores of 3.0 and above are unacceptable because adequate sugars cannot be leached from the tuber flesh to make an acceptable fry of good texture.

[2] Any stem end darker than the main fry is considered a sugar end in these evaluations, thus mirroring the worst case scenario. The processing industry defines a sugar end as a 3.0 or darker.

Attachment 7

April 5, 2017

To Whom It May Concern:

Our farm, Jonk Seed Farm Inc., is a small seed potato farm located in the eastern part of ND that grew Mondak Gold in the growing season of 2016. Mondak Gold has allowed us to create a few niche markets, and just recently this variety has proven to take hold some interest from a small chain of restaurants.

Mondak Gold is one of the best tasting varieties we have ever come across. It has a nice blocky appearance and has proven to store well with long storability. In a small chip/fry trial the chips fried up beautifully and the taste was excellent.
We are excited to further the production of this variety as we are seeing potential for the future.

Best regards,

Maria Jonk Carter
Jonk Seed Farm Inc.

Attachment 8

Improving Management of Early Blight in MonDak Gold Potatoes

Dimitri Fonseka, Audrey Kalil, Tyler Tjelde, Justin Jacobs, and Jerald Bergman

Introduction

Early blight, caused by the fungus *Alternaria solani,* is an important chronic foliar disease of potato present every growing season in North Dakota. Primary damage is attributed to premature defoliation of potato plants, resulting in tuber yield reduction. The pathogen may also cause dry rot of tubers, further reducing quality and shelf life of marketable potatoes. Damage to the tubers during harvest exacerbates the dry rot and should be prevented. Frequent application of protectant fungicides from early in the growing season until vine-kill is essential to manage early blight, but these are insufficient under high inoculum pressure and conducive environmental conditions. Therefore, the application of locally systemic and translaminar fungicides in rotation with protectant fungicides is important. The objective of this study was to improve control of early blight in MonDak Gold potatoes via imposition of a new fungicide spray schedule and to evaluate the role of harvest damage in worsening of tuber dry rot symptoms.

Methods

A non-replicated demonstration trial was conducted at the Nesson Valley Irrigated station in the 2016 season. The existing fungicide spray regime used in 2015 served as the control (A). The new spray schedule (B) design was based on fungicide testing results from the NDSU potato pathology group. The field was split into two equal sections and each half received either treatment A or B. Early blight foliar disease severity was rated visually on the same 10 plants at five locations within each treatment every 10 days for 10 weeks starting at row closure. Disease was assessed by estimating percentage of foliage showing chlorotic or necrotic lesions and senescent foliage typical of *A. solani* infections. Area under the disease progressive curve (AUDPC) was calculated by multiplying the average of pairs of disease severity observations by time. This provides a quantitative summary of total amount of disease over the season (higher AUDPC value = more disease). A representative leaf sample was collected from each treatment to verify pathogen infection. Laboratory culture plates confirmed the presence of the fungus. To evaluate harvest damage to tubers, samples from both treatments were collected from hand-harvested and mechanically harvested bins. Mechanically harvested samples had two sub samples with tubers being collected before and after entering the clod hopper. Tubers were cured by exposing them to temperatures between 50 and 60°F and 95% RH for 10 days in dark. After curing, the storage temperature was dropped to about 40 and 45°F. Tuber disease severity was rated visually monthly after storage.

Results

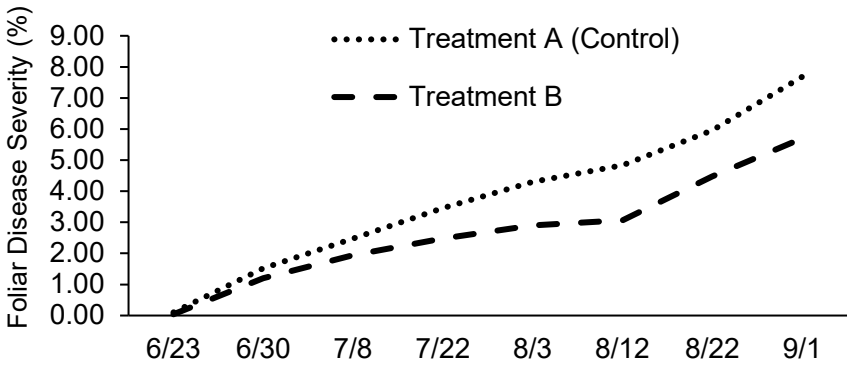


Figure 1. Early blight disease severity progress
Early blight foliar disease severity is plotted by time at each observation time point. Area under the line for each treatment is equal to the AUDPC.

Spray Schedule		Rate	Foliar Disease Severity (%)								AUDPC
			6/23	6/30	7/8	7/22	8/3	8/12	8/22	9/1	
A	Bravo WS (1) Bravo WS (2,9) Manzate (4) Quadris (7)	0.75 pt/a 1.5 pt/a 1 qt/a 15.5 fl oz/a	0.10	1.52	2.48	3.46	4.30	4.84	5.96	7.68	275.14
B	Bravo WS (1) Bravo WS (2,4,6,8,9) Headline + Manzate (3) Endura + Manzate (5)	0.75 pt/a 1.5 pt/a 9 fl oz/a + 2 qt/a 3 oz/a + 2 qt/a	0.04	1.20	1.94	2.48	2.90	3.06	4.48	5.70	197.11

Table 1. Foliar disease severity could potentially be reduced with modified fungicide schedule
Planted: May 02, 2016 Harvested: October 14, 2016
Spray Schedule: 1 = 6/22, 2 = 7/1, 3 = 7/7, 4 = 7/15, 5 = 7/22, 6 = 7/27, 7 = 8/2, 8 = 8/15, 9 = 8/19
AUDPC = Area Under the Disease Progressive Curve
Foliar Disease severity data was based on 10 plants at 5 locations in the field for each treatment (n = 50)

Treatment	Harvest Method	Tuber Disease Severity (%)					
		11/11/16	12/16/16	1/20/17	2/17/17	3/17/17	4/11/17
A	Hand	0	0	0	0	0	0
	Prior to clod hopper	0	0	0	0	0	0
	After clod hopper	0	0	0	0	0	0
B	Hand	0	0	0	0	0	0
	Prior to clod hopper	0	0	0	0.	0	0
	After clod hopper	0	0	0	0	0	0

Table 2. Tuber disease severity

Conclusion

In this demonstration trial, we observed a slight reduction of early blight foliar symptoms under the new spray regime (B) compared to the control (A) starting from July 22nd through September 1st. A lower AUDPC value also indicates less total early blight foliar disease over the growing season for the new spray regime (B). It should be noted that disease pressure was low, so it remains to be seen if such a slight reduction in disease will have any impact on yield or storability. For example, a rating of 90% for disease severity corresponds to a potential 20-30% yield reduction. The first monthly post-harvest tuber disease severity analysis was conducted on November 11th. Dry rot was not observed in stored tubers from any of the treatments at any of the time points. Further evaluations will be conducted to determine tuber infection caused by the dry rot phase of the pathogen.

Attachment 9

UNIVERSITY OF MINNESOTA

Twin Cities Campus

Department of Food Science and Nutrition
College of Food, Agriculture and Natural Resource Sciences

Room 145
1334 Eckles Avenue
St. Paul, MN 55108
Office: 612-624-3201

December 18, 2016

RESULTS OF ANALYSIS

Samples:

Yukon Gold potato
Red Norland potatoes
Mondak Gold potatoes

Sample preparation:

Potatoes were washed with distilled water, peeled and cut into slices with about 5cm thickness. The cut slices were washed again with distilled water and boiled in excess distilled water for about 6 min. The boiled potatoes were drained and rinsed with distilled water to cool. The cooked potatoes were then mashed with a mortar and pestle and used immediately for glycemic index determination.

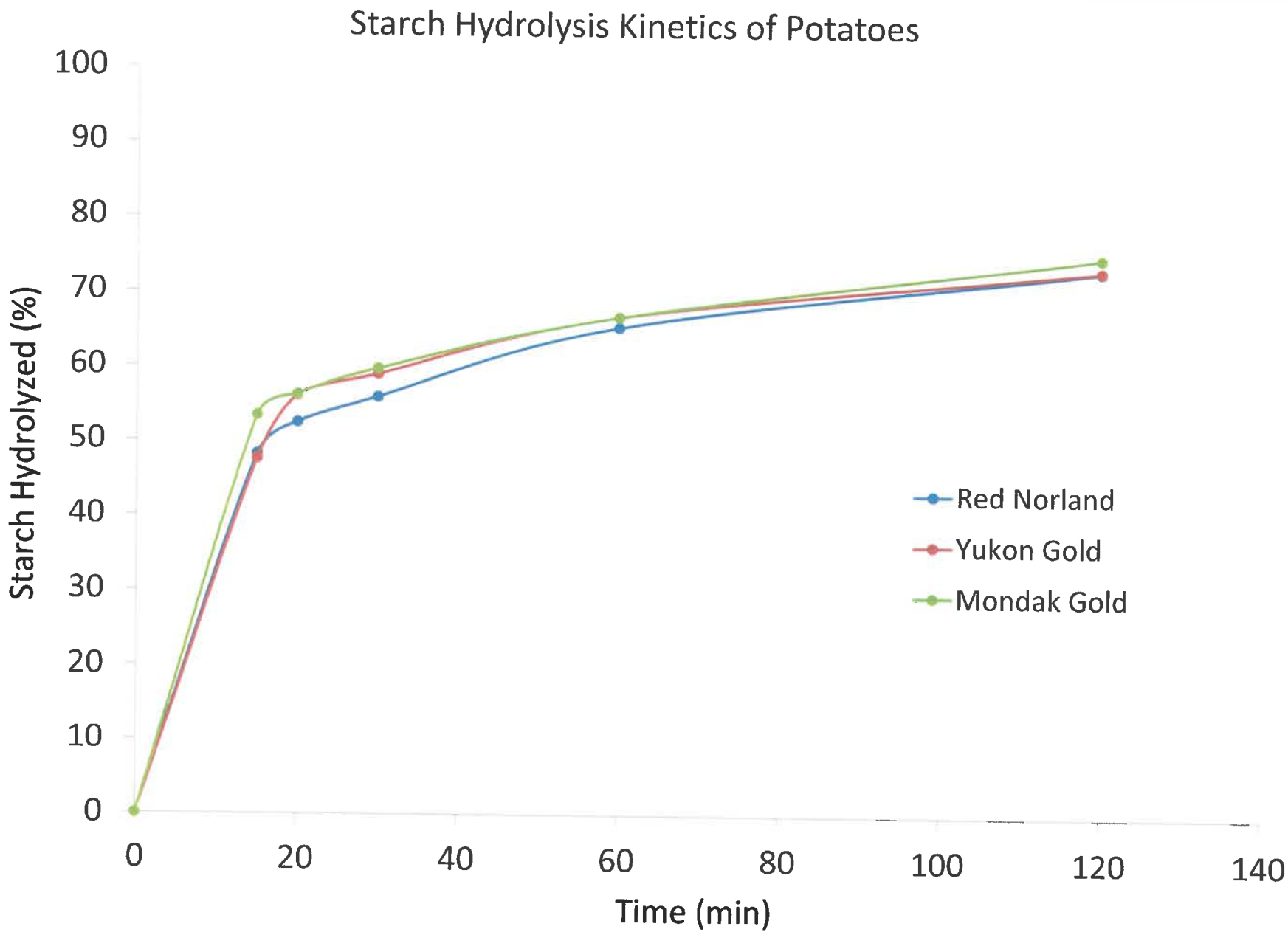
Analysis:

In-vitro starch digestibility and glycemic index. Sample containing about 100mg of starch was weighed and dispersed into 5mL of 0.1M sodium acetate buffer (pH 5.2). 1mL of freshly prepared enzyme mixture (porcine pancreatic α-amylase and amyloglucosidase) was added to the mixture and incubated for 2 hr at 37°C. The glucose content in 0.1 mL aliquots taken at time intervals were determined and converted to percent starch hydrolyzed. The glycemic index of the samples was calculated using the area under the curve method. All analysis was done in triplicates.

Sample	Expected glycemic index (eGI)
Red Norland	61.3±0.7
Yukon Gold	62.7±0.9
MonDak Gold	63.5±0.8

Sincerely,

George Annor
(Assistant Professor)


Starch Hydrolysis Kinetics of Potatoes
Starch Hydrolyzed (%)
100
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
120
140
Time (min)
Red Norland
Yukon Gold
Mondak Gold

Mondak Gold 3	Average Mondak Gold
0	0
51.62	53.42
54.21	56.26
57.91	59.78
65.24	66.79
75.95	75.01

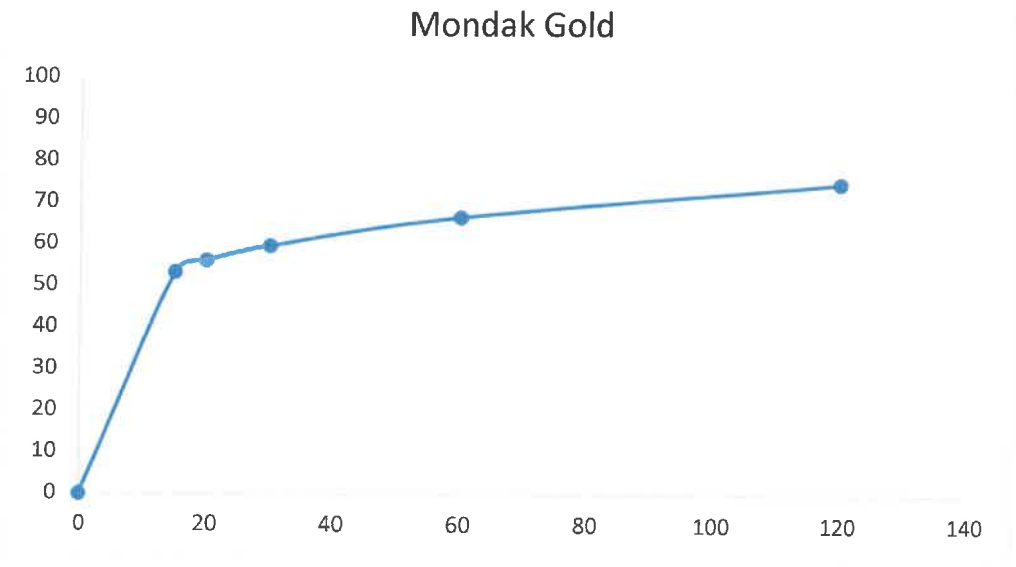


Table 1. Agronomic evaluations for advanced fresh market selections and cultivars, Nesson Valley, ND, 2016.

Clone	% Stand	Vine Size[1]	Vine Maturity[2]	Stems per Plant	Shape[3]	Color[4]	General Rating[5]
ND6002-1R	100	3.0	2.8	1.8	2.5	4.0	3.8
ND7132-1R	98	3.5	2.1	1.7	2.5	3.5	3.5
ND7818-1Y	99	4.0	1.8	2.1	2.0	Y	3.4
ND7982-1R	74	2.3	1.0	11.8	1.0	3.6	3.8
MonDak Gold	100	4.3	3.5	2.2	2.8	2.0	3.5
Dakota Ruby	100	3.5	1.6	2.4	1.0	4.0	2.4
Red Norland	98	2.3	1.5	2.2	2.5	2.6	3.0
Yukon Gold	99	3.0	1.0	1.4	2.5	Y	4.0
Mean	96	3.2	1.9	3.2	2.1	-	3.4
LSD ($\propto$=0.05)	23	0.7	0.9	9.7	0.3	-	0.3

[1] Vine size – scale 1-5, 1 = small, 5 = large.
[2] Vine maturity – scale 1-5, 1 = early, 5 = late.
[3] Shape = 1-5; 1 = round, 2 = oval, 2.5 = oblong, 3 = blocky, 4 = long, blocky, 5 = long.
[4] Color = 1-5; 1 = white/buff, 2 = pink, 3 = red, 4 = bright red, 5 = dark red, P = purple, Y = yellow.
[5] General Rating = 1-5; 1 = poor and unacceptable, 3 = fair, 4 = excellent, 5 = perfect.

Table 2. Yield and grade for advanced fresh market selections and cultivars, Nesson Valley, ND, 2016.

Clone	Total Yield Cwt./A	A Size Tubers Cwt./A	A Size %	0-4 oz. %	4-6 oz. %	6-10 oz. %	>10 oz. %	US No. 2 %	Culls %
ND6002-1R	358	204	57	11	37	20	29	1	2
ND7132-1R	394	239	60	12	40	21	27	1	0
ND7818-1Y	388	238	61	26	45	16	9	2	2
ND7982-1R	273	66	24	74	21	2	2	0	0
MonDak Gold	482	301	62	12	41	22	24	2	0
Dakota Ruby	353	166	47	51	39	8	2	0	0
Red Norland	389	239	61	8	38	23	28	0	3
Yukon Gold	297	148	50	12	33	17	35	2	1
Mean	367	200	53	26	37	16	19	3	1
LSD ($\propto$=0.05)	50	55	10	8	8	4	9	2	2

Table 3. Quality attributes, including specific gravity, internal disorders and bruise potential for advanced fresh market selections and cultivars, Nesson Valley, ND, 2016.

Clone	Scurf	Tubers per Plant	Specific Gravity[1]	% Hollow Heart[2]	Black-spot Bruise[3]	Shatter Bruise[4]
ND6002-1R	4.0	6.1	1.0799	1	3.5	2.9
ND7132-1R	2.3	7.1	1.0755	1	2.4	2.2
ND7818-1Y	4.3	9.5	1.0894	0	2.0	2.0
ND7982-1R	3.8	44.0	1.0896	0	2.4	2.0
MonDak Gold	3.0	8.9	1.0965	0	4.5	2.4
Dakota Ruby	4.0	11.7	1.0729	0	1.4	1.9
Red Norland	2.0	6.4	1.0724	0	2.1	2.3
Yukon Gold	3.8	5.1	1.0912	3	2.7	2.9
Mean	3.4	12.4	1.0834	1	2.6	2.3
LSD ($\propto$=0.05)	0.5	32.3	0.0054	2	1.1	1.1

[1] Determined using weight-in-air, weight-in-water method.
[2] Hollow heart includes brown center.
[3] Blackspot bruise determined by the abrasive peel method, scale 1-5, 1=none, 5=severe.
[4] Shatter bruise is evaluated using a bruising chamber with digger chain link baffles. Tubers are stored at 45F prior bruising. Shatter bruises are rated on a scale of 1-5, with 1 = none and 5 = many and severe.

Table 4. Agronomic and quality traits for advanced dual-purpose russet selections and check cultivars, full season, Nesson Valley, ND, 2016.

Clone	% Stand	Vine Size[1]	Vine Maturity[2]	Stems per Plant	Tubers per Plant	Shape[3]	Color[4]
AND97279-5Russ	95	2.8	2.5	2.1	6.0	3.0	7.1
AND00618-2RussY	98	3.0	3.0	1.4	3.6	3.4	8.1
ND7882b-7Russ	98	3.3	2.1	1.6	4.7	3.6	8.0
ND8068-5Russ	98	1.3	1.0	1.9	4.9	3.0	7.1
ND039194AB-1Russ	99	4.0	3.5	1.7	4.3	3.4	8.0
Ranger Russet	99	4.0	3.8	2.0	7.4	5.0	7.5
Russet Burbank	98	3.8	3.0	2.0	6.3	5.0	7.8
Russet Norkotah	98	3.0	1.6	1.9	5.6	4.0	8.0
Shepody	98	4.1	2.8	1.5	4.8	3.8	1.0
Umatilla Russet	99	4.3	3.5	1.7	7.3	4.0	8.0
Mean	98	3.3	2.7	1.8	5.5	3.8	7.1
LSD ($\propto$=0.05)	5	0.8	0.7	0.5	1.1	0.5	0.2

[1] Vine size – scale 1-5, 1 = small, 5 = large.
[2] Vine maturity – scale 1-5, 1 = early, 5 = late.
[3] Shape = 1-5; 1 = round, 2 = oval, 2.5 = oblong, 3 = blocky, 4 = long, blocky, 5 = very long.
[4] Color = 1-9; 1 = white/buff, 2 = pink, 3 = red, 4 = bright red, 5 = dark red, 6 = tan, flaky, 7 = light russet, 8 = medium russet, 9 = dark, heavy russet.

Table 5. Yield and grade for advanced processing selections and cultivars, full season, Nesson Valley, ND, 2016.

Clone	Total Yield Cwt./A	US No. 1 Cwt./A	US No. 1 %	0-4 oz. %	4-6 oz. %	6-10 oz. %	>10 oz. %	US No. 2 %	Culls %
AND97279-5Russ	260	200	77	21	47	19	11	1	3
AND00618-2RussY	352	330	94	2	14	9	72	1	3
ND7882b-7Russ	401	366	91	3	19	13	58	2	4
ND8068-5Russ	253	211	83	12	46	21	16	3	1
ND039194AB-1Russ	395	365	92	4	16	9	67	3	1
Ranger Russet	412	359	87	11	44	24	18	1	2
Russet Burbank	421	371	88	6	31	19	39	0	6
Russet Norkotah	365	338	92	6	32	17	43	0	1
Shepody	391	314	81	6	19	9	53	3	10
Umatilla Russet	427	351	82	12	38	20	25	2	4
Mean	368	320	87	8	31	16	41	2	3
LSD ($\propto$=0.05)	67	63	7	4	8	4	10	3	5

Table 6. Quality assessments including specific gravity, internal disorders, and bruising for dual-purpose russet selections, full season trial, Nesson Valley, ND, 2016.

Clone	Specific Gravity[1]	% Hollow Heart[2]	Black-spot Bruise[3]	Shatter Bruise[4]	General Rating[5]
AND97279-5Russ	1.1035	0	3.2	3.6	3.5
AND00618-2RussY	1.0974	9	1.8	3.8	3.9
ND7882b-7Russ	1.0903	3	4.5	1.7	4.3
ND8068-5Russ	1.0986	0	4.1	2.8	3.8
ND039194AB-1Russ	1.0899	1	3.3	3.0	3.5
Ranger Russet	1.1039	0	4.0	1.8	3.4
Russet Burbank	1.0909	3	2.4	1.7	3.1
Russet Norkotah	1.0848	0	3.6	2.3	4.8
Shepody	1.0945	0	2.4	2.1	3.3
Umatilla Russet	1.1004	3	2.7	2.4	3.6
Mean	1.0953	2	3.2	2.5	3.7
LSD ($\propto$=0.05)	0.0054	4	0.7	0.9	0.5

[1] Determined using weight-in-air, weight-in-water method.
[2] Hollow heart includes brown center.
[3] Blackspot bruise determined by the abrasive peel method, scale 1-5, 1=none, 5=severe.
[4] Shatter bruise is evaluated using a bruising chamber with digger chain link baffles. Tubers are stored at 45F prior bruising. Shatter bruises are rated on a scale of 1-5, with 1 = none and 5 = many and severe.
[5] General Rating = 1-5; 1 = poor and unacceptable, 3 = fair, 4 = excellent, 5 = perfect.

Table 7. French fry color, stem end color, and sugar end incidence, at harvest and after 8 weeks storage at 45F (7.2C), full season trial, Nesson Valley, ND, 2016.

Clone	Fry Color[1]	Stem-end Color	% Sugar End[2]	Fry Color[1]	Stem-end Color	% Sugar End[2]
	Field Fry			Following 8 wks. at 45F		
AND97279-5Russ	0.4	1.1	84	0.5	2.1	100
AND00618-2Russ Y	1.0	1.3	34	1.1	1.1	0
ND7882b-7Russ	0.8	1.2	50	1.3	2.8	75
ND8068-5Russ	0.5	0.7	17	0.6	1.0	67
ND039194AB-1Russ	1.0	1.4	17	1.1	1.3	25
Ranger Russet	0.6	1.3	75	1.0	1.3	25
Russet Burbank	0.5	1.7	92	0.5	2.8	100
Russet Norkotah	1.3	1.9	50	2.3	2.6	25
Shepody	0.5	1.1	58	0.8	2.1	75
Umatilla Russet	0.5	0.9	58	1.0	1.0	0
Mean	0.7	1.3	53	1.0	1.8	49
LSD (α=0.05)	0.4	0.6	45	0.5	0.7	41

[1] Fry color scores: 0.1 corresponds to 000, 0.3 corresponds to 00, 0.5 corresponds to 0, 1.0 equals 1.0; subsequent numbers follow French fry rating scale 000 to 4.0. Scores of 3.0 and above are unacceptable because adequate sugars cannot be leached from the tuber flesh to make an acceptable fry of good texture.

[2] Any stem end darker than the main fry is considered a sugar end in these evaluations, thus mirroring the worst case scenario. The processing industry defines a sugar end as a 3.0 or darker.

Managing Blemish Problems to Improve Marketing of Fresh Potatoes

Final Report

Partner Organization: North Dakota State University

Project Summary

The biggest challenge of providing a high quality potato is to produce tubers free from blemishes, because consumer will "buy with their eyes" as they naturally gravitate to tubers free from blemishes. Fresh market potato growers will cull 30% or more of their crop because of tuber blemishes. Blemishes can be defined as any flaw or imperfection that spoils the appearance of the tuber, thus making it unmarketable. Smooth-skinned tubers (red, white, and yellow) in particular are especially vulnerable to blemishes because they are easily observed. This project has three parts, first conduct a survey to determine the major blemish problems, second determine the effects of various chemistries on blemishes, and third evaluate the effects of blemishes on newer cultivars. From the survey the most prevalent blemishes were skin netting (on 40% of tubes evaluated) and external bruising (present on 35% of tubers evaluated). Some fungicides reduced black dot/silver scurf blemishes. Those that were most consistent were Emesto Silver, Quadris, Nubark Mancozeb + Moncot 70 DF, and Maxim 4 FS. The highest yielding red-skinned cultivars to mention were Red Endeavor, Dakota Ruby, Runestone Gold, and W8405-1R.

The purpose of this project was to reduce the loss of fresh market potatoes, primarily red- and yellow-skinned to blemish problems that make them unmarketable. Consumers are becoming more demanding and desire the perfect looking potato for cooking, thus potatoes with significant blemishes (silver scurf, black dot, scab, unattractive skin color, malformed tubers) are often rejected by grocery stores if they do not meet their strict expectations. Fresh potato growers report that many of their tubers become unmarketable because of various blemish problems. These blemish problems have been reported to be poor skin color, scab species (*Streptomyces* spp.), and silver scurf (*Helminthosporium solani*). In addition, when a high percentage of tubers are culled, this wastes food, energy, and our natural resources. A growing global population necessitates more food and by reducing culled potatoes, farmers in Minnesota and North Dakota can help feed more individuals. This timely project is very important to the stakeholders because there is greater pressure from other fresh potato growing regions. And improving fresh potato quality will also help stakeholders as most other crops are not profitable to grow, potatoes can help growers to be economically sustainable.

Project Approach

In 2015, at harvest the survey of blemishes was conducted. There were samples collected from 17 different potato lots. From these samples the most prevalent blemishes were skin netting (on 40% of tubes evaluated) and external bruising (present on 35% of tubers evaluated). Other blemished documented were superficial common scab (15%), black dot (14%), growth cracks (14%), lenticel spot (10%), silver scurf (7%), enlarged lenticels (6%), pink or light color (4%), vascular discoloration (3%), and unknown blemishes (3%). The interesting finding was the high

amount of external bruising. This is something that could be addressed through more carefully handling of the potatoes.

The cultivar trial plots were established in a commercial, non-irrigated, potato field near Crystal, ND. A randomized complete block design was utilized with four replicates. Seed tubers were hand cut to 2.0 oz. seed pieces and suberized for approximately 5-10 days at 55°F and 95% relative humidity prior to planting. Tubers were planted on May 21 at a 9 inch within-row spacing; rows were spaced 36 inches apart. Plots were single rows; measuring 25 feet long (33 seed tubers per plot were planted). Agronomic practices were typical of North Dakota non-irrigated production. Plots were harvested on September 15, and stored at about 55 °F until grading. The tuber size profile distribution was determined by sorting potatoes into C size (<1.875 inches), B size (1.875 to 2.25 inches), A size (2.25 to 3.5 inches), and Chef size (>3.5 inches).

The highest yielding red-skinned cultivars to mention were Red Endeavor, Dakota Ruby, Runestone Gold, and W8405-1R (Table 1). These cultivars are new releases or advancing selections that show promise. Many of the yellow-skinned cultivars yielded well. Of interest was the high yielding of the selection A05182-7Y (Table 2). These cultivars are being evaluated for blemish problems to determine which ones are having the most potential for high quality production in the Red River Valley. A small sample of tubers were inoculated with *Fusarium solani* and differences were found among the cultivars tested (Figure 1). However, the *Fusarium sambucinum* inoculation did not work properly, so only *Fusarium solani* data is shown (Figure 1).

The growth regulator trial was established on a non-irrigated site at the Northern Plains Potato Grower Association Potato Research farm in Grand Forks, ND. A randomized complete block design was utilized with four replicates. Seed tubers were hand cut to 2.0 oz. seed pieces and suberized for approximately 5-10 days at 55°F and 95% relative humidity prior to planting. Tubers were planted on June 15 at 9 inch within-row spacing; rows were spaced 36 inches apart. Plots were four rows wide, measuring 25 feet long (33 seed tubers per plot were planted) by 12 feet wide. Agronomic practices were typical of North Dakota non-irrigated production. Plots were harvested on September 22, and stored at about 55 °F until grading. The tuber size profile distribution was determined by sorting potatoes into C size (<1.875 inches), B size (1.875 to 2.25 inches), A size (2.25 to 3.5 inches), and Chef size (>3.5 inches).

The yield from the growth regulator trial had a difference in size class when ethylene (Ethephon 2SL) was applied at 4 oz./a twice (Table 3). However, the overall yield was similar to the non-treated and other treatments.

Trials conducted in Grand Forks, ND and Becker, MN showed that some fungicides reduced black dot/silver scurf blemishes. Those that were most consistent were Emesto Silver, Quadris, Nubark Mancozeb + Moncot 70 DF, and Maxim 4 FS (Table 4). Other treatments showed promise, but were not consistent as the previously mentioned products.

We have learned from this first year of study that blemishes were common from tubers sampled, and some can be controlled by management practices. Plant growth regulators generally did not

affect blemishes, except in the case of ethephon. Fungicides at planting reduced blemishes in some cases.

Reports have given on this work to stakeholders at the International Crop Expo in Grand Forks, ND in February 2016, at the Minnesota Area II Potato Council Short Course in March 2016, an article written in the Valley Potato Grower Magazine (March issue), and published on the NDSU / U of M Potato Extension website in April.

Goals and Outcomes Achieved

The objectives of this project were to 1) determine what are the major blemish problems causing potatoes to be unmarketable, 2) quantify the effect of various agronomic management practices for disease, skin color, and other blemish problems, and 3) conduct a trial with newer cultivars to determine their potential yield and marketability.

The major blemishes were recorded, and some of these can be addressed through the way the potatoes are handled. Future Extension work will focus on handling of potatoes to reduce bruising and skinning. Research can focus on specific diseases that are more common in fresh market potatoes. The effect of plant growth regulators did not seem to change quality much, but it did show that 2,4-D did not affect potato quality. Good baseline data was achieved through the cultivar trial. Some of the cultivars that have been tested in this trial are now being grown by stakeholders. Improving handling of potatoes from the field and through the wash plant, utilizing good fungicides that have consistently worked, and choosing the best cultivars will improve potato quality. The data generated from this project will help many stakeholders.

Our original goal was a bit lofty, but I believe that we have succeeded in helping potato growers understand what blemishes are causing losses. Blemishes are hard to quantify because there is so much variability from year-to-year. From conversations with potato growers they indicate that blemishes were only 20-25% of their crop in 2015, but in 2016 blemishes cause 10-15% loss of crop. Environmental conditions have a large role in blemishes, so it can be difficult to clearly determine from year-to-year how much success this work in having. It has been informative to potato growers to learn about the blemishes that are common. What we did not expect to find that the way potatoes are handled cause the most blemishes. Bruising and skinning were most common. We plan on performing more education on handling of potatoes to reduce blemishes.

Data has been published on the NDSU Potato Extension page https://www.ag.ndsu.edu/potatoextension/understanding-and-managing-blemish-problems-in-fresh-market-potato and https://www.ag.ndsu.edu/potatoextension/understanding-blemish-problems-to-improve-marketing-of-fresh-potatoes-potato-expo-poster. These posts were also put on Twitter and Facebook to notify growers of this information. The data was published in the Valley Potato Grower magazine in volume 81:245 p.12-13. Reports have been given on this topic at the Northern Plains Potato Growers meeting in Grand Forks, ND on 18 Feb 2016 and at the MN Area II Potato Council short course near St. Cloud, MN on 1 March 2016. An Extension bulletin for helping growers identify problems was published in September 2016 at https://www.ag.ndsu.edu/publicationS/landing-pages/crops/potato-diagnostics-clipboard-a-1817. This includes a number of the blemishes that were photographed during this study. The data was

also presented at the Potato Expo, 4 Jan 2017 in San Francisco, CA and at the Potato Association of America meeting in Grand Rapids, MI 31 Jul – 4 Aug 2016. We plan on publishing this work once all the data in gathered and analyzed.

Beneficiaries

The stakeholders that are benefitting from this project are the fresh market potato growers. There were approximately 140 potato growers at the winter meetings where this data was presented. They are benefitting by choosing better fungicide programs, new cultivars, and understanding what blemishes they need to improve management for to enhance quality.

Lessons Learned

We feel as the project generated much beneficial data and completed the purposes of the project. It could have been better if the research study could have been conducted over two growing seasons to provide more consistent data.

Contact Information

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Additional Information

NDSU/U of M website with publication:
https://www.ag.ndsu.edu/potatoextension/documents/ResearchonChippotatotrialresults.pdf

Valley Potato Grower magazine link:
http://www.valleypotatogrower.com/flipbookMarch2016Mag/flipbook/

Image of the growth regulator trial taken August 12, 2015.

Russeting or road-mapping on a red-skinned potato.

Skinning of a yellow potato.

Thumbnail bruise of red-skinned potato.

Black dot of potato.

Table 1. Red-skinned potato cultivar/selection trial agronomic characteristics and graded yield, conducted near Crystal, ND, in 2015.

Cultivar	Stand[1]	Stems per plant[2]	Vigor[3]	Total yield C	Total yield B	Total yield A	Total yield Chef	Total yield Total
	---------- number ----------			---------- cwt/a ----------				
CO00277-2R	25.8	6.5	3.5	46	122	182	0	349
CO00291-5R	25.3	4.4	2.0	23	82	166	1	272
CO98012-5R	26.3	4.0	3.8	39	149	213	1	402
CO99076-6R	25.0	4.3	3.0	23	87	231	7	349
Dakota Ruby	25.8	4.9	3.0	39	129	232	6	406
Dark Red Norland	24.3	4.3	3.5	12	57	301	22	393
MN10001PLWR-14R	24.5	2.8	1.8	33	58	50	0	142
MN10002PLWR-06R	24.5	3.4	1.5	12	54	231	16	314
MN10020PLWR-08R	24.5	3.7	2.8	20	60	115	2	198
ND6002-1R	26.0	2.7	2.8	11	52	276	18	355
ND7132-1R	23.3	2.9	3.0	14	86	201	9	309
ND7982-1R	24.8	6.0	3.8	41	201	156	0	398
NDA7985-1R	25.8	3.2	3.8	7	37	283	67	394
Red Pontiac	26.0	3.4	4.0	11	45	250	94	401
Red Endeaver	25.8	5.1	3.8	37	118	276	7	438
Red Norland	26.5	4.4	4.0	17	80	301	3	400
Runestone Gold	24.8	5.4	3.0	27	130	237	10	404
Sangre	17.3	1.4	1.0	22	70	160	26	278
Viking	25.3	2.0	2.7	7	26	241	99	372
W8405-1R	24.8	5.5	3.8	31	123	235	10	400
Mean	24.8	4.0	3.0	24	88	217	20	349
CV (%)	9.4	17.7	19	43	33	27	94	21

LSD (α = 0.10)	2.8	0.8	0.7	12	34	70	22	87
LSD (α = 0.05)	3.3	1.0	0.8	14	41	84	26	104

[1]Stand count was taken on June 30 by counting every emerged plant.
[2]Stems per plant was counted on all plants on June 30 and is shown as the average number of stems per plant.
[3]Vigor evaluation was completed on August 12. Rating compared to Red Norland where 1 indicates least vigor and 5 greatest vigor.

Table 2. Yellow-skinned potato cultivar trial agronomic characteristics and graded yield conducted near Crystal, ND in 2015.

Cultivar	Stand[1]	Stems per plant[2]	Vigor[3]	Total yield C	Total yield B	Total yield A	Total yield Chef	Total yield Total
	number			cwt/a				
A05182-7Y	25.8	4.2	3.8	77	201	138	6	422
Elfe	26.5	3.0	3.3	26	141	118	0	285
Gioconda	23.8	3.7	3.5	16	81	359	33	490
Julinka	25.3	3.7	3.8	32	118	167	4	322
MN02586	23.5	5.5	3.8	46	136	234	0	416
Milva	24.5	4.0	3.8	20	133	258	2	414
Satina	23.8	4.0	4.0	24	107	274	3	407
Soraya	25.3	4.3	4.0	24	137	266	5	432
Viviana	23.5	4.9	3.5	21	105	263	4	393
Yukon Gem	25.0	3.4	3.5	10	53	263	30	356
Mean	24.7	4.1	3.7	30	121	234	9	394
CV (%)	7.5	12	14	29	17	18	112	10
LSD ($\alpha = 0.10$)	2.2	0.6	0.6	10	25	51	12	48
LSD ($\alpha = 0.05$)	2.7	0.7	0.7	12	30	62	14	58

[1]Stand count was taken on June 30 by counting every emerged plant.
[2]Stems per plant was counted on all plants on June 30 and is shown as the average number of stems per plant.
[3]Vigor evaluation was completed on August 12. Rating compared to Red Norland where 1 indicates least vigor and 5 greatest vigor.

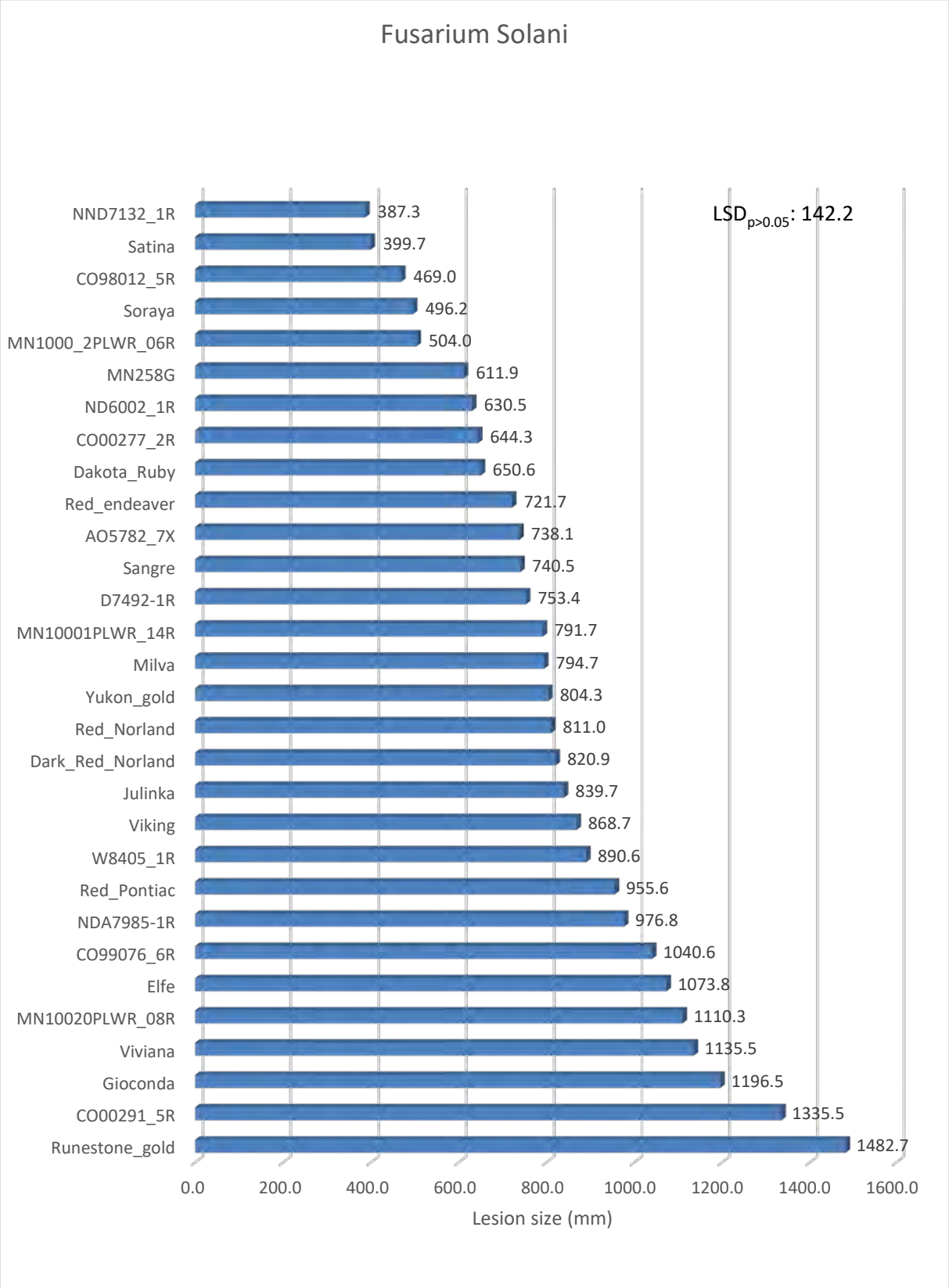
Table 3. Graded yield of Red Norland potato treated with various plant growth regulators at Grand Forks, ND 2015. Within columns means followed by the same letter are not significantly different according to Tukey pairwise comparison ($P \leq 0.05$).

	Treatment	Timing	Rate	Stand	Stems/plot		Stems/plant		C		B		A		Chef	Total yield
1	Non-treated			45	199	abc	4.5	abc	11	b	59	b	163	a	12	246
2	2,4-D LV6	Pre bud	1.58 oz./a	44	203	ab	4.7	ab	9	b	49	bc	187	a	3	248
	2,4-D LV6	14 days after pre bud	1.58 oz./a													
3	Ethephon 2SL	Pre bud	4 oz./a	46	200	ab	4.3	abc	33	a	128	a	86	b	1	249
	Ethephon 2SL	14 days after pre bud	4 oz./a													
4	Rejuvenate	Seed treatment	0.115 oz./ton seed	43	215	a	5.0	a	11	b	42	bc	203	a	12	267
	2,4-D LV6	2 WAE	0.55 oz./a													
	2,4-D LV6	Pre bud	0.82 oz./a													
5	Rejuvenate	Seed treatment	0.3 oz./ton seed	46	169	bc	3.7	bc	10	b	38	c	180	a	20	248
	2,4-D LV6	2 WAE	0.55 oz./a													
	2,4-D LV6	Pre bud	0.82 oz./a													
6	Rejuvenate	Seed treatment	0.115 oz./ton seed	44	225	a	5.1	a	12	b	40	bc	222	a	9	283
	Ethephon 2SL	2 WAE	2 oz./a													
	Ethephon 2SL	Pre bud	3 oz./a													
7	Rejuvenate	Seed treatment	0.3 oz./ton seed	48	156	c	3.3	c	10	b	42	bc	173	a	12	237
	Ethephon 2SL	2 WAE	2 oz./a													
	Ethephon 2SL	Pre bud	3 oz./a													

Table 4. Effect of fungicide treatments on black dot/silver scurf blemish of Red Norland and Yukon Gold potato seed in Becker, MN and Grand Forks, ND in 2015 and.

Treatment		**Rate**	**Schedule**	**Blemish %**			
				Becker, MN		Grand Forks, ND	
				Red Norland	Yukon Gold	Red Norland	Yukon Gold
1	Non-treated	-	-	68.5	48.8	22.0	5.2
2	Moncut 70 DF	1.1 lb / a	In-furrow	67.8	61.9	28.5	3.8
	Moncut 70 DF + Serenade	1.1 lb / a + 6.0 qt / a	In-furrow In-furrow	66.2	46.4	18.3	3.9
3	Quadris	11.6 fl oz. / a	In-furrow	50.0	43.9	13.8	3.4
4	Vertisan	23 fl oz. / a	In-furrow	65.3	51.5	21.1	3.8
5	Priaxor	8.1 fl oz. / a	In-furrow	67.3	55.2	18.9	4.7
6	Nubark Mancozeb Moncut 70 DF	1.0 lb / cwt 1.1 lb / a	Seed In-furrow	41.3	41.5	11.1	4.3
7	Emesto Silver	0.31 fl oz. / cwt	Seed	37.4	41.2	9.5	3.7
8	Omega 500F	3.0 pt / a	In-furrow	69.0	62.5	24.7	3.8
9	Luna Tranquility	11.2 fl oz. / a	In-furrow	66.8	65.3	18.1	5.1
10	Regalia	8.8 fl oz. / 1000 row ft	In-furrow	66.6	55.2	25.2	5.3
11	Maxim 4FS	0.08 fl oz. / cwt	Seed	33.4	35.4	6.7	5.2
LSD $_{p>0.05}$				7.90	6.70	5.80	1.15

Figure 1. Level of *Fusarium Solani* in inoculated potatoes that were grown in the cultivar trial in Crystal, ND in 2015.



Growth and fresh yield responses of sweet corn to mulch, planting date, and hybrid

Final Report

Partner Organization: North Dakota State University

Project Summary

Sweet corn is a high value crop that can be grown in North Dakota, but is often constrained with low yield and quality due to low temperatures and short season, and is not competitive with sweet corn from other regions. Plastic mulch can be used to increase the soil temperature, enable earlier planting, extend the growing season, and increase yield and quality. However, earlier planting can also create risk from frost and bird damage early in the season. In this study, three varieties of sweet corn with different maturity days (65, 75, and 84 day) were planted under four mulch films (black, clear, biodegradable and no mulch) on four different dates (from April 15 to June 1 at a 15 day interval when it is possible to plant). Plant emergence, plant height, growth stage and soil and near surface air temperature, and yield were measured in 2015 – 2016 growing season. We found that the emergence rate was the highest for the clear and biodegradable mulch treatments for all four planting dates. Mulch is effective in raising soil temperature, increasing early growth for the 84 day variety and improving yield for all mulch covers when compared to the no mulch treatment.

- Cold climates in ND only allow growth of short season crops that have lower yield potential. Mulch can improve soil temperature and allow longer maturity and earlier planting.
- Sweet corn growth and fresh yield differences were evaluated with four different mulch types (black, clear, biodegradable and no mulch) that were planted at four different planting dates (April 15, May 1, May 15, and June 1) for three hybrids with maturity dates of 65, 75, and 85 days.
- We also helped other specialty crops, such as Dr. Lee's vegetable crop project with mulch covers and instrumentation.

Project Approach

- According to the Work Plan and Expected Measurable Outcomes, we have accomplished our tasks and achieved our goals during the course of this project.

- **Experimental design and field preparation for 2015 and 2016:** A split plot randomized experiment was designed for both the 2015 and 2016 growing season. The four blocks will the four planting dates. The variables are the three hybrids and four different mulch types. For the 2015 growing season, one plot was ready for planting and the other needed some tillage work which ended up being done manually. For the 2016 growing season the plots were prepared by the NDSU Plant Science Department. Soil samples were collected in the research plots, for both seasons, at a grid pattern at two depths, 0-15 cm, and 15-46 cm. The samples were analyzed by the Soil Testing Lab for nitrate, phosphorus, potassium, pH, electrical conductivity, and organic matter. The results are directly used to guide the fertilizer application.

- **Ordered instruments, seed and fertilizer:** All the instruments in the proposal have been ordered. This includes Li-Cor LAI-2200C plant canopy analyzer, mulches, thermocouple wires, dataloggers, multiplexers, solar panels, accessary boxes, batteries, cameras, and radiation shields for temperature sensors. In addition, two hand planters have been ordered that were not in the original proposal, but they were much needed. They were ordered to make planting more consistent in depth and spacing, and to plant faster and easier. The fertilizer and three varieties of sweet corn were purchased, a 65 day (Sugar Baby), 75 day (Ambrosia) and 84 day (Delectable) varieties. There was enough seed and fertilizer from 2015, so they were used in the 2016 growing season.

- **Planted and installed instruments:** We planted three sweet corn hybrids under four different covers on four planting dates. For 2015 the planting dates were: April 15th, May 1st, June 4th and June 19th. For 2016 the planting dates were: April 22nd, May 6th, may 20th and June 9th.
  - Installed soil and air temperature sensors to measure temperature changes continuously at 30 min interval.
  - Installed 48 cameras to automatically take three photos per day and record crop growth stages under different cover and planting dates.
  - Installed rain gages at the plot.
  - For 2015, installed net radiometers to measure the radiation partitions on different covers.
- **Measurements taken:** Germination, number of leaves, growth stage, plant height and Leaf Area Index (LAI) were manually taken by the graduate and the undergraduate students.

- **Video:** NDSU Communication staff has signed a contract to create four videos for the project. The staff has been working with the team in the field on most of the important stages. The four videos are about (1) General introduction about the sweet corn mulch project; (2) The benefits about mulches for sweet corn; (3) Frequently asked questions about mulch applications; and (4) Field measurements for the project (will be available soon).

- **Field day:** A field day was held next to the plots with 24 attendees on August 14, 2015. We also provided some sweet corns for Dr. Lee's field day on September 18, 2015. For 2016 a field day was not held due to drought and hail damage to the sweet corn, and the sweet corn was not eatable due to mold growth after hail damages.

- **Harvesting:** Sweet corn was harvested with a goal of 78% moisture content from July 13 to September 18, 2015 and from August 1st – 23rd, 2016 because of differences in varieties, planting dates, and mulches. The total ear length, edible length, sugar content, moisture content, weight with husks and weight without husks were taken and measured.

- **Data analysis:** With help from a statistical graduate student, data analysis for three varieties, four mulches, four planting dates and three replicates was conducted. We found that:
  - Emergence was shortened by 2.6 days with clear plastic mulch
  - Early spring soil temperature at 5 cm below the surface under clear plastic mulch can increase the temperature by 5 ºC, the biodegradable by 3.4 ºC and the black plastic by 1.1 ºC, comparing to no mulch.
  - Fresh yield over any mulch covers was significantly higher than the no mulched plots.

- Sugar content was significantly higher when grown over mulches when compared to the no mulch.

Goals and Outcomes Achieved

- **Short term**
  - This project has demonstrated an important way to plant sweet corn earlier, harvest earlier and increase its market value.
  - Based upon the results, planting on April 15th is too early in this region due to potential frost, bird, and animal damages. With the use of the clear plastic mulch, you can safely plant two weeks earlier than fields without mulches.
  - With the use of black plastic, you can reduce the need for weed removal through herbicides and manual pulling.
  - We have reached the following numbers indirectly for this project:
    - Views to the research blog: 229 people total
    - Extension site videos: 100 visits
    - Extension workshop will present before December 2017, NDFMGA website currently has 349 followers on Facebook that can be reached once video is submitted, and one peer reviewed publication will be submitted before February 28, 2017
- **Midterm**
  - The midterm goal is to extend mulch application to other high value crops. We have helped Dr. Lee to use mulches on his vegetable trial experiments and installed soil and air temperature sensors on his plots.
  - We plan to present our results at field days and workshops to farmers, but were not invited to do it. However, we presented the results at NDSU Explore, and the ASABE international conference. There were more than 100 undergraduate students presenting at NDSU Explore, with more than 500 people attending. 1754 people attended the ASABE international conference. We will continue to seek opportunities to outreach this project.
  -
  - We have submitted a new proposal to the Specialty Crop and wished to collaborate with Carrington Research and Extension Center to continue the mulch application, but the project was not funded.
- **Long term**
  - Our long term goal is to extend the mulch application to a larger area and more specialty crops and establish a plastic culture program in North Dakota.
  - With the PI's background in irrigation, we will continue to pursue our goals of using mulch and smart sensor controlled irrigation for specialty crops in the region.

Beneficiaries

- The specialty crop groups that benefited from the project are gardeners, master gardeners, and several landowners in ND and MN.
- The corn growers are benefited from the completion of this project because the biodegradable mulch was made from corn starch.
- On the field day, we have outreached 24 gardeners and researchers. We have personally contacted three landowners and asked them to try mulch in their gardens and provide us with

their results. The PI is teaching several courses, and she has always showed her mulch project to the students (about 300 students in two years).

- A total of 636 people have benefitted from this project thus far. This number is expected to increase with the knowledge we have gained.
- Currently, the major benefit is the knowledge gain for the students.

Lessons Learned

- We have achieved our goals as we have set up in the proposal. We have evaluated the growth and yield difference among different mulches, varieties, and planting dates.

In 2016, since the weather was too dry, it is very important to have an irrigation under the mulch to combat the unpredictable weather.

- We also learned that we need to partner with others for any field experiments because of lack of resources.

Contact Information

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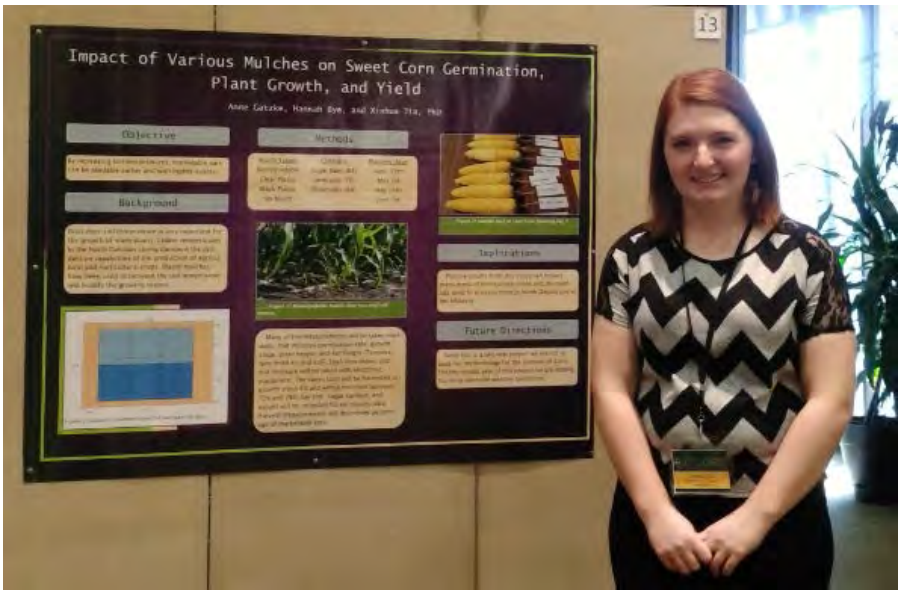
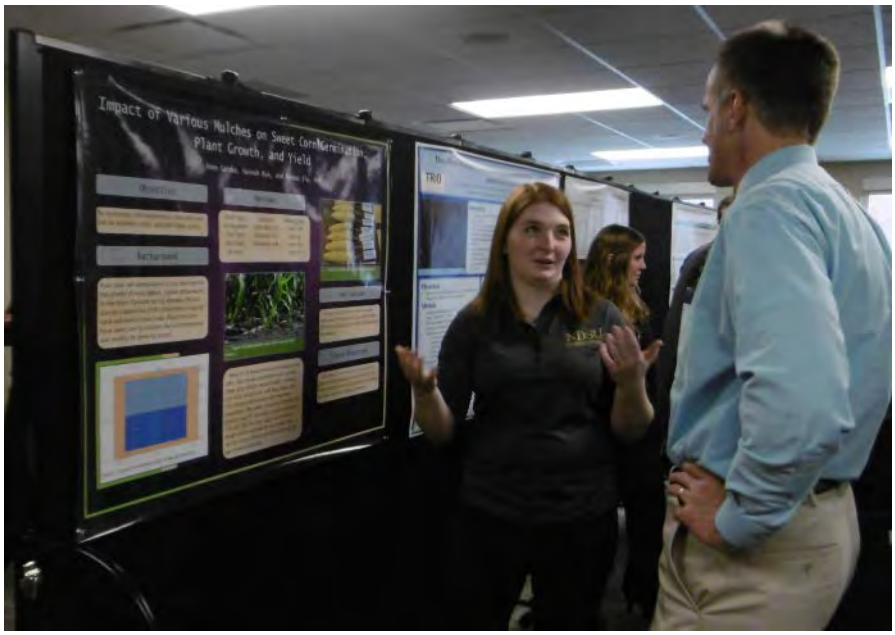
Additional Information

- **Publication**
  - Bye, H., A. Gaztke, Q. Li, and X. Jia. 2016. Sweet corn production with different mulches, varieties, and planting dates in North Dakota. ASABE Annual International Meeting, July 17-20, 2016. Orlando, FL. Paper No. 162458049.
  - Bye, H. 2016. Sweet corn production with different mulches, varieties, and planting dates in North Dakota. M.S. thesis, Fargo, North Dakota: North Dakota State University, Department of Agricultural and Biosystems Engineering. Will defend on October 25, 2016.
  - Bye, H., A. Gatzke, Q. Li, and X. Jia. 2016. Sweet corn production with different mulches, varieties, and planting dates in North Dakota. Transactions of the ASABE. In preparation with 70% done.
- **Award**
  - Anne Gatzke, $1,000, Duncan Scholar to attend the Annual International American Society of Agricultural and Biological Engineers Conference in July 2016.
- **Video (linked)**
  - General introduction about the sweet corn mulch project
  - The benefits about mulches for sweet corn
  - Frequently asked questions about mulch applications
  - Field measurements for the project (will be available soon)

- **Conference presentations**



Anne Gatzke presented a poster at NDSU Explore and won second prize in the morning session on her poster, "Impact of various mulches on sweet corn germination, plant growth and yield" on November 16, 2015, Fargo, ND. https://www.ndsu.edu/research/news/detail/22652/



Anne Gatzke presented her poster to show case ND agricultural research on April 12, 2016, Fargo, ND.

Xinhua Jia attended the High Tunnel Field Day and talked to several participants on using mulch inside high tunnels and sensor controlled irrigation for high tunnels on June 6, 2016.

Hannah Bye presented her poster at the International ASABE conference on July 18, 2016, Orlando, FL.

Anne Gatzke presented her poster at the International ASABE conference on July 17, 2016, Orlando, FL.

Hannah Bye, Anne Gatzke, and Xinhua Jia visited mulch and drip irrigation fields at Citra, University of Florida Research Center on July 21, 2016.

Determining the Causal Pathogen of a New Disease Affecting Dry Field Peas

Final Report

Partner Organization: North Dakota State University

Project Summary

Pea seedborne mosaic virus (PSbMV) can be a devastating disease of field peas if not managed by planting resistant cultivars or disease-free seed. The presence of virus variants not detectable using current methods makes seed testing and the evaluation of cultivars for resistance impossible. The research reported here not only characterizes the viral variant, but also provides a detection method to enable seed testing and cultivar evaluation. A previously unreported PSbMV variant was detected, confirmed to be a pathogen of field peas, and characterized using molecular techniques. Among 161 field samples tested from 2013 to 2014, 9% were positive for the known strains of PSbMV of which more than half of the samples also contained the new variant, while an additional 8% were positive for the new variant alone. The development of an immunocapture (IC) PCR assay allows for decreases in resources including time, financial and expertise when compared to RNA extraction and reverse transcriptase PCR typically needed for molecular detection of viruses. This IC-PCR assay can be utilized by diagnostic laboratories for detection of the new variant. Given the frequency of the new variant in samples collected from North Dakota, this is a very important outcome of this project.

Field and seed symptom evaluations for PSbMV in 2012 and 2013 led us to the hypothesis that another pathogen causing symptoms similar to PSbMV was present in field peas. Symptoms include leaf mottling, stunting, rosetting, decreased pod fill, pod distortion and seed scaring (Figure 1). Symptomatology suggests PSbMV, but serological testing was negative. Possible causal agents based on symptoms include virus or another phloem-limited (viroid, phytoplasma, or other bacteria) pathogen. Serological testing for other common pea viruses including, *Bean leaf roll virus* (BLRV), *Pea enation mosaic virus* (PEMV), *Pea mosaic virus* (PeMV) and *Pea streak virus* (PeSV), also were negative. Samples sent to a commercial laboratory for serological testing against 23 viruses known to infect dry peas were positive for *Lettuce mosaic virus* (LMV). In our experience, symptoms of the plants infected with the unknown pathogen are similar, but not identical to symptoms of LMV infection in peas previously described as chlorotic mottling, wrinkled leaflets, stunting, severe necrosis and eventual plant death (Provvidenti, 1973).

Symptoms presented in these plants are similar to those presented by other phloem limited pathogens including phytoplasmas, which have been described in dry peas (Zwolinska et al., 2012). They are found worldwide and have a large host range. Plants infected with phytoplasmas exhibit a range of symptoms indicative of disruptions to normal hormonal and growth patterns. These symptoms may include yellowing and reddening of the leaves, bunchy appearance, stunting, reduced vigor, shoot proliferation, floral abnormalities and leaf rolling (Lee et al., 2000; Bertaccini and Duduk, 2009). Phytoplasmas are single cell obligate parasites similar to bacteria but without a cell wall. They are spread by sap feeders such as leafhoppers, planthoppers, and psyllids (Lee et al., 2000). Phytoplasmas are not easily cultured in the laboratory, increasing the difficulty of detection. Phytoplasma detection is most commonly performed using a PCR-based assay in which nested PCR is performed followed by restriction digests for further classification.

Phytoplasma presence can be confirmed via sequencing of the PCR products or electron microscopy to visualize the organism within the phloem tissue. Historically, phytoplasmas are not considered to be seed transmitted. In a study on peas, seed was collected from infected fields and grown in the greenhouse. Phytoplasmas were found in these pea plants but were not found in the next greenhouse generation (Zwolinska et al., 2012). This leads us to believe that the pathogen is more likely a virus, but neither possibility can be excused at this point. The objectives of this project are to determine the identity of the causal pathogen of a new pea disease in North Dakota, develop a method to detect the pathogen and screen field pea samples for the presence of the pathogen.

Project Approach

- Evaluate serological assays from various vendors
- We completed testing samples for Lettuce Mosaic Virus (LMV) using kits purchased from AC Diagnostics, Bioreba and Agdia, Inc. Results from AC diagnostics kits were positive for LMV, but the background signal was high. Results from Bioreba kits were negative for LMV using two methods. Results from Agdia kits also came back negative for LMV.
- PSbMV testing performed using kits purchased from AC Diagnostics, Bioreba, Neogen, Loewe Biochemica and Agdia, Inc. gave negative results. PSbMV was not detected from some samples exhibiting symptoms typical of PSbMV.
- Collect greenhouse and field samples
- Seed and tissue samples were collected from the 2013 and 2014 field season. A total of 102 seed samples from 2013 were collected as well as 15 seed samples from 2014 and 44 leaf samples from 2014. Over 300 plants were grown from seed in the greenhouse. These plants displayed symptoms consistent with PSbMV, but tested negative for that virus.
- Optimize and validate phytoplasma PCR-based assay or viral RT-PCR and IC-RT-PCR assay for sample screening
- Samples were tested for general phytoplasma presence using a PCR-based assay with universal phytoplasma primers. Phytoplasma was successfully amplified from the positive control but no amplicon was observed in any samples. Therefore, all phytoplasma related testing has been eliminated from the work plan.
- A PCR-based assay was designed and developed based on the sequence comparison of the coat protein gene between obtained sequence of the proposed new variant and database sequences of the other characterized pathotypes.
- Present preliminary results at the Northern Pulse Growers Association Annual Meeting (224 meeting registrants)
- Preliminary results were presented at the Northern Pulse Growers Annual Convention in January, 2015.
- Analysis of preliminary serological and PCR-based data
- No serological assay was identified to detect the new pathogen.
- An RT-PCR assay was developed and validated via the presentation of typical symptoms to detect the new pathogen.
- Partial virus isolation preparations from four samples containing the unknown pathogen were completed. Virus particles were observed in all four samples evaluated using scanning electron microscopy by the electron microscopy lab at NDSU. RNA was extracted from these partial virus purification preparations. Serological (ELISA) testing again was negative, however,

these samples tested positive using the new RT-PCR assay, providing confirmation of the assays specificity.

- Inoculation of range of hosts for pathogen confirmation
- Delays in the completion of host-range studies were attributed to poor germination in many species. These studies were completed but are currently being repeated for confirmation.
- Inoculate (virus) or graft (phytoplasma) healthy plants for symptom development and observation (modified Koch's postulates)
- Successful mechanical transmission of the proposed new variant of PSbMV from infected to healthy field pea plants was confirmed via the newly developed IC-RT-PCR assay.
- Symptoms developed as typically observed in plants testing positive by RT-PCR for the new variant, but negative by PSbMV ELISA, confirming the variant as the previously undetected pathogen.
- Serologically test 2013 and 2014 samples
- Fourteen of 161 samples tested serologically positive for PSbMV.
- Virus isolation from inoculated plant samples
- Virus presence was detected in the inoculated plant samples using the developed RT-PCR assay, but virus isolation was only performed from the original plants, not inoculated plants.
- RNA extraction and partial viral genome sequencing
- RNA was extracted from the partial virus purification preparation obtained from four samples infected with the unknown pathogen. Primers identified from the literature and primers developed from conserved sequence regions were used to amplify parts of the viral genome. These PCR products were sequenced using Sanger sequencing.
- Sequence analysis
- Primer sequences were obtained from literature as well as from analysis of existing virus sequences. Two PCR products (totaling approximately 1,300 bp) from the unknown samples were sequenced using Sanger sequencing.
- Sanger sequencing and subsequent analysis provided ample evidence for a preliminary identification of the observed virus as a new pathotype of PSbMV.
- The sequence of the new variant is 94% similar to pathotype P4 and 86% similar to pathotype P1.
- Utilize viral RT-PCR and IC-RT-PCR assay for screening of 2013 and 2014 samples
- The viral RT-PCR and IC-RT-PCR assay was utilized to screen 102 samples from 2013 and 59 samples from 2014. Of the 2013 samples, 16 of 102 tested positive for the new variant and 6 of 59 samples from 2014 tested positive.
- Report preliminary results to the Pulse Crops Working Group
- Preliminary results were shared with the pulse research community at the Pulse Crops Working Group in March and August, 2015 with 12 in attendance.
- Preliminary analysis and compilation of Koch's postulates data, sequence data, host range data, and RT-PCR data (virus) or RFLP analysis (phytoplasma).
- Modified Koch's postulates were successfully completed and sequence data indicated the variant was homologous to PSbMV.
- Virus particles were observed in all samples by the electron microscopy lab, including four samples confirmed serologically negative for PSbMV, but RT-PCR positive.
- Present results at the Northern Pulse Growers Association Annual Meeting (240 meeting registrants)

- Preliminary results were presented to growers, extension professionals and other industry stakeholders at the Northern Pulse Growers Association in January, 2016 and the Pulse USA Trade Show in November, 2015 (26 in attendance).
- Final data analysis leading to identity confirmation of candidate pathogen
- Final analysis of the data resulted in the identification of a new variant of PSbMV. The identification was confirmed using two methods, electron microscopy and sequence analysis.
- Sequence analysis of two segments of the viral genome resulted in 86-94% sequence similarity to known pathotypes of PSbMV confirmed the identity of the pathogen.
- The pathogenicity was confirmed using a modified Koch's postulates procedure.
- The new variant of PSbMV was confirmed in 14% of the more than 100 samples from 2013 and the more than 50 samples from 2014.
- Share results with extension professionals
- This was accomplished through interaction at grower field days and annual meetings, as well as through personal interactions.
- Preliminary results were presented and the NDSU Research and Extension Center Conference in Fargo on March 3, 2016 by Julie Pasche with 25 in attendance.
- Present results at the North American Pulse Growers Association (NAPIA) meeting (81 meeting registrants)
- Preliminary results were presented to the scientific community at the North American Pulse Improvement Association in November, 2015.

We do not foresee the results of this project benefiting non-specialty crop commodities as PSbMV is a pathogen of pulse crops including peas, lentils, and chickpeas. Alfalfa can also carry the virus, but it is not known to cause damage to alfalfa.

Goals and Outcomes Achieved

Goal: to determine the identity of the causal pathogen of a new pea disease in North Dakota, develop a method to detect the pathogen and screen field pea samples for the presence of the pathogen.

The pathogenic organism causing the uncharacterized dry pea disease was identified as a variant of PSbMV. Serological screening was completed in attempt to preliminarily identify the pathogen. Early serological testing suggested LMV but the results were unverifiable via another manufacturer or another testing method resetting the baseline to 0. Testing for the presence of phytoplasm DNA in symptomatic samples produced negative results while the positive control produced positive results, thereby removing all phytoplasm related activities from the work plan. Successful serological testing requires an organism be detectable by available serological assays, therefore, the proposed measures to obtain an initial pathogen identification were inadequate. The lack of phytoplasm DNA suggested a viral presence undetectable by commonly used serological tests. A general virus isolation procedure was used on asymptomatic tissue, symptomatic tissue, and symptomatic tissue from serologically positive individuals. The resulting preparation was visualized using electron microscopy which provided the first method of confirmation of a viral presence. Nucleic acids were extracted and partially sequenced. The resulting sequence was highly similar to PSbMV suggesting a new variant of PSbMV and providing the second method of pathogen confirmation (**target** = 2; **target met**). The sequence was used to develop a detection assay based on RT-PCR for this new variant of PSbMV which

was used to complete the last of the proposed performance measures by confirming the pathogenicity of the newly identified pathogen using a modified Koch's postulates for non-culturable organisms.
The developed assay was utilized to determine the prevalence of the virus in samples collected in 2013 and 2014. One hundred and two seed samples had been collected from the 2013 field season (**target** > 100; **target exceeded**) and 16% tested positive for the new variant of PSbMV. An additional 15 seed samples as well as 44 symptomatic leaf samples were collected from the 2014 field season (**target** > 20; **target exceeded**). Of the 2014 samples, 13% of the seed samples tested positive and 9% of the leaf samples tested positive for the new variant of PSbMV. Information on the identity and potential importance of this novel pathogen has been shared with the field pea industry and research community using several formats. The research community met at the biennial North American Pulse Growers Association (NAPIA) meeting in November 2015 and results from this research were presented at that meeting. Additionally, Julie Pasche leads the Pulse Crops Working Group and has kept those pulse researchers informed using the web blog, via conference calls and at in-person meetings, which typically occur twice annually. The North Dakota pea industry was updated on research progress at the Northern Pulse Growers Association annual meeting in January 2015 and January 2016. NDSU extension professionals have been kept informed on any results that have impacts to grower management decisions. Results are currently being prepared for publication in in the American Phytopathological Society Journal Plant Disease.

Beneficiaries

Nearly 300,000 acres of peas were planted in North Dakota in 2013, 265,000 acres in 2014, 385,000 acres in 2015 and 640,000 acres in 2016. Field pea production in North Dakota has generated as much as $141 million in yearly revenue. This project will directly benefit the approximately 2,500 pulse producers in North Dakota, processors and all others involved in pea production. The new variant of PSbMV was present in over 10% of the field pea samples tested to date. Yield losses from PSbMV of up to 82% have been documented. Plants displaying symptoms of this disease also produce fewer seeds, and seeds may be scarred. The use of clean seed is the most effective way to manage this disease in the absence of host resistance. The new RT-PCR assay allows the detection of this pathogen to reduce planting infected seed and for use in developing resistant varieties. Integrating this assay into seed testing labs is the next step in reducing the losses caused by PSbMV. Additionally, cultivars and breeding material can now be more accurately evaluated for the presence of the disease. Other pea growing regions such as neighboring Montana also will benefit from this project.

Lessons Learned

- The execution of the research and the administration of this project went smoothly.
- The development and execution of the work plan aided in keeping the project on-time and in efficient completion of the described goals and outcomes.

Figure 1. Symptoms of unknown pathogen in greenhouse-reared plants; A and B) seed scaring; C) healthy plant on the left and diseased plant on the right showing irregular growth including stunting, mottling and rosetting; D) rosetting; E) closeup showng dieback of new growth and rosetting; F and G) incomplete pod filling and irregular pod development.

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

Final Report

Partner Organization: North Dakota State University

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 measurable 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 trials were conducted in 2015 in a field known to be infested with PMTV. In total, sixty three cultivars were screened. Forty seven of these cultivars expressed symptoms of PMTV-induced tuber necrosis. Tubers from the remaining 16 cultivars that did not show PMTV symptoms were randomly assayed for the presence of the virus by RT-PCR. Two cultivars (Yukon Gold and Russet Norkotah 296) that showed no visual symptoms tested positive for PMTV while the remaining 14 symptomless cultivars did not test positive for the virus. These cultivars may be resistant to PMTV. All 63 cultivars will be retested in 2016.

The specific objective of this project is:

- 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 purpose of this project is 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.

Project Approach

- Pathogen-free seed consisting primarily of minitubers derived from tissue culture of 63 potato cultivars were planted in a field known to be infested with *Potato mop top virus* on May 1, 2015.
- Emergence data for each clone was collected on July 21. We had good emergence from every clone planted.

- Foliar symptoms of PMTV were monitored biweekly from July 21 until vine desiccation on September 12.
- Field plots were harvested on September 14-15, 2015.
- Post-harvest evaluations were conducted twice during the storage period. The first evaluation commenced on September 21-25. The second evaluation took place January 4—February 5, 2016. Tubers of each clone from every replication were sliced and scored for tuber necrosis.
- In the first evaluation, significant differences in PMTV-induced tuber necrosis were found among the potato cultivars evaluated. The incidence PMTV tuber necrosis ranged from 0 to 21.9%, and severity (on a 0-1 scale, with 1 being total necrosis) ranged from 0 to 0.13.
- Tuber necrosis in red-skinned cultivars was significantly higher than for any other market class of potato. PMTV-induced tuber necrosis was highest in in Red Endeavor (21.9%) and was significantly higher than the internal susceptible control, Dakota Jewel, for this market class.
- Tuber necrosis in white-skinned cultivars was highest in the internal susceptible control cultivar Nicolet (12.2%) and was significantly higher than any other cultivar for this market class.
- Tuber necrosis in yellow- and russet-skinned cultivars were very low and significantly lower than red- or white-skinned cultivars.
- Overall, expression of PMTV-induced tuber necrosis was much lower than expected and much lower than we have experienced during our preliminary studies. We have a plan to resolve this issue in 2015 and this is discussed in more detail below.
- 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 2016.
- The second grading of tubers for mop-top disease was initiated on January 12, 2016. The tubers 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 33.4%. PMTV tuber necrosis severity ranged from 0 to 0.49. Out of 63 clones, 47 clones have PMTV infection, while the remainder (16) did not show disease symptoms. This was a dramatic increase in the incidence of PMTV-induced tuber necrosis as only ten clones showed symptoms at during the first grading while 47 clones were observed to have tuber necrosis during the second evaluation.
- Forty symptomless tubers from across the 16 cultivars that did not show PMTV symptoms were randomly assayed for the presence of the virus by RT-PCR. Two cultivars (Yukon Gold and Russet Norkotah 296) that showed no visual symptoms tested positive for PMTV while the remaining 14 symptomless cultivars did not test positive for the virus.
- 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.

Goals and Outcomes Achieved

- Tuber necrosis in red-skinned cultivars was significantly higher than for any other market class of potato. PMTV-induced tuber necrosis was highest in in Red Endeavor (21.9%) and was significantly higher than the internal susceptible control, Dakota Jewel, for this market class. Tuber necrosis in white-skinned cultivars was highest in the internal susceptible control cultivar Nicolet (12.2%) 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 lower than expected and much lower than we have experienced during our preliminary studies. We have a plan to resolve this issue in 2016 by insuring that soil moisture in the field trial is maintained at a high level by applying additional water through supplemental irrigations. Since this plot is in a grower's field, we simply allowed the cooperator to apply the irrigation applications as he determined. However, after explaining the influence of high soil moisture on the vector of PMTV (powdery scab pathogen), he agreed that he will apply 2X the rate of water on our field plot than he applies on the remainder of the field.
- No recommendations or conclusions can be made at this time based upon our data since we have only completed the first year of evaluations. We will be able to make definitive recommendations to the potato industry regarding the susceptibility of potato cultivars to PMTV-induced tuber necrosis in each market class after the completion of our research trials in 2016.
- The second field experiment of this research was planted on April 28, 2016. Emergence was excellent, and we anticipate that we will be able to obtain excellent data that can be combined with the data from 2015.

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 121 people in attendance.
- 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 ensure 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 ensure 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.

Farm Food Safety Training for Local Vegetable and Fruit Growers to Increase Sales and Success

Final Report

Project Summary

The project addressed the need for farm food safety training and technical assistance to small scale growers of specialty crops to assist in expanding production and sales. Within its timeframe, project staff completed a needs assessment of small-scale specialty crop producers, delivered three farm food safety courses based on the identified needs to 32 producers, 15 of whom completed farm food safety plans, surveyed participants to ensure needs were met and provided technical assistance to specialty crop producers that resulted in expanded production and sales. Additionally, a locally purchased produce safety presentation was delivered to 70 produce managers from around the state to assist them in purchasing safe, fresh, locally grown produce.

Ensuring the safety of locally grown vegetables and fruits is important and timely as the national Food Safety Modernization Act is now being implemented and producers and growers must be prepared to comply. Additionally, more consumers are interested in buying local food. Although many small specialty crop producers are exempt from the FSMA regulations, their businesses are growing and they must be ready to comply when they reach non-exempt levels. Even if exempt, producers must be aware of the food safety guidelines for the safety of their customers. Most outbreaks of food-borne illness have originated at large distribution centers and locally grown produce provides consumers a safer alternative. Decentralization of food production in the produce industry makes it easier to pinpoint and isolate issues. Direct-to-consumer sales of vegetables and fruits also provide consumers opportunities to ask farmers what food safety measures are in place.

This project didn't directly build upon a previously funded project, but is a natural outgrowth of the Entrepreneurial Center for Horticulture's current work. This project is not funded by and was not submitted to any other Federal or State grant programs. While the Entrepreneurial Center for Horticulture does receive funding from other sources, including federal grants, there were no other funding sources supporting the activities of this project.

Project Approach

This project addressed the need for farm food safety training and technical assistance to help small-scale specialty crop growers expand production and sales of vegetables and fruits in a safe manner. The Entrepreneurial Center for Horticulture (ECH), located at Dakota College at Bottineau, provided classes for farmers in farm food safety, including Good Agricultural Practices, Good Handling Practices and training in creating a Food Safety Plan. The ECH also provided farmers with one-on-one technical assistance including training in crop production, season extension, farm management, and marketing.

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More households are choosing to spend grocery dollars at local farmers markets or purchase CSA shares, creating opportunities for small-scale vegetable and fruit growers to expand enterprises. The number of farmers markets nationally more than tripled from 1,755 to 6,132 between 1994 and 2010 and the North Dakota Department of Agriculture reports that in our state the number grew from five markets in 2004 to over 50 in 2015. In a recent national consumer research study, over 40% of respondents said they purchase local food on a weekly basis.

The Entrepreneurial Center for Horticulture's objectives for this project was to increase the number of farmers trained in farm food safety and who have created Food Safety Plans and to increase the number of farmers starting new direct-to-consumer sales enterprises. The ECH provided farm food safety classes including training on Good Agricultural Practices and Good Handling Practices. Farmers also learned how to prepare a Farm Food Safety Plan that would be compliant with the national Food Safety Modernization Act. The ECH also provided one-on-one technical assistance to farmers including training in farm business management, marketing, crop production, and season extension, thereby training producers in methods that are safe and compliant with local, regional, and national protocols. New producers were also assisted in growing their enterprises and doing such in a manner that helped ensure success and compliance with regulations.

During the grant period, the tasks accomplished from the work plan include; an initial project needs assessment to determine specific course topics to be addressed that was delivered via internet and paper, development of class materials and food safety plan templates, creation of four different promotional materials to inform farmers and producers of the food safety learning opportunities, scheduling and delivery of three farm food safety courses that were taught to thirty-two (32) farmer/producers, creation and delivery of a post food safety course survey to all course participants to gauge the understanding of food safety concepts and determine the number of producers completing farm food safety plans, and delivery of one-on-one technical assistance to producers in over 90 visits to assist in the safe production of specialty crops and growth in marketing and sales by new and expanding producers.

The grant funds for this project were used solely to benefit specialty crop growers. This was ensured through use of a survey instrument that asked the farmers what crops they produced and then cross checking those answers with the USDA list of specialty crops.

Goals and Outcomes Achieved

The first goal of the project was to increase the number of small to mid-sized vegetable and fruit growers trained in farm food safety. Measurement of this goal began with development of a benchmark of the needs of specialty crop producers in regards to food safety. The benchmark was developed through use of a survey instrument to determine areas of need and current levels of knowledge among producers. Results of the benchmark survey were shared with internal and external stakeholders via online newsletter, social media and print newsletters that reached over 500 individuals. Of the respondents to the benchmark survey, which was posted online and open to all, all non-specialty crop producer responses were discarded and not evaluated as a part of the project. The majority of specialty crop producers who responded, indicated a mid-level knowledge of farm food safety, rating themselves either a '3' or '4' (80% of respondents

combined) on a five-point scale where five equaled very knowledgeable. Over one third had previously taken a food safety course or training and had a food safety plan written for their production business. The majority of those with already in place food safety plans, indicated they felt the need for refresher courses. This indicated that course materials on farm food safety should be arranged with advanced curriculum for most producers, which was carried out in the curriculum development phase.

Through the delivery of three courses, thirty-two (32) vegetable and fruit producers were trained in three separate classes. The original goal for this project was 25 producers. Of these participants, fifteen (15) completed farm food safety plans that were reviewed by project personnel and found to be compliant with Good Agricultural Practices and local/regional regulations. This met the project goal of fifteen producers writing plans.

The second goal for the project was an increase in vegetable and fruit farmers beginning new direct-to consumer sales venues. The benchmark for this goal was determined through initial needs assessment survey by asking participants their current markets and determining the percent of which were selling wholesale versus direct to consumer. It was also measured by tracking the number of producers receiving one-on-one technical assistance that were new to growing and selling specialty crops and whose market was direct to consumer. The target for this goal was eight (8) farmers beginning new direct to consumer sales venues. Technical assistance visits began in the 1st quarter of 2015. Project personnel provided technical assistance to 69 producers in over 90 visits and two technical support workshop. The workshops were provided to a group of Aronia Berry producers who were looking for markets for their product and to a group of 70 grocery store produce employees who needed information on how to work with local producers to ensure safe, quality produce was purchased.

Evaluation of documentation on technical assistance visits showed fourteen (14) farmers or producers of specialty crops who began new direct to consumer sales venues within the project timeframe. This exceeded the goal of eight (8) originally planned as a target for the project.

Based on review of technical assistance provided, post farm food safety course participant surveys, and the initial benchmark needs assessment; it can be concluded that most producers have at least a base knowledge of farm food safety but may not be aware of the specific tasks involved in monitoring and record keeping for FSMA or state and regional regulations. Most new producer farmers of specialty crops have the desire and base knowledge of production but need assistance in finding the market venue that will work for their operation. Most producers have the desire to work hard at making their product safe.

Beneficiaries

The initial goal for the project was at least 40 farmers impacted, assuming 30 farmers participate in farm food safety training and receive one-on-one technical assistance and an additional ten farmers receive technical assistance only. Final results of the project show over 101 producers impacted with thirty-two (32) in farm food safety courses, fourteen (14) that received technical assistance beginning or expanding into direct to consumer venues, fifty-five (55) additional producers receiving technical assistance, and twelve (12) Aronia growers receiving assistance to

cooperate as a group and expand their markets. Almost all farmers served fall within the definition of "Beginning Farmers." Sixteen (16) participating farmers were new farmers and most participants already raising vegetables and fruits have been farming for less than ten years.

The ECH anticipated that at least eight (8) of the farmers receiving farm food safety training would begin new direct-sales vegetable and/or fruit enterprises when in fact final tally shows fourteen (14) producers in new direct sale venues. This will result in more locally grown produce available to consumers and should increase farmers' income, which in turn should positively impact the communities where farmers live and spend money. This has potential for significant impact as nationally, nearly 150,000 farmers and ranchers are selling their products directly to consumers, and 50,000 are selling to local retailers. Nationally, local food sales were valued at $7 billion in 2011, according to industry estimates.

Lessons Learned

At least one scheduled farm food safety class had to be rescheduled due to lack of registrations. Producers and farmers are reluctant to participant in activities that take them away from their production of specialty crops during the growing season. All educational opportunities for producers should be held during 'off-season' times. Scheduling grant activities in a timeline that supports the growing season and reaches out to producers and farmers during winter months is recommended.

This is not true however of technical assistance to new and beginning farmers who seek out advice and assistance once their crop is in the ground. Being new to the industry, these beginning producers tend to think more in the moment rather than plan to far ahead. Most technical assistance provided during this project was in response to a request from a producer who already had fruits or vegetables growing but was just then thinking about where to sell them or seeking solutions to problems at hand.

Contact Information

Holly Rose Mawby
Dakota College at Bottineau Entrepreneurial Center for Horticulture Director
105 Simrall Blvd.
Bottineau, ND 58318
701-681-0252
HollyRose.Mawby@dakotacollege.edu

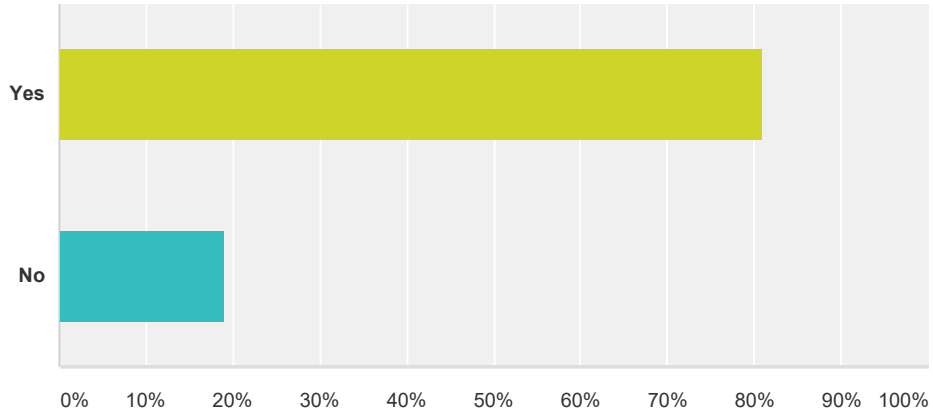
Additional Information

Attached with this report are the following documents:

- The report from the online needs assessment survey used as a part of the benchmarking,
- A copy of the paper needs assessment that was distributed, this same tool was used as a pre-course measure of producer knowledge and to assure specialty crop producers were being served through this project,
- The 'post' farm food safety course survey instrument, and
- Marketing brochures from two of the three food safety courses held
- A copy of a farm food safety plan that was used as a template during course

Q1 Do you raise and sell specialty crops or plan to do so in the next two years?

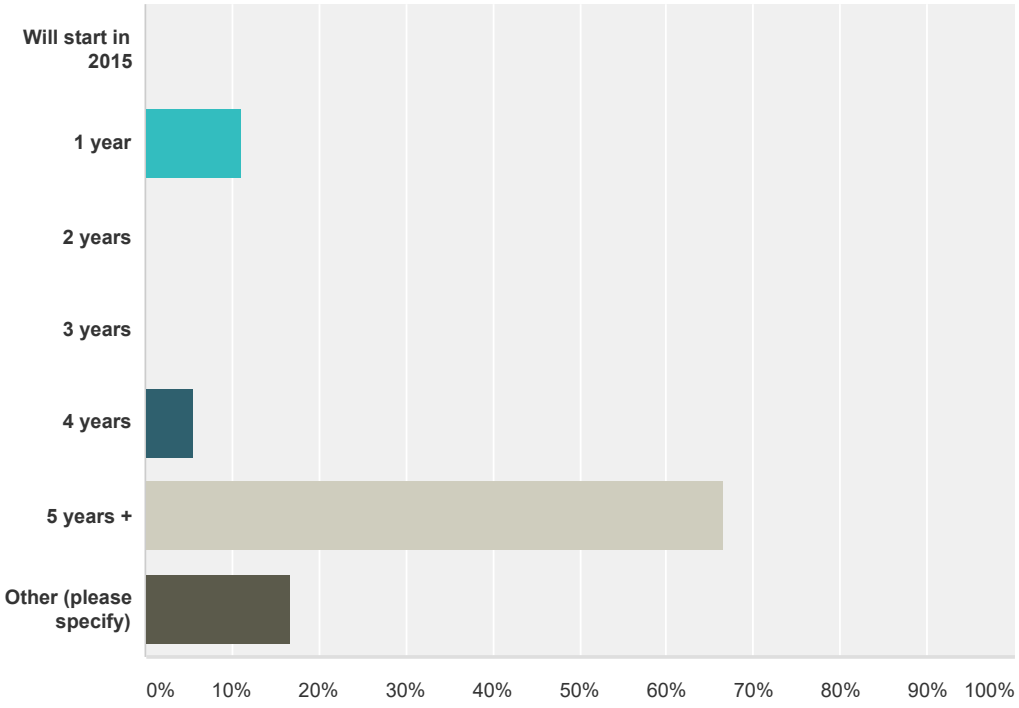
Answered: 21 Skipped: 0



Answer Choices	Responses	
Yes	**80.95%**	17
No	**19.05%**	4
Total		**21**

Q2 How many years have you produced specialty crops?

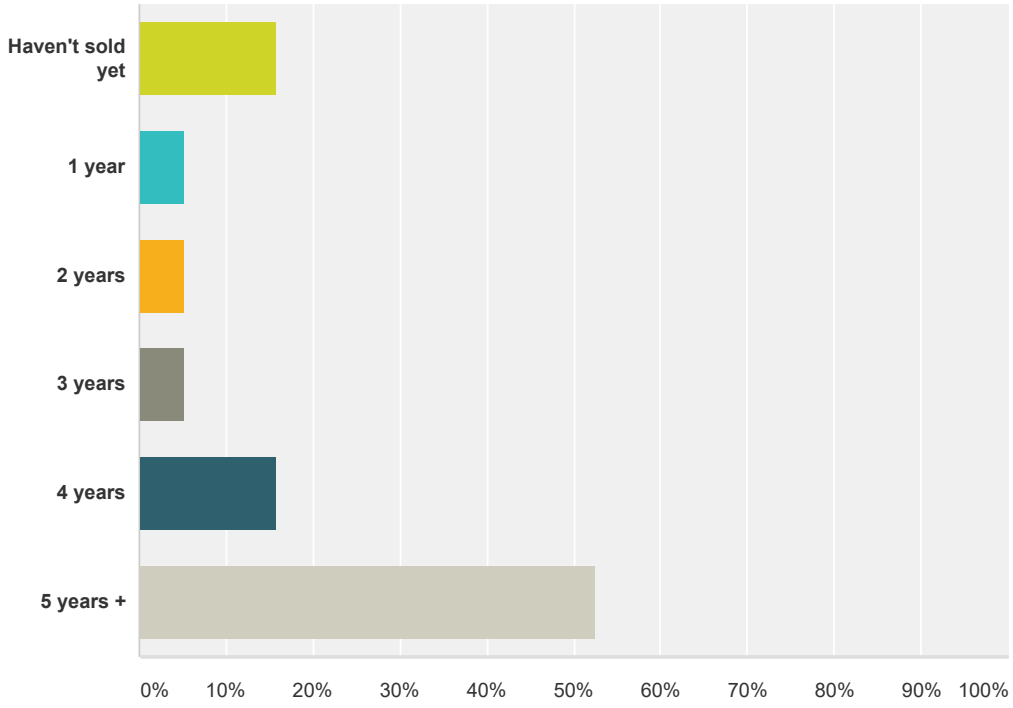
Answered: 18 Skipped: 3



Answer Choices	Responses	
Will start in 2015	**0.00%**	0
1 year	**11.11%**	2
2 years	**0.00%**	0
3 years	**0.00%**	0
4 years	**5.56%**	1
5 years +	**66.67%**	12
Other (please specify)	**16.67%**	3
Total		**18**

Q3 How many years have you sold specialty crops?

Answered: 19 Skipped: 2



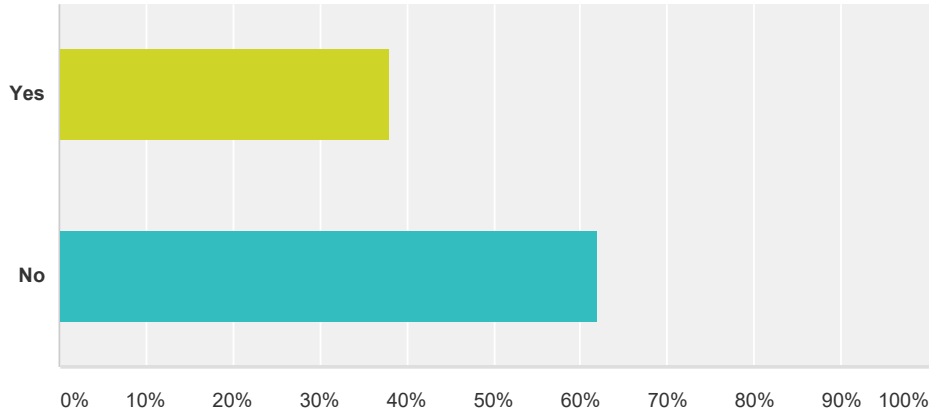
Answer Choices	Responses	
Haven't sold yet	**15.79%**	3
1 year	**5.26%**	1
2 years	**5.26%**	1
3 years	**5.26%**	1
4 years	**15.79%**	3
5 years +	**52.63%**	10
Total		**19**

Q4 Specifically which specialty crops do you raise/sell?

Answered: 17 Skipped: 4

Q5 Have you had any contact or communication with your local health district about selling your specialty crop products?

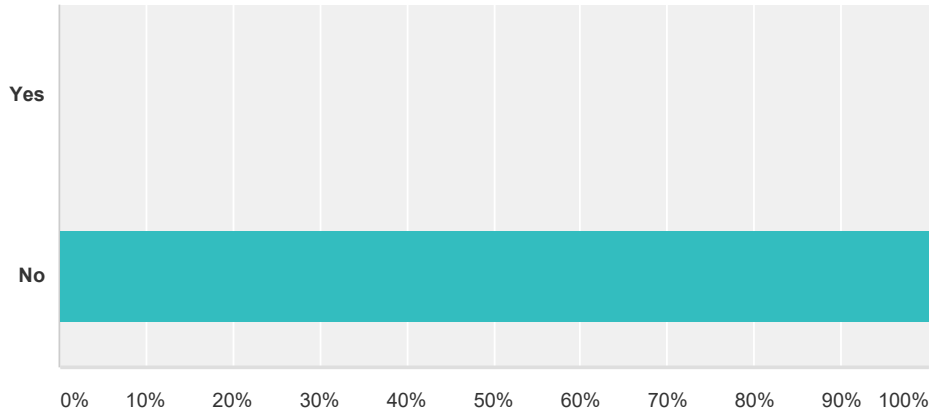
Answered: 21 Skipped: 0



Answer Choices	Responses	
Yes	**38.10%**	8
No	**61.90%**	13
Total		**21**

Q6 Are you (your operation) currently inspected by any Health regulatory operation?

Answered: 21 Skipped: 0



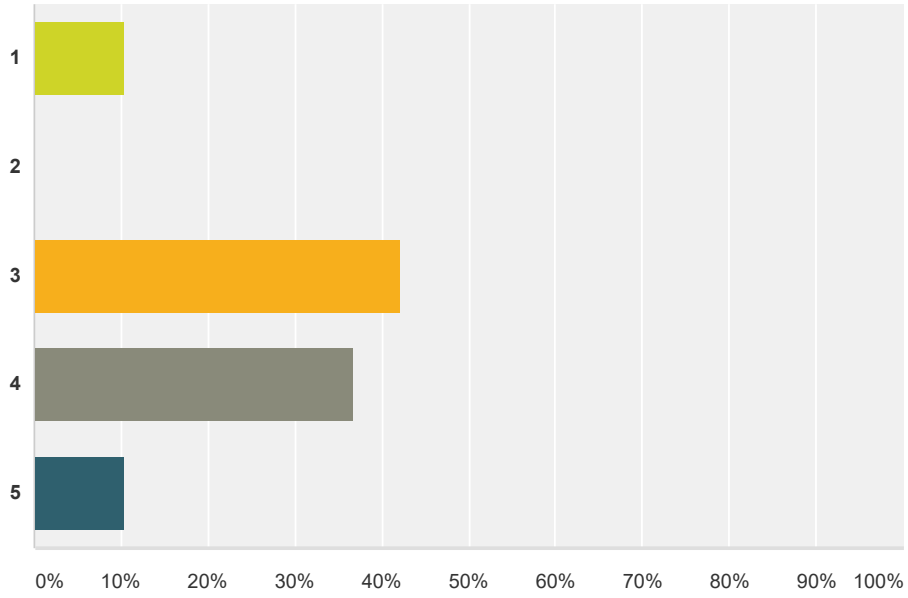
Answer Choices	Responses	
Yes	**0.00%**	0
No	**100.00%**	21
Total		**21**

Q7 If you answered ‘yes’ to the question above, please tell us which Health agency/district inspects your operation, if you answered ‘no’ please skip to the next question.

Answered: 2 Skipped: 19

Q8 What level of knowledge do you have about farm food safety practices? (ranking – 1 to 5 with one being none and 5 being very knowledgeable)

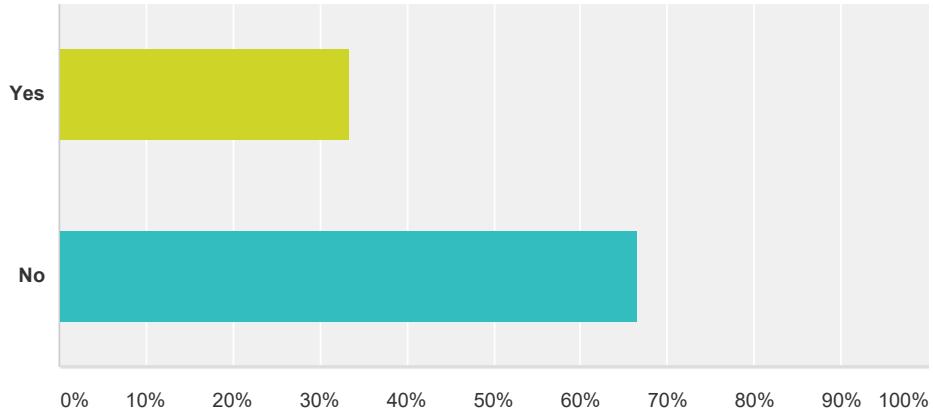
Answered: 19 Skipped: 2



Answer Choices	Responses	
1	**10.53%**	2
2	**0.00%**	0
3	**42.11%**	8
4	**36.84%**	7
5	**10.53%**	2
Total		**19**

Q9 Have you ever taken a food safety course?

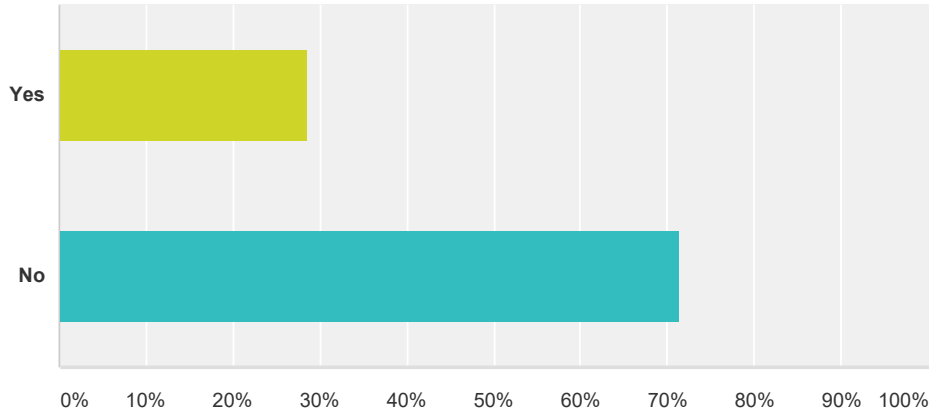
Answered: 21 Skipped: 0



Answer Choices	Responses	
Yes	**33.33%**	7
No	**66.67%**	14
Total		**21**

Q10 Do you have a food safety plan?

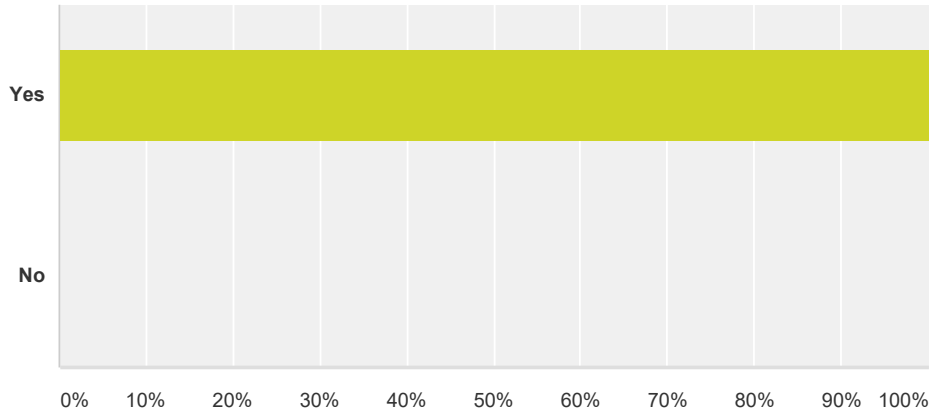
Answered: 21 Skipped: 0



Answer Choices	Responses	
Yes	**28.57%**	6
No	**71.43%**	15
Total		**21**

Q11 If you answered 'yes' to the previous question, please answer the following. If you answered 'no' to the previous question please skip this one.If you have a food safety plan for your farm or operation, do you feel that periodic updates to that plan or updated training would be useful to you to keep abreast of changing regulations or practices?

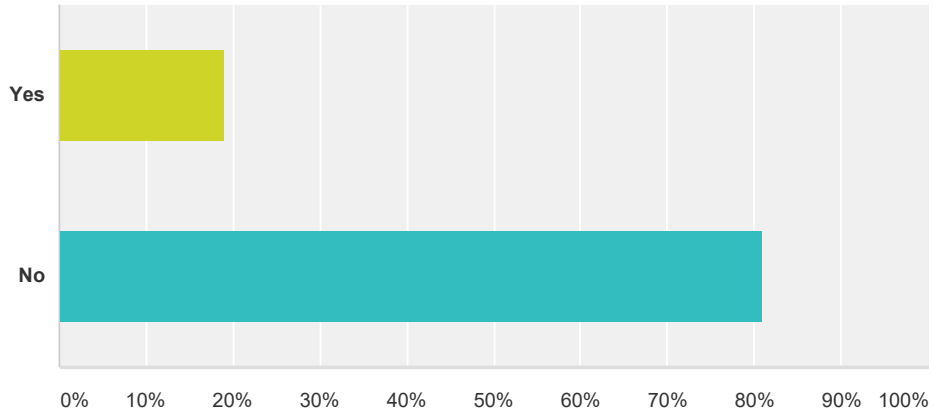
Answered: 7 Skipped: 14



Answer Choices	Responses	
Yes	**100.00%**	7
No	**0.00%**	0
Total		**7**

Q12 Do you know of any other producer that has a farm food safety plan designed for their farm?

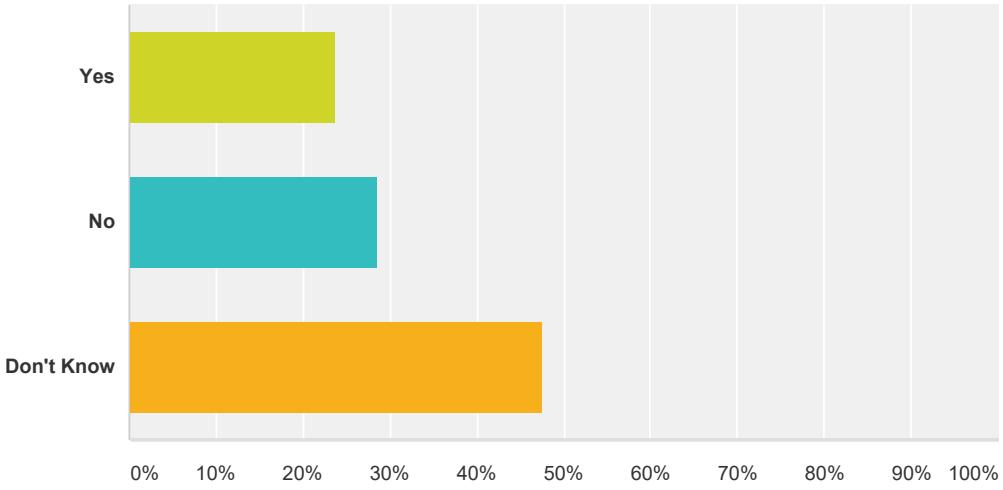
Answered: 21 Skipped: 0



Answer Choices	Responses	
Yes	**19.05%**	4
No	**80.95%**	17
Total		**21**

Q13 Are you using any farm food safety practices in your operation that you know of?

Answered: 21 Skipped: 0



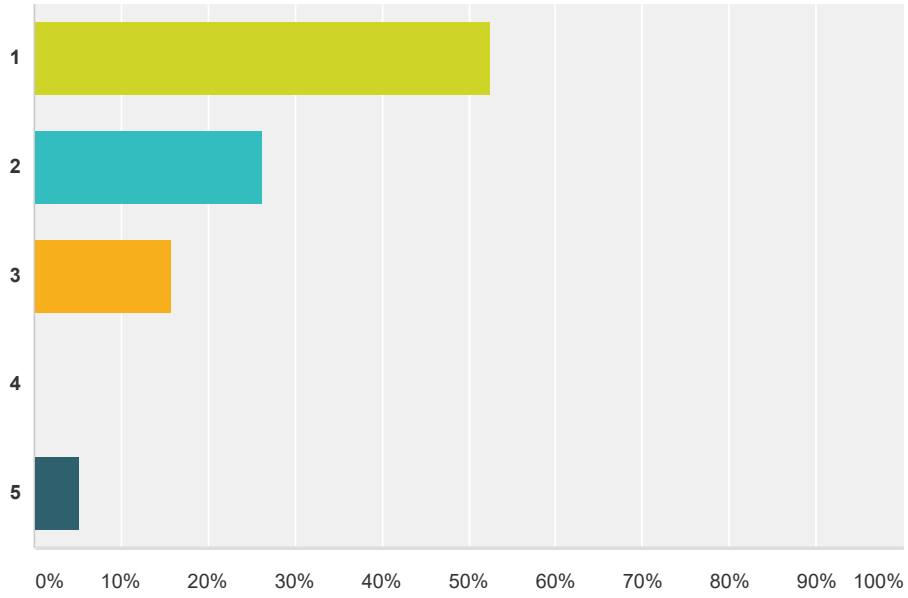
Answer Choices	Responses	
Yes	**23.81%**	5
No	**28.57%**	6
Don't Know	**47.62%**	10
Total		**21**

Q14 If you answered 'yes' to the previous question, please tell us a little about the actions you take that constitute farm food safety practices. If you answered 'no' please skip to the next question.

Answered: 6 Skipped: 15

Q15 How much do you know about the Food Safety Modernization Act? (ranking scale 1 to 5 with 1 being nothing and 5 being very knowledgeable)

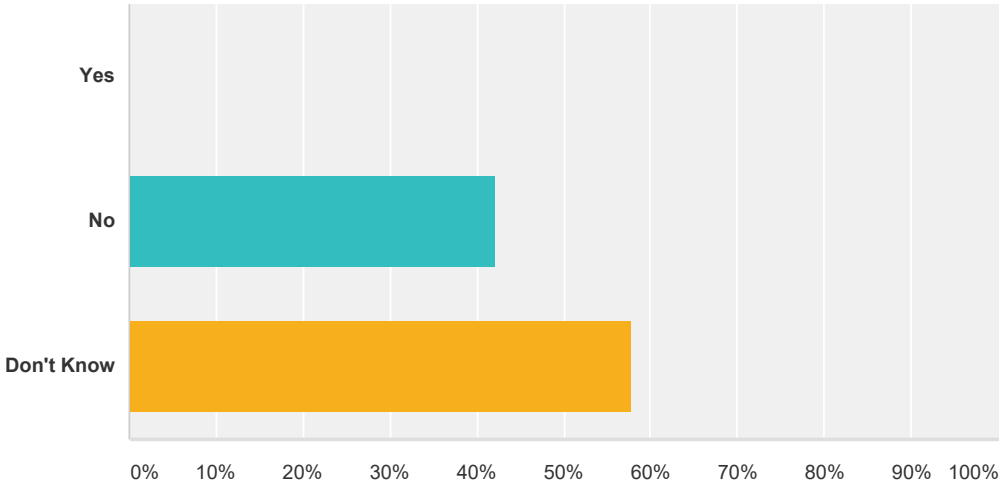
Answered: 19 Skipped: 2



Answer Choices	Responses	
1	**52.63%**	10
2	**26.32%**	5
3	**15.79%**	3
4	**0.00%**	0
5	**5.26%**	1
Total		**19**

Q16 Do you think the Food Safety Modernization Act will make it easier for you to raise and sell your specialty crop products?

Answered: 19 Skipped: 2



Answer Choices	Responses	
Yes	**0.00%**	0
No	**42.11%**	8
Don't Know	**57.89%**	11
Total		**19**

Q17 If you sell specialty crops, what market/s do you use, as an example; CSA, Farmers Market, Online sales, wholesale, farm to school, etc.?

Answered: 17 Skipped: 4

Q18 What markets would you like to enter as an example; CSA, Farmers Market, Online sales, wholesale, farm to school, etc.?

Answered: 11 Skipped: 10

Entrepreneurial Center for Horticulture

Specialty Crop Producer Farm Food Safety Survey

Thank you for helping the Dakota College at Bottineau Entrepreneurial Center for Horticulture with this survey!

Your answers will help us plan for farm food safety courses that teach what you need to know. Please be sure that you are a specialty crop producer as defined by the USDA. Specialty crops are defined by the USDA as fruits, vegetables, tree nuts, dried fruits, horticulture, and nursery crops. Eligible plants must be intensively cultivated and used by people for food, medicinal purposes, and/or aesthetic gratification to be considered specialty crops. For a complete list of specialty crops, please check out this link: http://www.ams.usda.gov/AMSv1.0/scbgpdefinitions.

The survey should only take a few minute of your time.
Thank you!

Questions:

Do you raise and sell specialty crops or plan to do so in the next two years?
Yes No

How many years have you produced specialty crops?

How many years have you sold specialty crops?

Specifically which specialty crops do you raise/sell?

Have you had any contact or communication with your local health district about selling your specialty crop products?

Are you (your operation) currently inspected by any Health regulatory operation?

If you answered 'yes' to the question above, please tell us which Health agency/district inspects your operation, if you answered 'no' please skip to the next question.

What level of knowledge do you have about farm food safety practices? (ranking – 1 to 5 with one being none and 5 being very knowledgeable)

Have you ever taken a food safety course?
Yes No

Do you have a food safety plan?
Yes No

If you answered 'yes' to the previous question, please answer the following. If you answered 'no' to the previous question please skip this one.
If you have a food safety plan for your farm or operation, do you feel that periodic updates to that plan or updated training would be useful to you to keep abreast of changing regulations or practices?
Yes No

Do you know of any other producer that has a farm food safety plan designed for their farm?
Yes No

Are you using any farm food safety practices in your operation that you know of?
Yes No Don't know

If you answered 'yes' to the previous question, please tell us a little about the actions you take that constitute farm food safety practices. If you answered 'no' please skip to the next question.

How much do you know about the Food Safety Modernization Act? (ranking scale 1 to 5 with 1 being nothing and 5 being very knowledgeable)

Do you think the Food Safety Modernization Act will make it easier for you to raise and sell your specialty crop products?
Yes, No Don't know

If you sell specialty crops, what market/s do you use, as an example; CSA, Farmers Market, Online sales, wholesale, farm to school, etc.?

What markets would you like to enter as an example; CSA, Farmers Market, Online sales, wholesale, farm to school, etc.?

Producer/Learner Post-course Survey

1. Were you using any farm food safety practices in your operation before this training?

   Yes No

2. Please rate your level of knowledge about farm food safety **BEFORE** starting this course:

I knew a Lot		I knew some			I knew very little			I knew nothing	
10	9	8	7	6	5	4	3	2	1

3. Do you feel that you know more about farm food safety than you did before the course?

   Yes No

4. Do you plan to implement **at least one <u>new</u>** food safety action in your operation?

   Yes No

5. Do you plant to write a farm food safety or GAP's plan for your operation?

   Yes No

6. Do you feel that having a food safety plan or following good farm food safety protocols will help you market your product?

   Yes No

7. Do you plan to use your farm food safety training or farm food safety plan to market your product?

   Yes No

8. What was the most valuable thing you learned during this course?

_____

_____

_____

_____

9. Do you feel you received answers to your questions or that the instructor will follow up with you on any unanswered questions?

   Yes No

10. If farm food safety regulations change in the future, would you be interested in a refresher or update course?

    Yes No

11. Would you like assistance in writing a full farm food safety plan?

    Yes No

If you answered yes to either of the last two questions, Please list give us your name and contact information:

_____

_____

What is a …

Food Safety Plan?

A food safety plan is your farm's guide to minimizing the potential for contamination of your produce. It is your farm's policies and practices to keep the produce safe for your consumers. YOU will get to set the policies and practices within it. This workbook will help you create the plan, follow the policies, and record your actions.

PLACE STAMP HERE

Spring Workshop INFO

What: Farm Food Safety Course Development- *planning.writing.using*

Where: Prairie Den, 122 ½ Broadway, Fargo, ND 58012 701.371.6073

When: Saturday, April 9, 2016

Time: 9 am to 4 pm

Get in touch to register

Crystal Grenier, Admin Assistant
Dakota College at Bottineau
105 Simrall Blvd. M 22
Bottineau, ND 58318
Office: 701.228.5649

crystal.grenier@dakotacollege.edu

Farm Food Safety Course 2016

For Producers of Fruits, Vegetables and Other Specialty Crops

Presenter:
Erin Williams, BSN, RN
Dakota College
at Bottineau, ECH

Nature, Technology & Beyond
Dakota College at Bottineau
An affiliate of Minot State University

Interesting Insights

In recent years, there has been growing interest in locally produced fresh fruits, vegetables, and other food products. Farmers' markets have become a common feature of local efforts to encourage community economic development by promoting locally grown products. The health benefits of eating lots of fresh fruits and vegetables have been clearly demonstrated and encouraged by national and international nutrition and health authorities. Given the choice, many consumers prefer locally grown products, and are often very willing to support the hard work of local growers who bring fresh and wholesome products into their communities.

Food safety is a good idea and can be good for your business. At this time most North Dakota growers do not need to have their farm and produce GAP audited unless their customer– typically a distributor, grocery store, school, or restaurant–requires it. If you need to have an audit, the very first thing you need is to have a written food safety plan.

Even if you are direct marketing your produce and don't need an audit, having a food safety plan and following good hygiene and sanitation practices can benefit your operation. It will assure your customers that you are proactively reducing the risk of microbial contamination on your produce. This workshop and accompanying food safety plan templates are designed to help North Dakota fruit and vegetable growers create a food safety program on their farm. The goal is to provide farmers with a straightforward way to document, plan and approach food safety on their farm to minimize the chance of a food safety issue or contamination of their produce.

The material provided at the workshop is guidance and not regulation and should be applied as appropriate and feasible to your fruit and vegetable operations. Keep in mind that this is **YOUR** plan and should fit **YOUR** farm. Don't make it harder than you need to.

We will discuss the major sections of the Food Safety Modernization Act and assist you in addressing each topic.

Spring Workshop

Where: Prairie Den, 122 ½ Broadway, Fargo, ND 58012
701.371.6073

When: Saturday, April 9, 2016
Time: 9 am to 4 pm

Get in touch to register

Crystal Grenier,
Admin Assistant,
Dakota College at Bottineau
Office: 701.228.5649
crystal.grenier@dakotacollege.edu

Your Instructor

Born and raised in a farming family in Bottineau, North Dakota. I graduated from Bottineau Public School and then continued my education at Dakota College at Bottineau with an Associate of Science Degree. I transferred to Medcenter One College of Nursing in Bismarck and graduated with a Bachelor of Nursing Science Degree in 1998. I am currently the student health services nurse and an instructor at Dakota College at Bottineau, as well as the Simulation Technician and clinical/lab instructor for the practical nursing program at Dakota Nursing Program at Bottineau. I began working with the Dakota College at Bottineau Entrepreneurial Center for Horticulture demonstration gardens and on campus CSA in 2015. I have a passion for growing vegetables, healthy eating, and food safety.

Contact INFO

Erin Williams, BSN, RN
Office: 701.228.5460
email:
Erin.beth.williams@dakotacollege.edu

What is a …

Food Safety Plan?

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PLACE
STAMP
HERE

To all Local Producers!

Dakota College at Bottineau
105 Simrall Blvd.
Bottineau, ND 58318

Food Safety Planning 2015

For Producers of Fruits, Vegetables and Other Specialty Crops

Presenter: Keith Knudson
Dakota College at Bottineau, ECH

Nature, Technology & Beyond
Dakota College at Bottineau
An affiliate of Minot State University

Interesting Insights

In recent years, there has been growing interest in locally produced fresh fruits, vegetables, and other food products. Farmers' markets have become a common feature of local efforts to encourage community economic development by promoting locally grown products. The health benefits of eating lots of fresh fruits and vegetables have been clearly demonstrated and encouraged by national and international nutrition and health authorities. Given the choice, many consumers prefer locally grown products, and are often very willing to support the hard work of local growers who bring fresh and wholesome products into their communities.

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The material provided at the workshop is guidance and not regulation and should be applied as appropriate and feasible to your fruit and vegetable operations. Keep in mind that this is **YOUR** plan and should fit **YOUR** farm. Don't make it harder than you need to.

We will discuss the major sections of the Food Safety Modernization Act and assist you in addressing each topic.

Fall Workshop Date-

What: Food Safety Planning and Writing a Food Safety Plan
Where: Dakota College at Bottineau Main Building – Thatcher in Holmberg Rm. Bottineau, ND 58318
When: Tuesday, November 17th
Time: 9 am to 4 pm

Contact Information to Register:

Crystal Grenier,
crystal.grenier@dakotacollege.edu
Dakota College at Bottineau – ECH
105 Simrall Blvd.
Bottineau, ND 58318
Ph: 701-228-5649

Keith Knudson

Contact

Keith Knudson
Farm Business Management Instructor

Dakota College at Bottineau
701-228-2160 or 701-228-5130
Keith.Knudson@dakotacollege.edu
www.dakotacollege.edu

Aspen Hills Farm
Food Safety Plan

Revised 04/02/13

Contact Information
Keith Knudson
10191 Lake Road
Bottineau, North Dakota 58318
Food Safety Officer
Keith Knudson, Farm Manager
In case of a food safety emergency, contact: Keith Knudson @ 701-871-7304
Farm Manager
Keith Knudson
701-263-4685
Cell 701-871-7304 (does not work on the farm)
kknudson@srt.com

FARM INTRODUCTION

Aspen Hills Farm grows approximately 2 acres of vegetables and herbs, in Bottineau County of North Dakota. We have been farming on this land since 1995. There are a total of 170 acres on this farm. Portions are maintained as woodland, grassland, lake, hay land and roads.

The home farm is located on the property at 10191 Lake Road, Bottineau, North Dakota 58318. The legal description of this land is the NE1/4 of Section 4, T162N, R75W, Bottineau County, North Dakota; it is located 48.891805 degrees N latitude, 100.381394 W longitude.

On farm maps and in field records, we refer to this ground as "**Farm 6776**" per the NRCS/FSA maps. Prior to Aspen Hills Farm, the property was owned by an individual living in Alaska who used it as an investment. The land was in the USDA Wetland Reserve Program from 1975 through 1995.

Aspen Hills Farm also rents ground adjacent to this property on the north and west sides. This land is known as the Earl Kamrud farm. On farm maps and in field records, it is identified as **"Farm 7880"** This ground has been under our management since 2005, and in 2007 a portion of the Earl Kamrud Farm was put back in to the Wetland Reserve Program.

Map

Map shows the topography of the farm and locations of fields, high tunnel and compost pile. Each area is designated as follows:

- C = Compost Area
- F = Field
- H = High Tunnel

See Attached Map

STANDARD OPERATION PROCEDURES

Traceability
Aspen Hills Farm utilizes a paper-based traceability system that allows us to trace product back to the field from which it was harvested and the date on which it was harvested. (See attached Harvest/Trace Information Log)

Product shipped has a label with the lot code. The lot codes are also printed on the customer invoice.

Lot Code Description
Harvest information is logged with the date and location from which it was harvested, and harvest tote bins are identified with the lot code, as described below.

When a crop is harvested more than one location or employee on a calendar date, each lot will have a tag with the information listed below. Aspen Hills Farm uses a six digit lot code that looks like this: DDDDLLR. This number uniquely identifies every batch of product.

DDDD = The first 2 digits are the month and the next two are the day of the month.
LL = The general location from which the produce was picked.(high tunnel or Field)
# = Picked by

Example of lot code: **Lot Code 0910H11** harvested September 10th from high tunnel # 1 by Keith.

Worker Health and Hygiene
All employees are required to follow good hygienic practices. Workers will be trained per the guidelines listed in this manual. All training will be recorded on the Employee Training Log

Visitor Health and Hygiene Policy
All visitors will sign in at the farm office and are informed of farm policies regarding health and hygiene. Visitors are defined as anyone arriving at the farm who is not presently employed by Aspen Hills Farm. See Visitor Log (Guest Book)

Training
All employees receive training when they start work on the farm and a refresher course at least once a year. Employees include those that work on the farm that plant, care for, harvest, scout pests, process, and pack fresh produce.

Training includes instruction on all company policies related to worker health and hygiene and where appropriate specialized training related to specific jobs such as anyone who applies pesticide sprays as required by law. All worker training is documented in the worker training log.

Hand Washing
Everyone must wash their hands before beginning work and returning to work after taking breaks, going to the restroom, eating, smoking, or otherwise compromising the sanitary nature of their hands. Signs are posted in bathrooms and eating areas to instruct employees to wash their hands before beginning and returning to work.
Proper hand-washing technique includes the following:

- Wet hands with clean water (warm is preferred if available), apply soap and work up lather.
- Rub hands together for at least 20 seconds.
- Clean under the nails and between the fingers.
- Rub fingertips of each hand in suds on palm of opposite hand.
- Rinse under clean, running water.

- Dry hands with a single-use towel.

It is important to remember to wash hands after touching any potentially unsanitary surface. When possible, turn off the faucet with the single-use towel instead of directly with the hand. **DO NOT** use a paper towel more than once or share towels with others.

Toilet and Hand Washing Facilities
Clean and well-maintained toilet and hand washing facilities are provided for employees and customers. All toilet/restroom facilities are properly supplied with single-use towels, soap, and toilet paper. It is the employee's responsibility to notify the supervisor about any cleaning or restocking issues. Restroom facilities are serviced and cleaned every week. See Restroom Logs.
Employees working more than a ten-minute walk from the restroom will have a farm vehicle to use to return to the restroom facilities at the farm. Upon returning to the field, employees must wash at the portable hand cleaning field station.

Designated Eating Areas
Food may not be consumed while harvesting crops. Drinks may be consumed from lidded containers.

Smoking and Tobacco Use Not Allowed
Aspen Hills Farm endeavors to provide a healthy environment; in addition, tobacco can serve as a disease vector to economically important crops. Therefore, any form of tobacco consumed on the farm, including leased property, vehicles, and equipment, is prohibited. Employees who use tobacco outside of work must wash their hands immediately upon arrival at Aspen Hills Farm.

Sick Employees Don't Work with Food
Sick employees represent a hazard to fellow employees and customers in the potential transmission of food-borne illnesses. Sick employees are not to come to work, and may be asked to leave. Any employee who becomes sick should notify their supervisor immediately and not handle fresh produce. If an employee does not self-report and is found to be sick by the supervisor, the employee will be dismissed from work and not allowed to return until they are symptom free. These symptoms preclude and employee from working and handling fresh produce:

- Diarrhea
- Fever
- Vomiting
- Jaundice
- Sore throat with fever
- Lesions containing pus (including boils or infected wounds, however small) on the hand, wrist, or any exposed body part.

If an employee is recognized as having any of the conditions listed above, these conditions will be recorded on an Illness and Injury Report Log.

If Blood or other Body Fluids
If blood or other bodily fluid should come in contact with the field or the produce, it will be addressed immediately. If a person is not able to immediately deal with the contamination due to injury, that person will mark the area if able and immediately notify his/her supervisor who will take appropriate action. If an employee is injured in the field, their supervisor after assuring their safety, will immediately inspect the area where the injuring occurred to be certain no blood or bodily fluids have contaminated the area. If there is blood or other bodily fluids in the field, all contaminated material will be removed to a plastic bag with a shovel or gloved hands and placed in a trash can. All affected soil will be shoveled up around and under the area and will be

removed. All affected produce will be discarded as well as all packing materials. Food contact surfaces will be cleaned and sanitized before using them again. All actions will be documented by in writing to the Farm Safety Officer.

First Aid
If someone is injured at the farm, there are first aid kits available for use in the employee restroom and field vehicle. The first aid supplies are checked and updated monthly. All workers are instructed during training to attend to injuries immediately. This includes any cuts, abrasions, or other injury incurred while working. Employees should notify their supervisor and fill out an Illness and Injury Report Log. If the injury is critical or life threatening, employees are instructed to call 911 for proper care. If a hand or finger wound requires a bandage, a glove should be worn over the wound.

Pre-Harvest and Post-Harvest Materials Application
Anyone who applies pesticide sprays will be trained as required by law. All worker training is documented in the worker training log. The application of non-regulated materials will be conducted only by workers and contracted personnel who have received appropriate training.

Drinking Water Policy
Potable drinking water is provided and available for employees at the farm. All employees are notified of this policy during training and instructed to notify their supervisors if water is not available. Drinking water will be provided in the form of purchased plastic bottle water.

Clothing, Jewelry, and Cell Phone Policy
Employees will wear clean clothing to work every day. No jewelry is permitted in the field and around machinery. Cell phones are not allowed unless they are required for farm business. All cell phones will be kept in a belt holster or pants pocket when required for farm business.

FARM REVIEW

Water Quality Assessment

Our water comes from the water well on our farm. Our crops are irrigated by drip irrigation and sprinklers.
We test water annually for nitrites, nitrates and total bacteria and these records are kept on file in the farm home office for 3 years. If any water test is outside our normal range, we do an observational review of the water source area to see if there are any obvious problems or situations that can be mitigated. We then take recommended actions to mitigate contamination and retest water as needed. All observational reviews are documented and any mitigation actions are documented in our Water Test Results Log.

Protection of Water Sources
Livestock is kept away from the well head to prevent contamination. The well head is located west of the farm shop, and east of the house.

Animals/Wildlife/Livestock and Manure Lagoons

Two-3 acre pastures with horses near the high tunnel. The fence line is approximately 80 feet away from the high tunnel and water drains away from the high tunnel. Field # 1 has a 6 foot fence around it to prevent large animals from entering the field. Field # 2 is open and a 10 foot strip is cultivated around the perimeter to reduce wildlife. Our dog, Reggie and his friends are not allowed in our growing areas.

Raw Manure and other Composting

We use composted materials on our fields and in the high tunnel. Raw manure and organic waste such as leaves, grass, hay, straw, woodchips and other materials grown on our farm may be part of our composting process. Our composting policy is to breakdown manure and organic matter by properly piling, mixing and moistening to achieve high heat decomposition. Our steps are as follows:

1. Site Selection – The manure is move to an identified location in the farmstead. The area selected will not drain to areas where crops are grown or toward the well head.
2. Windrowed – Manure is piled in rows that is 10 to 14 feet wide and up to 4 to 6 feet tall.
3. Moisture Management – A manual technique is used to determine moisture.
    a. The “wet rag” test is used to determine proper moisture content. The compost is squeezed by hand. If water drips from the compost, then there is too much moisture. If the compost looks like a wet rag and no water drips after squeezing, then the proper amount of moisture is present.
    b. When it is too wet, straw or other dry material will be added. If it is too dry water will be sprinkled over the compost pile.
4. Temperature – Compost pile temperature is monitored with a probe-type thermometer. Temperature should rise above 120 F within 2 or 3 days. Monitor temperature every 4 days and turn when it falls below 110 F. Normally it should be turned about every 2 weeks. If temperature exceeds 150 F, open pile up to lower temperature with the loader.
5. Compost Turning – Turn when Temperature cycle drops to 110 F and turn 5 times during the composting process
6. Compost pile should be moved and stored near the area it will be applied.
7. We keep a Compost Turning and Temp Log to document these procedures.

Soil Assessment

Our crop production land has been mixed grasses and legumes for 16 years. There are no concerns about previous land use related to microbial contamination of crops. There are no feedlots, municipal water treatment facilities, adjacent to our property. Land used for vegetable production is not susceptible to flooding

The history of this farm has been natural forest, wetlands, cropping, and hay land with a majority of it in a wetland reserve program for the past 35 years. Soil and tissue analysis are used as required.

FIELD HARVESTING AND PACKING PROCEDURES

Pre-harvest risk assessment statement

We conduct a pre-harvest risk assessment of our fields. When there are signs of dumping, flooding, garbage, unusual or excessive human or animal presence, corrective measures are taken. Any produce that may be contaminated is left in the field untouched and will be removed after current harvesting activities have ceased. Contaminated produce will be disposed per Farm Manager's instructions.

Field Harvesting and Transportation

All objects that come into contact with produce must be clean and in good working condition. This includes, for example, hands, harvesting equipment (knives, pruners, etc.); harvesting containers, transportation equipment; bulk hauling vehicles; processing equipment and storage equipment. Be sure to wash your hands before handling any produce. When handling produce gloves should be used when practical. Gloves are available in the farm truck and at the portable wash station. If you wear gloves, you must still wash your hands before putting on the gloves.

Water for Cleaning and Sanitizing

Potable water is used during harvesting for hand-washing and cleaning of tools and equipment. The following describes how we clean various items:

1. When washing totes and hand equipment fill right-hand sink (to mark on sink) with hot water. The water should be at least 100 F. Add 1/8 cup of soap. (Cleaning Sink)
2. In the left-hand sink fill to the mark with hot water and add 1/4 cup of chlorine. (Sanitizing Sink)
3. Wash items in the right side and then completely submerse in the left side. Put the cleaned items on the table. Be sure the table has been cleaned and sanitized prior to washing the equipment. If item cannot be fully submersed, hand wipe item with water from the sanitizing side.
4. After cleaning all items drain water in both sides of sink and rinse all items with hot water.
5. Let items air dry. Do not put any items on the floor.
6. Put items back on storage shelves.

The water in the portable wash station should be filled from the hot side. Water needs to be replaced when water is cool to the touch. Hand sanitizer should also be used after cleaning hands. Be sure to thoroughly spread on both hands.

Harvesting Tools

Any tool used for harvesting produce, such as a knife or scissors, will be cleaned daily or when it contacts a contaminated surface. If this is not done, that tool may not be used for harvesting.

Harvesting totes/containers

The harvest containers are kept in good repair and damaged ones are immediately discarded or repaired. Harvesting tote bins will be cleaned and disinfected before each harvest season and whenever needed.

Harvesting machinery
Harvesting machinery and equipment is inspected before harvest and is in good repair. Mechanical harvesting equipment is inspected daily during harvest as well and any foreign objects such as rocks, glass, metal, or other items are removed.

Broken glass policy
If broken glass or plastic is found, during harvest, stop harvesting in that area. That area must be inspected and all shards are picked up, placed in a plastic container. The area is further inspected and the equipment is checked for any other shards and they are disposed of as well.

Gas and Petroleum Spills or Leaks Policy
Petroleum products of any kind are not be stored or used within the perimeter of the farm fields. If gas or oil is spilled in the field, immediate attention will be taken to stop the spill by turning off valves or plugging the source of the leak. If the source is a tank or any other kind of container and it is punctured, the leakage is minimized and the container is removed from the field area. After stopping the spill, the contaminated soil will be removed from the ground and contained in a bucket, pail, or other non-permeable container. All soil that has visible oil stains or petroleum odor will be dug out and contained. The contaminated soil will be moved away from the field and disposed of according to manufacturer's guidelines. If equipment or engines are leaking, immediately contain the spill area and notify the supervisor.

Harvest Container Use Policy
If something other than produce is placed in a harvesting tote, that tote must be cleaned and disinfected. Before moving produce from the field, excessive dirt and mud will be removed from the tote bins as much as possible.
The farm vehicles used to move produce from the field are clean and in good repair. The farm vehicle used for carrying cases of produce out of the field will be cleaned prior to use or as needed.
It is our **policy** that any product that is being moved from the field will be covered.

Storage and Transportation

Storage area housekeeping
Storage areas are kept clean and tidy. Produce will be harvested, cleaned if necessary, and transported as quickly as possible to market. This effort will allow produce to be kept at high quality and prevent the growth of harmful agents.

Storage Coolers
Aspen Hills Farm does not use storage cooling facilities. A policy and procedure will be implemented prior to it use. We harvest our vegetables and deliver it before its quality compromised.

Vehicles for Produce Transportation to Market
All delivery trucks and vehicles used to transport produce to market are inspected for odors and signs of unsanitary conditions before loading. If a vehicle is found to be unsanitary, it will be cleaned before produce is loaded.

Equipment used to carry animal products or other potentially hazardous items including carcasses, manure, or pesticides will not be used. Any contracted truck operators must state the last load that was hauled in the vehicle and provide a cleaning schedule and temperature log for the vehicle before loading, as well as a log of previous loads.

Invoices and shipment manifests are kept on file for the period of one year. Produce will be loaded carefully so that risk of damage will be minimized. Only employees who are trained in loading produce will be allowed to do so.

Allergens
Aspen Hills Farm recognizes the following products as potential allergen of our customer: peanuts, soy, wheat, tree nuts, milk, fish, crustacean shellfish and eggs.
Fish are the only item listed that is produced on our farm. We take every precaution to not intermingle fish products with our vegetable produce. Fish are processed in a location separate from other products.

Evaluation of Dry Bean Germplasm for Tolerance to Waterlogging

Final Report

Partner Organization: North Dakota State University

Project Summary

Because of the severe susceptibility of common bean (*Phaseolus vulgaris* L.) to flooding, the Dry Bean Genetics and Breeding program at North Dakota State University assessed the genetic architecture and physiological responses of this crop to the stress. The ~700 genotypes of the Middle-American and Andean diversity panels of common bean that includes several market classes was evaluated under both flooding and non-flooding conditions at two early developmental stages (germination and seedling) in the greenhouse. A genome-wide association study (GWAS), using ~150K SNPs, was performed to discover genomic regions and candidate genes associated with these multiple physiological responses. Black and small red beans were the least affected to excess water at germination stage. At the seedling stage, pinto and great northern genotypes were the most tolerant. These genotypes will be used in breeding programs for the improvement of waterlogging tolerance. A subset of contrasting genotypes was further evaluated in the field to assess the relationship between greenhouse and field data under flooding condition. A good correlation was found between greenhouse and field results. The GWAS analysis revealed Pv08/1.6 Mb and Pv02/41 Mb as two regions associated with root weight and germination rate, respectively. These regions are syntenic with two QTL reported in soybean (*Glycine max* L.) that contribute to flooding tolerance, suggesting a conserved evolutionary pathway involved in flooding tolerance for these related legumes.

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

Project Approach

The objectives of the project were:

1. To identify dry bean genotypes with tolerance to waterlogging conditions during early growth stages (germination, emergence, and vegetative growth), by using a diverse panel of 700 germplasm accessions (varieties, breeding lines, and landraces) in the greenhouse.

2. To evaluate a selected group of genotypes for waterlogging tolerance, especially during crop emergence and establishment in the field.

3. To identify genomic regions associated with waterlogging tolerance using a Genome- Wide Association Mapping (GWAS) approach.

North Dakota ranks first for dry bean production in the U.S. in terms of area planted, area harvested, production, and total value (~$250 million in 2013). Pinto, navy, and black are the main market classes produced in the state. In combination with Minnesota (ranked 3rd in total production and first for kidney bean), the ND/MN region accounts for nearly 50% of all dry edible beans produced in the U.S. Estimated yield losses due to waterlogging and flooding are

between 25-30%. In addition, 4 million acres were affected by prevented planting due to flooding/waterlogging just in 2013 alone. Among the economically important crops grown in North Dakota, dry bean is the most sensitive to waterlogging and flooding. Contrastingly, breeding efforts towards the genetic improvement for waterlogging tolerance is almost non-existent worldwide. Genetic improvement has the potential to produce resilient varieties that tolerate these harsh conditions thus minimizing the economic impact of yield losses. Plant breeding is a long term process that requires between 7-10 years from crossing to release, so varieties will not be the direct outcome of this project. However, the evaluation of this large set of germplasm will be a first step to identify genotypes that can withstand waterlogging. These genotypes will be intensively used in the breeding pipeline in order to gain tolerance to this abiotic stress. In addition, the genomic mapping efforts will allow having a better understanding of the genetic control of waterlogging tolerance. The genomic tools developed by this project will accelerate the breeding process for waterlogging tolerance in dry beans. In the near future, dry bean growers in North Dakota will have access to this technology which will be in a form a new improved variety. The Northarvest Bean Growers Association estimates there are approximately 2,000 dry bean growers in the region, so this would have the potential to impact 2,000 farms.

Greenhouse screening of the Mesoamerican Diversity Panel (MDP) was completed and at least 15 tolerant genotypes were identified, mostly belonging to pinto, small red/pink, and black market classes. Greenhouse screening of the Andean Diversity Panel (ADP) was completed and at least 10 tolerant genotypes were identified. The scientific manuscript is currently under review. Field trials with selected genotypes were completed at one location due to low number of seeds available and lack of adequate irrigation facilities at the second location. Statistical analyses of greenhouse experiments and field trials were reported at meetings and presentations during this time. Genome-Wide Association Studies have been completed and reported for MDP. ADP is currently under review for publishing. The first round of crosses with elite and commercial genotypes have also been completed. All results were presented to bean growers at Bean Day 2016. Please see the following section for results.

Goals and Outcomes Achieved

To evaluate the effect of waterlogging on germination rate, we employed the same experimental design as explained in previous reports. Briefly, a Randomized Complete Block Design with split plot arrangement was selected for this experiment. We also considered three replications. Ten seeds from each genotype were planted in the loamy sand soil. To induce the flooding, sown seeds in the soil were submerged in the water for one day. After submergence, water was drained. The same set of seeds were planted and germinated at well-drained condition. After 15 days, the germination rate was evaluated based on the number of germinated seeds from initial ten sown seeds.

The results indicate that, in general, excess water in germination level can reduce the germination rate of bean varieties by almost 60 %. However, the existence of a significant treatment x genotype interaction indicate that the response of all varieties is not the same and there is a genetic diversity in the Middle-American panel for the tolerance to excess water in germination stage. Mean separation revealed that flooding effect on germination rate is

significantly different between race Durango and Mesoamerica (Figure 1a). Among market classes, germination rate of black market class was less affected by the excess water, followed by small red. On the other hand, navy were the most susceptible market class to excess water at this stage (Figure 1b). Interestingly we could also detect significantly different germination rates among market classes in control condition. In control condition, Pintos, Blacks, Pinks and Small reds had the highest germination rate (73-76%), however Great Northern and Navys with 69.7 % and 60 % germination rates had the lowest rate.

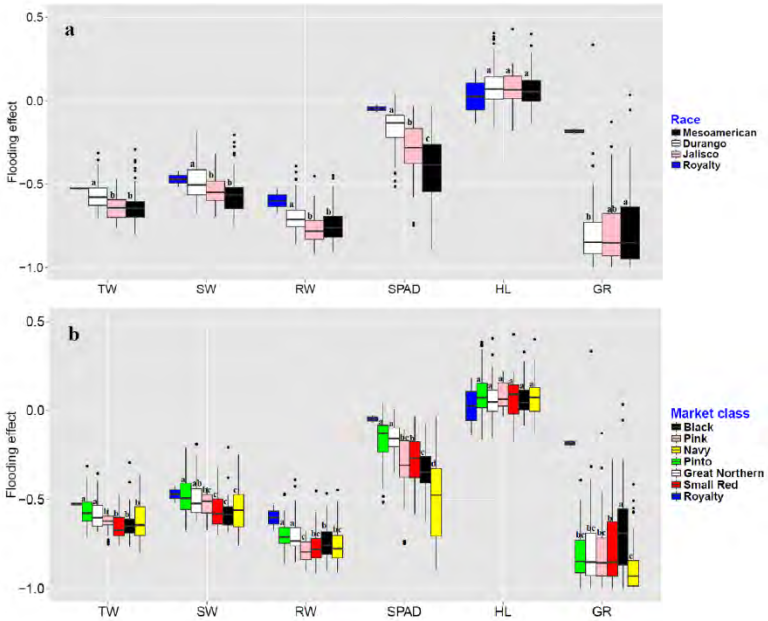
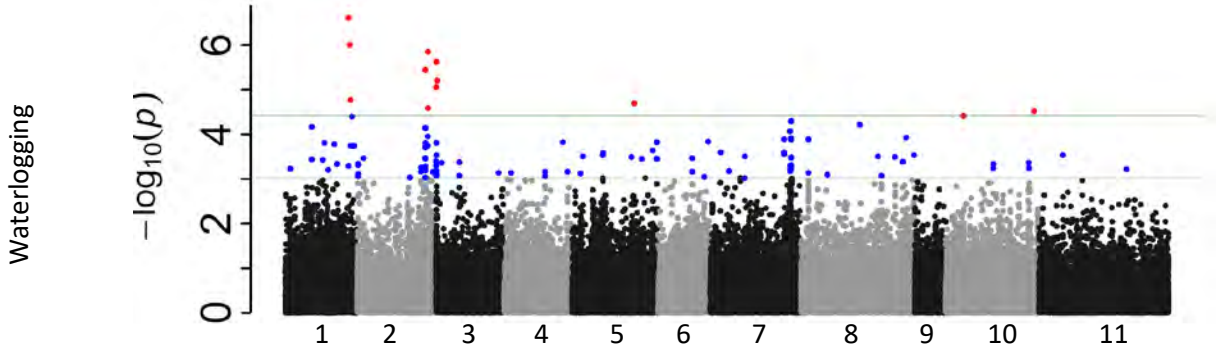


Figure 1. Flooding effect on different races (Figure 1a) and market classes (Figure 1b) for different traits. TW: Total weight, SW: Shoot weight, RW: root weight, HL: hypocotyl length, GR: germination rate.

To identify the genomic loci, associated with the germination rate in each condition, we used a Genome-wide Association Study (GWAS). In total, four genomic loci were identified that control germination rate in waterlogging condition (Figure 2). These include Pv01/42.4, Pv02/36.9, Pv02/41.1 and Pv03/0.1. On the other hand, five genomic loci were identified that control germination rate in control condition; Pv01/32.6, Pv05/23.9, Pv08/28.9, Pv11/24.4 and Pv11/35.6. We couldn't detect any major locus that control germination rate in both waterlogging and germination rate which indicates the genetic architecture of this trait is different in these conditions.



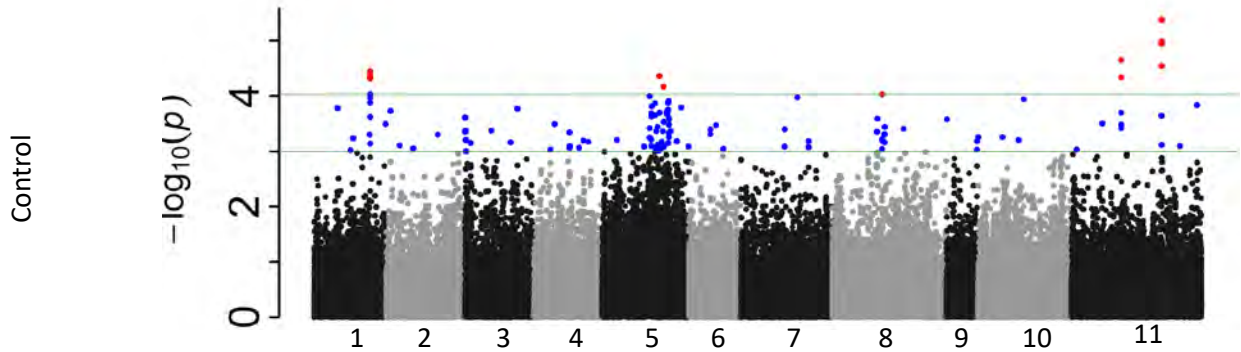


Figure 2. Manhattan plots showing the genomic loci associated with germination rate in control and waterlogged conditions.

Through this experiment we were able to identify the best lines with the highest germination rate in each market class (Table 1). Our results indicated that our check (Royalty) with the 70%, possess the highest germination rate in waterlogging condition. The best lines will be used in the breeding pipeline to improve the tolerant level of the germplasm.

Table 1. List of the best lines in terms of germination rate in waterlogged condition.

Pinto		Great northern		Pink		Small red		Black		Navy	
ID	GR[a]	ID	GR	ID	GR	ID	GR	ID	GR	ID	GR
Sonora	53	Ui_123	40	i9365_25	58	Dehoro	60	Blackmagic	63	Avalanche	35
92us_1006	37	Ne1_09_20	38	i9365_5	53	Inta_precoz	60	Cdc_jet	60	Morales	25
CDC_Camino	36	Ui_59	37	Pr_0401_259	40	Rojo_chiquito	58	Black_hawk	58	Navigator	25
Fargo	30	Ne1_09_19	28	Rog_312	28	Jamaican_Red	57	Midnight	50	Verano	21

a. indicates the germination rate in percentage

In addition during the summer, a subset of genotypes that were previously evaluated under greenhouse conditions were tested in the field. The main goal of this step was to assess if the greenhouse results were a reliable reflection of the field condition. For this step, 40 genotypes were evaluated in the field using a RCBD (randomized complete block design) with split plot arrangement. In this design waterlogged and non-waterlogged treatments were two levels of main plot and genotypes were considered as sub-plots. Out of these 40 genotypes, 20 belong to Middle-American gene pool (MDP) and another half belonged to Andean gene pool (ADP). Furthermore, from each gene pool, ten best and ten worst lines (based on chlorophyll content under waterlogging condition in greenhouse) were chosen to be evaluated in the field.

Plant materials were sown using four row planter in a drained condition in both main plots. After plants reached to V2 developmental stage, waterlogged designated plots were flooded. To induce waterlogging condition in the designated field plot, water was delivered from a close by fire hydrant and sprinkled on the field (Figure 3).

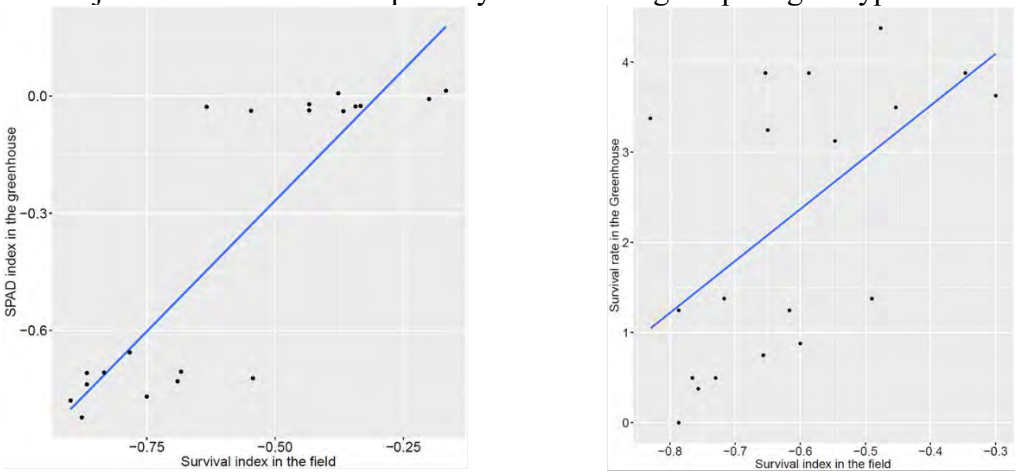
Waterlogged Control

Figure 3. Performing waterlogging experiment in the field. Water was delivered from a fire hydrant and sprinkled on the designated plot (left). Plants in control plots were rainfed (right).

Waterlogging duration was continued for ten days. Meanwhile, non-flooded plot were rainfed. After that, parameters such as SPAD index and survival rate were evaluated from both waterlogged and non-waterlogged plots. Flooding indices for each genotype and trait were further estimated by following equation:

$$Flooding\ index = \frac{(Value\ of\ trait\ in\ flooding\ condition\ -\ value\ of\ trait\ in\ control\ condition)}{value\ of\ trait\ in\ control\ condition}$$

Correlation and regression values between greenhouse and field data were subsequently calculated (Figure 2). Based on this analysis we could detected a high regression (R^2= 0.74) in MDP. A significant but weak correlation was also detected in ADP (R^2= 0.27). These results indicate that although for both panels greenhouse results are significantly correlated with the field results, this correlation is much stronger for the Middle-American gene pool. Several factors can be involved in the observed difference between MDP and ADP in terms of regression between greenhouse and field results. One possibility can be the higher susceptibility of ADP to root rot which can be present in the waterlogged field condition. Moreover, lower genetic diversity in ADP might result in marginal differences between susceptible and tolerant group. Nevertheless, the interaction between soil-borne pathogens, waterlogged soil and plants could be the subject of future studies especially for Andean gene pool genotypes.



MDP ADP

Figure 2 Correlation analysis between greenhouse and field results for Middle-American Diversity Panel (MDP) and Andean Diversity Panel (ADP).

There was no previous information about the tolerance level of dry bean genotypes to waterlogging conditions. However, current experiment reveals the most tolerant and susceptible lines to this stress. These lines will be used extensively in the breeding program to improve the tolerance level of commercial lines, grown in the North Dakota. Furthermore the GWAS results reveals several genomic regions that are associated with stress tolerance. These information will be used to develop markers that will subsequently use in marker-assisted selection.

- A new greenhouse screening protocol for waterlogging tolerance at early stages: The new protocol will facilitate extensive screening of new germplasm.
- Identification of genotypes with tolerance to waterlogging: Approximately 30 genotypes with tolerance have been identified which will be used in future crosses by breeding programs. This exceeds our goal of 10.
- Identification of genomic regions controlling waterlogging tolerance: New molecular markers can be designed that will aid in the improvement of this trait. Also, we gained a better understanding of the physiological aspects of waterlogging tolerance.

All the goals initially established for this project have been accomplished, except the publication of final results for the evaluation of the Andean dry bean genotypes, which is currently underway (draft manuscript under review).

Results of this project were presented to bean growers during the 2016 annual Bean Day in Fargo, ND. Results were not presented back in 2015 because the project leader was asked to present on other topics of major importance during that year and lack of time. In addition, there were only preliminary data at the beginning of 2015.

Beneficiaries

Approximately 2000 dry bean growers and 100 bean scientists have benefited from this new knowledge as well as the new germplasm identified with tolerance to waterlogging. New commercial varieties will be released in the near future with improved tolerance to waterlogging.

Lessons Learned

The development of a reliable greenhouse protocol took longer than expected. However, multiple experiments were run together in order to save time.

Field experiment was difficult to set up even for a small number of genotypes, this is the reason why genotypes need to be evaluated in the greenhouse first and only promising material should be evaluated in the field. Field experiments should include no more than 30 genotypes.

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Additional Information

Please contact for other images, figures, and tables. In the same way, all posters and PowerPoint presentations are available under request.

Fort Yates Helping Hands Community Garden and Cannon Ball Garden to Table Greenhouses

Final Report

Partner Organization: Dakota Prairies Resource Conservation & Development Council

Project Summary

This project benefited more than 1,000 socially-disadvantaged tribal members of the Standing Rock Sioux Reservation. It accomplished this by growing and strengthening the previously created Helping Hands Community Gardens and farmers market in Fort Yates and establishing two new gardens and a farmers market in Cannon Ball. One of those gardens in Cannon Ball is the first school garden on the Standing Rock Reservation. There, every one of the about 120 students in grades kindergarten through 6 received hands-on learning in planting, growing, and harvesting fresh and healthy specialty crops. These same students, along with more than 900 adults, increased their gardening and nutritional knowledge and consumed more locally-grown specialty crops through this project.

As a result of this project, more than 1,000 tribal members and tribal youth are learning valuable and lasting lessons of how to develop and implement locally-gown sustainable food production systems. This will not only help make them more self-sufficient, but will eventually improve their health. Standing Rock Sioux Tribe suffers from one of the highest rates of obesity in the country. In Sioux County, where the project is located, the obesity rate is 43 percent, much higher than North Dakota average of 29.7 percent. With the rapid rise of obesity, comes obesity-related health issues such as diabetes, hypertension, heart disease and obesity-related cancers.

The two main objectives of this project were to: 1) Provide training and education to students and others on the Standing Rock Reservation to increase nutritional and gardening knowledge; and 2) provide more access to fresh, nutritious fruits and vegetables on the Standing Rock Reservation.

Located in a food dessert, the lack of fresh fruits and vegetables on the Standing Rock Sioux Reservation has led to high rates of obesity, diabetes, heart disease and other health issues. This project successfully provided fresh specialty crops to tribal residents not only in Fort Yates, but in the Cannon Ball area, where there is no grocery store and the median household income is about $18,500. The Cannon Ball area, with its population of about 500, has an unemployment rate of 31.4 percent and many residents rely on government subsidies and processed foods, which adds greatly to the obesity and diabetes problems.

The previous project work in Fort Yates, where the tribal offices are located, created interest and demand for local fresh specialty crops throughout the reservation. Because many of the low-income residents of Cannon Ball do not have the means to travel to Fort Yates, the school and community gardens and the resulting farmers market created fresh food opportunity for them and their children and grandchildren. The Cannon Ball school students learned firsthand how to plant, grow and harvest fresh specialty products. This learning will help put into place sustainable local food growing practices that could last for a lifetime for these 120 students and their families.

As a result of this project, there is a much more enthusiastic awareness of local and high-quality food now, especially with the prevalence of obesity and diabetes on the reservation. People want to know where their food comes from and want to play a role in its production. Local food also creates good local economics. It offsets other economic costs and keeps dollars local.

Previous completed work established community specialty crop gardens, a weekly summer/fall farmers market and nutritional education in Fort Yates. This project complemented and enhanced this solid groundwork by expanding into the impoverished tribal community of Cannon Ball. A school garden and a large community garden east of the Cannon Ball elementary school were established under this project. In addition, a weekly farmers market was established in Cannon Ball. Because the majority of the Cannon Ball residents do not have money to pay for the fresh fruits and vegetables, a bartering system was used at the farmers market. Instead of using cash to gain fresh produce, some volunteered to help weed and water the new gardens. Or, if their health did not allow for this, they were given the produce totally free. This allowed those most in need of it, to secure fresh produce to nourish themselves and their families.

This project, like the one before it, helped instill a sense of pride and dignity and raised awareness of local food. It continued the previously completed work of involving the youth and expanded their food choices and educated them on how to plant and start their own food for self-sufficiency and better nutrition.

Project Approach

The new school and community garden sites at Cannon Ball were seen as a huge success, with hundreds of children and adults gaining access to fresh specialty products for their diets. In addition, all 120 students at the Cannon Ball elementary school received both in-classroom plant and gardening education and hands-on learning that include planting and tending their own specialty crops. Through this personalized education, these 120 students were more apt to taste all the different specialty crops. Many students had not had an opportunity to taste many of the specialty crops they helped to grow. The majority of them discovered they liked the taste of produce such as kale, peppers, cucumbers and other produce they got to sample and take home to their families. This project has resulted in thousands of meals prepared with fresh locally-grown specialty crops, which has resulted in a rise in the use of specialty crops and a rise in health benefits. This project also has the unseen psychological benefits of open minds and the sense of pride and self-sufficiency.

We recommend this school garden model that was established at the Cannon Ball School be eventually used throughout the Standing Rock Reservation and reservations throughout the country. This will ensure every reservation child at every reservation community school has the same opportunity to grow, taste and eat their own fresh and nutritional specialty crops. These students, in turn, can help teach their family members of the many benefits of growing and eating fresh produce.

This project was unique in a highly visible way. It resulted in the renovation and use of an historic root cellar that has a long and favorable history to the tribal members. It was built in the 1930 and the elders of the community remember using it when they were younger. It has become

a destination place in Cannon Ball and has instilled a sense of pride and community and helped raise awareness of the importance of fresh local food and how to use and store it.

This project also introduced culturally significant food production like the planting of the Three Sisters (corn, beans and squash) and an in-ground high tunnel greenhouse that is fashioned after a walipini, or "place of warmth."

According to Indian legend, corn, beans and squash are three inseparable sisters who only grow and thrive together. This tradition of interplanting corn, beans and squash in the same mounds, widespread among Native American farming societies, is a sophisticated, sustainable system that provided long-term soil fertility and a healthy diet to generations. Growing a Three Sisters garden also is a way to feel more connected to the history of the land.

This project has integrated modern, intensified food production such as hoop houses, raised beds, mulching techniques and micro irrigation with traditional ways, making it meaningful for both youth and elders.

Activity 1: Present gardening/nutrition classes to students and residents at Cannon Ball, Fort Yates and Solen: All of the 120 students in grades kindergarten through 6 received gardening and nutrition classes during 2015 and 2016 under this project. During the first year of this project, a gardening-related school enrichment program was established at the Cannon Ball Elementary School. Thirty- to 40 minute classes were held as part of the school curriculum on Mondays, Tuesdays and Wednesdays, with a food composting and recycling initiative on Thursdays. Some of the classes covered: 1) Plant growth and development; 2) plant classification; 3) plant parts; and 4) plant needs. Students also learned about the types of soil and soil health and learned about plant root structure. A successful component of the educational classes was having each student develop a journal to document the results of their growing activities.

As part of the gardening classes, students started specialty crops in the classroom and then transplanted them to the garden that was established just behind the school. The learning continued into the summer months as many of the students gathered at the school every work day to assist with the gardening activities such as weeding and watering. They not only learned hands-on gardening skills, but learned about the nutritional value of what they helped to grow. In addition to the students, we estimate about 50 adults also participated in helping with either the school garden or community garden in Cannon Ball.

To help reach the adult population, nutritional information and cooking ideas were provided at the weekly farmers markets held in Fort Yates, Cannon Ball and McLaughlin. Taste-testing was also used at the farmers markets, which increased the usage of specialty crops such as Swiss chard and beet greens.

On Oct. 24, 2015, a "Traditional Harvest Festival" held in Fort Yates drew about 50 reservation residents. While there, they learned more about the specialty crops that are being grown under this project.

Sue Isbell, the project director, and other staff also shared information on seeds, planting and food preparation to individuals and small groups where they were gathered. This informal way of teaching proved to be the most successful way of reaching the tribal residents. By finding and talking to small groups already gathered for other events, information was able to be shared quickly and inexpensively.

Isbell also led several presentations to students and community members at events held at Sitting Bull College in Fort Yates and other locations on the reservation.

Activity 2: ***Develop and distribute project promotional materials:*** Cindy Dunn, clerical technician, created and distributed promotional materials for all the farmers markets and special events that were held during the 2015 and 2016 growing seasons. Flyers listed what fresh specialty crops would be available were posted throughout Fort Yates, Solen and Cannon Ball. In addition, announcements were given on the tribal radio station and information was shared in person with individuals and groups.

Isbell and Dunn also posted flyers, pictures and community garden information on the Sioux County Extension Service Facebook page at www.facebook.com/SiouxCountyExtension/

Activity 3: ***Research and order commercial food dehydrator and portable walk-in cooler:*** As the result of additional research, it was determined a food freeze dryer would be better than a food dehydrator to preserve specialty crops for the winter months. A commercial-grade food dehydrator would have required 220 wiring, which is not readily available, and would have cost up to $16,000. In addition, sources show that freeze-dried food lasts up to eight times longer than canned or dehydrated food and retains 60 percent of the nutritional value.

Therefore, a Harvest Right food freeze dryer was purchased for $4,000 by the Sioux County Commission and leased to this project in February 2016. It uses regular 110 wiring, so it can be used at any location. It is 30 inches tall, 20 inches wide and 25 inches deep and weighs just a little more than 100 pounds, making is easy to use on a countertop, cart or table.

Project staff experimented on freeze drying specialty crops, especially those that will be used for a prototype dried soup mix for those in need of nutritional food during the winter months. While a final soup prototype has not yet been completed, we found that all the specialty crops tested to date can be successfully freeze dried and should work well in a soup mix or other uses.

Rather than purchasing a portable walk-in cooler, during 2015 this project secured and restored a historic root cellar at the community garden site just east of the Cannon Ball school. The root cellar was built in the early 1930s by the Civilian Conservation Corp (CCC) Indian Division Program. Perched a bluff overlooking the Missouri River, the root cellar is about 22 feet wide and 60 feet long. The root cellar is made of cast-in-place concrete and the walls are 15 inches thick and 8 feet tall. There are tapered columns along the long walls spaced at 20 feet on center. The roof is about 12 inches thick and is gabled and sloped to the exterior walls. The bottom of the peak is about 9 feet tall. The floor is not finished and consists of native soil. The root cellar's exterior walls are encased in about 6 feet of soil supported by a rock wall. There is about 32

inches of soil on top of the roof structure. The front of the cellar faces east and overlooks the Missouri River.

Project staff worked closely with Dr. Kelly Morgan, interim tribal archaeologist for the Standing Rock Sioux Tribe's Historic Preservation Office, on restoring the root cellar and fencing and using the surrounding area for the new Cannon Ball community garden. The Bismarck engineering firm of KLJ donated staff time to conduct a structural assessment of the root cellar in June 2015. The foot cellar was found to be structurally sound. The root cellar only required minor renovations, which included cleaning out debris, painting the inside walls white, sweeping the packed earth floor and adding a door. Garden Director George "Sunny" Keller built and installed a wood door on the only opening on the east side. He replicated the door as close as possible to the original one by looking at old photographs.

Grant funds were not used for the restoration of the historic root cellar. However, we wanted to include this work as it greatly complements the entire project and has created a sense of community garden project ownership in the Cannon Ball community.

Activity 4: ***Research and order laptop/tablets, printer and digital camera:*** Two laptops and one tablet were purchased during the first year of this project. The printer and digital camera were not purchased as costs were higher under personnel than planned and photos could be taken with the tablet.

The two Dell Inspiron 11.6-inch HD touch Screen Notebooks and tablet offered portability as they were used for activities such as communication, garden planning, scheduling and tracking garden work tasks and taking photographs of the project.

Activity 5: ***Research and order two high tunnel greenhouses and install at Cannon Ball:*** During this project, it was determined that one high tunnel greenhouse would be sufficient to begin with. It was ordered from Growers Solution during the early part of 2015. Work was conducted to erect this 20-by-20-foot high tunnel greenhouse just south of the historic foot cellar in Cannon Ball. The greenhouse frame was erected in July 2015 after the soil under it was excavated to allow it to be used almost year round. By combining the extra heat from the high tunnel greenhouse and the earth, the growing season is expected to be greatly extended in this walipini-like creation. The greenhouse will also capture extra heat from the sun, as its two ends face north and south

In 2015 sand bags were used to line the sides and terraces of the excavated hole under the greenhouse. However, the sand bags deteriorated and needed to be removed. Therefore, during the summer of 2016, the sandbags were dug out and project staff used packed tires to line and stabilize the walls of the in-ground part of the greenhouse. During the early part of spring 2017, the plastic will be put on the high tunnel and it will be used for the first time for growing specialty crops. Besides increasing the production of specialty crops, this structure is expected to be used as a year-round living science center for the Cannon Ball school students and community members.

Activity 6: ***Research and order 8-foot chain link fencing for around Cannon Ball garden site and install:*** A commercial-grade chain link fence for the east Cannon Ball garden site was

installed during July 2015. The purpose of the fence is to keep animals and vandals out of the site. The 580 linear feet of chain link fence surrounds the root cellar and high tunnel area. It also leaves ample room on the sides for fruit trees and additional outdoor gardens. The fence is 6 inches high with three strands of barb wire across the top. The fence includes one 10-foot wide double gate and two 4-foot wide walk-through gates.

Dr. Kelly Morgan helped determine the location of the fence. This property, owned by the Standing Rock Sioux Tribe, was surveyed by Dr. Morgan in early 2015 and the project was allowed to continue.
Despite the new fence surrounding the site, the restored and locked root cellar was broken into during March 2016 and many project-related items stolen and the newly rebuilt wood door to the root cellar was destroyed. Among the items stolen was a new generator and a tiller. Tools missing include hoses, rakes, hoes, weeding tools, pruners, spades, shovels, a post hole digger, a wheelbarrow, a pull dump cart, a utility cart and water timers. The value of the loss is estimated at almost $1,800, which does not include the cost to rebuild the door for the root cellar. The cost to repair the fence was almost $150, plus labor.

Activity 7: ***Order wood and construct raised beds for Cannon Ball gardens:*** During March 2015, lumber was secured and wood growing beds for the Cannon Ball school garden were constructed on site. In addition, galvanized metal growing beds were also ordered and constructed for the garden directly behind the Cannon Ball school. The rest of the planting was done in straw and other mulch.

Lumber for the east Cannon Ball community garden site was purchased in July 2016 and garden beds built and placed to the east of the historic root cellar.

On Oct. 11, 2015, a prairie fire swept through the Cannon Ball area, destroying every wood growing box at the school garden site and damaging one wood box at the Cannon ball community garden site. Replacement lumber was purchased during the spring of 2016 and all the wood planter boxes were replaced at the Cannon Ball school garden and the one damaged one at the Cannon Ball community garden site was repaired. Although they received some fire damage, the galvanized steel planter boxes were able to be salvaged and reused. They have some blackened areas from the smoke and fire, they are still in good structural condition.

Activity 8: ***Hire and train new Cannon Ball garden director:*** George "Sunny" Keller was hired in June 2015 as the new garden director of the Cannon Ball school and community gardens. Little training was needed as Keller already has a wealth of knowledge and experience with conventional horticulture. Keller grew up on an organic orchard and has experience with urban gardens and landscape architecture. He previously grew gardens in underserved communities, including Chicago.

When Sam Rough Surface, the Fort Yates, garden director left this project during late summer of 2015, Keller became the project's overall garden director and assumed the oversight of the Fort Yates gardens as well. He worked closely with Sue Isbell throughout this project.

When hired for this project in 2015, Keller lived on the South Dakota side of the Standing Rock Sioux Reservation, in McLaughlin. During the second year of this project (in 2016) Keller and his family moved to Solen. This made it easier for him to travel to the garden and farmers market sites in both Fort Yates and Cannon Ball.

As part of his duties, Keller worked with Sue Isbell, this project's director, on the gardening planning work and scheduled and supervised all the part-time gardening help. He also supervised all the farmers market work.

Activity 9: ***Order soil and have delivered to Cannon Ball site:*** Although straw and other mulch were used at the Cannon Ball sites, some composted soil was necessary as the existing soil contains a great deal of clay and some sand. A 2-ton truckload of composted soil was delivered to the east Cannon Ball garden site during the spring of 2015. Some of it was transferred to the Cannon Ball school garden. Then, three more 2-ton truckloads of soil was delivered to the Cannon Ball community garden site in September 2015. Project staff manually filled the wood planter boxes with the rich composted soil. Some soil was saved to fill the planter boxes in in-ground high tunnel greenhouse at this site. Straw was also used to preserve nutrition and build soil health.

It is anticipated that enough compost soil has been secured and no additional soil should be needed. Keller spent some time this quarter adding amendments to the soil.

Activity 10: ***Research and order rain gutters and rain barrels and install on Cannon Ball greenhouses for rainwater collection:*** When developed in 2015, this site had no well or piped water. Therefore, we initially planned to use rain gutters and rain barrels on the high tunnel greenhouse to collect rainwater for the Cannon Ball community garden. However, when all the planted fruit trees withered and died from lack of water, we found and it was imperative to have a reliable water source.

After securing approval, we were able to extend a water line to the site for less than $5,000. After visiting with tribal leaders, including tribal Chairman Dave Archambault II, the project was granted access to a nearby Standing Rock Municipal, Rural and Industrial Water System main water line in Cannon Ball. The Standing Rock Tribe offered to provide the water used free of charge if the project just needed to pay for the excavation and water pipeline costs.

Activity 11: ***Develop plan for gardens at Fort Yates and Cannon Ball:*** Numerous planning meetings were held throughout the project on what specialty crops to plant during 2015 and 2016 and where to plant them. Intentional planting helped ensure the right specialty crops were being planted and grown in the best location.

It was determined that the large outdoor garden spot in Fort Yates near the Church of Jesus Christ of Latter-day Saints is best suited for vining crops to cut down on the weeding time. During the spring of 2016, fabric was laid down on this entire large site and timers were used for watering. The crops at this site consisted mostly of melons and squash.

The multiple high tunnel greenhouses at Fort Yates at the site of the offices of the Sioux County Extension Center at Fort Yates, housed a variety of crops, but included most of the tomatoes, cucumbers and peppers. Vertical growing was used in these high tunnels in 2016. Posts were erected and cables we used for support for the tomatoes and cucumbers. These greenhouses were also used for early cool-weather specialty crops such as radishes, lettuce and kale.

A wide array of produce was planted and grown at the two Cannon Ball garden sites. Plans carried out also included erecting a small 10-by-12 foot greenhouse at the school garden to germinate seeds in.

During this project, planning and work was conducted to number all the planter boxes and create a digital grid to help schedule maintenance. This way, students could be assigned certain planter boxes to weed and water. When they completed a job they could sign off on it.

Activity 12: ***Order gardening and farmers market supplies for Cannon Ball:*** The majority of the gardening supplies were secured during 2015 for the first year of the two new gardens in Cannon Ball. Most of the supplies were garden essentials such as hoses, sprinklers and garden tools. In addition, some learning aids were ordered for the educational work at the Cannon Ball School.

During the spring of 2016, almost all the garden supplies needed to be replaced, which added to the cost of the Cannon Ball gardens. Supplies at the Cannon Ball School were destroyed during the October 2015 prairie fire and those at the Cannon Ball community garden were stolen during the March 2016 vandalism and thefts.

Activity 13: ***Order and plant bedding plants at Fort Yates and Cannon Ball:*** Some specialty crop seeds were ordered during this project as not as many seeds were able to be saved following past harvests as anticipated. When possible, heirloom seeds were used.

Some starter plants were seeded at the Sioux County Extension Center office in Fort Yates and some were purchased. About 150 fruit trees and bushes were purchased in 2015 at a reduced cost from the Cedar Soil Conservation District at Selfridge. Unfortunately, most of these needed to be replaced in 2016 due to the prairie fire at the Cannon Ball School and lack of adequate rainfall and no piped water source at the Cannon Ball community garden site.

Activity 14: ***Active gardening activities at both sites (weeding, watering, fertilizing, etc.):*** Much of the personnel costs involved with this project came during the active gardening seasons during 2015 and 2016. Time was spent on general cleanup and preparation for the growing seasons. Then, staff worked to plant, weed, water and fertilize the specialty crops. Garden Director Keller led the work with help from many different garden assistants. When possible, Native American students performed some of this work and received either an hourly rate or a small stipend for their help.

Petra Reyna One Hawk, a graduate student in public health at North Dakota State University, contributed to this project both years by serving as the youth and community mentor and focusing on the cultural and historical aspects of gardening on the Standing Rock Reservation.

This project has also had a great deal of "buy-in" from local communities, with donated time, supplies and equipment coming from many individuals and businesses.

Activity 15: ***Harvest fruits and vegetables and sell at weekly farmers markets at both sites:*** Harvesting and selling/distributing specialty crops occurred from early June to mid-October of both the 2015 and 2016 growing seasons. The weekly schedule for farmers markets was Wednesdays in Fort Yates; Thursdays in Cannon Ball; and Fridays in McLaughlin. The new Cannon Ball farmers market featured a barter system for those who could not afford to pay for the specialty crop produce. No one at any of the farmers market was denied produce if they had no way to pay for it.

Activity 16: ***Dehydrate extra produce and vacuum seal for preservation and distribution:*** During the harvest of 2015, some small-scale dehydration was conducted using home-type dehydrators. Produce used included tomatoes, kale, onions, green peppers and hot peppers. Dehydrated sunchoke chips were given away for tasting to show they are a good, nutritional snack and can also be used in a variety of ways, including as a substitute for potatoes in soups and side dishes. Kale chips also proved to be popular.

As the 2016 specialty crops were harvested, some were freeze dried in the new HarvestRight food freeze dryer. The freeze dried produce was used for taste testing to help plan and prepare for a freeze-dried soup mix that would eventually provide a nutritional meal for the winter months.

Activity 17: ***Provide information at the farmers markets on how to cook the fresh produce:*** This project featured cooking and nutritional information sheets at the farmers markets in Fort Yates, Cannon Ball and McLaughlin. In addition, taste testing was also conducted at some of the weekly farmers markets. This introduced reservation residents to fresh specialty crops they have not tasted before, and ways they could cook and incorporate them into their meals.

This project and its funds were used to solely enhance the competitiveness of specialty crops.

Goals and Outcomes Achieved

Goal 1: Provide training and education to K-12 students and others on the Standing Rock Reservation to increase nutritional and gardening knowledge: This goal was successfully achieved by providing hands-on and classroom training and education to more than 1,000 reservation residents. This included all 120 students at the Cannon Ball Elementary School. Besides receiving formal school classes both in 2015 and 2016, the Cannon Ball students all were provided hands-on gardening lessons with the newly created school garden. As part of the education, Cannon Ball students learned to plant, tend and harvest their own specialty crops, which they took home to help feed their own families. Hands-on training and education was also provided to about 30 adults in Cannon Ball by helping with the school and community gardens, either as paid part-time project staff or parents or other family members who volunteered to help. In addition, about 900 Standing Rock tribal members received nutritional and gardening information during the weekly farmers markets at Fort Yates and Cannon Ball. Although no formal evaluations were used, we estimate there was at least a 50 percent increase in knowledge

and more than 70 percent who indicated they changed their eating behaviors to incorporate fresh, nutritional food. While we were unable to implement the "interactive quiz" monitoring indicator, we estimate we distributed 2,500 or more pieces of information containing nutritional information and the locations and times of the farmers markets. During each of the farmers markets, nutritional information was handed out to tribal and other community members seeking fresh, nutritional food. We estimate a total of 60 farmers markets were held in Fort Yates, Cannon Ball and McLaughlin during the 2016 growing season and 40 farmers were held in Fort Yates and McLaughlin during the 2016 growing season. Before each event, flyers were distributed via e-mail and posted in tribal communities on the time and date of each event and what specialty crops were available. We estimate the average attendance at each farmers market at 25, making the total number of at least 2,500 pieces of nutritional information, recipes and other materials were available.

Goal 2: Provide more access to fresh, nutritious fruits and vegetables on the Standing Rock Reservation: We estimate more than 1,000 residents of the Standing Rock Reservation gained access to the fresh specialty crops that were grown and distributed through this project. Weekly farmers markets were held at Fort Yates and Cannon Ball beginning in June and lasting until late September or early October of 2015 and 2016. In addition, the grade school students at Cannon Ball ate fresh produce right out of their school garden, as well as taking produce ho for their families. This project resulted in the two new gardens at Cannon Ball, which we estimate added about 50 percent more total pounds of specialty crops produced. The majority of the specialty crops grown in Cannon Ball were distributed for free. The Cannon Ball area is one of the most poverty stricken tribal communities in the nation, so little to no money is available for fresh produce. The barter system that was established at the Cannon Ball farmers market, allowed those in need of fresh produce to trade such things as volunteer hours (if they could) or take home the produce for free.

The number of "farmers market vouchers" issued by tribal members by the Standing Rock Senior Farmers Market Nutrition Program rose to about 3,000 in 2015 and 3,200 in 2016. For the 2015 season, this is an increase of 23.7% from 2012 and an increase of 32.0% from 2014. For the 2016 season, this is an increase of 14.3% from 2012 and an increase of 21.9% from 2014. A total of 2,425 vouchers were issued in 2012 and a total of 2,625 in 2014.

Although produce was not all consistently weighed before being distributed, we estimate that specialty crop production totals were more than 2,600 pounds in 2015 and more than 5,000 pounds in 2016. These are low estimates and the numbers could be much higher. When staff was short and lines were long for the produce, many times produce was not weighed.

Some of this large increase is due to the two new gardens and the resulting farmers market established at Cannon Ball beginning in 2015. Many of these low-income tribal members were not served previously as many did not have transportation to attend the farmers markets in Fort Yates. In addition, informal surveying of the elderly using vouchers at the Fort Yates farmers market showed a rise in new voucher uses as more people find out about the program and the fresh produce that is available through this project.

There is a growing demand for additional fresh specialty crops on the Standing Rock Reservation. It will be imperative to complete the in-ground high tunnel at the Canon Ball community garden site in early 2017 to help meet this demand. Additional school gardens will also help meet the demand for more fresh produce as well as transfer the successful student learning model (both classroom and hands-on gardening) that was conducted at the Cannon Ball school during this project.

We also recommend engaging more tribal members in either growing specialty crops for their own consumption and/or to sell at the farmers markets in Fort Yates, Cannon Ball and possibly other tribal communities. This may require some additional gardening training and education as it appears many tribal members have become far removed from growing their own food.

By establishing the first tribal school garden at Cannon Ball and the plans to establish three more tribal school gardens during the next two years, this project leaves an achievement that we hope will last for many years to come. Even if funding does not continue under this program, we hope and expect that the students, teachers and community members will take "ownership" of these gardens and continue to use them to teach, learns and help the students and others eat more healthy food. The Cannon Ball School teachers and administrators have shown great interest in the special classroom food/growing lessons and we anticipate this to remain in place in Cannon Ball and expand to additional tribal schools.

The same is true for the community garden sites that have been established in Fort Yates and Cannon Ball. These gardens are presently called "community gardens" because the project staff grows specialty crops in them that are distributed to help the community. In the future, the goal is to either make these true "community gardens," in the sense that community are assigned a plot of their own to raise their own food, or develop true community garden sites for those who want to learn how to grow their own specialty crops.

Beneficiaries

Benefitting from this project and previous work is the entire population of the Standing Rock Reservation, but especially the 120 children attending the Cannon Ball Elementary School. This future generation proved to not only be hungry for fresh local foods, but hungry for learning on how they can plant, protect and eat the very food that will nourish them to better health and a better life.

The elders of the reservation also benefitted by receiving free farmers market vouchers they could use at the Fort Yates and Cannon Ball farmers markets for free fresh produce. While we do not know the exact number of elders who benefitted, we estimate it to be at least half of the approximately 300 tribal members age 65 or older on the North Dakota side of the reservation.

In general, the entire reservation community benefitted as this project, the previous work and the upcoming work is creating a new legacy of food production on the reservation.

Other groups, stakeholders and partners included the Sioux County Extension Service; the Sioux County Commission; the Nutrition for the Elderly Program, the Standing Rock Diabetes Program; Sitting Bull College; the Tribal Historic Preservation Office; North Dakota State

University; the Cannon Ball School; Standing Rock Sioux Tribe, Bureau of Indian Affairs, North Dakota State University; and the Church of Latter-day Saints.

We estimate more than 1,000 tribal members of the Standing Rock Sioux Reservation benefited from this project. In addition, toward the end of this project (during the late fall of 2016) Native Americans from many other tribes learned about this project when they were fed squash and other late fall produce from the Fort Yates and Cannon Ball gardens while staying in the water protector camps near Cannon Ball.

In addition, as word has gotten out about the successful specialty crop gardens on the Standing Rock Reservation, numerous groups have traveled to the reservation from across the country. They want to tour the project's gardens, especially the school garden at Cannon Ball, to try to duplicate this successful model.

Through this project, the 120 students at the Cannon Ball Elementary School have made a solid connection to food production through the creation of their own school garden. This legacy of food production is an invaluable learning tool that can and should be modeled and duplicated throughout the reservation. The cultural and nutritional education these 120 students learned has been multiplied many times over as they share their local foods experience with their families and other community members.

Other community members, especially the elderly and families in need of food, benefitted by being able to nourish themselves with fresh, healthy food. We conservatively estimate this number to be about 1,000, but it could be much more than that.

Lessons Learned

This project continues to struggle to find reliable ways to document outcome measurements. Because of the high demand for the specialty crops, it is frequently not feasible to weigh the produce before it is distributed to hungry families. Lack of time and staff results in this step not being conducted. In addition, the number of farmers market vouchers issued by the Nutrition for the Elderly Program are not consistency tracked, making it difficult to accurately document exact outcome data.

We also did not fully achieve our goal of creating a prototype dried soup mix to help feed the hungry of Standing Rock Reservation in the winter. We did a great deal of experimentation with freeze drying a wide variety of specialty crops, but due to a lack of time and staff, did not finalize a soup product.

If starting a similar project, others would be cautioned to keep things on a manageable scale. Although a school garden sounds relatively easy on paper, it still takes a lot of time and labor to plan and maintain one.

This project has found that many more personnel than expected are needed to help plant, maintain and harvest specialty crops. Tasks such as weeding and watering are labor intensive. This project has been diligent in making it a priority to involve the youth of the community. Therefore, many grade-school and high-school tribal students have been hired to help with the

gardening. While they were first eager to volunteer their time and help, we found that even a small stipend or low hourly rate helped instill pride in the job they were doing. It also helped them learn the responsibility of showing up when they were scheduled and expected and getting the work done and done well.

We also found that it is critical to plan a good balance in the varieties of specialty crops that are produced. While there will also be the standard most common and popular specialty crops such as tomatoes and cucumbers, it is important to introduce different varieties such as kale, celery and beets, which many would not think of planting, but provide a variety of nutritional choices when eating and cooking.

Along with this, we have learned that we need to put more emphasis on food preparation and preservation. Many do not know how to cook with some of the produce, so it is not being used to its full potential. And, with more priority given to specialty crop preservation, tribal members will have more nutritional meal choices year-round, not just three or four months out of the year.

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Additional Information

Photographs of the project are included in Appendix A (attached). Additional photographs, farmers market flyers and other additional information are available on the Sioux County Extension Service Facebook page at www.facebook.com/SiouxCountyExtension/

APPENDIX A

Following the prairie fire in October 2015, the Cannon Ball school garden was rebuilt with new planter beds and mulch.

Project Director Sue Isbell, center, helps tribal members choose fresh produce at a Cannon Ball farmers market.

Above: The new wood planter boxes near the historic root cellar at the Cannon Ball community garden site were filled with composted soil and grew many specialty crops in 2016. Left: Cannon Ball students prepare to plant their soil pods. Above left: People attend one of the farmers markets held every Wednesday at Fort Yates.

A new chain link fence was erected around the Cannon Ball community garden site to protect if from animals and vandals.

Ryan Taylor, USDA Rural Development North Dakota, and Elvis Cordova, USDA-DC, tour the Cannon Ball school garden.

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

Final Report

Partner Organization: North Dakota State University

Project Summary

Six research trials were established in 2015 to evaluate the effect of glyphosate and dicamba drift onto dry beans, field peas, and potatoes. The goal of the research was to determine the amount of glyphosate and dicamba drift that can translate into yield loss for each crop by identifying visual cues and lab-tested plant residue levels. The rates used to simulate drift ranged from 0.4-17% of typical full application rates for the products, and in the same ratio as new commercial products that will be sold. Field peas were the most tolerant of the herbicides, with yield responses only with the combination of the products. Dry beans were the most sensitive with yield responses even to the products applied alone. Potatoes yields were affected by dicamba and the combination of glyphosate and dicamba at the high rates. When testing the biomass for herbicide, glyphosate tests were often poor predictors of yield response. Dicamba residue levels provided more insight into expected yield loss when tissue was collected 10 days after treatment, but neither test was as accurate as visual estimates of injury, particularly 20 days after treatment.

Soybean continues to be one of the top commodities produced in many areas of the United States, and is the second largest crop by acres planted in North Dakota. This project will coincide with the likely release of dicamba-resistant soybean varieties, resulting 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.

New formulations of dicamba alone and in combination with glyphosate are being developed with the goal of reducing the volatilization of dicamba. Even so, it is likely that instances of herbicide spray and vapor drift and injury are going to increase after dicamba-resistant crops are released even if producers follow Best Management Practices. Meanwhile, glyphosate drift injury has occurred regularly since the release of glyphosate-resistant crops in the late 1990s. Crops such as potato, in particular, cannot only be affected by yield loss due to glyphosate drift, but potatoes grown from tubers that were exposed to glyphosate application are severely inhibited as well.

Primary objective: to correlate plant injury in dry beans, field peas, and potatoes to results a producer would receive from lab analysis of leaf tissue.

-New analytics equipment can detect trace amounts of herbicide in a sample, but the effect of those trace amounts on plant health remains uncertain in many cases.

-Page 115 of the 2014 North Dakota Weed Control Guide directs people to laboratories that test for herbicide residues and also contains information about the safe levels of chlorimuron, clomazone, dinitroanalines, imazethapyr, and atrazine that can exist in plant samples from several crops. Definitive information on interpreting glyphosate and/or dicamba lab results from dry beans, field peas, and potatoes is rare or non-existent.

Project Approach

All activities of the work plan were completed. Each co-PI for the project successfully established, executed, and completed the individual tasks defined by the proposal. Several outreach activities will be ongoing past the termination of the project, as well as continued research into this topic area. Due to the complexity of the work plan, the results of the work will be covered in this section that represents the culmination of the completed activities for each researcher. Note that all tables are added at the end of the report due to the size.

Field Peas:

There were very similar trends noted at both field pea research locations. In both cases, very little injury was noted across the treatments (Table 1). Maximum visual injury occurred with the combination of glyphosate and dicamba at the two highest rates. Minor injury was also noted with the highest rate of dicamba alone, and at Carrington, minor injury was seen with the high rate of glyphosate as well. However, no product alone caused yield reductions in field peas. The highest rate of the combination caused a yield reduction in both locations, and the middle rate caused a yield reduction in Minot. At both sites, the glyphosate residue testing produced non-significant results, meaning that no glyphosate was detected at statistically greater quantities than the check plots. With the dicamba treatments the Minot location had non-significant results but the Carrington site had statistically significant but relatively minute amounts of dicamba in the residue. Even so, the dicamba residues correlated really well with crop yields (R^2 of 0.74 and 0.84 at Carrington and Minot) 10 DAT. By 20 DAT the correlation had dropped and visual estimates of damage correlated much better.

Dry Beans:

Different results occurred with the two dry bean locations. Visual injury ratings were relatively similar for both sites (Table 2). Unlike the field peas, injury levels remained the same or worsened between 10 and 20 DAT. Yields differed greatly between the sites. At Carrington, the highest two rates of dicamba alone and in combination caused complete yield loss where the plant growth stagnated and never recovered. A frost finally caused the plants to mature but without seeds. At the Thompson location, severe damage still occurred from the highest rate of dicamba alone and in combination but yield was still collected from those treatments. The yield results correlate well with residue testing of dicamba. Even though the magnitude of the residue testing results were different, the pattern was a good predictor of yield loss for both sites. Glyphosate residue testing did not provide any insight into crop response at Thompson, while the Carrington dry bean residues had a good relationship to yield loss.

Potatoes:

Once again different results occurred between the two locations for potato research. At Inkster, there was very little visual injury that occurred (Table 3). For this location, glyphosate applications at the highest rate alone, and at all three rates in combination, caused significant injury symptoms. At Oakes, only the two lowest rates of glyphosate alone did not injure the potatoes. This translated to yield response of the potatoes, where there were no differences in yields at Inkster, but Oakes had injury from the highest rate of dicamba alone and in combination. Dicamba residues had a good relationship to yield response at Oakes. At both sites, there was a similar dicamba residue trend, however since there was no yield response at Inkster,

that correlation was non-existent. Glyphosate residues at both locations were not good predictors of yield response even though there was a significant increase in residue levels as the rate increased. Tubers harvested from treated potatoes also had glyphosate and dicamba residues present. Continued efforts are being undertaken to test the germination and vigor of the daughter tubers.

Conclusions:

At the lowest dose, no crop had yield reductions from either herbicide. Overall, field peas were the most tolerant of products with a 20-30% yield loss but only when the products were combined at the highest rates. When the products were applied alone, there was no yield loss at any rate. Dry beans had the most severe response to the herbicides with 68-100% yield loss from the highest rates of the combination as well as the products applied alone. Potato yields also declined from the highest rate of the combination as well as with dicamba alone, but only in one of the two locations.

Glyphosate residue testing did not provide clear results that translated to a consistent yield response. Dicamba testing often showed a good relationship to yields when sampled at the 10 days after application, but often not 20 days after application. However, the magnitude of the residue levels varied significantly between crops, locations, and sometimes timings. This calls into question the relevance of residue testing from a single affected plant or area, at least at when minor drift is suspected in a field. A second year of testing is being conducted in 2016 which will generate a similar dataset. Comparison between multiyear datasets will be essential to determining whether residue tests can be relied upon for yield predictions.

Workshops were held to serve as in-service training to NDSU Extension educators as well as crop consultant training sessions. Results from this research were also presented at the North Central Weed Science Society, Western Society of Weed Science (both of which are for national weed science professionals) and the Wide World of Weeds Workshop (for farmers and consultants). Results will also be presented at future field days, workshops, and professional meetings.

Goals and Outcomes Achieved

Primary objective: to correlate plant injury in dry beans, field peas, and potatoes to results a producer would receive from lab analysis of leaf tissue.

The ultimate goal was to see if visual injury was related well to residues found through lab analysis of the tissue. After a year of working with the project we do have some positive outcomes with this objective. It is safe to say that plant tissue testing should be done soon after suspected drift. Our data shows that in almost every case, the residue data was more reflective of expected yields ten days after the application event versus twenty days. Most of the time, visual injury evaluation was the most accurate method of determining yield loss, particularly by twenty days after application. However other environmental conditions can sometime cause symptoms similar to herbicide damage which is why the residue data is very useful. The glyphosate residues were much more unpredictable than dicamba. But dicamba was nearly always

influencing yields more than glyphosate. This is good for growers who would have received drift from soybeans treated with dicamba, but at least from one year of data, drift from glyphosate alone appears to be difficult to detect in tissues.

While we are not yet able to determine the parts per million/billion that would cause yield loss from dicamba, I believe that a sampling procedure can be put together that will help estimate damage done from drift. By sampling plants from several parts of a field starting from the most affected to least/not affected areas, a specialty crop producer can get similar trends to ours. These results could then be used after harvest to determine if the damage was in fact, herbicide-related. Though another year of data will be needed to solidify any official methodology related to this.

Overall, the baseline data that we generated from this study is a very valuable cornerstone of information. With multiyear data we can develop the tools that producers need to protect themselves from inadvertent damages caused by herbicides. Even with the data we currently have, we are able to educate producers and consultants about the potential damage caused by dicamba and glyphosate in combination. There appears to be a synergistic affect between the two products that causes more damage together than either product alone. In the potatoes more dicamba showed up in residue treated with both products indicating potentially more uptake of dicamba when glyphosate is applied also. Dry beans, in particular, seem to be very sensitive to the product. One of the important components of our outreach will be educating about the dangers of these products to nearby sensitive crops. It seems that our caution about these dangers was warranted since it only takes about 4% of applied product to cause significant yield loss to dry beans. This same information tells us that field peas and potatoes (not including tuber residues) may still be safe at that same level.
This project will continue another year with research efforts. Our outreach with this data began this winter/spring and will continue throughout the duration of the research efforts as well as beyond the duration of the research. Next year is slated to be the first time that dicamba will be applied to soybeans so this coming winter and subsequent years will be critical in education applicators about the dangers.

16,000 weed control guides were distributed last year. The NDSU Weed Science page (https://www.ag.ndsu.edu/weeds) is one of the primary places this was published. That site had 8287 unique viewers last year, with people visiting an average of 3.8 pages/visit (total of 62580 page views).

Beneficiaries

This project primarily affects the potato, dry bean, and field pea producers directly. This is data, particularly the visual injury data (for now), will be used primarily to protect the producers from damages caused by nearby spraying operations. This has the potential to affect many producers due to the volatility of dicamba, which puts it at high risk for drift. When the use of dicamba goes up, the rate of herbicide drift calls is likely to go up as well. As mentioned above, after a second year of data, more concrete recommendations can be put together regarding the residue testing for herbicide damage but the visual injury data can be used immediately to aid producers, consultants, insurance agents, and others in determine threat of yield damage.

The number of people that would have directly received the results from this study are estimated at 470 for the duration of the grant (field tours and future trainings will also include results from this project). For reference, there are roughly 420,000 dry bean acres from 1567 farms, 80,000 potato acres from 189 farms, and 290,000 field pea acres from 789 farms.

Lessons Learned

We had hoped that the residue data would be more conclusive. While this data will still be useful as the new project moves forward, it also helped us refine our sampling techniques which may aid in getting clearer results in the future. Unfortunately the glyphosate sampling, in particular, was ineffective at drawing meaningful conclusions. In some cases glyphosate residues were showing up in check plots or plots treated only with dicamba. This may indicate contamination from the surrounding area or perhaps inaccuracy of the test from the lab. Either way, it provides important information about which residue tests may be more relied upon when sending tissue samples to a lab.

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Additional Information

This information will be published and made into posters and presented at several meetings after the conclusion of this project in the fall and winter of 2016.

Table 1. Field pea herbicide residue, plant injury, and grain yield from two sites in 2016.

		Dicamba Residue				Glyphosate Residue				Injury				Yield	
		Carrington		Minot		Carrington		Minot		Carrington		Minot			
Treatment	Rate	10 dat	20 dat	10 dat	20 dat	10 dat	20 dat	10 dat	20 dat	10 dat	20 dat	10 dat	20 dat	Carrington	Minot
	fl oz/a	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb	%	%	%	%	bu/a	bu/a
non-treated	0	3	0	0	1	47	18	31	0	0	0	0	0	57.8	67.8
glyphosate	0.1	1	0	0	1	33	13	32	0	3	3	0	0	59.3	68.9
glyphosate	0.5	1	0	0	1	53	11	38	7	3	5	0	0	51.8	68.0
glyphosate	1	3	0	0	1	36	16	45	22	9	8	0	0	56.9	67.2
dicamba	0.05	0	0	0	8	28	15	36	27	3	0	1	0	58.7	68.4
dicamba	0.25	5	2	0	30	32	13	18	0	3	3	4	4	58.9	65.8
dicamba	0.5	4	10	0	57	46	9	32	2	8	8	8	6	59.6	61.3
dicamba + glyphosate	0.05 + 0.1	0	0	0	11	66	13	13	4	0	0	1	1	62.1	74.0
dicamba + glyphosate	0.25 + 0.5	5	5	0	37	35	10	38	4	5	4	14	7	59.1	54.9
dicamba + glyphosate	0.5 + 1	11	8	0	52	71	16	35	20	15	13	21	11	47.0	46.7
LSD (0.05)		6	6	NS	NS	NS	NS	NS	NS	5.1	3.3	2.1	1.2	8.6	9.2

Table 2. Dry bean herbicide residue, plant injury, and grain yield from two sites in 2016.

		Dicamba Residue				Glyphosate Residue				Injury				Maturity	Yield	
		Carrington		Thompson		Carrington		Thompson		Carrington		Thompson				
Treatment	Rate	10 dat	20 dat	10 dat	20 dat	10 dat	20 dat	10 dat	20 dat	10 dat	20 dat	10 dat	20 dat	Carrington	Carrington	Thompson
	fl oz/a	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb	%	%	%	%	J date	lb/a	lb/a
non-treated	0	0	11	3	0	78	15	2100	84	0	0	0	0	240	2090	928
glyphosate	0.1	0	12	9	1	201	25	5700	88	3	2	2	20	240	2097	1446
glyphosate	0.5	0	25	2	0	1835	349	4700	92	17	17	0	12	282	657	1009
glyphosate	1	1	17	8	0	3197	453	790	89	22	22	15	25	282	787	1277
dicamba	0.05	3	264	14	1	84	8	4400	94	8	8	10	27	274	1640	1492
dicamba	0.25	8	1190	37	4	57	11	2900	88	30	22	13	37	282	0	1464
dicamba	0.5	13	6360	520	275	60	16	3000	100	39	35	35	55	282	0	406
dicamba + glyphosate	0.05 + 0.1	1	452	7	1	261	90	4900	99	8	17	6	20	258	1665	1134
dicamba + glyphosate	0.25 + 0.5	10	3050	46	14	2095	285	2600	95	37	32	23	43	282	0	1508
dicamba + glyphosate	0.5 + 1	12	3905	270	311	2580	189	2000	96	48	42	52	73	282	0	304
LSD (0.05)		9	2606	NS	170	885	281	NS	NS	21	20	7	9	6	1277	375

Table 3. Potato herbicide residue, plant injury, and tuber yield from two site in 2016.

		Dicamba Residue				Glyphosate Residue				Injury			Marketable Yield		Daughter Tuber Residues			
		Inkster		Oakes		Inkster		Oakes		Inkster		Oakes			Dicamba		Glyphosate	
Treatment	Rate	10 dat	20 dat	10 dat	20 dat	10 dat	20 dat	10 dat	20 dat	10 dat	20 dat	20 dat	Inkster	Oakes	Inkster	Oakes	Inkster	Oakes
	fl oz/a	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb	%	%	%	cwt	cwt	ppb	ppb	ppb	ppb
non-treated	0	9	2	29	119	30	39	88	22	0 a	0 a	0 e	268	362 ab				
glyphosate	0.1	9	0	45	131	0	55	126	4	0 a	0 a	0 e	184	385 a				
glyphosate	0.5	4	0	121	208	87	19	232	55	1 ab	0 a	0 e	272	372 ab				
glyphosate	1	149	0	50	13	765	129	822	224	11 d	12 b	10 d	255	373 ab				
dicamba	0.05	158	110	559	217	0	22	51	39	3 bc	9 b	30 bc	208	358 ab				
dicamba	0.25	576	221	1180	769	22	480	40	21	4 bc	12 b	31 b	177	328 b				
dicamba	0.5	1755	496	3736	693	0	29	62	3	4 abc	10 b	49 a	238	238 c				
dicamba + glyphosate	0.05 + 0.1	294	77	462	114	0	34	45	5	4 bc	9 b	30 bc	227	379 ab	0		0	
dicamba + glyphosate	0.25 + 0.5	509	173	770	628	107	75	93	24	7 cd	11 b	28 c	236	340 ab	75	0	21	0
dicamba + glyphosate	0.5 + 1	2647	1280	5570	1177	928	790	700	111	13 d	14 b	50 a	224	191 c	115	0	50	19
LSD (0.05)		715	369	1246	816	314	NS	245	128				NS					

Hop Selections for North Dakota

Final Report

Partner Organization: North Dakota State University Williston Research Extension Center

Project Summary

Hop Selections for North Dakota enabled NDSU researchers to investigate hops grown in North Dakota for production. This project evaluated different hop varieties across two regions of the state in order to determine best management practices, and top yielding varieties. We found that hops can be successfully grown in North Dakota, but more research is warranted in the areas of production, processing, economic feasibility and marketing.

To look at North Dakota as a growing region with limited or reduced disease incidence and possibly even a region with opportunities to grow organically. The diseases that plague the major growing regions in the US include various viroids, viruses (apple mosaic virus), fungal infections (downy and powdery mildew) and wilt diseases (Verticillium). Pests include leafhoppers, mites and aphids which serve as vectors for some of these diseases.
Another reason for this project was to bridge the gap between research and practical application for growers to consider hops as a potential niche crop in our region for both agro-tourism and farm diversification prospects. Demand for locally sourced and/or organically grown hops has been on the rise with the popularity of craft and micro-breweries opening within the state. Home brewing also continues to increase the interest in growing hops. Both home and craft brewers find locally sourced ingredients to be a major marketing opportunity for creating uniquely distinct products.

Project Approach

The work activities involved two project sites, one located at the Williston Research Extension Center in Williston, ND and the other located at the Dale E. Herman Research Arboretum in Absaraka, ND. Both sites effectively established hopyards similar to commercial yards located in either the Pacific NW or Great Lakes Regions. Attending the American Hops Conference allowed the researchers to take in seminars containing specific information about hops, such as disease and breeding work being done and networking with large scale growers.
The activities throughout the grant duration included soil sampling to meet the plant nutrient requirements, installation of a trellis structure that allows stringing for the hops to grow on, installation of irrigation supplies, ordering and planting live plants, and weed management using different mulching techniques and herbicides. Once the hop plants have grown on the strings that have been hung from the trellis, the plants are cut down at the time of harvest. This required the trellis to be re-strung each spring as well as emerging hop shoots to be trained onto the string. Hops send up many shoots from their crown, but only 2 or 3 of the best looking shoots are trained to grow on to the string. The other shoots must be kept under control either by chemical or mechanical methods; if left to grow unchecked the shoots will grow onto one another in a tangled mess that creates an environment conducive disease and insect prevalence.
Harvest was accomplished with a mechanical harvester ordered from Hops Harvester LLC. With age, hop plants increase production each year until about year five. Hand picking hops is

incredibly time consuming, and the purchase of this harvester significantly increased the efficiency of harvest, drying and compiling data.

During the duration of this grant, this project was presented at numerous events including field days for both Williston and Absaraka during both 2015 and 2016 summers, an open house farm tour in Carrington, ND, and various workshops including one in Minot, ND and another in Rapid City, SD. Research from this project was also presented to other university researchers in the Great Lakes region at the Great Lakes Hops Working Group spring meeting in Burlington, VT.

Goals and Outcomes Achieved

One of the performance goals stated in the project proposal has been to identify high-yielding varieties that produce high quality product from the eastern and western regions of North Dakota. There are hundreds of hop varieties available on the market, some of which are desirable to the brewer and some that are not. The varieties that were chosen for this project had to do with their popularity, grower availability, brew usage and growing region from where they were hybridized. The top yielding variety for Williston was the variety *Challenger*, from the UK breeding program. This variety isn't the most popular variety requested by breweries in the region. The variety *Cascade*, however, is a desirable variety because of its alpha acid and beta acid profiles. *Cascade* came in third in the Williston variety trials and is a popular choice for many breweries because of its flavor profile and versatility. Cascade was, however, the top yielding variety in the Absaraka trial.
Because hops are a perennial crop that do not meet full production until years three to five, it's difficult to draw any conclusions at this point. We do intend, however, to continue data collection and monitoring in the future of this project even though the grant duration has concluded.
Management practices need further research in order to make recommendations specific to each growing region in which we will be doing with a continuation grant from the Specialty Crop Block grant program that was awarded in the fall of 2016 which will allow us to further the research with a training date trial. Continuing this trial will allow us to draw further conclusions for other management practices including watering, fertilizing, pruning, pesticide application as well as training techniques.

Beneficiaries

The project beneficiaries of this project's accomplishments have been existing ND growers, other university researchers, various microbreweries in the region who wish to source locally grown ingredients, and those who wish to start growing but needed more information. Approximately 150 people were reached at the outreach events.

There have also been several articles written and published about this project. These include interviews with the Sidney Herald, Williston Herald, Dakota Farmer, Farm and Ranch Guide and The Dirt - an NDSU Extension Master Gardener publication. There is also a book that is being written about the history and future of hops in North Dakota. All of these publications garner a regional audience and it is hard to say how many people have truly been reached through these outlets, but the amount of phone calls, emails and inquiries regarding this project dramatically

increased from the tours, workshops and news articles.

There are approximately 5 producers that we are aware of who have reported their acreage to the North Dakota Department of Agriculture to be included in the National Ag Statistics Service. The number that has been reported is 2.18 acres.

This project has helped generate a lot of interest in the areas of growing and producing hops in a state where no information or acreage reports were known previously.

Lessons Learned

As a researcher, I've learned that the sky really is the limit when it comes to problem solving. There are no set rules as to how you set up a trellis. However, there are ways to grow them successfully and I believe that each grower has to identify his or her own unique skill set, abilities and time, and determine how they can make the growing process work for their own operation. With that being said, there are ways to quickly ruin a hop crop and consideration should be given to the time and labor needed for harvest, and most importantly where the final product will end up. Hops need to be dried in order to be packaged and stored properly. There is specialized equipment needed for processing whether that includes having the cones reduced into pellets, or having them packaged as whole cone.

One of the goals of this project was to purchase and build a commercial sized drying system. After doing a little research, it became evident that a drying system such as the one originally considered for this project was unnecessary, but would likely be needed for a large commercial hop yard. We were able to utilize basic technology such as a box fan and screens to dry our hops to the correct moisture. This is an area where a producer can come up with a system that will work for their operation. In our first attempt to dry the hops, we used heated grain dryers. This turned out to have negative results-over dried and uneven drying- considering that we could not adjust the temperature and ambient air flow of the dryers.

It was determined that a pelletizer should be looked into for the processing end. However, due to the inefficiency of smaller sized pelletizers and from the recommendation of a Certified Hops Agronomist in Quebec, we looked into other areas of problem solving. Small scale pelletizers are expensive and run at temperatures capable of degrading the important chemical components that give the hops their desired flavors and aromas. Canada utilizes a large pelletizing operation that can effectively process an entire growing region, as opposed to having each grower trying to work independently to produce a top quality product.

Also, we looked into various ways to safely re-string our hop trellis without having to rent or purchase expensive lift equipment. The solution utilized was a series of brake winches and cables that allowed us to lower the top wire to a workable height. This allowed us to effectively tie off our string, raise the top wire back up and train the hops accordingly.

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Additional Information

https://www.ag.ndsu.edu/WillistonREC/horticulture-program-1/current-research-1/hops

Ornamental Woody Plant Breeding

Final Report

Partner Organization: North Dakota State University

Project Summary

This grant provided the support to develop and strengthen an ornamental breeding program at North Dakota State University in conjunction with the NDSU Woody Plant Improvement Program. Historically there has not been a breeding program for woody ornamentals at NDSU. This project collected germplasm from multiple resources for inclusion into the current germplasm collection at the NDSU Research Arboretum (Absaraka, ND). Breeding programs were developed for lilac (*Syringa* spp.), maple (*Acer* spp.) and magnolia (*Magnolia* spp.). Inter and intraspecific crosses were successful conducted with resulting progeny planted at the NDSU Research Arboretum for long-term evaluations including winter hardiness.

The results of this project are to develop new ornamental cultivars suited for use in North Dakota and the Northern Great Plains.

The purpose of this project is to increase the breeding efforts and germplasm collections for ornamental woody plants suited for the harsh North Dakota landscapes.

Increasing woody plant species for use in landscaping applications would have a significant impact on residents specifically in North Dakota and throughout the region.

There would also be a positive financial impact to the nurseries growing woody plants in North Dakota by providing new plant materials that has been bred, selected and evaluated for the Northern Great Plains to be produced for sale.

The objectives of this study are to:

- Develop a strong ornamental breeding program to include improvements with magnolia, maple and lilac germplasm.
- Develop new ornamental cultivars suited for use in North Dakota and the Northern Great Plains.

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

Project Approach

Work Plan: Project Activity

- Potential germplasm will be identified in 2014 to be used in breeding efforts of magnolia, hybrid maple and tree form lilac. Germplasm was identified and included three key NDSU selections; Spring Welcome® magnolia (*Magnolia x loebneri* 'Ruth'), Northern Spotlight® (*A. pseudosieboldianum* 'KorDak'), and Copper Curls® Pekin lilac (*Syringa reticulata* ssp. *pekinensis* 'SunDak'). These three selections are the hardiness selections of the known available cultivars within the commercial ornamental nursery trade. They are excellent selections as parents for an ornamental woody plant breeding and improvement program. All

three selections were utilized in subsequent breeding efforts within the NDSU Woody Plant Improvement Program.

- Crosses will be made in 2015 and 2016 with desirable parentage of magnolia, hybrid maple and tree form lilac. Successful crosses were made with respect to utilizing the three key NDSU breeding parents (previously mentioned). There were three different locations involved with making sexual crosses with the three different NDSU breeding parents. Magnolia breeding was conducted at the NDSU Research Arboretum and at Green Bay, WI in collaboration with Dennis Ledvina, a private magnolia breeder. Maple breeding was conducted at the NDSU Research Arboretum utilizing pollen collected from the University of Wisconsin – Madison Arboretum from Northern Glow® maple (*A. pseudosieboldianum* x *palmatum* 'Hasselkus'). Lilac breeding was conducted at the NDSU Research Arboretum and within the NDSU Woody Plant Improvement Tissue Culture Lab.
- Seeds from crosses will be collected, prepared for germination and germinated. Hybridized seeds were successfully collected in 2015 and 2016 from magnolia and maple. All the seed were stratified and germinated and placed in greenhouse for increased seasonal growth in preparation for field planting evaluations. Lilac crosses were collected as immature embryos and placed in tissue culture for embryo rescue. Embryo rescue efforts of lilac were unsuccessful.
- Germplasm will be catalogued and evaluated once selection criteria can be obtained. Hyridized plants grown in greenhouse were field planted in spring 2017 for long-term evaluations to determine environmental adaptability.
- NDSU Research Field Day Updates. Updates were given at the 2015 and 2016 NDSU Horticulture Research Farm Field Day on breeding efforts of magnolia, maple and lilac. Attendance for NDSU Horticulture Research Farm Field Day is 125 individuals per year and comprises of commercial nursery producers, retail garden center owners/managers and residential homeowners.
- Research Update at NDNGA annual meeting. Research updates were presented at the 2015 and 2016 NDNGA annual meeting to provide beneficiaries with updates on breeding efforts with respect to magnolia, maple, and lilac. Attendance at NDNGA annual meeting is 50 individuals and comprises of commercial nursery producers, retail garden center owners/managers and landscape architects/contractors.

This project did not benefit commodities other than specialty crops.

Goals and Outcomes Achieved

Goal 1. Develop a strong ornamental breeding program to include improvements with magnolia, hybrid maple and tree form lilac germplasm.

- Performance Measure: Increase available germplasm through breeding efforts of magnolia, hybrid maple and tree form lilac.
- Benchmark: There are no breeding efforts currently being done with the three hardy NDSU selections of Spring Welcome® (or Bismarck magnolia), Korean maple (no hybrid maple recommended) – Northern Spotlight® and Pekin lilac – Copper Curls®.

- Target: To increase the germplasm possibilities for use in breeding programs of magnolia, hybrid maple and tree form lilac utilizing hardy germplasm already released by NDSU and incorporating other sources of plant germplasm.
- Performance Monitoring Plan: Germplasm increases will be cataloged and uniquely identified based on breeding parentage. The crosses will be monitored to ensure greater potential for increased hardiness and ornamental traits suitable for future cultivar selections.
- Outcomes Achieved: A strong ornamental breeding program has been developed at NDSU with respect to the NDSU Woody Plant Improvement Program and focus on magnolia, maple and lilac breeding. This project increased the germplasm possibilities by increasing germplasm resources to be used as parents for hybrid crosses. Numerous hybrid magnolias with high aesthetic value were propagated (grafted or cuttings) to produced plants that can be and were used in making sexual crosses. Protocols were developed for protoplast fusion of lilac to be able to produce interspecific crosses with Pekin lilac and shrub forms to be able to incorporate flower color into tree species. Protoplast fusion of lilac is possible but has limitations based on expense, requirement of specific laboratory equipment and unknown of successful recombination of genes which include flower color in a tree form selection. Long-term evaluations are being conducted with field plantings for evaluation of environmental tolerances.

Goal 2: Develop new ornamental cultivars suited for use in North Dakota and the Northern Great Plains.

- Performance Measure: Increase available cultivars through breeding efforts for commercial nursery production as well as landscape use of magnolia, hybrid maple and tree form lilac.
- Benchmark: There are limited cultivars available of magnolia, hybrid maple and tree form lilac that are suitable for ND hardiness zone 3-4.
- Target: To increase the number of magnolia, hybrid maple and tree form lilac cultivars suited for the harsh North Dakota landscapes. This is a long-term goal as a result of woody plant material requiring long-term evaluations to determine if desired characteristic is expressed and with multiple winter hardiness trials. Short-term selection of potential new cultivars can be performed based on ornamental traits with hybrid maples, flowering characteristics can be more long-term because the plants needs to reach sexual maturity before it will flower.
- Performance Monitoring Plan: Plants will be identified as potential cultivar releases based on winter survival as well as desirable ornamental characteristics such as leaf shape, leaf color, plant height and form and flowering characteristics. These characteristics will segregate and will be uniquely identified within the germplasm collection.
- Outcomes Achieved: There were over 500 hundred successful hybrid crosses obtained from magnolia resulting in seedlings for long-term evaluations. Initial selections of hybrid maples have been conducted based on ornamental value of leaf form and color suitable for landscape use. A wide array of leaf forms was achieved with the hybridization between Northern Spotlight® Korean maple and Northern Glow® hybrid maple. These leaf forms include deep incised margins, to

color form including red leaf and green leaf forms. Several of the potential future selections received many positive comments from commercial nursery growers. Hybridized lilac cultures are still in the developmental phase. All the hybrid crosses will be subjected to long-term evaluations including direct field evaluations as well as commercial nursery evaluations to determine production requirements. These evaluations will determine if the specific cross has value to the ornamental nursery trade for the Northern Great Plains. This will have a direct impact on the ornamental nursery trade by increasing the availability of

Beneficiaries

The specialty crop beneficiaries of this project are commercial nursery producers and retail garden centers across the state of North Dakota and professional landscape installation and management companies. Many of these beneficiaries are members of the North Dakota Nursery and Greenhouse Association (NDNGA). Both groups will benefit from this project by providing new superior ornamental woody plants to be utilized in landscapes. The expected number of specialty crop beneficiaries is 100. Residents of North Dakota will also benefit from this project with the availability of new superior ornamental woody plants to be enjoyed in their landscapes across the state.

Outcomes: As new cultivars are developed and made commercially available, commercial nurseries in ND (100 beneficiaries) will benefit from having new cultivars available for them to sell and utilize in ND. They will benefit from being able to grow and sell more ornamental plants that are ideally suited for environmental conditions of the Northern Plains increasing their competitiveness and profits.

Updates were given at the 2015 and 2016 NDSU Horticulture Research Farm Field Day on breeding efforts of magnolia, maple and lilac. Attendance for NDSU Horticulture Research Farm Field Day was 125 individuals per year and comprised of commercial nursery producers, retail garden center owners/managers and residential homeowners.

Research updates were presented at the 2015 and 2016 NDNGA annual meeting to provide beneficiaries with updates on breeding efforts with respect to magnolia, maple, and lilac. Attendance at NDNGA annual meeting was 50 individuals and comprised of commercial nursery producers, retail garden center owners/managers and landscape architects/contractors.

Lessons Learned

Protoplast fusion is not an ideal method for obtaining new cultivars or increasing germplasm resources. It is an expensive method of hybridizing and results are not clear with respect to successful incorporation of desired genetic traits.

Maple breeding is ideally suited for short-term evaluation goals as compared to flowering traits as found with magnolia and lilac. Maples are selected primarily on leaf form, overall habit and bark quality. Leaf form can be identified very quickly within the first growing season of the hybrid progeny. This cannot be done with flowering traits with magnolia which may take 5 – 10 years to determine flowering traits.

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Additional Information

Figure 1. Magnolia germplasm obtained from Dennis Ledvina's private collection to increase germplasm resources for breeding efforts. Plants depicted here were propagated either with grafting or vegetative cuttings.

Figure 2. Leaf color and form diversity resulting from cross between Northern Spotlight® Korean maple and Northern Glow® hybrid maple

Figure 3. Hybrid maple selection resulting from cross between Northern Spotlight® Korean maple and Northern Glow® hybrid maple with red foliage color.

Development of Low Glycemic Products Using Pulse Ingredients

Final Report

Partner Organization: Northern Pulse Growers Association

Project Summary

Pulse ingredients (pea and lentil flour, pea protein and fiber) were incorporated into 91 different food products. Products included pastas, breads, crackers, extruded snacks, cookies, cereal bars and muffins. Products were screened for estimated Glycemic Index (eGI) using an *in vitro* method. Based on the screening results, five products (pasta, bread, cracker, granola bar, and cookie) were selected for *in vivo* GI testing. For each control (containing 100 % wheat flour), a pulse variant (containing up to 50 % of pulse flour) was developed. Ten healthy subjects consumed each test meal (five control and five pulse variants) in addition to three control dextrose meals on separate days during the *in vivo* GI testing. All pulse variants had lower GI except granola, which did not lower the GI value of non-pulse variant. The pulse variants were all categorized as low GI. The greatest GI reduction occurred in pasta and bread products containing 50 % and 16 % of lentil flour, respectively. Palatability scores showed no significant difference (r=0.05) between control and pulse variant. The data supports substituting wheat flour with pulse ingredients to reduce the GI value without changing palatability of the products.

Pulses are valuable crops for the region's agriculture and are primarily grown in rotation with wheat and other cereal crops. Pulses provide producers with rotational benefits such as increasing yields and breaking disease cycles commonly found in cereal grains. North Dakota and Montana comprise the largest pulse producing region in the United States and currently account for approximately 80% of the pulse crop production. Pulses are well recognized by health organizations globally and are recommended as part of healthy eating.

Pulse ingredients, namely pulse flour, protein, starch, and fibers, have established itself in the market due to the nutritional value, clean-label, low-allergenicity, and wide range of product applications. Among the pulse ingredients, pulse flour ranked highest in terms of usage (Mintel GNPD, 2013). It is used in thirteen different food categories including snacks (33%), followed by bakery (21%), soup (14%), meat substitute (10%), baby food (8%), dessert and ice cream (4%), sauces and seasonings (3%), and pasta and noodles (3%) (Mintel GNPD, 2013). Even though the usage of pulses is growing, we still see challenges in promoting nutritious pulses to United States and international consumers.

Cooked whole pulse are known for their nutritional value and low glycemic response. The glycemic index (GI) is a measure of the power of foods to raise blood sugar (glucose) levels after being eaten. Low GI foods have been associated with decreased risk of type 2 diabetes, obesity, cardiovascular disease metabolic syndrome, stroke, and others. Testing GI in food is expensive. It is conducted by feeding healthy people a portion of the food and then measuring the effect on their blood glucose levels over time (in vivo test). In vitro test, or test tube experiments, is a less expensive test, however can only be used to estimate the glycemic responses and the results cannot be used on the food labeling claimed in many countries. It is important to note that GI cannot be guessed by understanding the composition of the food. The nutritional values and GI of pulse flours, when incorporating into food products, on the other hand, is not well understood. The biggest challenge when promoting pulse flours for its nutritional values is the lack of evidence to support the claims. This project will help the pulse industry to overcome this challenge by promoting new product

development of pulse based food products, and by providing strong evidence on the GI benefits to the food industry. This project has not been submitted to or funded by another Federal or State grant program.

Project Objectives:
1) Develop new low GI products with pulse flours including pasta, cookies, muffins, bread, crackers, quick bread, and extruded snack and snack bars.
2) Provide in vivo evidence showing the benefit of pulse flour to glycemic response.
3) Promote the benefit of pulse flours to the food industry.

Project Approach

The project comprised of 3 sections; 1) product development, 2) in vitro GI testing, 3) in vivo GI testing.

1) Product development
Product development was conducted by Natsuki Fujiwara, Food scientist at the Northern Crops Institute and Cheryl Doherty, Doherty Food Advisory LLC. Four types of pulses were chosen as a raw material which are 1) red lentil, 2) green lentil, 3) yellow pea and 4) green pea. Ingredients used in the project include a) raw flour, b) pre-gelatinized flour, c) pulse flakes, d) pea protein and e) pea fiber, and the ingredients were supplied and purchased by i) AGT Foods (Regina, SK, Canada), ii) Harvest Innovations (Indianola, IA), iii) Inland Empire Foods (Riverside, CA) and iv) Roquette Corp (Lestrem, France). A summary of pulse ingredients and suppliers are provided in Table 1 of technical report attached. Seven different products were developed including pasta, extruded snacks, bread, crackers, cookies, bars and muffins, and a total of 94 samples were tested for *in vitro* GI, total starch (TS) and resistant starch (RS).

2) *In vitro* GI testing
In vitro GI test was performed by Dr. Clifford Hall, NDSU professor and his technical team. The objective of the section was to screen food products containing different types of pulse ingredients at different levels according to its *in vitro* GI and product characteristics. All 94 samples developed were analyzed.

3) *In vivo* GI testing
In vivo GI testing was conducted by GI Laboratories Inc. led by Dr. Alexandra Jenkins, Director of Research. Based on the *in vitro* GI test result, 10 samples listed below were chosen to be sent to in vivo testing lab.

1. Pasta control (100% semolina) (serving size = 56g)
2. Pasta Red lentil – semolina blend 50:50 (serving size = 56g)
3. Focaccia control (serving size = 50g)
4. Focaccia lentil (serving size = 50g)
5. Shortbread chocolate cookie control (serving size = 30g)
6. Shortbread chocolate yellow pea + protein + fiber (serving size = 30g)
7. Baked granola bar control (serving size = 40g)
8. Baked granola bar protein puff (serving size = 40g)

9. Seedy crisp cracker control (serving size = 15g)
10. Seedy crisp cracker pea (serving size = 15g)

Prior to the test, nutritional analysis was performed which provided carbohydrate content and available carbohydrate. The information was necessary to determine serving portion for the *in vivo* test. *In vivo* GI was determined following the protocol described in ISO 26642:2010 – "Food products – Determination of the glycemic index and recommendation for food classification". A total of 10 males and females, aged 18-75 years with good health condition were used as subjects. After consuming a test meal, the subjects rated the palatability of the test meal in hedonic scale with a line showing "very unpalatable" to "very palatable".

Result:

In vivo GI test resulted in a reduction of GI on all pulse variant except granola bar with which no change of GI was observed. GI category moved from medium to low with pasta and focaccia products whereas the GI category for the rest of samples category remained at low (Table 25 on technical report). Even though the statistical analysis result indicated that there was no statistical difference between controls and pulse variants, an average of 5 GI unit reduction was found. Palatability test result showed that there was no difference between control and pulse variant (Figure 3 on technical report). Short bread cookies gained the highest palatability score followed by granola bars.

Product photograph:

Photoshoot was conducted at NCI laboratory. Products selected for *in vivo* testing were used in the shoot. These photos are used in the brochure and fact sheet prepared by Dr. Julie Garden-Robinson at NDSU.

Goals and Outcomes Achieved

Increased Production of U.S. Peas, Lentils and Chickpeas

In 2015, pulse acres in the region increased dramatically. From 2014 to 2015, lentil acres increased by 54% and dry pea acres increased by 20%. Both acreage and production for dry peas are at record highs for the United States industry. Preliminary numbers reported from USDA/NASS show that U.S. pulse crop acreage in 2016 has doubled from 2015. Chickpeas (garbanzo beans) experienced a slight drop in 2015 acreage due to weather issues. The industry does forecast that in 2016 there will be an increase in chickpea acreage particularly in Montana driven by food manufacturer demand.

Increased Awareness among Participants in Pulse Product Development Course

The USA Dry Pea & Lentil Council (USADPLC) and the Northern Pulse Growers Association (NPGA) hosted a Pulse Bakery & Snack Product Development Course September 11-13, 2015 in Hyde Park, New York. The three day course hosted thirty two company representatives from across the United States and Canada. The event included a three day conference and hands on training that featured pulse flours, proteins and fiber for use in the snack food and bakery products. The majority of the funding event was from the Idaho Department of Agriculture's Specialty Crop Block Grant. In addition, the Northern Pulse Growers Association was a partial sponsor of the event. This was an invitation only event which included discussion, baking demonstrations and bake shop sessions providing attendees with the opportunity to incorporate pulses as ingredients in new menu items and food products.

Attendees of the course were asked to rate their knowledge of pulse ingredients prior to coming to the course. Sixty-seven percent stated that they were somewhat knowledgeable, seventeen percent very knowledgeable and eight percent extremely knowledgeable or not at all knowledgeable respectively. After completing the course, attendees were again asked to rate their knowledge on pulse utilization. Sixty-seven percent stated that they were very knowledgeable and sixteen percent stated somewhat or extremely knowledgeable respectively.

The Northern Pulse Growers Association attended a producer/industry meeting December 9, 2015 in Great Falls, Montana. Information and publications on utilizing pulses were distributed to the 250 producers and industry in attendance.

Increase in Number of U.S. Pulse Flour Processors
According to data issued by Mintel, since 2010 there has been a significant increase in products utilizing pulse ingredients in the United States. The categories increasing the most include meat substitutes, bean-based snacks and vegetable snacks. Because these product categories all utilize pulse fractionations, it is expected that the number of U.S. processors will increase to meet the demands of increasing markets.

Increase in the number of Commercial Products Using Flour
Companies represented at the Pulse Bakery & Snack Product Development Course in September included flour, milling and ingredient companies as well as major food manufacturers. Attendees were surveyed on whether or not their companies were likely to incorporate dry peas, lentils and chickpeas as ingredients in new products, a current value added product line, an ingredient offering or foodservice menu item. Fifty-two percent stated that they were extremely likely, forty-four percent were very likely and four percent were somewhat likely.

Beneficiaries
The primary beneficiaries of the project will be the region's pulse producers who grow dry pea, lentil and chickpea crops. In 2014-15 total sales of U.S. pulse crop represented more than $504 million dollars. The industry is successful in marketing pulses through food aid and commercial channels internationally. Over the past several years, domestic interest in pulses has increased dramatically as a result of a more health conscious approach to the U.S. consumer diet. Low glycemic diets have recently gained consumer and media attention because of the success with long term weight loss. Instead of restricting carbohydrates in diets, nutritionists are advising people to focus on the quality of carbohydrates that they eat or those that are slowly digested and absorbed, keeping you fuller longer and making it easier to burn fat. Pulses and pulse–based foods fit perfectly into a low-GI diet because they are a plant based protein that is rich in fiber and other essential nutrients. Because of this peaked interest in healthy diets, there are new opportunities to expand the domestic markets for pulse crops.
An expanded domestic market may create more opportunities for processing companies in the region creating a higher net value back to the producers. An example of this economic development is the recent addition of a large milling facility in northwest North Dakota which sources pulse products from North Dakota and Montana. In 2015, the NPGA collaborated with North Dakota State University to publish the first economic analysis for the regions pulse industry. The data

showed that the pulse industry has made a significant economic contribution of nearly $300 million in total business activity to the state of North Dakota.

Lessons Learned

The project provided a positive outcome on using pulse ingredients in food products to reduce glycemic response. Also the significance of the project is to use *in vitro* as a screening method and use *in vivo* GI testing to confirm the *in vitro* result. The project did not aim for testing the degree of GI reduction relative to pulse addition. In addition, it did not determine individual effects of different ingredients including protein, fiber and starch. The information would be useful to further investigate the effects of pulses on glycemic response. Furthermore, testing the degree of GI reduction by adding pulse ingredients without affecting sensory profile would be a good information. It is critical to prepare a proper experimental design and conduct a study according to the experimental design in order to scientifically prove the effect of pulse ingredients.

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Additional Information

- Attachment:
  - Technical report prepared by NCI
  - Technical brochure prepared by Julie Garden-Robinson, Professor NDSU
  - Final report provided by GI Laboratory Inc.
  - Power point presentation prepared by Julie Garden-Robinson, Professor NDSU
  - Project manuscript submitted to Cereal Chemistry

- Poster presentation
  The project was accepted as a poster at the Experimental Biology conference held at San Diego on April 2 through April 6, 2016. Natsuki Fujiwara will be presenting the poster.

- Manuscript Submission
  The project will be submitted for peer review. Upon acceptance, the article will be available at the Cereal Chemistry Pulse issue which will be issued late 2016/early 2017.

- Information/photos will be posted on the Northern Pulse Growers Association website

- Banners were created with photos to use at food ingredient/manufacturer trade shows & events to promote the utilization of pulses

Development of Low Glycemic Index Products Using Pulse Ingredients

Technical Report

Prepared by

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I. Project objectives

1) Develop new low GI products with pulse flours including pasta, cookies, muffins, bread, crackers, quick bread, and extruded snack and snack bars.
2) Provide in vivo evidence showing the benefit of pulse flour to glycemic response.
3) Promote the benefit of pulse flours to the food industry.

II. Project background

Pulses are valuable crops for the region's agriculture and are primarily grown in rotation with wheat and other cereal crops. Pulses provide producers with rotational benefits such as increasing yields and breaking disease cycles commonly found in cereal grains. North Dakota and Montana comprise the largest pulse producing region in the United States and currently account for approximately 71% of the pulse crop production. Pulses are well recognized by health organizations globally and are recommended as part of healthy eating.

Pulse ingredients, namely pulse flour, protein, starch, and fibers, have established itself in the market due to the nutritional value, clean-label, low-allergenicity, and wide range of product applications. Among the pulse ingredients, pulse flour ranked highest in terms of usage (Mintel GNPD, 2013). It is used in thirteen different food categories including snacks (33%), followed by bakery (21%), soup (14%), meat substitute (10%), baby food (8%), dessert and ice cream (4%), sauces and seasonings (3%), and pasta and noodles (3%) (Mintel GNPD, 2013). Even though the usage of pulse is growing, we still see challenges in promoting nutritious pulses to United States and international consumers.

Cooked whole pulse is known for their nutritional values and low glycemic response. The glycemic index (GI) is a measure of the power of foods to raise blood sugar (glucose) levels after being eaten. Low GI foods have been associated with decreased risk of type 2 diabetes, obesity, cardiovascular disease metabolic syndrome, stroke, and others. Testing GI in food is expensive. It is conducted by feeding healthy people a portion of the food and then measuring the effect on their blood glucose levels over time (in vivo test). In vitro test, or test tube experiments, is a less expensive test, however can only be used to estimate the glycemic responses and the results cannot be used on the food labeling claimed in many countries. It is important to note that GI cannot be guessed by understanding the composition of the food. The nutritional values and GI of pulse flours when incorporating into food products, on the other hand, is not well understood. The biggest challenge when promoting pulse flours for its nutritional values is the lack of evidence to support the claims. This project will help the pulse industry to overcome this challenge by promoting new product development of pulse based food products, and by providing strong evidences on the GI benefits to the food industry.

III. Glycemic index

The concept of GI was introduced by Jenkins et al. (1981) to classify food based on blood glucose response upon consumption of food (Goni et al. 1997). The method to determine the GI calculates the incremental glycemic area upon consumption of a meal expressed as a percentage corresponding white bread or glucose as a reference food. Due to its high cost and time it takes to conduct a test using human subjects, determination of GI in vitro has developed. A few studies investigate the correlation between in vivo and in vitro GI methods and good correlations

between in vivo result and reference GI as well as in vitro result and reference GI were found (Goni et al. 1997).
Factors known to affect rate of digestions include particle size, heating and chewing, processing methods and conditions, product composition and nature of food. In addition to that, in vitro analysis is largely affected by the methodology and enzymes used, and since there are no standard methods established, these methodological variations largely differentiate the numbers reported in literatures. Composition of starch and presence of dietary fiber greatly affect the rate of digestion and legumes are known to slow down the rate of digestions. Numbers of researches conducted investigating the background of its effect. Physical form of legumes were found to affect the slow digestion process as fine lentil powder increased the rate by 7-fold (Wong et al. 1985). Presence of high fat was proved to reduce glucose response but not insulin response, hypothesizing the fat delays absorption of blood glucose thus gastric emptying (Collier et al. 1983). Ratio of amylose and amylopectin is considered to take a larger role in rate of digestion. Legumes contain relatively high amylose content than cereals and it contributes to the slower digestion as larger surface area of amylopectin accelerate the enzyme digestion (Thorne et al. 1983). State of starch is equally important as disruption of starch cell wall and granule structure by cooking would increase the availability of starch to enzyme digestion (Thorne et al. 1983). In addition, presence of anti-nutrient, such as enzyme inhibitors and phytates, which often found in legumes, decreases its digestibility as well. Starch-protein interaction can affect the rate as tighter interaction tends to inhibit enzyme access. Furthermore, gelatinization of starch accelerates the digestion rate therefore the glycemic response.

IV. Product development

1. Pulse Ingredients

Pulse materials used and its suppliers are summarized in Table 1. Four types of pulse were chosen as a raw material which are 1) red lentil, 2) green (yellow) lentil, 3) yellow pea and 4) green pea. Note that green lentil flour used in this part was decorticated which appears as yellow lentil flour. Ingredients used in the project include a) raw flour, b) pre-gelatinized flour, c) pulse flakes, d) pea protein and e) pea fiber, and the ingredients were supplied and purchased by i) AGT foods (Regina, SK, Canada), ii) Harvest innovations (Indianola, IA), iii) Inland empire foods (Riverside, CA) and iv) Roquette corp. (Lestrem, France).

Table 1. Pulse ingredients and suppliers

Ingredient	Supplier
Décor green lentil flour (raw)	AGT
Décor Red lentil (raw)	AGT
Green pea flour (raw)	AGT
Yellow pea flour (raw)	AGT
Pea Protein concentrate	AGT
Pea fiber (bran)	AGT
Pre-gel Yellow pea	Harvest Innovations
Pre-gel red lentil	Harvest Innovations
Green pea flakes	Inland Empire
Yellow pea flakes	Inland Empire

Pea protein isolate	Roquette

2. Products Formulations

Seven categories of product developed were pasta, extruded snacks, bread, crackers, cookies, bars and muffins. A total of 94 samples were developed and tested for *in vitro* GI, total starch (TS) and resistant starch (RS). A list of samples along with its GI, TS and RS are shown in Table 10 and Table 11, respectively.

Pasta

Macaroni shape pasta with 14 different formulations were processed. The formulations included pulse flour at four different levels (30, 50, 80 and 100%) blended with semolina and pea protein and pea fiber added to semolina. In addition, gluten free (GF) pasta was developed. Pasta quality was tested including optimum and overcook cook time, percent weight gain, cook loss and firmness (Table 2). Overcook pasta was tested by adding five minutes to optimum cooking time.

Pasta cooking and test procedure

Cooking test was conducted by adding 25 g of pasta sample into 300 ml of boiling distilled water. Pasta was stirred 10 seconds at 0, 1, 3, 5, 7, 9… minutes until achieving optimal cook time. The sample was poured into the funnel and rinsed with distilled water for 30 seconds then drained for 60 seconds and cooked weight was recorded.

Weight gain was calculated as $\frac{(Final\ weight - initial\ weight)}{Final\ weight} x100$. Cooked pasta was tested for firmness using a texture analyzer (TA-XTplus, Stable Micro System, Godalming, UK). Cook loss was determined according to AACC method 66-50.

Table 2. Cook time, weight gain, cook loss and firmness of pasta products

Product	Cook time (min)	Weight gain (%)	Cook loss (%)	Firmness (g)
Optimum				
Control semolina 100%	5.5	124	4.3	559
Semolina: RL 70:30	5.0	122	4.7	755
Semolina: GL 70:30	5.0	119	5.0	679
Semolina: YP 70:30	5.0	120	5.1	635
High protein (semolina 90: PP 10)	5.25	117	6.8	700
High fiber (semolina 90: PF 10)	5.0	119	5.3	497
GF control pre-gel rice and corn	4.0	87	10.1	761
GF GL : Xanthan gum 99:1	3.5	108	8.9	443
GF Pregel Rice: Corn: YP 35:35:30	4.5	74	21.1	342
GF high protein	3.5	72	20.3	284
Semolina: RL 50:50	5.5	122	7.2	655
Semolina: RL 20:80	4.5	119	7.2	595
Semolina: YP 50:50	5.0	118	7.1	631
Semolina: YP 20:80	4.5	119	7.4	539

Overcook				
Control semolina 100%	10.5	185	6.5	410
Semolina: RL 70:30	10.0	174	7.1	530
Semolina: GL 70:30	10.0	182	7.4	510
Semolina: YP 70:30	10.0	179	7.8	470
High protein (semolina 90:PP 10)	9.25	171	10.1	554
High fiber (semolina 90:PF 10)	10.0	173	13.2	362
GF control pregel rice and corn	9.0	119	22.5	163
GF GL : Xanthan gum 99:1	8.5	161	15.4	292
GF Pregel Rice: Corn: YP 35:35:30	9.5	78	40.7	101
GF high protein	8.5	84	45.3	68
Semolina: RL 50:50	10.5	179	10.1	424
Semolina: RL 20:80	9.5	169	11.0	461
Semolina: YP 50:50	10.0	181	10.8	442
Semolina: YP 20:80	9.5	182	10.5	375

YP - Yellow pea, GP - Green pea, GL - Green lentil, RL - Red lentil, PP - Pea protein, PF - Pea fiber, GF - Gluten free

The result indicated that adding pulse flour to semolina improved texture and cooking property. Addition of protein at 10 % improved firmness compared to 100 % semolina even overcooked. GF products in general had a higher cook loss and lower firmness. 100 % green lentil and 1 % xanthan gum had relatively lower cook loss and higher firmness compared to the rest of gluten free products.

Extruded snacks

Multigrain chips

Formulations of multigrain chips are shown on Table 3. Extrusion was conducted with a pilot scale Wenger TX-52 twin screw extruder (Wenger Manufacturing, Sebath, KS). Extruded product was collected then deep fried with vegetable oil for 1 minute each side. The image shows the extruded product before frying (Figure 1).

Table 3. Multigrain chips formulation of control, yellow pea and green pea (%)

Ingredient	Multigrain chips control	Multigrain chips YP	Multigrain chips GL
Corn flour	40	29	29
Rice flour	24	17.4	17.4
Oat flour	15	10.9	10.9
Whole wheat flour	12	8.7	8.7
Sugar	5	5	5
Pregel starch	4	4	4
Split yellow pea flour	0	25	0
Décor green lentil flour	0	0	25
Total	100	100	100

Figure 1. Multigrain chips extrude before frying

Curls and puffs

Formulations of curl and puff products are shown in Table 4. In addition to pulse and corn flour combination, addition of pea protein and fiber were tested at three different levels; high at 30 %, medium at 20 % and low at 10 % and all contains 5 % of pea fiber. Also different types of pulse including decorticated green lentil, red lentil and green pea flour were tested. Immediately after collecting, the products were placed in an impingement oven and roasted for 1 minutes at 350 F.

Table 4. Curl and puff products formulation (%)

	Curl Control	Curl: GL (Med)	Curl : GL (High)	GL puff: control	GL Puff: PP&PF (High)	GL Puff: PP&PF (Med)	GL Puff: PP&PF (Low)	RL Puff: PP&PF	GP Puff: PP&PF
Corn cones	99.5	49.5	24.5	99.5	29.5	34.5	40.7	34.5	34.5
Décor Green lentil	0	50	75	0	35	40	45.3	0	0
Décor Red lentil	0	0	0	0	0	0	0	40	0
Green pea	0	0	0	0	0	0	0	0	40
Pea protein	0	0	0	0	30	20	9	20	20
Pea fiber (bran)	0	0	0	0	5	5	4.5	5	5
Calcium carbonate	0.5	0.5	0.5	0.5	0.5	0.5	0.45	0.5	0.5
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

YP - Yellow pea, GP - Green pea, GL - Green lentil, RL - Red lentil, PP - Pea protein, PF - Pea

Bread

Focaccia bread

Focaccia bread, GF focaccia bread, white pan bread and whole wheat pan bread with pea protein and fiber were formulated and tested. A summary of product formulation is shown in Table 5.

Table 5. Focaccia bread formulation of control, yellow pea and green lentil in gram and bakers percent

	Control		YP 30 %		GL 30 %	
Ingredient	Gram	%	Gram	%	Gram	%
Flour	700.0	51.3	490.0	36.2	490.0	36.2
Pea flour	0.0	0.0	210.0	15.5	0.0	0.0
Lentil flour	0.0	0.0	0.0	0.0	210.0	15.5
Water	478.5	35.1	469.0	34.6	469.0	34.6
Olive oil	109.4	8.0	109.4	8.1	109.4	8.1
Instant Yeast	16.4	1.2	16.4	1.2	16.4	1.2
Salt	16.4	1.2	16.4	1.2	16.4	1.2
Garlic powder	4.8	0.4	4.8	0.4	4.8	0.4
Black pepper, fine	2.5	0.2	2.5	0.2	2.5	0.2
Dried herb	1.5	0.1	1.5	0.1	1.5	0.1
Olive oil (topping)	30.0	2.2	30.0	2.2	30.0	2.2
Sea salt fine	5.0	0.4	5.0	0.4	5.0	0.4
Total	1364	100	1355	100	1355	100

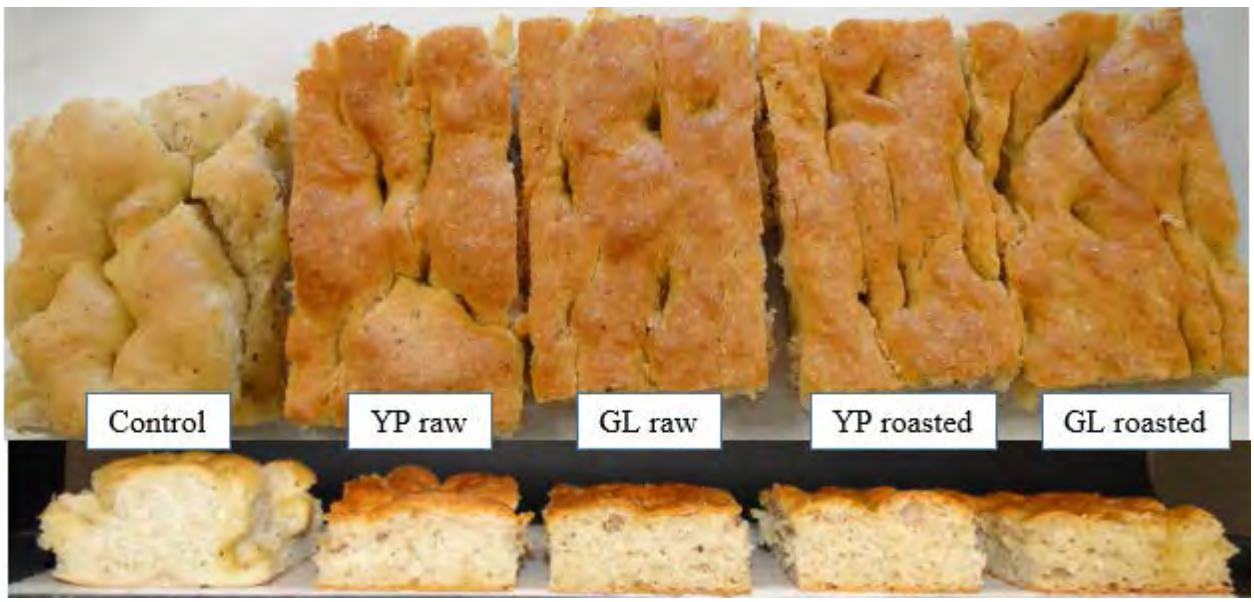

Figure 2: Image of focaccia control, yellow pea and green lentil raw flour, yellow pea and green lentil roasted flour.

White pan bread and whole wheat bread

White pan bread and whole wheat bread were developed and the formulation is shown in Table 6 and 7, respectively. 4 % and 2 % of pea protein and pea fiber were used in whole wheat bread.

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Table 6. White pan bread formulation of control, yellow pea and green lentil in gram and percent

	Control		Yellow Pea		Green Lentil	
	gram	%	gram	%	gram	%
Flour	800	55.2	640	43.5	640	44.2
Yellow pea flour	0	0.0	160	10.9	0	0.0
Green lentil flour	0	0.0	0	0.0	160	11.0
Shortening	48	3.3	48	3.3	48	3.3
Salt	12	0.8	12	0.8	12	0.8
Sugar	48	3.3	48	3.3	48	3.3
Emplex	4	0.3	4	0.3	4	0.3
Yeast	16	1.1	16	1.1	16	1.1
Water	520	35.9	544	37.0	520	35.9
Total	**1448**	**100**	**1472**	**100**	**1448**	**100**

Table 7. Whole wheat formulation of control and pea protein and pea fiber fortified in gram and percent

	Control		PP&PF	
Ingredients	**Gram**	**%**	**Gram**	**%**
Whole wheat flour	800	48.9	800	47.1
Pea protein	0	0.0	32	1.9
Pea fiber	0	0.0	16	0.9
Vital wheat gluten	40	2.4	40	2.4
Yeast	24	1.5	24	1.4
Salt	16	1.0	16	0.9
Light brown sugar	80	4.9	80	4.7
Granulated sugar	40	2.4	40	2.4
Dependox	3.2	0.2	3.2	0.2
Canola oil	24	1.5	24	1.4
Vinegar	8	0.5	8	0.5
Honey	32	2.0	32	1.9
Water	568	34.7	584	34.4
Total	1635	100	1699	100

Crackers

Pita chips, seedy crisps, wheat cracker and GF cracker were developed. Yellow pea flour was used in pita chips and pea protein isolate was used in seedy crisps and wheat crackers. Formulation are shown in Table 8 and Table 9.

Table 8. Formulation of pita chips, seedy crisps and wheat cracker in gram and percent

	Control		Pulse variant	
Pita bread	g	%	g	%
Flour (50:50 mix of all purpose and pastry flour)	700	60.2	560	48.2
Yellow pea flour	0	0.0	140	12.0
Instant yeast	3.5	0.3	3.5	0.3
Salt	10.5	0.9	10.5	0.9
water	420	36.1	420	36.1
Shortening	28	2.4	28	2.4
Ascorbic acid	0.7	0.1	0.7	0.1
Total	1162.7	100.0	1162.7	100.0
Seedy crisp	g	%	g	%
Whole wheat flour	142	23.8	114	18.0
All-purpose flour	135	22.7	107	16.9
Pea protein isolate	0	0.0	55	8.7
Poppy seed	40	6.7	40	6.3
Sesame seed	50	8.4	50	7.9
Salt	8	1.3	8	1.3
Garlic powder	2	0.3	2	0.3
Baking soda	6	1.0	6	0.9
Olive oil	43	7.2	43	6.8
Water	170	28.5	210	33.1
Total	596	100	635	100
Wheat cracker	g	%	g	%
Whole wheat flour	142	53.0	100	32.5
Pea protein isolate	0	0.0	42	13.6
Agave	40	14.9	40	13.0
Canola oil	20	7.5	20	6.5
Salt	3	1.1	3	1.0
Baking soda	3	1.1	3	1.0
Water	60	22.4	100	32.5
Total	268	100	308	100

Table 9. Formulation of gluten free cracker in grams and percent

	g	%
Corn flour	30	11.5
Corn starch	16	6.1
Potato starch	18	6.9
Lentil flour	80	30.6
Minced dry onion	0.7	0.3
Salt	3	1.1
Italian seasoning	0.07	0.0
Water	60	22.9
Oil	28	10.7
Chopped sunflower seed	16	6.1
Sesame seed	10	3.8
TOTAL	261.77	100

Cookies

Oatmeal and shortbread cookie formulas were developed. Pulse flours replaced 100 % of the pastry flour in oatmeal cookies and up to 50 % of the pastry flour in chocolate and vanilla shortbread formulas. Flavor and texture were compare to the controls. Average height and diameter (of 5 cookies) were recorded to evaluate spread.

Oatmeal Cookies

All oatmeal variants had a good flavor and were comparable to the control in flavor and texture. Variants generally browned more and had more surface cracks than the control. Cookies made with GP and RL flours had less spread than the control. Formulations and spread data are shown in Tables 10 and 11.

Vanilla Shortbread Cookie

RL and GL flours substituted up to 40 and 45 % of the pastry flours respectively generated the best tasting cookie. Spices were increased in these variants to cover slight beany notes. Beany notes tended to dissipate over 3-5 days. GL variant with added pea protein (PP) and pea fiber (PF) had the most off flavor initially however after 5 days these notes dissipated. All variants spread less than the control. The RL variant had texture closest to the control. The GL variant with added pea protein and bran was slightly harder than the control and other variants. Formulations are shown in table 12.

Chocolate Shortbread Cookie

Because of their flavor and color, YP and GP flours were better suited in a chocolate formula. These flours could be substituted up to 35 % of the pastry flour. Beany notes were evident and stronger than in the vanilla but dissipated with time as in the vanilla cookie. This formula could also accommodate more GL flour (50 % of pastry flour), pea protein and pea fiber without effecting flavor. As in the vanilla shortbread cookie, all pulse variants spread slightly less. Textures were similar to the control except for the GL variant was a bit harder. Formulations are shown in table 13.

Table 10. Control and variant oatmeal cookie formulations

	Control		Pulse Variant	
	g	%	g	%
Unsalted butter	57.00	9.51	57.00	9.51
Vegetable shortening	50.00	8.34	50.00	8.34
Light brown sugar	106.00	17.69	106.00	17.69
sugar	50.00	8.34	50.00	8.34
Vanilla	4.00	0.67	4.00	0.67
Salt	3.25	0.54	3.25	0.54
White vinegar	4.00	0.67	4.00	0.67
Cinnamon	3.20	0.53	3.20	0.53
Nutmeg	0.32	0.05	0.32	0.05
Whole egg	57.00	9.51	57.00	9.51
Baking soda	2.60	0.43	2.60	0.43
Rolled oats	142.00	23.69	142.00	23.69
Pastry flour	120.00	20.02	0.00	0.00
Pulse flour*	0.00	0.00	120.00	20.02
Total	599.37	100.00	599.37	100.00

*Flours evaluated: GL, RL, GP and YP at 100 % replacement of pastry flour.

Table 11. Oatmeal cookies spread sheet result

cookie variant	Height	Length
	cm	cm
Oatmeal Control	6.1	33.8
Oatmeal with RL	6.8	34.0
Oatmeal with GL	6.9	35.5
Oatmeal with GP	7.7	32.6
Oatmeal with YP	7.0	35.5

Data is expressed as an average measure of 5 cookies.

Table 12. Vanilla shortbread formulation

	Control		RL (40 % sub)		GL (45% sub)		GL (45 % sub)+ PB& PP	
	g	%	g	%	g	%	g	%
Unsalted Butter	227.00	33.69	113.50	33.81	113.50	33.81	113.50	33.58
Salt	6.75	1.00	3.38	1.01	3.38	1.01	3.38	1.00
Sugar	149.00	22.12	74.50	22.19	74.50	22.19	74.50	22.04
Vanilla	5.00	0.74	2.50	0.74	2.50	0.74	2.50	0.74
Pastry flour	283.75	42.12	82.60	24.61	75.60	22.52	70.88	20.97
Pulse flour	0.00	0.00	57.00	16.98	64.00	19.07	64.00	18.94
Pea bran (PB)	0.00	0.00	0.00	0.00	0.00	0.00	5.00	1.48
Pea protein (PP)	0.00	0.00	0.00	0.00	0.00	0.00	2.00	0.59
Cinnamon	1.50	0.22	1.50	0.45	1.50	0.45	1.50	0.44
Nutmeg	0.50	0.07	0.50	0.15	0.50	0.15	0.50	0.15
Cloves	0.20	0.03	0.20	0.06	0.20	0.06	0.20	0.06
Total	673.70	100.00	335.68	100.00	335.68	100.00	337.96	100.00
Height of 5 cookies in cm	4.00		5.50		5.20		5.20	
Length of 5 cookies in cm	36.50		34.50		34.70		35.70	
Bake time (minutes)	20.00		22.00		22.00		22.00	

Table 13. Chocolate shortbread formulations

	Control		YP (35% sub)		GP(35 % sub)		GL (50% sub) + PB PP	
	g	%	g	%	g	%	g	%
Unsalted butter	113.50	33.71	113.50	33.61	113.50	33.61	113.50	34.63
Salt	3.38	1.00	3.38	1.00	3.38	1.00	3.38	1.03
Sugar	99.32	29.49	99.32	29.41	99.32	29.41	99.32	30.30
Vanilla	2.50	0.74	2.50	0.74	2.50	0.74	2.50	0.76
Dutched cocoa powder	14.20	4.20	14.20	4.20	14.20	4.20	14.20	4.33
Pulse flour	0.00	0.00	36.50	10.81	36.50	10.81	36.50	11.14
Baking Powder	1.95	0.29	1.95	0.58	1.95	0.58	1.95	0.59
Pastry Flour	102.88	30.55	66.38	19.65	66.38	19.65	51.40	15.68
Pea Bran (PB)	0.00	0.00	0.00	0.00	0.00	0.00	3.00	0.92
Pea Protein (PP)	0.00	0.00	0.00	0.00	0.00	0.00	2.00	0.61
Total	337.73	100.00	337.73	100.00	337.73	100.00	327.75	100.00
Height of 5 cookies in cm	4.00		5.30		4.80		5.20	
Length of 5 cookies in cm	39.50		36.50		36.30		35.70	
Bake time (minutes)	35.00		30.00		30.00		22.00	

Bars

Pulse flours, puffs (table 4) and/or pulse flakes were evaluated in five formulations shown in tables 14-18. In the baked granola bars pastry flour and rice crispy were replaced with pulse flours and pulse puffs then baked at 350 F for 9-10 minutes.

After cooling bars were cut into 35 mm by 110 mm rectangles and bottomed with melted cinnamon chips. All variants had similar final characteristics flavor to the control.

Table 14. Finished baked granola bar formulas with cinnamon chip coating on bottom.

	Control		YL		RL		GP	
	g	%	g	%	g	%	g	%
Coconut oil	60.00	7.45	60.00	7.45	60.00	7.45	60.00	7.45
Agave Syrup	123.50	15.34	123.50	15.34	123.50	15.34	123.50	15.34
Sugar	70.00	8.69	70.00	8.69	70.00	8.69	70.00	8.69
Vanilla	5.00	0.62	5.00	0.62	5.00	0.62	5.00	0.62
Cinnamon	4.00	0.50	4.00	0.50	4.00	0.50	4.00	0.50
Rice Crispies	35.00	4.35	0.00	0.00	0.00	0.00	0.00	0.00
Pulse Puff*								
GL Puff PP + PF High	0.00	0.00	35.00	4.35	0.00	0.00	0.00	0.00
RL Puff PP+ PF	0.00	0.00	0.00	0.00	35.00	4.35	0.00	0.00
GP Puff PP + PF	0.00	0.00	0.00	0.00	0.00	0.00	35.00	4.35
Grape nuts cereal	35.00	4.35	35.00	4.35	35.00	4.35	35.00	4.35
Pastry flour	70.00	8.69	0.00	0.00	0.00	0.00	0.00	0.00
Pulse flour	0.00	0.00	70.00	8.69	70.00	8.69	70.00	8.69
Sunflower Seeds	42.00	5.21	42.00	5.21	42.00	5.21	42.00	5.21
rolled oats	160.00	19.86	160.00	19.86	160.00	19.86	160.00	19.86
cinnamon chips	201.00	24.95	201.00	24.95	201.00	24.95	201.00	24.95
Total	805.50	100.00	805.50	100.00	805.50	100.00	805.50	100.00

*See table 4.

Two formulations of peanut butter crispy treats were made using the pulse puffs. In the syrup base formula, a peanut butter syrup was prepared and folded into the puffs in a 70:30 ratio respectively. The resulting mixture was slabbed to ½ inch. Upon cooling bars were cut into 35 mm by 110 mm rectangles and bottomed with a chocolate coating. Flavor of the variant was good however the slab did not pack similar to the control as the extruded puff was more uniform and rounded than the rice crispy used in the control. To get better packing upon slabbing a mixture of puffs and flake were used in the fat based formula. Bars were made in a similar fashion except melted peanut butter chips were used as the binder.

Chocolate chewy bars were made by cooking all ingredients except the flour to 238 F. Syrup was then cooled to 185 F and the flour was stirred in. The mixture was slabbed to 3/8 inch. Upon cooling bars were cut into rectangles 30 mm by 110 mm. Grassy flavors were more evident in the YL+PB variant however these grassy flavors became stronger for both variants

once the bars became softer and crystallized. Green lentil flour was substituted for pastry flour in the peanut butter center for the peanutty cups. All ingredients were blended together in a dough. The dough was then rolled into 1" balls and dipped in a chocolate coating. Grassy flavor was evident in the variant.

Table 15. Finished peanut butter crispy treat formulations (syrup based)

	Control		Variant	
	g	%	g	%
Water	12.50	2.07	12.50	2.07
Sugar	34.96	5.79	34.96	5.79
Vegetable glycerine	26.21	4.34	26.21	4.34
Corn syrup	101.33	16.77	101.33	16.77
Peanut butter	114.69	18.98	114.69	18.98
Salt	4.06	0.67	4.06	0.67
Canola Oil	6.25	1.03	6.25	1.03
Rice Crispies	123.00	20.35	0.00	0.00
Pulse Puff*	0.00	0.00	123.00	20.35
Chocolate Coating	181.29	30.00	181.29	30.00
Total	604.29	100.00	604.29	100.00

*Pulse Puff used: GL PP&PF High, RL PP%PF, GP PP&PF (see Table 4).

Table 16. Finished peanut butter crispy treat formulations (fat based)

	Control		Variant 1		Variant 2		Variant 3	
	g	%	g	%	g	%	g	%
Peanut butter chips	250.00	55.94	250.0	55.94	250.0	55.94	250.0	55.94
Paramount flakes	7.50	1.68	7.50	1.68	7.50	1.68	7.50	1.68
Rice crispies	75.00	16.78	0.00	0.00	0.00	0.00	0.00	0.00
Pulse Crisp*								
GL Puff PP&PF High	0.00	0.00	75.00	16.78	0.00	0.00	0.00	0.00
RL Puff PP&PF	0.00	0.00	0.00	0.00	75.00	16.78	0.00	0.00
GP Puff PP&PF	0.00	0.00	0.00	0.00	0.00	0.00	75.00	16.78
Corn flake	25.00	5.59	0.00	0.00	0.00	0.00	0.00	0.00
Green pea flake	0.00	0.00	0.00	0.00	0.00	0.00	25.00	5.59
Green lentil flake	0.00	0.00	25.00	5.59	25.00	5.59	0.00	0.00
Compound coating (choc flavored)	89.38	20.00	89.38	20.00	89.38	20.00	89.38	20.00
Total	446.88	100.00	446.88	100.00	446.88	100.00	446.88	100.00

Table 17. Finished chocolate chewy bar formulations

	Control		YL		YL + PB	
	g	%	g	%	g	%
Sugar	205.00	29.73	205.00	29.73	205.00	29.73
Light corn syrup	195.73	28.39	195.73	28.39	195.73	28.39
Heavy whipping cream solids	97.28	14.10	97.28	14.10	97.28	14.10
Dark chocolate (72%)	70.00	10.15	70.00	10.15	70.00	10.15
Butter	15.00	2.18	15.00	2.18	15.00	2.18
Pastry flour	100.00	14.50	0.00	0.00	0.00	0.00
Yellow lentil flour	0.00	0.00	100.00	14.50	90.00	13.05
Cocoa powder	6.50	0.94	6.50	0.94	6.50	0.94
Pea bran	0.00	0.00	0.00	0.00	10.00	1.45
Total	689.50	100.00	689.50	100.00	689.50	100.00

Table 18. Peanutty cup formulation

	Control		Green lentil	
	g	%	g	%
Peanut butter	128.20	35.36	128.20	35.36
Powdered sugar	76.93	21.22	76.93	21.22
Pastry flour	30.78	8.49	0.00	0.00
Pulse flour	0.00	0.00	30.78	8.49
Butter	12.83	3.54	12.83	3.54
Salt	5.05	1.39	5.05	1.39
Chocolate coating	108.76	30.00	108.76	30.00
Total	362.55	100.00	362.55	100.00

Muffins

All pulse flours were evaluated at levels up to 50 % of the pastry flour in a standard one egg muffin formula. Good muffins comparable to the control in crumb, color and volume were made at the levels shown in Table 19. Adjustments made to the pulse formula included increasing leaving by 15 %, reducing baking time by 10 % and adding spices to mask grassy, beany flavor notes. Adding spices was successful in making flavors in GL, GP and RL variants however these notes were still evident in the YP variant. Use of a roasted YP flour did not reduce these flavor notes.

Table 19. Muffin formulations

	Control		RL		YL		GL		GP	
	g	%	g	%	g	%	g	%	g	%
Pastry flour	314.0	36.73	188.0	21.95	188.0	21.95	204.0	23.82	204.0	23.82
Pulse flour	0.00	0.00	126.0	14.71	126.0	14.71	110.0	12.84	110.0	12.84
Sugar	109.0	12.75	109.0	12.73	109.0	12.73	109.0	12.73	109.0	12.73
Salt	3.50	0.41	3.50	0.41	3.50	0.41	3.50	0.41	3.50	0.41
Baking powder	13.40	1.57	15.00	1.75	15.00	1.75	15.00	1.75	15.00	1.75
Whole milk	246.0	28.78	246.0	28.72	246.0	28.72	246.0	28.72	246.0	28.72
Vegetable oil	48.60	5.69	48.60	5.67	48.60	5.67	48.60	5.67	48.60	5.67
Whole egg	116.0	13.57	116.0	13.54	116.0	13.54	116.0	13.54	116.0	13.54
Cinnamon	2.60	0.30	2.60	0.30	2.60	0.30	2.60	0.30	2.60	0.30
Nutmeg	1.25	0.15	1.25	0.15	1.25	0.15	1.25	0.15	1.25	0.15
Cloves	0.50	0.06	0.50	0.06	0.50	0.06	0.50	0.06	0.50	0.06
Total	854.9	100.0	856.4	100.0	856.5	100.0	856.5	100.0	856.5	100.0

V. In vitro testing

All 94 samples developed were analyzed for *in vitro* GI. Result summary is shown in Table 20 along with standard deviations (SD) and coefficient of deviations (CV).

1. Analytical method

Sample preparation

Sample was prepared by freezing immediately after cooling followed by freeze drying using a laboratory freeze dryer (FreeZone, Labconco, Kansas City, MO). Then samples were milled into a particle size through 0.5 mm mesh. Pasta samples were boiled to an optimum cooking time and then freeze dried. Samples with low moisture content including crackers, extruded snacks, cookies and bar samples omitted freeze drying process and directly milled into powder using a coffee grinder.

Estimated GI assay

eGI assay was conducted using 300 mg of ground sample obtained by passing through a 5 mm screen. Enzyme solution containing 9 ml, 6 ml and 81 ml of amyloglycosdiase (140 AGU/ml; Megazyme, Brey, Ireland), Ivertase (≥ 300 unites/mg; Sigma Aldrich, St. Louis, MO) and Pancreatin (8x USP; Sigma Aldrich, St. Louis, MO), respectively, was added to samples. The solution was prepared fresh daily and used immediately. Acetic acid buffer (pH 5.2) containing 4.1 g sodium acetate, 125 ml benzoic acid (0.5 g/100 ml) and 2 ml 1M calcium chloride was prepared. The buffer (20 ml) was added to a test tube containing sample (300 mg), guar gum (50 mg) (Sigma Aldrich, St. Louis, MO), five beads (1 cm diameter) and the enzyme solution mixture (5 ml). The mixture was incubated in a shaking water bath (200 strokes/min) (VWR International; Radnor, PA) for 180 minutes at 37 °C. A sample was taken every 20 minutes into absolute ethanol (5 ml) and centrifuged. Supernatant (0.1 ml) was placed in a tube and GOPOD

solution (3 ml) was added for glucose determination. The sample was incubated in a 50 ºC water bath for 20 minutes and color development was read in spectrophotometer against a blank at 510 nm. Glucose standard curve was prepared from glucose solution at 6 different concentration levels (0.1, 0.2, 0.5, 1.0, 2.0 and 5.0 mg/ml) to obtain the equation and percent glucose released was calculated by plugging the absorbance into the equation. From that, area was obtained and hydrolysis index (HI) was calculated comparing the area under hydrolysis curve (AUC) with the AUC of white bread. The eGI was then calculated using the following equations:

$$AUC = \{C_\infty (t_f - t_0) - (\frac{C_\infty}{k}) [1 - exp [-k (t_f - t_0)]]\}$$

Where C_∞ = equilibrium % of starch hydrolysis after X min, t_f = final time, t_0 = initial time and k = kinetic constant.

$$Hydrolysis\ Index\ (HI) = \frac{AUC}{AUC\ with\ white\ bread\ control}$$

$$eGI = 8.198 + (0.862)\ HI$$

Starch analysis

Total starch assay

Total Starch (TS) and Resistant Starch (RS) assay kits were purchased by Megazyme International (Brey, Ireland). Ethanol (0.2 ml) was added to samples (100 mg) in a glass test tube and α-amylase (3 ml) was added followed by incubation in a boiling water bath for 6 minutes. Then, amyloglucosidase (0.1 ml) was added and incubated for 30 minutes in a 50 ºC water bath. The solution was diluted to 100 ml with distilled water and centrifuged at 1500 g for 10 minutes (Multifuge X1R, Thermo Scientific, Waltham, MA). Glucose Oxidase-Peroxidase (GOPOD) reagent (3 ml) was added to the supernatant (0.1 ml) and incubated for 20 minutes at 50 ºC. D-glucose was used as a standard and analyzed along with blank. Spectrophotometer (GENESIS 10s UV-Vis, Thermo Scientific, Walthama, WA) was used to determine the color development by reading the absorbance against a blank at 510 nm. TS was calculated using an equation as follows:

$$TS\ (\%) = \Delta A \times F \times \frac{FV}{0.1} \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180}$$

Where ΔA = Absorbance, F = $\frac{100\ (\mu g\ of\ D-glucose-)}{absorbance\ of\ 100\ \mu g\ of\ glucose}$, FV = final volume, 0.1 = volume of sample analyzed, $\frac{1}{1000}$ = conversion from μg to mg, $\frac{100}{W}$ = Factors to express starch as a percentage of flour weight, W = dry sample weight in mg, $\frac{162}{180}$ = factors to covert from free D-glucose to anhydro D-glucose.

Resistant starch assay

Enzyme solution containing α-amylase (3 Ceralpha Units/mg) (1 g), amyloglucosidase (300 U/ml) (1 ml) and sodium maleate buffer (100 ml) was prepared. Bread sample (100 mg) and enzyme solution (4 ml) were placed in glass test tubes. Then the tubes were incubated in shaking water bath (200 strokes/min) at 37 ºC for 16 hours. After 16 hours, ethanol (4 ml) was added to the solution followed by centrifugation at 1500 g for 10 minutes. The pellet was removed and re-suspended in ethanol (8 ml) followed by centrifugation at 1500 g for 10 minutes. This procedure was repeated twice. The pellet was suspended in potassium hydroxide solution (2 ml) and stirred

with a magnetic stirrer bar in ice water bath for 20 minutes. Then, sodium acetate buffer (8 ml) and amyloglucosidase (0.1 ml) were added to the solution and incubated for 30 minutes at 50 ºC water bath. GOPOD reagent (3 ml) was added to supernatant (0.1 ml) and incubated for 20 minutes at 50 ºC water bath and absorbance was read against a blank at 510 nm.
Resistant starch was calculated using the equation as follows;

$$RS = \Delta E \times F \times \frac{100}{0.1} \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180}$$

Where ΔE = Absorbance, F = conversion from absorbance to micrograms, $\frac{100}{0.1}$ = volume correction, $\frac{1}{100}$ = conversion from μg to mg, W = dry sample weight in mg, $\frac{100}{W}$ = factor to present RS as a percentage of sample weight, $\frac{162}{180}$ = factor to convert from free D-glucose to anhydro D-glucose.

Raw Ingredients

Raw materials including raw flour, pregelatinized flour and starch were tested for *in vitro* GI, TS and RS. The result suggested that pregelatinized flour has slightly lower GI than the raw flour. Comparing pea and lentil flour, lentil flour appeared to have lower GI than pea flour. Lentil tends to have the higher dietary fiber than peas even though their starch content is similar. The dietary fiber affects the digestibility of starch therefore GI. When the flour is incorporated into the product, however, there was no consistency in the result indicating lentil flour is lower GI than pea flour. The variability probably came from different processing methods used and food system. Amount of starch greatly affects the *in vitro* GI as pea starch exhibited extremely high GI (Table 21).

Bread

Comparing different types of bread, focaccia bread showed the lowest GI followed by whole wheat bread and white pan bread. This was unexpected as whole wheat contained higher dietary fiber than the focaccia which was made with refined flour. In focaccia bread, difference between raw pulse flour and roasted pulse flour were tested. As shown in Figure 2, there was not much difference between two in terms of appearance and *in vitro* GI. However, there was a noticeable difference in the dough and flavor as the raw flour made the dough much stickier and stronger flavor even after baked. Both white pan bread and whole wheat bread did not show noticeable difference between control and pulse treatments indicating 20 % pulse flour was acceptable and it did not provide negative attributes. Pea protein did not have a strong flavor when used in dough system.

Pasta

All pasta *in vitro* GI result showed unusually high GI values except a GF pregel rice: corn: YP blend. This was completely unexpected because pasta products are in general known as low GI. According to International table of GI and GL, all the cooked macaroni products listed had lower than GI 70 and most of the spaghetti products had similar or lower GI.

Crackers

Crackers generally had lower *in vitro* GI possibly due to a lower moisture content allowing minimum gelatinization of starch. The results showed lowered *in vitro* GI by addition of pulse flour in general even though the International table of GI indicates relatively higher GI on crackers. This might be due to higher carbohydrate content of crackers increasing the glycemic response. Total starch content was lowered with addition of pulse ingredients which supports the GI results.

Extruded snacks

Extruded snacks showed higher GI in general which agrees with literatures. Simon et al. (2012) concluded that extruded snacks made with 100 % bean flour exhibited high *in vitro* GI even though rate of hydrolysis was much lower than that of reference white bread. Yong et al. (2011) also indicated that extruded products are digested much more than non-extruded products even with the presence of high protein due to high temperature and shear of extrusion process causing loss of native starch and protein structure, increasing starch gelatinization resulting in higher digestion of starch.

Comparing fried and baked multigrain chips, fried snacks had a lower GI. Researches proved that high fat delays gastric emptying therefore glycemic response. Similar theory can be made with *in vitro* test that a higher fat content reduces the relative starch content and interrupts enzymatic starch digestion therefore lowers GI. Pulse variants reduced GI compared to control with all the extruded snacks. Comparing shapes of snacks, curl shape had the higher GI than crisp shape. Among crisps, the higher the protein addition the more reduction of GI was observed.

2. Results

Results obtained from *in vitro* GI testing, total starch and resistant starch testing are summarized in Table 20 and 21, respectively.

Table 20. Estimated glycemic index, standard deviation and coefficient of variation result summary

Products	eGI	SD	CV
Raw ingredient			
Yellow Pea flour (raw)	92.6	2.827	3.053
Pregel pea flour	82.3	0.818	0.994
Yellow lentil flour (raw)	84.6	6.659	7.869
Pre-gel lentil flour	59.8	3.292	5.507
Red lentil flour (raw)	84.9	2.080	2.451
Green pea flour (raw)	82.2	0.462	0.562
pea starch	172.7	5.953	3.446
Bread			
Focaccia Control	81.2	0.295	0.364
Focaccia YP raw	78.9	1.220	1.546
Focaccia GL raw	74.7	5.327	7.136

Focaccia YP roasted	74.6	0.450	0.603
Focaccia GL roasted	75.5	11.263	14.921
Pan bread control	89.1	1.591	1.785
Pan bread + GL 20%	98.1	8.138	8.299
Pan bread + YP 20%	89.6	4.197	2.975
GF Focaccia	90.2	4.686	3.298
Whole Wheat control	80.9	0.597	1.542
Whole wheat PP & PF	79.7	0.737	1.935
Pasta			
Control semolina 100%	110.9	3.112	2.806
Semolina : RL 70:30	113.3	3.115	2.751
Semolina: GL 70:30	102.9	5.946	5.777
Semolina :YP 70:30	109.6	5.391	4.918
High protein (semolina 90:PP 10)	104.5	2.405	2.300
High fiber (semolina 90:PF 10)	105.5	4.637	4.395
GF control pregel rice and corn	128.5	7.754	6.034
GF GL : Xanthan gum 99:1	103.0	5.865	5.694
GF Pregel Rice:Corn:YP 35:35:30	83.0	0.657	0.792
GF high protein	103.5	0.603	0.582
Semolina :RL 50:50	101.9	1.279	1.255
Semolina: RL 20:80	95.6	2.050	2.144
Semolina :YP 50:50	103.3	1.907	1.847
Semolina :YP 20:80	92.5	1.193	1.290
Crackers			
GF cracker GL	70.4	3.846	5.464
Baked pita chips Control	108.9	10.499	9.640
Baked pita chips YP	89.8	1.956	2.177
Seedy crisp control	74.8	7.346	9.822
Seedy crisp YP	56.2	2.542	4.521
Wheat thin control	71.2	0.409	0.574
Wheat thin YP	47.6	0.137	0.288
Extruded Snack			
Multigrain chips control	108.2	4.135	3.823
Multigrain chips fried YP	94.6	1.721	1.818
Multigrain chips fried GL	94.8	2.438	2.572
Multigrain chips baked YL	112.8	7.088	6.285
Multigrain chips baked YP	115.1	5.487	4.766
Puff YL	101.8	7.345	7.217
Puff YL High	101.8	0.284	0.280

Puff corn control	139.1	0.540	0.388
Crisp control 100% Corn	131.3	2.686	2.045
Crisp fiber & protein (High)	87.9	2.101	2.390
Crisp fiber & protein (Med)	90.8	5.900	6.494
Crisp Protein & Fiber (low)	104.9	15.271	14.563
Crisp Protein & Fiber Red Lentil	89.6	3.651	4.074
Crisp Protein & Fiber Green Pea	96.2	9.808	10.200
Short Bread cookies			
SB Vanilla cookie control	66.9	7.569	11.308
SB cookie Vanilla YL 45%	54.8	1.261	2.302
SB cookie Vanilla RL 40%	57.2	6.023	10.534
SB Vanilla cookie YL 45% +PP+PF	56.4	4.291	7.601
SB cookie chocolate control	70.2	4.803	6.846
SB cookie chocolate 35% GP	61.1	0.996	1.630
SB Cookie chocolate YP 50%+PP+PF	57.5	2.022	3.519
SB cookie chocolate YP 35%	57.8	1.343	2.324
Muffins			
Muffin GP 35%	73.9	3.743	5.066
Muffin YP 35%	80.5	1.117	1.387
Muffin RL 40 %	82.4	10.946	13.277
Muffin GL 40%	73.0	3.066	4.202
Muffin Control	87.9	3.759	4.274
Oatmeal cookies			
Oatmeal Cookie 100% GP	61.1	0.594	0.973
Oatmeal Cookie 100% RL	59.9	3.719	6.208
Oatmeal Cookie YP 100%	61.4	1.012	1.649
Oatmeal Cookie GL 100%	66.4	3.253	4.896
Oatmeal Cookie Control	83.1	4.596	5.533
Rice crispy bars			
Rice crispy control (rice 100%)	61.4	1.777	2.893
Rice crispy RL (75:25 RL:Corn) crisp	69.3	2.383	3.441
Fat base crispy bar			
Fat base crispy bar control	70.5	7.628	10.819
Fat based crispy bar w high PP crisps	63.1	0.666	1.055
Fat based crispy bar w RL crisps	69.1	2.600	3.764
Fat based crispy bar w GL crisps	68.5	0.839	1.225
Baked granola bar			
Baked granola bar control	59.5	5.875	9.880
Baked granola bar w high PP crisps	47.8	0.444	0.930

Baked granola bar w RL crisps	57.5	0.807	1.404
Baked granola bar w GL crisps	53.8	0.513	0.953
Peanut butter crispy treat			
Peanut butter crispy treat control	72.8	10.286	14.126
Peanut butter crispy treat w high PP crisps	59.5	1.512	2.540
Peanut butter crispy treat w RL crisps	67.4	1.524	2.261
Peanut butter crispy treat w GL crisps	67.5	3.131	4.638
Chewy chocolate bar			
Chewy chocolate bar control	85.1	8.974	10.541
chewy chocolate bar GL	74.0	5.838	7.885
Chocolate GL + PF	76.3	1.616	2.118
Peanut butter cup			
Peanut butter cup control	64.7	9.083	14.046
peanut butter cup GL	60.5	6.806	11.250

YP - Yellow pea, GP - Green pea, GL - Green lentil, RL - Red lentil, PP - Pea protein, PF - Pea fiber, GF - Gluten free, SB - short bread, SD – Standard deviation, CV – Coefficient of variance

Table 21. Total starch and resistant starch along with standard deviation and coefficient deviation result summary

Products	Mean	SD	CV	Mean	SD	CV
Raw ingredient						
Yellow Pea flour (raw)	5.56	0.03	0.01	59.3	13.18	0.22
Pregel pea flour	0.97	0.19	0.20	44.8	0.78	0.02
Yellow lentil flour (raw)	3.93	0.40	0.10	47.2	2.81	0.06
Pre-gel lentil flour	3.12	0.03	0.01	45.8	0.88	0.02
Red lentil flour (raw)	3.64	2.07	0.57	47.3	0.01	0.00
Green pea flour (raw)	2.86	0.38	0.13	40.6	6.75	0.17
pea starch	6.51	1.87	0.29	81.0	2.14	0.03
Bread						
Focaccia Control	1.10	0.11	0.10	58.8	8.43	0.14
Focaccia YP raw	2.07	0.01	0.01	50.4	1.57	0.03
Focaccia GL raw	1.76	0.36	0.21	47.8	2.18	0.05
Focaccia YP roasted	1.83	0.04	0.02	51.1	3.13	0.06
Focaccia GL roasted	1.88	0.42	0.23	48.3	1.49	0.03
Pan bread control	0.51	0.18	0.36	58.7	0.05	0.00
Pan bread + GL 20%	2.70	2.16	0.80	54.6	0.93	0.02
Pan bread + YP 20%	1.29	0.36	0.28	54.6	1.44	0.03
GF Focaccia	2.37	0.15	0.06	57.3	2.89	0.05
Whole Wheat control	0.63	0.13	0.21	44.9	1.54	0.03
Whole wheat PP & PF	0.64	0.15	0.23	43.7	1.24	0.03

Pasta						
Control semolina 100%	0.57	0.04	0.06	70.0	1.79	0.03
Semolina :RL 70:30	1.32	0.48	0.36	64.7	0.63	0.01
Semolina : GL 70:30	1.22	0.22	0.18	63.2	0.81	0.01
Semolina :YP 70:30	1.90	0.76	0.40	66.5	1.40	0.02
High protein (semolina 90:PP 10)	0.77	0.36	0.47	61.7	1.32	0.02
High fiber (semolina 90:PF 10)	0.59	0.33	0.57	63.0	1.75	0.03
GF control pregel rice and corn	0.29	0.21	0.73	75.8	0.09	0.00
GF GL : Xanthan gum 99:1	0.76	0.74	0.97	65.8	1.61	0.02
GF Pregel Rice:Corn:YP 35:35:30	1.01	0.57	0.57	54.7	0.81	0.01
GF high protein	0.83	0.19	0.23	61.8	0.79	0.01
Semolina :RL 50 50	1.68	0.54	0.33	57.8	2.03	0.04
Semolina : RL 20 80	2.48	0.46	0.19	56.2	0.46	0.01
Semolina :YP 50 50	2.66	0.95	0.36	60.5	0.55	0.01
Semolina :YP 20 80	2.92	0.29	0.10	57.7	1.24	0.02
Crackers						
GF cracker GL	1.14	0.08	0.07	46.3	1.04	0.02
Baked pita chips Control	0.26	0.20	0.76	62.2	1.38	0.02
Baked pita chips YP	0.61	0.49	0.81	57.2	1.73	0.03
Seedy crisp control	0.12	0.13	1.06	47.0	3.87	0.08
Seedy crisp YP	0.33	0.05	0.15	34.2	2.64	0.08
Wheat thin control	0.31	0.02	0.07	40.8	2.79	0.07
Wheat thin YP	0.22	0.01	0.04	26.0	1.61	0.06
Extruded Snack						
Multigrain chips control	0.44	0.28	0.64	63.7	2.50	0.04
Multigrain chips YP	0.52	0.19	0.37	56.8	0.76	0.01
Multigrain chips GL	0.38	0.15	0.40	57.1	1.19	0.02
Multigrain chips baked YL	0.50	0.21	0.42	60.8	0.84	0.01
Multigrain chips baked YP	0.55	0.23	0.41	60.6	2.84	0.05
Puff YL	0.65	0.21	0.32	55.0	3.42	0.06
Puff YL High	0.15	0.06	0.41	60.6	3.56	0.06
Puff corn control	0.33	0.09	0.28	77.0	6.17	0.08
Crisp control 100% Corn	0.55	0.20	0.37	76.1	4.51	0.06
Crisp fiber & protein (High)	0.32	0.08	0.26	50.1	3.03	0.06
Crisp fiber & protein (Med)	0.50	0.15	0.30	51.2	2.17	0.04
Crisp Protein & Fiber (low)	0.62	0.22	0.36	30.9	36.16	1.17
Crisp Protein & Fiber Red Lentil	0.63	0.22	0.34	36.9	14.45	0.39
Crisp Protein & Fiber Green Pea	0.66	0.24	0.36	39.6	17.06	0.43
SB cookies						
SB Vanilla cookie control	0.18	0.01	0.08	33.8	0.27	0.01
SB cookie Vanilla YL 45%	0.85	0.47	0.55	27.6	2.62	0.09

SB cookie Vanilla RL 40%	0.40	0.13	0.32	27.2	3.40	0.12
SB Vanilla cookie YL 45% +PP+PF	0.85	0.54	0.64	27.4	1.38	0.05
SB cookie chocolate control	0.32	0.19	0.61	24.9	0.46	0.02
SB cookie chocolate 35% GP	0.48	0.05	0.10	21.7	0.46	0.02
SB Cookie chocolate YP 50%+PP+PF	0.49	0.15	0.31	19.9	0.04	0.00
SB cookie chocolate YP 35%	0.61	0.22	0.36	21.5	1.54	0.07
Muffins						
Muffin GP 35%	0.72	0.12	0.17	35.8	1.43	0.04
Muffin YP 35%	0.78	0.13	0.16	37.8	0.70	0.02
Muffin RL 40 %	1.01	0.01	0.01	36.0	2.28	0.06
Muffin GL 40%	0.93	0.03	0.03	38.2	0.64	0.02
Muffin Control	0.56	0.08	0.14	42.4	0.14	0.00
Oatmeal cookies						
Oatmeal Cookie 100% GP	0.88	0.41	0.47	27.4	0.53	0.02
Oatmeal Cookie 100% RL	0.52	0.01	0.01	27.5	1.13	0.04
Oatmeal Cookie YP 100%	0.98	0.05	0.05	29.4	2.08	0.07
Oatmeal Cookie GL 100%	0.67	0.12	0.18	28.1	0.39	0.01
Oatmeal Cookie Control	0.22	0.01	0.03	32.7	0.01	0.00
Rice crispy bars						
Rice crispy control (rice 100%)	2.09	2.44	1.17	28.2	0.73	0.03
Rice crispy RL (75:25 RL:Corn) crisp	0.15	0.04	0.28	19.2	1.23	0.06
Fat base crispy bar						
Fat base crispy bar control	0.20	0.03	0.14	19.7	1.32	0.07
Fat based crispy bar w high PP crisps	0.30	0.16	0.55	17.0	0.47	0.03
Fat based crispy bar w RL crisps	0.36	0.01	0.02	15.7	1.12	0.07
Fat based crispy bar w GL crisps	0.36	0.00	0.00	17.1	0.32	0.02
Baked granola bar						
Baked granola bar control	0.25	0.01	0.06	24.5	0.86	0.04
Baked granola bar w high PP crisps	0.20	0.06	0.28	21.2	0.10	0.00
Baked granola bar w RL crisps	0.16	0.11	0.71	21.7	0.42	0.02
Baked granola bar w GL crisps	0.16	0.11	0.71	22.7	0.01	0.00
Peanut butter crispy treat						
Peanut butter crispy treat control	0.12	0.06	0.47	19.6	2.30	0.12
Peanut butter crispy treat w high PP crisps	0.12	0.06	0.47	15.2	2.69	0.18
Peanut butter crispy treat w RL crisps	0.08	0.11	1.41	14.5	0.60	0.04
Peanut butter crispy treat w GL crisps	0.04	0.06	1.41	16.1	1.48	0.09

Chewy chocolate bar						
Chewy chocolate bar control	0.08	0.00	0.00	12.6	2.78	0.22
chewy chocolate bar GL	0.20	0.06	0.33	11.1	5.05	0.46
Chocolate GL + PF	0.16	0.11	0.71	11.0	4.70	0.43
Peanut butter cup						
Peanut butter cup control	0.16	0.11	0.71	8.5	0.44	0.05
peanut butter cup GL	0.16	0.11	0.68	6.7	0.28	0.04

YP - Yellow pea, GP - Green pea, GL - Green lentil, RL - Red lentil, PP - Pea protein, PF - Pea fiber, GF - Gluten free, SB - short bread, SD – Standard deviation, CV – Coefficient of variance

VI. In vivo testing

Based on the result from in vitro GI test, 10 samples were chosen to be sent to in vivo testing lab which are:

1. Pasta control (100% semolina) (serving size = 56g)
2. Pasta Red lentil – semolina blend 50:50 (serving size = 56g)
3. Focaccia control (serving size = 50g)
4. Focaccia lentil (serving size = 50g)
5. Shortbread chocolate cookie control (serving size = 30g)
6. Shortbread chocolate Yellow pea + protein + fiber (serving size = 30g)
7. Baked granola bar control (serving size = 40g)
8. Baked granola bar protein puff (serving size = 40g)
9. Seedy crisp cracker control (serving size = 15g)
10. Seedy crisp cracker pea (serving size = 15g)

1. Test Procedure

Available carbohydrate which computed by subtracting a total dietary fiber content by a total carbohydrate were determined by nutrition analysis and shown in Table 22. Based on available carbohydrate, serving size was determined which is shown in Table 23.

A total of 10 male and female, aged 18-75 years with good health condition were tested. The protocol follows the methods described in ISO 26642:2010 – "Food products – Determination of the glycaemic index and recommendation for food classification". Each subject was required a 10-14 hour overnight fast before coming into the test. The subjects were weighed and two fasting blood samples were obtained by finger-prick five minutes apart before consuming a test meal with 15 minutes. Further blood samples were obtained at 15, 30, 45, 60, 90 and 120 minutes after the start of the test meal. Test meal consisted of a portion of the test food or white bread containing 50 g of available carbohydrate based on nutritional composition shown in Table 23. Each subject was allowed to have a beverage of choice (water, coffee, or tea with 30 ml of 2% milk if desired) and this was kept the same for the rest of test. The control meal was tested 2 times by all subjects. Blood glucose analysis was conducted using YSI (Yellow Spring

Instruments, OH) analyzer within five days of collection. Blood samples were taken in duplicate and each meal was consumed in replicate.

After consuming a test meal, subjects rated the palatability of the test meal in hedonic scale with a line showing "very unpalatable" to "very palatable".

Data Analysis

Incremental area under the blood glucose curve (iAUC) was calculated using the trapezoid rule, and fasting glucose was taken to be the mean of the first measurement of the glucose concentration at times -5 and 0 min. The glycemic index was calculated by expressing each subject's glucose iAUC for the test food as a percentage of the same subject's mean response after reference meal and, if required, adjusting the GI to the glucose scale where glucose = 100 and white bread = 71.

2. Result

Results from *in vivo* test is shown Table 24 and a summary of results from *in vitro*, *in vivo*, glycemic load (GL) and glycemic index category is shown in Table 25. GL was calculated as multiplying glycemic index by available carbohydrate and divided by 100.

According to the report provided, no statistical significance between control products and pulse variance were found. However, reduction of GI was found in all products except granola bars in which there was no difference on GI. This is possibly due to the use of extruded puffs in which high *in vitro* digestibility as well as estimated GI (Simon, et al. 2012). It is noticeable that GI category moved from medium to low in both pasta and focaccia products which indicates the high potential of pulse flour on GI lowering effect.

Palatability score showed that there was no statistical difference between control and pulse variant products (Figure 3). The scores between controls and pulse variants were similar except cracker and pasta, with which pulse variants scored slightly less than the controls. Among five different products, focaccia, granola bars and shortbread cookies had higher scores on palatability. Even though sensory testing was not conducted as a part of the study, this result indicates that the products developed had desirable sensory characteristics.

Table 22. Nutritional analysis results

ID	Method	Facaccia bread control	Focaccia GL raw	Pasta control	Pasta RL 50:50	Seedy crisp control	Seed crisp YP	Baked granola bar control	Granola bar w high PP crisp	SB control	SB Cookie chocolate YP 50%+PP+PF
Serving size (g)		50	50	56	56	15	15	40	40	30	30
Moisture (%)	AOAC 325.06	35.9	35	9.2	10.9	2.5	2.6	4.9	5.1	0.9	1.2
Protein (%)	AOAC 992.15	8.6	10.8	14	19.3	15.6	23.9	7	9.7	5.2	6.8
Fat (%)	AOAC 922.06	13.2	13.4	3.2	2.9	22.6	22.5	19.3	19.1	29.6	30.7
Ash (%)	AOAC 93.06	2	2.2	0.6	1.3	3.7	4.5	1.1	1.4	1.7	1.8
Carbohydrate (%)	Calculation	40.3	38.6	73	65.6	55.6	46.5	67.7	64.7	62.6	59.5
Total Dietary Fiber (%)	AOACV 991.43	2.2	4.5	3.7	5.4	11.6	11.7	4.1	6	2.5	3.1
Calories (Cal)/serving	21 FR 101.9	160	160	210	200	70	70	190	190	160	160
Sodium (ppm)	AOAC 2011.14	5510	5520	14	16	8700	9840	1240	1050	4180	4870

Table 23. Available carbohydrate content from each product

Test Meal	Abbr	Weight (g)	Protein (g)	Fat (g)	T CHO (g)	Fibre (g)	AvCHO (g)
White bread control	WB	117.6	10.1	1.1	52.3	2.6	50
PA 1 - Pasta Control	PA01	72.2	10.1	2.3	52.7	2.7	50
PA 14 - Pasta Red lentil	PA14	83.1	16.0	2.4	54.5	4.5	50
BR 1 - Focaccia Control	BR01	131.2	11.3	17.3	52.9	2.9	50
BR 3 - Focaccia Lentil	BR03	146.6	15.8	19.6	56.6	6.6	50
#12 - Shortbread Chocolate Cookie Control	SB12	83.2	4.3	24.6	52.1	2.1	50
#16 - Shortbread Chocolate Pea Protein and Fiber	SB16	88.7	6.0	27.2	52.7	2.7	50
#23 - Baked Granola Bar Control	GB23	78.6	15.2	15.2	53.2	3.2	50
#24 - Baked Granola Bar Protein Puff	GB24	85.2	16.3	16.3	55.1	5.1	50
CRK 4 - Seedy Crisp Cracker Control	CR04	113.6	17.7	25.7	63.2	13.2	50
CRK 5 - Seedy Crisp Cracker Pea	CR05	143.7	34.3	32.3	66.8	16.8	50

Provided by GI laboratory Inc. as a part of report.

Table 24. Summary of glycemic index result of 10 samples and white bread control

Test Meal	Glycemic Index*	GI Category**
White bread control	71	High
PA 1 - Pasta Control	61±5	Medium
PA 14 - Pasta Red lentil	55±8	Low
BR 1 - Focaccia Control	61±6	Medium
BR 3 - Focaccia Lentil	53±5	Low
#12 - Shortbread Chocolate Cookie Control	42±4	Low
#16 - Shortbread chocolate Yellow Pea Protein and Fiber	38±3	Low
#23 - Baked Granola Bar Control	35±4	Low
#24 - Baked Granola Bar Protein Puff	35±5	Low
CRK 4 - Seedy Crisp Cracker Control	46±4	Low
CRK 5 - Seedy Crisp Cracker Pea	42±3	Low

* The listed GI value(s) is/are only valid as long as product ingredients, formulation, processing, and/or any other material production factors remain unchanged. ** Using the classification of ISO 26642:2010, products with a glycemic index (GI) less than or equal to 55 are classified as being low GI, those with a GI of 56 to 69 are classified as medium, while those with a GI equal to or greater than 70 are high GI.

Provided by GI laboratory Inc. as a part of report.

Table 25. Summary of estimated glycemic index, glycemic load and glycemic index category

Product	Estimated GI	Glycemic index	Glycemic load	GI category
White Bread control	NA	71	36	High
Pasta control	111	61	31	Medium
Pasta red lentil	96	55	28	Low
Focaccia control	81	61	31	Medium
Focaccia lentil	75	53	27	Low
SB Chocolate cookie control	70	42	21	Low
SB chocolate yellow pea protein and fiber	58	38	19	Low
Baked granola bar control	60	35	18	Low
Baked granola bar protein puff	48	35	18	Low
Seedy crisp cracker control	75	46	23	Low
Seedy crisp cracker protein	56	42	21	Low

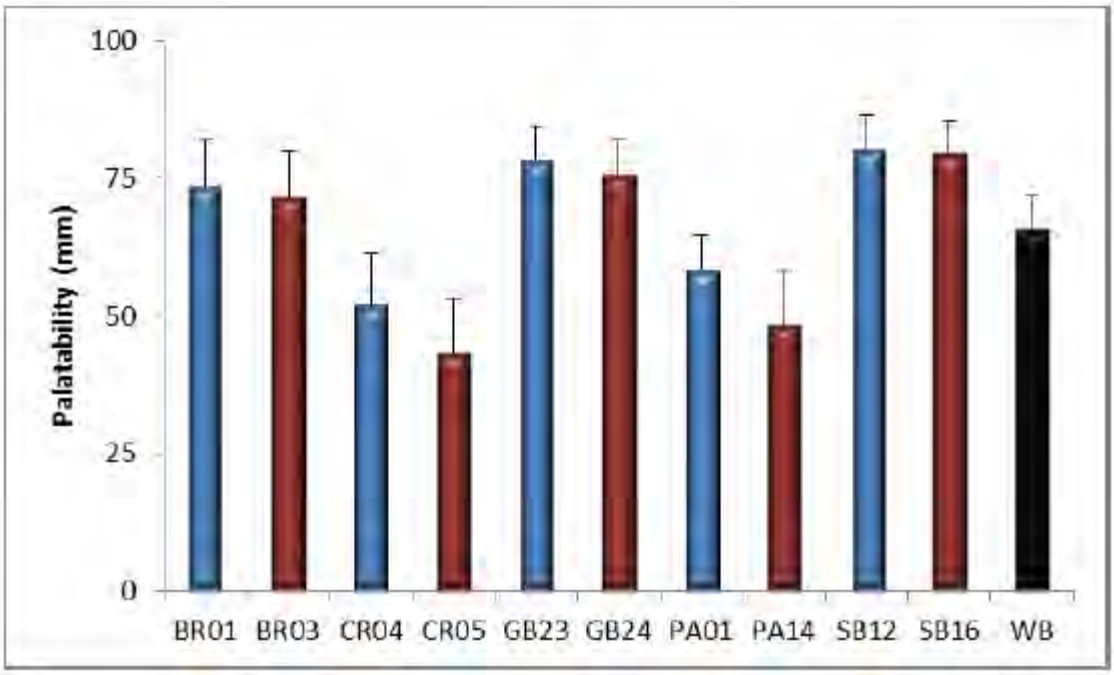

Figure 3. Palatability scores after consumption of focaccia control (BR01), focaccia lentil (BR03), seedy crisp cracker control (CR04), seedy crisp cracker pea (CR05), baked granola bar control (GB 23), baked granola bar protein puff (GB24), pasta control (PA01), pasta red lentil (PA14), shortbread chocolate cookie control (SB12), shortbread chocolate cookie yellow pea + protein + fiber (SB 16). The control products are in blue, pulse variants are in red and the white bread control is in black. Data are expressed as Mean + SEM.

VII. Conclusion

The project demonstrated the effects of pulse flour on lowering GI without altering palatability of products. Using *in vitro* GI as a screening method was shown to be a good indicator of starch digestibility. Combination of in vitro and in vivo GI tests provides advantages on saving time and cost of running a GI test. GI testing both in vitro and in vivo is a complicated process with its result affected by more than one factor. The presented test exhibited the unique system of each product affecting its starch digestibility and thus it is critical to study each system closely to determine the major factors affecting glycemic response.

VIII. References

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DEVELOPMENT OF LOW-GLYCEMIC INDEX PRODUCTS USING PULSE INGREDIENTS

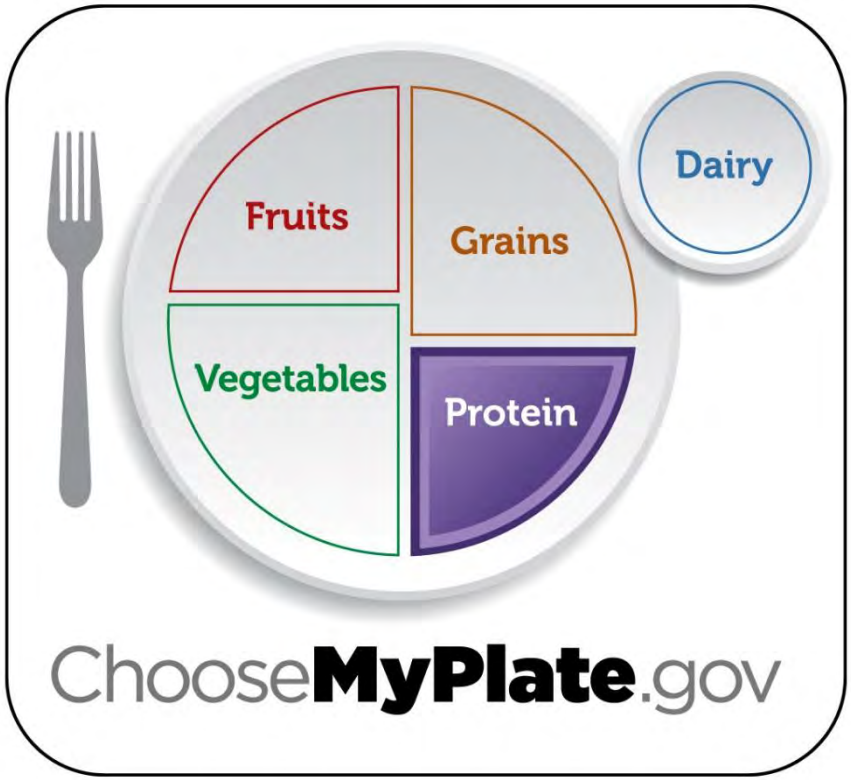
Pulses - the nutrient rich seeds of legumes

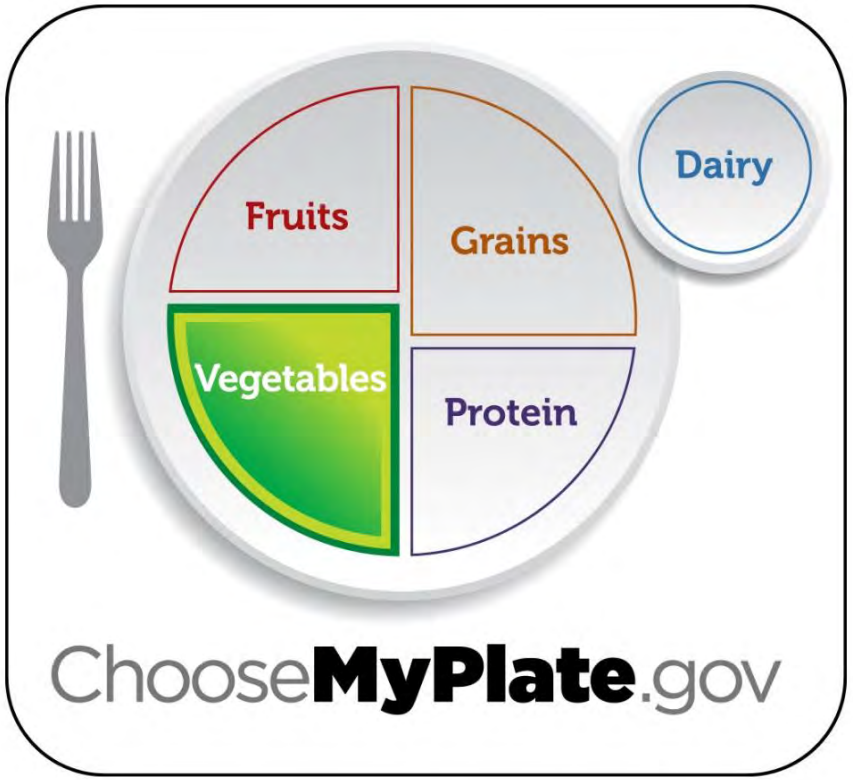
PULSE NUTRITION

- ✓ Excellent source of fiber
- ✓ Excellent source of fiber
- ✓ Excellent source of
- ✓ Good source of protein
- ✓ Good source of iron

Nutrition experts recommend 2 ½ to 3 cups of pulses per week.

PULSES COUNT AS EITHER A PROTEIN OR AS A VEGETABLE ON THE U.S. DIETARY RECOMMENDATION





PULSES AND GLYCEMIC RESPONSE

- Glycemic index (GI) is a measure of the effect of foods in raising blood sugar (glucose) levels after a meal

- Pulses ingredients are recognized for their low glycemic response, or effect on blood sugar levels.

FOOD APPLICATIONS OF PULSE INGREDIENTS

COMPONENTS

Flour

Protein

Starch

Fiber

USED IN

Snacks

Bakery products

Soup

Meat substitutes

Baby food

Ice cream

Sauces

Pasta

Other applications

OBJECTIVES OF STUDY

- To develop new low-GI products with pulse flours including pasta, cookies, muffins, bread, crackers, quick bread, and extruded snack and snack bars

- To provide *in vivo* evidence showing the benefit of pulse flour on glycemic response.

PARTNERS

- Northern Pulse Growers Association

  (Bismarck, ND)

- North Dakota State University (Fargo, ND)

- Glycemic Index Labs (Toronto, ON)

METHODS

- Pasta, extruded snacks, bread, crackers, cookies, bars and muffin formulations were developed including one or more pulse ingredients:
  - Yellow pea flour
  - Green or red lentil flour
  - Pea protein
  - Pea fiber (bran)

- 94 samples tested for *in vitro* Glycemic Index, total starch, resistant starch

STEPS TAKEN AFTER PRELIMINARY TESTING

- 10 samples selected for GI testing
- Proximate analysis conducted
- Palatability testing/sensory evaluation
- *In vitro* GI testing (human study)

Formulation

FOCACCIA CONTROL AND FOCACCIA GREEN LENTIL

	Control		Pulse variant	
Ingredient	g	%	g	%
Flour	700	51.3	490	36.2
Decorticated green lentil flour	0	0.0	210	15.5
Water	479	35.1	469	34.6
Extra virgin olive oil	109	8.0	109	8.0
Instant yeast	16	1.2	16	1.2
Salt	16	1.2	16	1.2
Garlic powder	5	0.4	5	0.4
Black pepper, fine	2	0.1	2	0.1
Dried herb	2	0.1	2	0.1
Olive oil (topping)	30	2.2	30	2.2
Sea salt, fine	5	0.3	5	0.3
Total	338	100	328	100

Formulation

CRACKERS

	Control		Pea Protein	
	g	%	g	%
Water	170	28.5	210	33.1
Whole wheat flour	142	23.8	114	18
All-purpose flour	135	22.7	107	16.9
Sesame seed	50	8.4	50	7.9
Olive oil	43	7.2	43	6.8
Poppy seed	40	6.7	40	6.3
Salt	8	1.3	8	1.3
Baking soda	6	1	6	0.9
Garlic powder	2	0.3	2	0.3
Pea protein	0	0	55	8.7
Total	**596**	**100**	**635**	**100**

Formulation

Granola Bar

	Control		High PP	
	g	%	g	%
Cinnamon chips	201	25	201	24.95
Rolled oats	160	20	160	20
Agave Syrup	123.5	15.3	123.5	15.3
Pastry flour	70	8.7	0	0
Coconut oil	60	7.5	60	7.5
Sugar	70	8.7	70	8.7
SF Seeds	42	5.2	42	5.2
Rice Crispies	35	4.4	0	0
GL Puff + PF High	0	0	35	4.4
Grape Nuts cereal	35	4.4	35	4.4
Pulse flour	0	0	70	8.7
	338	100	328	100

Formulation

Puffs Used in the Granola

Décor green lentil	35.0
Pea protein	30.0
Corn cones	29.5
Pea fiber (bran)	5.0
Calcium carbonate	0.5
Décor Red lentil	0
Green pea	0
Total	**100**

Formulation

PASTA CONTROL AND PASTA RED LENTIL

	Control	Pulse variant
Ingredient	%	%
Semolina	100	50
Decorticated red lentil flour	0	50

CHOCOLATE SHORTBREAD COOKIES

Formulation

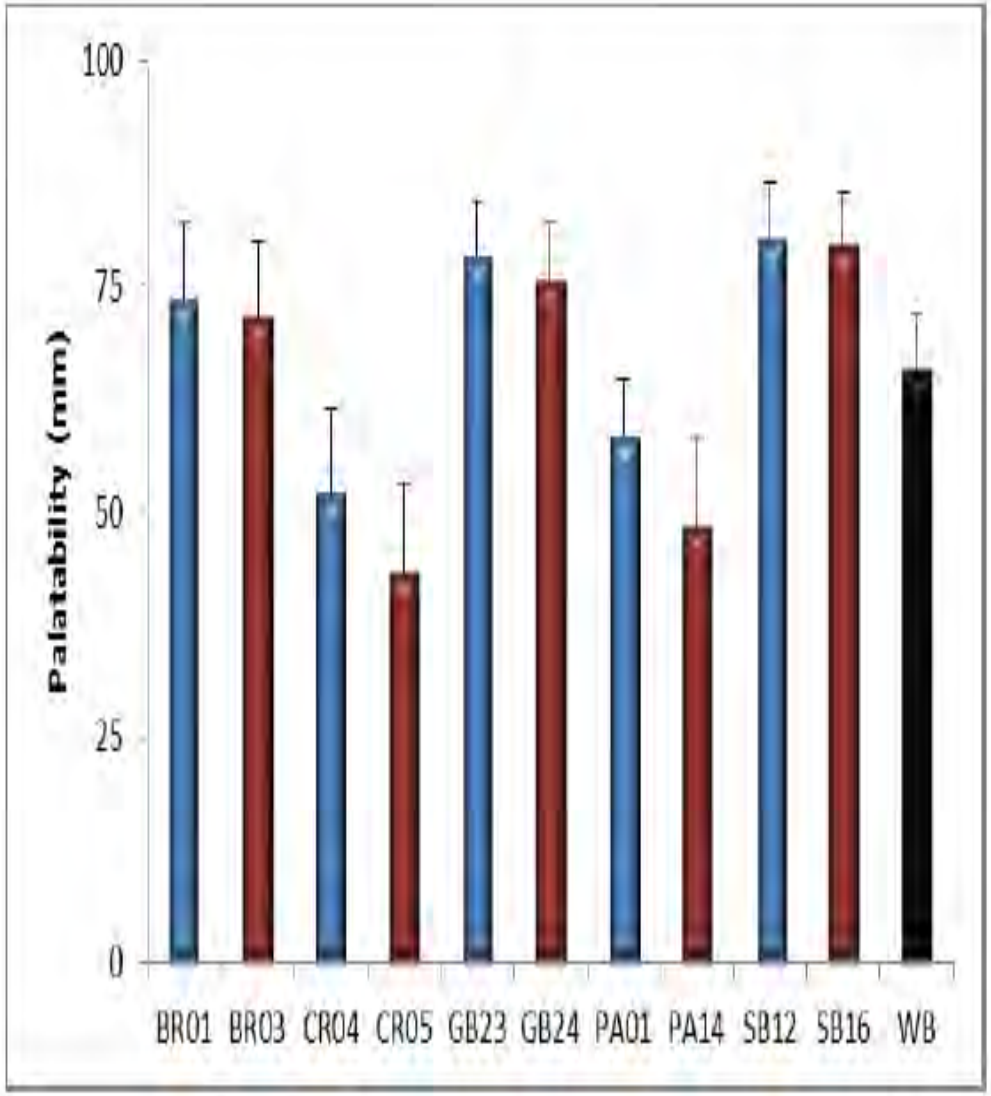
	Control		GL (50% sub) + PB PP	
	g	%	g	%
Unsalted butter	113.5	33.7	113.5	34.6
Pastry flour	102.9	30.6	51.4	15.7
Sugar	99.3	29.5	99.3	30.3
Dutched cocoa powder	14.2	4.2	14.2	4.3
Salt	3.4	1	3.4	1.0
Vanilla	2.5	.7	2.5	0.8
Baking powder	2.0	.3	1.9	0.6
Pulse flour	0	0	36.5	11.1
Pea bran (PB)	0	0	3	0.9
Pea Protein (PP)	0	0	2	0.6
Total	**338**	**100**	**328**	**100**

IN VIVO GLYCEMIC INDEX TESTING

- 10 subjects (ages 18 to 75) in good health
- 10- to 14-hour overnight fast
- Fasting blood samples obtained
- Test meal: white bread or glucose as reference
- Blood samples obtained at 15, 30, 45, 60, 90 and 120 minutes

RESULTS

Palatability Results



Blue = Control

Red = pulse variant

Black = white bread control

No significant differences in palatability were shown between samples with and without pulse ingredients.

Nutrition Analysis Results

Test Meal	Weight (g)	Protein (g)	Fat (g)	Total CHO (g)	Fiber (g)	Available CHO (g)
White Bread Control	117.6	10.1	1.1	52.3	2.6	50
Pasta Control	72.2	10.1	2.3	52.7	2.7	50
Pasta Red Lentil	83.1	16.0	2.4	54.5	4.5	50
Focaccia Control	131.2	11.3	17.3	52.9	2.9	50
Focaccia Lentil	146.6	15.8	19.6	56.6	6.6	50
Shortbread Cookie Control	83.2	4.3	24.6	52.1	2.1	50
Shortbread Chocolate Pea Protein and Fiber	88.7	6.0	27.2	52.7	2.7	50
Baked Granola Bar Control	78.6	15.2	15.2	53.2	3.2	50
Baked Granola Protein Puff	85.2	16.3	16.3	55.1	5.1	50
Seedy Crisp Cracker Control	113.6	17.7	25.7	63.2	13.2	50
Seedy Crisp Cracker Pea	143.7	34.3	32.3	66.8	16.8	50

Results

Product	Estimated GI	Glycemic index	Glycemic load	GI category
White Bread Control	NA	71	36	High
Pasta Control	111	61	31	Medium
Pasta Red Lentil	96	55	28	Low
Focaccia Control	81	61	31	Medium
Focaccia Lentil	75	53	27	Low
Shortbread Chocolate Cookie Control	70	42	21	Low
Shortbread Chocolate Yellow Pea Protein and Fiber	58	38	19	Low
Baked Granola Bar Control	60	35	18	Low
Baked Granola Bar Protein Puff	48	35	18	Low
Seedy Crisp Cracker Control	75	46	23	Low
Seedy Crisp Cracker Protein	56	42	21	Low

SUMMARY

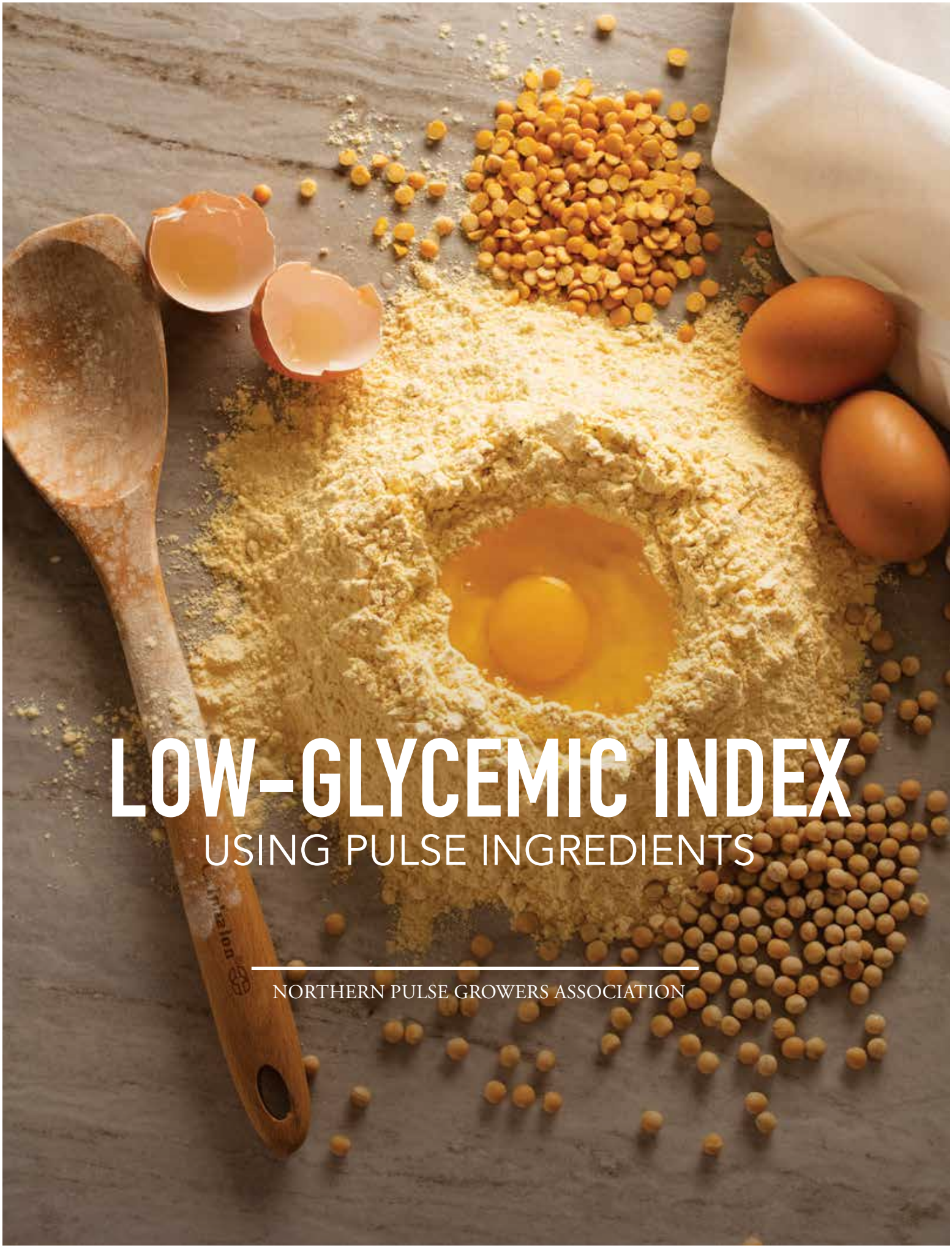
Adding pulse ingredients:

- Did not affect the palatability scores
- Can improve the nutritional profile of food products by increasing fiber and protein content.

in vitro testing showed decreases in estimated GI in all products with added pulse ingredients except granola bars.

- *According to in vivo* testing, two of the ten food products showed a reduction in their GI category from medium to low
  - Pasta with added decorticated red lentil flour
  - Focaccia bread with added decorticated red lentil flour

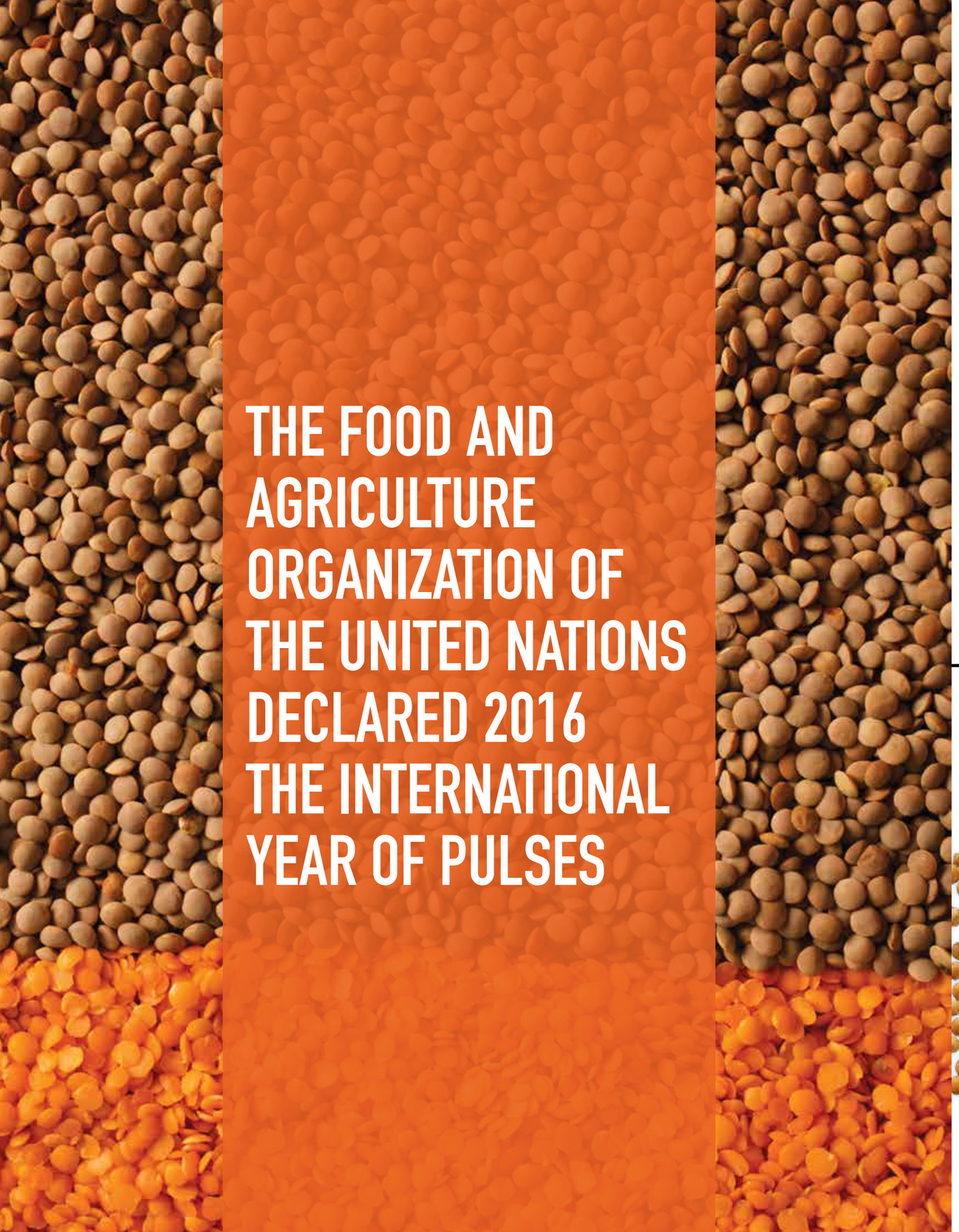
DEVELOPMENT OF LOW-GLYCEMIC INDEX PRODUCTS USING INGREDIENTS



LOW-GLYCEMIC INDEX

USING PULSE INGREDIENTS

NORTHERN PULSE GROWERS ASSOCIATION

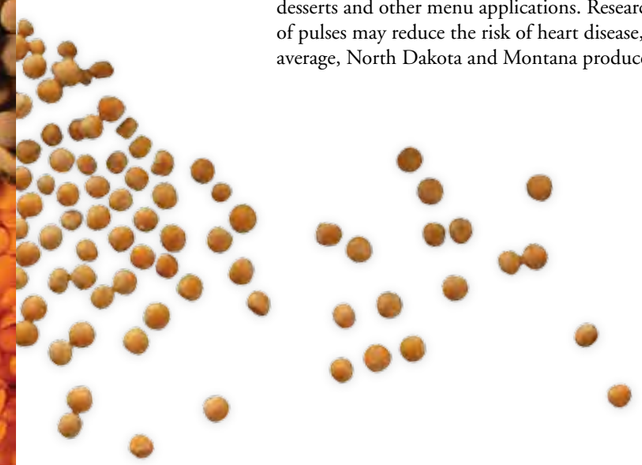


THE FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS DECLARED 2016 THE INTERNATIONAL YEAR OF PULSES

DEVELOPMENT OF LOW-GLYCEMIC INDEX PRODUCTS USING PULSE INGREDIENTS

Pulses, which are members of the legume family, include lentils, chickpeas, and dry peas. On a global level, pulses increasingly are being recognized for their role as a nutritious food source that promotes good health. As a result of their role in providing nutrition and promoting food security, the Food and Agriculture Organization of the United Nations declared 2016 as the International Year of Pulses.

Pulses are versatile, easy-to-prepare ingredients that can be used in entrees, salads, breads, desserts and other menu applications. Researchers have reported that regular consumption of pulses may reduce the risk of heart disease, diabetes and certain types of cancer. On average, North Dakota and Montana produce 71% of the pulse crop in the United States.



PULSE INGREDIENTS HAVE BEEN USED IN SNACKS, BAKERY PRODUCTS, SOUP, MEAT SUBSTITUTES, BABY FOOD, ICE CREAM AND DESSERTS, SAUCES AND SEASONING, PASTA AND NOODLES.

FOOD APPLICATIONS OF PULSE INGREDIENTS

Pulses can be fractionated into their components through special processing. Pulse ingredients, such as flour, protein, starch, and fiber, have a wide range of product applications. Pulse ingredients have been used in snacks, bakery products, soup, meat substitutes, baby food, ice cream and desserts, sauces and seasoning, pasta and noodles. For example, in gluten-free applications, pulse flour can improve the nutritional profile as a result of its protein, fiber and vitamin and mineral content.

GLYCEMIC RESPONSE

Along with their nutritional value, pulse ingredients are recognized for their low glycemic response or effect on blood sugar levels. Jenkins (1981) introduced the glycemic index (GI) system for foods. The GI is a measure of the effect of foods in raising blood sugar (glucose) levels after a meal. Some research has shown an association between low-GI foods and decreased risk of type 2 diabetes, obesity, cardiovascular disease, metabolic syndrome, stroke, and other chronic diseases.

RESEARCH OBJECTIVES AND METHODS

The Northern Pulse Growers Association, Bismarck, ND, collaborated with the Northern Crops Institute, Fargo, ND, North Dakota State University, Fargo, ND, and Glycemic Index Labs, Toronto, ON, to conduct research related to the influence of pulses on the GI of food formulated with pulse ingredients.

The objectives of the project were 1) to develop new low-GI products with pulse flours including pasta, cookies, muffins, bread, crackers, quick bread, and extruded snack and snack bars; and 2) to provide *in vivo* evidence showing the benefit of pulse flour on glycemic response.

Pasta, extruded snacks, bread, crackers, cookies, bars and muffin formulations were developed to contain pulse ingredients, including yellow pea flour, green or red lentil flour, pea protein, and/or pea fiber (bran). Ninety-four samples were tested for *in vitro* GI, total starch (TS) and resistant starch (RS) using standard methods.

After preliminary testing, 10 samples, including five controls without added pulse ingredients, were selected for further GI testing. Proximate analysis was conducted using official AOAC methods. Glycemic load (GL) was calculated by multiplying GI by available carbohydrate and dividing by 100.

GI testing and palatability testing were conducted on the following products: White Bread Control, Pasta Control, Pasta Red Lentil, Focaccia Control, Focaccia Lentil, Shortbread Chocolate Cookie Control, Shortbread Chocolate Yellow Pea Protein and Fiber, Baked Granola Bar Control, Baked Granola Bar Protein Puff, Seedy Crisp Cracker Control, and Seedy Crisp Cracker Pea.

The Glycemic Index Labs, Toronto, ON, conducted the *in vivo* GI testing. Ten subjects (ages 18 to 75) in good health participated in the *in vivo* testing. After a 10- to 14-hour overnight fast, the subjects were weighed and two fasting blood samples were obtained. After having a test meal with white bread or glucose as a reference, blood samples were obtained at 15, 30, 45, 60, 90 and 120 minutes. A limitation of the research was the relatively small number of subjects in the *in vivo* study.

For more information about the preliminary results, please contact the Northern Pulse Growers Association (www.northernpulse.com) and the Northern Crops Institute (www.northern-crops.com).

FORMULATIONS

PASTA CONTROL AND PASTA RED LENTIL

Ingredient	Control %	Pulse variant %
Semolina	100	50
Decorticated red lentil flour	0	50

FOCACCIA CONTROL AND FOCACCIA GREEN LENTIL

Ingredient	Control		Pulse variant	
	g	%	g	%
Flour	700	51.3	490	36.2
Water	479	35.1	469	34.6
Extra virgin olive oil	109	8.0	109	8.0
Olive oil (topping)	30	2.2	30	2.2
Instant yeast	16	1.2	16	1.2
Salt	16	1.2	16	1.2
Garlic powder	5	0.4	5	0.4
Sea salt, fine	5	0.3	5	0.3
Black pepper, fine	2	0.1	2	0.1
Dried herb	2	0.1	2	0.1
Decorticated green lentil flour	0	0.0	210	15.5
Total	1364	100	1354	100

SHORTBREAD CHOCOLATE COOKIE CONTROL, SHORTBREAD CHOCOLATE YELLOW PEA PROTEIN AND FIBER

Ingredient	Control		Pulse variant	
	g	%	g	%
Unsalted butter	113.5	33.7	113.5	34.6
Pastry flour	102.9	30.6	51.4	15.7
Sugar	99.3	29.5	99.3	30.3
Dutched cocoa powder	14.2	4.2	14.2	4.3
Salt	3.4	1	3.4	1.0
Vanilla	2.5	0.7	2.5	0.8
Baking powder	2.0	0.3	2.0	0.6
Decorticated green lentil flour	0	0	36.5	11.1
Pea bran (PB)	0	0	3	0.9
Pea protein (PP)	0	0	2	0.6
Total	337.8	100	327.8	100

BAKED GRANOLA BAR CONTROL AND BAKED GRANOLA BAR PROTEIN PUFF

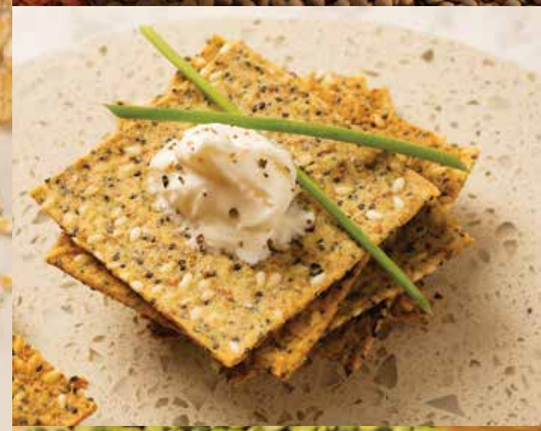
Ingredient	Control		Pulse variant	
	g	%	g	%
Cinnamon chips	201	24.9	201	24.9
Rolled oats	160	19.9	160	19.9
Agave syrup	123.5	15.3	123.5	15.3
Sugar	70	8.7	70	8.7
Pastry flour	70	8.7	0	0
Coconut oil	60	7.5	60	7.5
Sunflower seeds	42	5.2	42	5.2
Rice Krispies	35	4.4	0	0
Grape nuts cereal	35	4.4	35	4.4
Vanilla	5	0.6	5	0.6
Cinnamon	4	0.5	4	0.5
Pulse Puff*				
GL Puff PP + PF High	0	0	35	4.4
Decorticated green lentil flour	0	0	70	8.7
Total	805.5	100	805.5	100

*PULSE PUFFS USED IN THE GRANOLA

Ingredient	GL Puff PP + PF High
Decorticated green lentil	35
Pea protein (PP)	30
Corn cones	29.5
Pea fiber (bran) (PF)	5
Calcium carbonate	0.5
Total	100

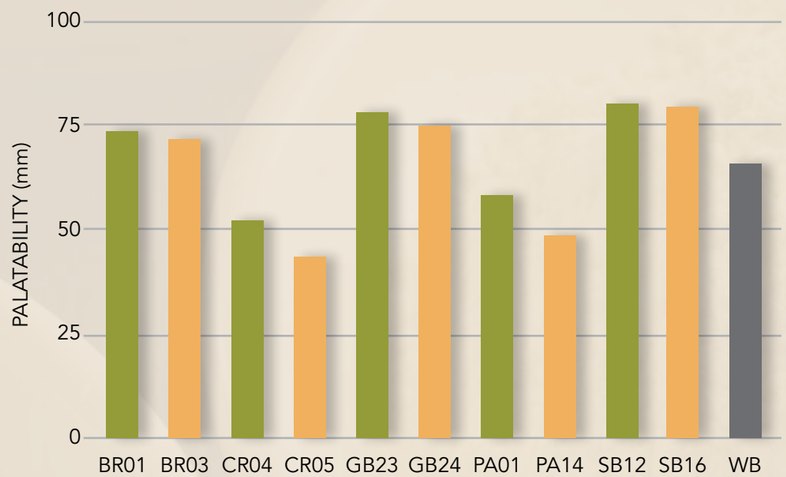
SEEDY CRISP CRACKER CONTROL AND CRACKERS WITH PEA PROTEIN

Ingredient	Control		Pulse variant	
	g	%	g	%
Water	170	28.5	210	33.1
Whole wheat flour	142	23.8	114	18
All-purpose flour	135	22.7	107	16.9
Sesame seed	50	8.4	50	7.9
Olive oil	43	7.2	43	6.8
Poppy seed	40	6.7	40	6.3
Salt	8	1.3	8	1.3
Baking soda	6	1	6	0.9
Garlic powder	2	0.3	2	0.3
Pea protein isolate	0	0	55	8.7
Total	596	100	635	100



PALATABILITY RATINGS

The graph below shows the palatability results of samples with and without the addition of pulse ingredients. Overall, no significant differences were shown in palatability when pulse ingredients were added to control formulations.



Palatability scores after consumption of Focaccia Control (BR01), Focaccia Lentil (BR03), Seedy Crisp Cracker Control (CR04), Seedy Crisp Cracker Pea (CR05), Baked Granola Bar Control (GB 23), Baked Granola Bar Protein Puff (GB24), Pasta Control (PA01), Pasta Red Lentil (PA14), Shortbread Chocolate Cookie Control (SB12), Shortbread Chocolate Cookie Yellow Pea + Protein + Fiber (SB 16). The control products are in green, pulse variants are in yellow and the white bread control is in gray. Data are expressed as Mean + SEM.

NUTRIENT ANALYSES

The proximate analyses (protein, fat, carbohydrate (CHO), fiber and available carbohydrate) of the samples used in *in vivo* testing indicate differences among samples (shown below). The test meal weights were standardized to provide 50 grams of available carbohydrate (total carbohydrate minus fiber).

Nutrient analysis of food samples used in vivo GI testing. (All measurements in grams.)

Test Meal	Weight	Protein	Fat	Total CHO	Fiber	Available CHO
White Bread Control	117.6	10.1	1.1	52.3	2.6	50
Pasta Control	72.2	10.1	2.3	52.7	2.7	50
Pasta Red Lentil	83.1	16.0	2.4	54.5	4.5	50
Focaccia Control	131.2	11.3	17.3	52.9	2.9	50
Focaccia Lentil	146.6	15.8	19.6	56.6	6.6	50
Shortbread Chocolate Cookie Control	83.2	4.3	24.6	52.1	2.1	50
Shortbread Chocolate Pea Protein and Fiber	88.7	6.0	27.2	52.7	2.7	50
Baked Granola Bar Control	78.6	15.2	15.2	53.2	3.2	50
Baked Granola Protein Puff	85.2	16.3	16.3	55.1	5.1	50
Seedy Crisp Cracker Control	113.6	17.7	25.7	63.2	13.2	50
Seedy Crisp Cracker Pea	143.7	34.3	32.3	66.8	16.8	50

WITH THE EXCEPTION OF THE GRANOLA BARS, A REDUCTION OF GI WAS FOUND IN ALL 10 FOODS THROUGH THE IN VITRO GI INDEX TESTING.

IN VITRO AND IN VIVO GLYCEMIC INDEX DETERMINATION

Using the classification of ISO 26642:2010, products with a glycemic index (GI) less than or equal to 55 are classified as being Low GI, those with a GI of 56 to 69 are classified as Medium GI, while those with a GI equal to or greater than 70 are High GI. The estimated GI, GI and GL were predominantly in the Low GI category (shown below).

With the exception of the granola bars, a reduction of GI was found in all 10 foods through the *in vitro* GI index testing. Specifically, the GI category changed from Medium to Low in both pasta and focaccia products.

Summary of *in vitro* and *in vivo* glycemic index testing of 10 samples and white bread control.

Product	Estimated Index	Glycemic Index	Glycemic Load	GI Category
White Bread Control	NA	71	36	High
Pasta Control	111	61	31	Medium
Pasta Red Lentil	96	55	28	Low
Focaccia Control	81	61	31	Medium
Focaccia Lentil	75	53	27	Low
Shortbread Chocolate Cookie Control	70	42	21	Low
Shortbread Chocolate Pea Protein and Fiber	58	38	19	Low
Baked Granola Bar Control	60	35	18	Low
Baked Granola Bar Protein Puff	48	35	18	Low
Seedy Crisp Cracker Control	75	46	23	Low
Seedy Crisp Cracker Pea	56	42	21	Low

SUMMARY: RESEARCH TO PRACTICE

In vitro testing showed decreases in estimated GI in all products except granola bars. According to the results of *in vivo* testing, two of the ten food products (pasta with added decorticated red lentil flour and focaccia bread with added decorticated red lentil flour) showed a reduction in their GI category from medium to low. In all cases, adding pulse ingredients did not affect the palatability scores and can improve the nutritional profile of food products by increasing fiber and protein content.

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ADDING PULSE INGREDIENTS DID NOT AFFECT THE PALATABILITY SCORES AND CAN IMPROVE THE NUTRITIONAL PROFILE OF FOOD PRODUCTS BY INCREASING FIBER AND PROTEIN CONTENT.

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Developing Cold-Hardy Wine Grapes with Early Acclimation Stability

Final Report

Project Summary

- The NDSU grape germplasm enhancement project was initiated in 2009 in response to grape growers' difficulties with consistent fruit production using the cultivars currently available. Microvinification is the only way to compare accessions with fruit characteristics (soluble solids, pH, and titratable acidity) within an enologist's minimum requirements and to determine which vines should proceed to advance testing. After evaluating fruit characteristics of approximately 400 accessions, approximately 75 accessions possessed fruit characteristics suitable for microvinification and enological evaluation. Of these, only seven were considered for secondary testing. These selections have been propagated and transplanted at four locations to further evaluate enological properties.
- Proper vine acclimation before severe cold conditions in the fall is key to vine winter survival and for fruitfulness of one-year-old wood. A selection procedure based on fall photoperiod-based acclimation stability was developed using controlled environmental conditions using growth chambers and validated using the same standard cultivars in the field. The procedure identified acclimation characteristics in 'Marquette' that contribute to its lack of winter hardiness in North Dakota.

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

Project Approach

One of the key selection or screening criteria for grape seedlings developed under the germplasm enhancement project is their potential for wine. Grape seedlings from the project will vary widely in their suitability for wine. Some can be screened out of further consideration by means of their high acidity and low pH, or by the presence of herbaceous wild grape flavors in the fresh fruit. Many seedlings, however, will pass these initial screening tests. Understanding their full potential for wine will require microvinification. Microvinification of fruit from these seedlings will reveal the organoleptic (aroma, flavor, mouthfeel) characteristics, both good and bad, that are released during maceration and yeast fermentation. These characteristics are central to assessing their wine potential and making well-informed seedling selection decisions.

Seedlings will be screened for wine potential in their very first year of fruiting. Even very small amounts of fruit from a young seedling can be evaluated to help make selection decisions. For example, 200-300 grams of fruit from a seedling can be microvinified and the resulting small wine sample analyzed and organoleptically evaluated by a trained tasting panel. It is anticipated that approximately 100+ accessions of the 1,000+ transplanted seedlings each year will produce fruit and be scored with favorable composition (soluble solids, pH, and titratable acidity) for winemaking. However, these components do not constitute the sole basis of grape and wine potential quality, even though they are often used as quality indicators because they are easily measured. Other components, less easily quantified that interact with quantitative measurements of these grapes, play a significant role in defining the grape and future wine quality. Thus,

microvinification is a necessary step to begin to understand the visual, taste, and aroma characteristics of a particular grape for wine.

Funding for the microvinification of the mature seedling crosses will compliment funding received by the ND Grape and Wine Grant by enabling us to select biotypes for advancement not only due to plant physical characteristics and fruit yield and quality parameters, but to also include organoleptic characteristics that are released during maceration and yeast fermentation.

Plants depend on environmental cues to signal life history events. Climactic cues, such as temperature and moisture, may be less reliable in unpredictable climates when compared to orbit based cues such as photoperiod. Increased stringency in response to reliable cues should stabilize year to year performance in northerly latitude. Both field and growth chamber experiments will be used to relate findings in controlled conditions to field level outcomes. Field data has been collected for almost three years at two locations on three cultivars (St. Croix, Marquette, and Frontenac Gris) using three replications. Data probes have been tracking soil moisture content as well as ambient air temperatures. Following the longest day of the year, measurements were taken as indicators of plant responses. Indicators included growth rate, shoot tip abscission, lateral shoot development, periderm development rate, and bud maturation rate. Relative indicator values will be correlated with relative winter injury rates as well as climatic conditions to determine cultivar differences. Results and conclusions from the field study will be compared to those found in the growth chamber study to determine if the conclusions drawn from the controlled condition study are representative of real field observations.

The growth chamber study will evaluate differences in environmental responses of several important germplasm sources. Forty-nine accessions will be tested in combinations of ambient temperature and soil moisture regimes under decreasing photoperiod. Deviations of individuals given favorable acclimation conditions (cold and dry) will be compared to those given unfavorable conditions (hot and wet) using the same indicators utilized in the field experiment. Important individual comparisons will be made including, reactions of different species, reactions of different related families, and reactions of parents versus their offspring. Selections of progeny will be based on the study as well as incorporation of the selection procedure into the NDSU hybrid grapevine germplasm enhancement program upon successful determination between individuals in the stability of their response.

Project Activity	**Who will do the work?**	**When will the activity be accomplished?**
Test fruit quality and microvinification characteristics of ND accessions that fruited in 2014.	John Stenger, Brittany Olson, 9 mo. researcher, Harlene Hatterman-Valenti, Tom Plocher, and private enologist	Oct 2014 –May 2015
Twenty-nine ND accessions were microvinified and sensory evaluated using a modified scoring sheet used by enologists in the Northern Grape Project. The results are presented in the table on the following page. From the evaluation, three show promise and will be propagated so that larger samples can be made.		
Collect field and growth chamber acclimation data.	John Stenger and 9 mo. researcher	Oct 2014 – Oct 2015

A growth chamber to compare acclimation response of grapevine under reducing photoperiod in two static temperatures through the quantification of seven predictor variables. Singular value decomposition (SVD), Tucker decomposition, and Multidimensional scaling (MDS) were compared for their effectiveness in interpreting the interaction among cultivars, traits, photoperiodic times, and temperatures. Overall similar results were obtained from either SVD or Tucker decomposition. Each identified three axes relating to the genotypes investigated. Most SVD and Tucker models, with the exceptions of the non-rotated SVD and core-rotated Tucker models, identified significant variation in axes relating cultivars to rates of tip abcission and the transition from active growth to tissue maturation. Tucker decomposition was better able to separate wild parental types (*Vitis riparia*) from other genotypes, was more consistent in the subspace defined, and was more readily interpretable as modes could be evaluated separately, thus was preferred over SVD. Field grown plants of three locally important cultivars were evaluated under five environmental conditions for similar acclimation traits along with fruit maturation traits under naturally decreasing photoperiod and temperature regimes. Reductions of phenotypic and temperature trends lead to a correlation between axes contrasting 2012 with other years. In both studies, differences in response to differing temperatures were found in either tissue maturation or in tip abcission progress along with effects on additional related traits. Adapted types, including *V. riparia* and 'Frontenac', showed increased tip responsiveness to temperature while *V. riparia* and 'MN 1131' had increased growth rate of growth cessation and tissue maturation when compared with more marginal types including 'Marquette'. The commonality of *V. riparia* and all tested checks in acclimation timing lead to the conclusion that one temperature adaptive response in addition to early acclimation timing was required for successful adaptation to the Northern Plains Region. Unique responses to temperature reduction were found in all individuals in the field, while 'Marquette' additionally was found to be more responsive under temperature increases, as it comparatively reverted to an active growth state. These alterations were speculated to be caused by differential partitioning of phloem resources within the plant through control of stomatal conductance. Overall, the investigated qualities were found to be detectable in small plants under controlled conditions allowing for controlled testing to increase the efficiency of adapted temperature responses into quality backgrounds. Lastly, a unique genotype, 'ND mutant 1', was discovered while conducting this research. The unique genotype was determinate in both growth and reproduction in contrast to the indeterminate vining growth habit that defines members of *Vitis*. Further testing of the unique phenotype and its derived progeny for any difference in expression of the homologous flowering genes of those well studied in *Arabidopsis* spp. and *Populus* spp. may give more insight into the unique nature of flowering and indeterminate vining growth form of found within *Vitis*. Overall, insight into the contrasts among acclimation processes occurring within *Vitis* hybrids during fall was gained, and the use of growth chamber based evaluations of *V. riparia* derived progeny for background selection may lead to more rapid introgression of adaptive traits into favorable quality backgrounds in cold-climate *Vitis* breeding.		
Assess accessions for wine potential from organoleptic (aroma, flavor, mouth-feel) characteristics and basic fruit quality characteristics.	Tom Plocher and private enologist	Feb - May 2015

Sixteen people were invited to Bear Creek Winery to conduct sensory evaluations on the microvinified wine. The 16 invited people were experienced in sensory evaluation of cold-hardy wines and all participated. Evaluation sheets were compiled for the advancement decision on each accession.		
Present progress report at NDGWA annual meeting	Harlene Hatterman-Valenti	Feb 2015
Dr. Hatterman-Valenti presented progress report to audience at the NDWGA annual conference.		
Clonally propagate potential advance selections	Collin Auwarter and 9 mo. researcher	March - June 2015
Individuals selected for further testing from both phenotypic data and organoleptic evaluations were propagated and placed into a replicated field trial at two locations (west and east) with current standards.		
Crossing high-quality early-ripening *V. vinifera* and near *vinifera* cultivars with wild grape and extremely hardy hybrid sources to create F1 progeny	John Stenger, Collin Auwarter, Tom Plocher, undergraduate students	Oct 2014-April 2016
Germinated approximately 8,000 seeds from greenhouse and field crosses and maintain over 5,000 seedlings (weeding and watering) each year until transplanting into field nurseries.		
Collect field data on seedlings planted in nurseries	Chris Asmundson, John Stenger, Collin Auwarter, Harlene Hatterman-Valenti, and 9 mo. researcher	May - Oct 2015
Numerous hours have been used by all to transplant seedlings, maintain new and older seedlings, collect phenotypic data on all seedlings and collect fruit quality data on individuals that produce fruit. Over 15,000 individual plants from both locations have data collected throughout the growing season.		
Present progress reports during the NDSU Horticulture Field Day and NDGWA Fall Tour.	Harlene Hatterman-Valenti	July-Sept 2015
Dr. Hatterman-Valenti made two field day presentations along with an update at the "wine to dine" NDGWA fall event.		
Test fruit quality and microvinification characteristics of ND accessions that fruited in 2015.	John Stenger, Brittany Olson, Collin Auwarter, Harlene Hatterman-Valenti, Tom Plocher, and private enologist	Oct 2015 – April 2016

Twenty-three new ND accessions along with 12 repeated from the previous year were microvinified and sensory evaluated using a modified scoring sheet used by enologists in the Northern Grape Project. From the evaluation, two show promise and will be propagated so that larger samples can be made.		
Present results at VITINORD (International Northern Grape Conference)	Harlene Hatterman-Valenti	November 2015
Both John Stenger and Harlene Hatterman-Valenti were invited speakers at VITINORD		
Analyze acclimation data and prepare manuscripts with results	John Stenger	Jan-April 2016
John completed the two chapters for his dissertation which are available through the NDSU library dissertation and thesis database		
Prepare progress reports, annual report and final report	Harlene Hatterman-Valenti	Jan 2015 – April 2016
Quarterly reports were completed and this is the final report.		

Goals and Outcomes Achieved

Goal #1. To identify at least two accessions for advance testing. These accessions not only have to have the fruit composition measurements that winemakers want and need, they must also have good enological characteristics (visual, taste, and aroma). Accomplishing this goal will get our program one step closer to the long term goal of the introduction of a red and white wine grape for North Dakota.	Goal #2. To identify at least one native *V. riparia* biotype with a stringent and stable photoperiodic acclimation response. This biotype will be used as a parent and crossed with short season, high quality V. vinifera cultivars that do not have a stable photoperiodic acclimation response. Under the developed controlled test, individual populations of seedlings will be tested for their relative ability to stringently acclimate in the fall. Selections will be made on this criterion dramatically reducing the number of seedling needed to be planted and its associated cost in certain situations.
PERFORMANCE MEASURE #1: Percent of siblings from each cross that have the minimum fruit composition measurements (soluble solids, pH, and titratable acidity) that winemakers want and need was collected. Percent of siblings from each cross that have the minimum enological measurements (visual, taste, and aroma) that winemakers want and need was collected. Number of siblings from each cross that have	PERFORMANCE MEASURE #2: A new method to select cold-hardy accessions was developed and validated and now can be used to determine the number of biotypes with stable photoperiodic acclimation and the number of siblings from a particular cross with stable photoperiodic acclimation even though there was not enough time to initiate these comparisons.

both fruit composition and enological measurements was collected.	
BENCHMARK #1: Fruit from the University of Minnesota cultivars (Frontenac, LaCrescent, and Marquette) was included as the benchmarks for the fruit composition measurements (soluble solids, pH, and titratable acidity) and enological measurements (visual, taste, and aroma). Five individuals have been propagated and placed in replicated field trials on the east and west half of the state along with industry standards.	BENCHMARK #2: A *V. vinifera* cultivar, known not to be stable for photoperiodic acclimation response and *V. riparia* biotypes collected from North Dakota that are considered to have a rather stable photoperiodic acclimation response were compared in the growth chamber trial. The *V. riparia* had varied responses to temperature declines followed by temperature increases as daylength was reduced indicating that the response is not stable for the species. 'Marquette' responded similar to the V. *vinifera* cultivar in comparison to *V. riparia* biotypes and other *V. riparia*-based cultivars indicating that part of the winter injury with 'Marquette' is due to reallocation to plant sinks with warmer temperatures in the fall even if they are temporary.

The target of having 10% of the accessions tested with better fruit composition and enological measurements in comparison to 'Marquette' or 'LaCrescent' was met if all fruit composition and enological measurements did not have to be surpassed. The accessions that have been selected for advanced testing have similar and better fruit composition that the two UM cultivars used for comparison. However, due to small samples (microvinification), even trained sensory panelists wanted these accessions propagated so that more typical small batch wines could be made and evaluated.

Beyond early photoperiod based induction, the overall rate of transition from active growth to tissue maturation was a discernable trait of *V. ripara*. Additionally, the trait that contributed greatest to the variation in the dataset was the differences in tip abscission progress rate between the two temperatures over the photoperiodic time. *V. riparia*-like accessions tended to be temperature sensitive in their responses, having greater increases in reaction across the two temperatures when compared to the average vine, where non-*V. riparia* types tended to be less responsive. 'MN 1131' was found to be an adaptive type that combined early acclimation response with rapid progression of the transition from active growth to tissue maturation, while 'Frontenac' displayed an alternative method as it had early induction of acclimation response coupled with temperature adaptive tip abscission rate across the tested temperatures. 'Marquette' did not differ from *V. riparia* in acclimation initiation timing where the trait was found to statistically differ across genotypes, however it did differ for the additional adaptive responses involving rate of progress of either tip progression and the transition from active growth to tissue maturation across the two temperatures. The conclusions of this study indicate, in part, that the inclusion of either a rate adaptive response or an early acclimation initiation was sufficient for cultivars in North Dakota to be productive. However, the exclusion of both rate adaptive responses, even with early initiation, may leave cultivars unreliable in the region. The only

native *V. riparia* biotype that did not significantly differ from *V. riparia* across all axes having genotypic variation across tested all models was '917'. Other entries that tended to have the fewest deviations from *V. riparia* across axes and models were 'Frontenac', '911' and '913'. Other accessions that did not differ along any axes showing genotypic variation in select models were '64', '73', '906', '920', '924', '937' and '938'. The tested genotype that tended to significantly differ from *V. riparia* along two evaluated axes most was 'Marquette', while '906', '909', '914', '936', '937', '939', and '940' also differed from *V. riparia* along two axes for specific models.

Beneficiaries

The project beneficiaries were originally thought to be commercial growers and wineries that buy the fruit. With the local foods initiatives all over ND, it became evident that homeowners wanting to plant an edible landscape would also benefit from grapes that will thrive in North Dakota due to stable acclimation processes. Field days had approximately 400 people over the 2-year span. Approximately 30% indicated that they knew NDSU was working on wine grape cultivars for North Dakota. The number of field day participants that new about grape efforts was lower than we originally thought, which means field day efforts helped to disseminate information.

Lessons Learned

The lessons learned are numerous, but three really stand out. First of all, the time to collect all the phenotypic and fruit quality data was greatly underestimated. Luckily, Tom Plocher and Rod Ballinger did not want the funding for microvinification and we could rebudget to collect all the needed data. Secondly, the variables involved in the acclimation process were more than anticipated, but we now know the main measurements that need to be recorded in order to determine acclimation stability. Lastly, we thought that winter injury to 'Marquette' was due to lack of overall hardiness compared to other *V. riparia*-based cultivars. However, we found that unlike other *V. riparia*-based cultivars, 'Marquette' stops the acclimation process when temperatures increase after a chilling event, which makes it very susceptible to injury if temperatures drop quickly after the temperature increase.

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Additional Information

Five presentations were made at national and international conferences. One dissertation was completed from this research, and three publications are planned from this research.

Development of Superior Juneberry Cultivars

Final Report

Partner Organization: North Dakota State University

Project Summary

Saskatoon serviceberry (*Amelanchier alnifolia* Nutt.), or simply, serviceberry, is an ornamental shrub that produces high quality, berry-like pome fruit, and is often compared to blueberry (*Vaccinium* spp.) in appearance along with its richness in nutrients and flavor. A member of the *Rosaceae* family, serviceberry produce delicate, white flowers that create an aesthetic and naturalistic look in plantings. Most serviceberry are propagated in Canada. However, imported stocks are often expensive and reduced quality. In 2007 and 2008, clones were accessed from over 70 locations throughout North Dakota and proliferated in tissue culture. Following establishment, North Dakota State University (NDSU) associates planted replicate field trials of these biotypes and named cultivars at two locations with the intent of introducing edible-ornamental North Dakota strains. Yield data were gathered from harvests in July of 2015 and 2016. Native plants were comparable to and often out-yielded market cultivars. As per 2015 data, some of the highest-yielding biotypes were ND 48-2, ND 1-7, ND 1-2, ND 1-5, ND 1-6, ND 1-4; comparable with market cultivars Success, Parkhill, and Regent. Other qualities that were measured included growth habit, flower showiness, and presence of disease and insect pests. Of the six that have consistently performed as well as if not better than named cultivars, one will be selected for release this upcoming year after consultation with Kyla Splichal at the Williston REC. The remaining will be evaluated for another year before deciding on additional releases.

Project Approach

Project Activity	Who will do the work?	When will the activity be accomplished?
-Add difficult to tissue culture selections into field nurseries. Collect phenology data.	**Graduate student – Jorden; Kim and Jorden – data collection**	**spring 2015**

Flower data was collected along with spring frost injury both years. A graduate student (Jorden) was hired to assist with data collection at NDSU and to initiate tissue culture of the missing biotypes.

-Maintain nurseries, water plants, weed control, and note any pests or pathogens. Collect plant growth and fruit yield and quality data.	**Summer employees, Jorden, Hinrichsen, Harlene Hatterman-Valenti and Kim Holloway**	**Summer 2015 and 2016**

Plants did not need supplemental water, but a netting system was installed to reduce bird predation as a lot of fruit was lost the previous year without netting. A big storm went through the Absaraka trial over a weekend during mid-July, breaking wood posts and tearing the netting. Plant growth, flower abundance, and frost injury were collected each spring. Fruit yield and quality data were also collected and are provided in tables at the end of this report.

-Develop surveys for field days. Distribute and collect surveys.	**Hatterman-Valenti and Kim Holloway/Kyla Splichal**	**Summer 2015 and 2016**

A survey was provided at the Williston field days since the field days were scheduled when the shrubs had ripe fruit. The field day at the NDSU Horticulture Research and Arboretum site near Absaraka, ND were in September, well after the shrubs had produced fruit. Over 75% of the attendees knew about juneberries and would purchase an introduction from NDSU.

-Bring fruit to grocery markets and collect taste and marketing data.	**Hatterman-Valenti and Kim Holloway/Kyla Splichal**	**Summer 2015 and 2016**

Spotted winged drosophila was identified in juneberries at the Carrington REC, which concerned us since neither nursery applied insecticides to make sure fruit was free of larvae and no pheromone traps were located at either location in 2015 and traps located at each nursery showed may flies in 2016.

-Present preliminary results at state and regional small fruit meetings	**Harlene Hatterman-Valenti and Jorden Hinrichsen**	**Summer and Fall 2015**

Presented results at NCCC212 multi-state small fruit and viticulture meeting that was held at Auburn University in 2015 (42 in attendance) and at Virginia Tech in 2016 (35 in attendance). Jorden presented results at the International Woody Landscape Plant Symposium in August of 2016 (100 in attendance). There is no state small fruit organization in North Dakota, but I have been asked to talk at the South Dakota Specialty Producers Association meeting.

-Identify advance selections for testing at NDSU REC's with Juneberries.	**Hatterman-Valenti and Kyla Splichal**	**Summer/Fall 2016**

Biotypes ND 48-2, ND 1-7, ND 1-2, ND 1-5, ND 1-6, ND 1-4; have good ornamental characteristics and yields were comparable with market cultivars Success, Parkhill, and Regent. They out-yielded all other named cultivars. Since Kyla Splichal worked with the plants at the Williston REC, her opinion on the advancement of a single accession will be considered.

Goals and Outcomes Achieved

Outcome Measure #1	Outcome Measure #2
Goal:	**Goal:**
Increase access to superior native juneberry biotypes in ND	**Increase awareness of fruit availability and health benefits**
Performance measure:	**Performance measure:**
Number of native biotypes that perform as well as the standard cultivars commonly grown in Canada	**Number of growers and customers familiar with juneberries and familiar with health benefits from project surveys. Number of customers willing to purchase fresh juneberries versus juneberry products.**
Benchmark:	**Benchmark:**
Yield and performance measurements of standard cultivars commonly grown in Canada	**Initial level of knowledge on surveys prior to sampling and results reporting**

Data for the native biotypes will be monitored after collection in the spring and after fruit harvest in July.
Plant growth, flower abundance, and frost injury were collected each spring and suggested that most biotypes were Amelanchier hybrids as flowering responses were similar to those of 'Parkhill' 'Success', and 'Reagent'. We collected yield and performance measurements in 2015 and 2016 and feel that a number of biotypes are as good as the standard cultivars commonly grown in Canada.

Data for monitoring survey performance will be generated using pre- and post-project surveys. Pre-surveys will be given to field day attendants prior to presentations. Post-project surveys will be either mailed one week following the field days or handed out at the end of the field days. Pre- and post-surveys for the customers will be given prior to fruit sampling and explanation of health benefits. Data will be used to identify areas where grower and customer knowledge is lacking, which will help guide the development of final project report.
Field day attendees were surveyed pre and post at the field days and a majority (60%) seemed to know about juneberries and the value-added products available from juneberries, but would only purchase one or two shrubs for their landscape. This was because the horticulture field days are primarily getting homeowners instead of commercial growers to attend. Originally, we wanted to increase the number of commercial producers, but after further consideration, greater return would occur if releases were for the homeowner as edible landscape plants. We decided not to survey customers at grocery stores due to the identification of SWD in juneberry at the Carrington REC and the lack of insecticide applications to protect fruit. The concern that there would be SWD larvae in the fruit and that consumers unfamiliar with juneberry fruit would be completely turned off from ever trying the fruit again.
Target (for both #1 and #2):
Increase the juneberry growers by 30% and increase the survey scores of growers by 40% and increase the scores of customers by 80%.
The grower target was originally for commercial growers, but as growth and flowering data became available, there was the realization that there may be greater market potential to homeowners. Since more than two years of data is wanted by the NDAES, before cultivar release, the top accession wasn't submitted for release. We also underestimated the number of people and potential growers that knew about juneberries, so the increase by 40% was unrealistic. Even though the grower targets were not reached, we would rather see NDSU juneberry cultivars purchased instead of Canadian cultivars and am hopeful that this will occur in the future. The infestation of juneberry fruit by SWD was completely unexpected as we really thought the fruit skin thickness would be a deterrent to these fruit flies. The number of pick your own juneberry establishments has increased from three to four, with the newest operation near Kindred, ND. Due to the original small number of commercial growers, the additional grower would increase the grower percentage by 33%. The greatest deterrent to growth in this area has been the lack of access to plant material. A company has opened in Minnesota (Honeyberry USA), which sells the main cultivars available in Canada. The survey of growers at the Williston REC field day that knew about juneberries and had tasted the fruit, indicated that all (30 attendees) knew about juneberries and had tasted the fruit, so it was hard to increase juneberry identity in the western part of the state. At the Absaraka field day about half of the

attendees knew what a juneberry was and approximately one-third had tasted a juneberry. Since the Absaraka field day is scheduled after the typical harvest dates, we have made plans to freeze fruit samples so that attendees can taste the fruit in August. Freezing the samples will also stop SWD development, the main reason we didn't bring the fruit to a grocery store for sampling. Recent research (2016) has also shown promising control of SWD with an organically approved insecticide + surfactant.

Beneficiaries

The project beneficiaries were originally thought to be commercial growers and customers that buy the fruit. With the local foods initiatives all over ND, it became evident that homeowners wanting to plant an edible landscape would also benefit from the release of these biotypes. Field days had approximately 400 people over the 2-year span. Approximately 60% indicated that they knew what a juneberry was. The number of field day participants that new about juneberries was higher than we originally thought, exceeding the expectations of the survey score prior to the explanation of what plants were what.

Lessons Learned

The biggest obstacle will not be to educate customers about what a juneberry is. Instead the biggest obstacle will be the propagation of the accessions wanted. We still haven't been able to get at least two accessions into tissue culture so that we could field evaluate. Post-rooting dormancy and other micro-propagation issues most likely will cause the greatest challenge.

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Additional Information

Table 1. Flower showiness ratings for Absaraka orchard.

Cultivar/ accession	Flower rate		Cultivar/ accession	Flower rate		Cultivar/ accession	Flower rate	
ND 1-7	99.5	a*	Parkhill	95.0	ab	ND 41-1	57.5	de
ND 1-4	98.0	a	ND 15-2	92.0	ab	ND 5-1	53.0	def
Regent	98.0	a	ND 9-1	89.5	ab	Buffalo	52.0	def
Success	98.0	a	ND 18-1	82.5	ab	Par90	49.5	def
ND 1-6	97.5	a	ND 16-1	78.5	bc	JB30	42.5	ef
ND 1-5	97.0	a	ND 71-1	63.5	cd	Northline	41.5	ef
ND 1-2	96.5	a	ND 17-2	62.0	cd	Kelner	38.5	f
ND 48-2	96.5	a	ND 12-1	61.5	cd	Honeywood	36.0	f

*lower case letters represent no significant difference between same letters at p=0.05.

Table 2. Berry size for 2015 and 2016 harvest of Absaraka planting.

Cultivar/ accession	Mean diameter		Cultivar/ accession	Mean diameter		Cultivar/ accession	Mean diameter	
	--------mm-----			--------mm-----			--------mm-----	
Par90	13.2	a*	ND 9-1	11.1	edf	ND 16-1	10.5	h
Honeywood	12.0	b	ND 71-1	11.1	efg	Buffalo	10.5	h
ND 1-2	12.0	b	ND 1-6	11.0	efg	ND 12-1	9.7	i
JB30	11.7	bc	ND 41-1	11.0	efg	ND 5-1	9.7	ij
ND 1-7	11.6	bcd	Parkhill	11.0	fg	ND 15-2	9.5	ij
ND 48-2	11.5	cde	ND 1-5	10.6	gh	ND 17-2	9.5	ij
ND 1-4	11.4	cdef	Regent	10.5	h	ND 18-1	9.2	j
Success	11.2	edf	Northline	10.5	h	Kelner	8.0	k

*lower case letters represent no significant difference between same letters at p=0.05.

Table 3. Soluble sugar content for 2015 and 2016 Absaraka planting.

Cultivar/ accession	Soluble sugars		Cultivar/ accession	Soluble sugars		Cultivar/ accession	Soluble sugars	
	----%---			----%---			----%---	
Northline	18.7	a*	Honeywood	14.9	ef	Parkhill	13.2	jklm
ND 17-2	18.5	a	ND 18-1	14.6	efg	ND 1-7	13.1	klm
ND 15-2	18.0	ab	JB30	14.2	fgh	ND 1-4	13.1	klm
ND 71-1	17.3	bc	Par90	14.1	ghi	ND 48-2	13.1	klm
ND 5-1	16.9	c	ND 1-6	13.9	ghij	ND 1-2	13.0	klm
ND 12-1	16.6	cd	ND 41-1	13.7	hijk	ND 1-5	12.9	lm
ND 16-1	16.0	d	Success	13.6	hijkl	Regent	12.5	m
ND 9-1	15.1	e	Buffalo	13.3	ijkl	Kelner	9.2	n

*lower case letters represent no significant difference between same letters at p=0.05.

Table 4. Marketable weight for 2014, 2015, and 2016 Absaraka planting.

Accession/ Cultivar	Weight		Accession/ Cultivar	Weight		Accession/ Cultivar	Weight	
	--------g-----			--------g-----			--------g-----	
ND 1-2	1768	a*	Parkhill	1043	d	ND 12-1	463	gh
ND 1-7	1653	ab	Regent	1036	d	ND 41-1	383	ghi

ND 1-4	1611	ab	ND 71-1	937	d	JB30	381	ghi
ND 48-2	1609	ab	Buffalo	708	e	Honeywood	365	ghi
ND 1-6	1605	ab	ND 18-1	661	ef	ND 17-2	283	hij
ND 9-1	1574	b	ND 15-2	540	efg	ND 5-1	231	ijk
Success	1375	c	ND 16-1	483	fg	Kelner	149	jk
ND 1-5	1275	c	Par90	471	fgh	Northline	77	k

*lower case letters represent no significant difference between same letters at p=0.05.

Increasing Consumption of Edible Beans by Creating Bean Flours

Final Report

Partner Organization: North Dakota State University

Project Summary

The goal of this project was to optimize the milling of edible beans into different flour types (i.e. whole grain flour, cotyledon flour and seed coat rich flour). Whole grain flour and cotyledon flour were evaluated as ingredients in cookies and cakes. Whole grain flour was milled using a centrifugal mill. Rotor speed and aperture screen size affected the level of heat generated during milling. Size reduction of black beans was more difficult and starch damaged was enhanced when the bean moisture was above 10%. Burr mill was identified as being effective in separating seed coat from cotyledon. Results strongly indicated that a moisture treatment was needed for enhanced separation of seed coat from cotyledon. The best moisture treatment was to temper the seeds to 40% moisture and dried at 90°C to ambient moisture content. Application studies showed that the cotyledon flour performed best among the bean flour in both cookies and cakes. When using cotyledon flour alone, additional eggs may be required, specifically in cakes. The cotyledon flour made very good cookies and the only concern was that some of the cookies had a greyish color. The whole bean flour had a more intense bean flavor compared to the cotyledon flour.

Milling of dry beans in general and black beans in particular have not been studied extensively. Even less is known about methods to separate seed coat from cotyledon. Thus, the first objective was to develop a dry milling system capable of grinding dry bean into a whole-grain flour and capable of removing and separating the seed coat from the cotyledon, which would result in a seed-coat-rich flour and a cotyledon-rich flour. The seed-coat-rich flour and cotyledon-rich flour represent two additional ingredients that could be incorporated in food products.
The trend in the food industry to produce foods that are gluten free and high in protein and fiber demonstrates a need to incorporate more bean ingredients into food products. However, beans have not been traditional ingredients in food products due to the limited knowledge available regarding the functionality of bean flours and incorporation of beans into food products beyond traditional products (i.e. soups). Incorporation of beans into non-traditional products such as baked goods and snack products would greatly expand the healthful consumption of beans. The second objective was to produce baked products such as cakes, cookies and bread from different types of bean flours obtained during milling and assess the sensory properties of these products.

Food industry needs more information and successful examples of milling and utilization of edible bean flour. The third objective was to increase the knowledge of dry bean utilization through dissemination of results at professional trade organizations and by hosting two workshops on dry bean milling and the application of bean flours in non-traditional food application.

Project Approach

The development a dry milling system capable of grinding dry bean into a whole-grain flour and capable of removing and separating the seed coat from the cotyledon, which would result in a

seed-coat-rich flour and a cotyledon-rich flour, was phase one of the project. In this project, key factors that affect black bean milling were evaluated using a centrifugal mill. Factors that affect black bean milling were grouped into those associated with the mill (rotor speed, screen aperture size, and feed rate) and those associated with the grain (seed conditioning treatments).

Burr mill, disk mill, oat thresher, first break rolls on a roller mill, and a corn cracking roller mill were evaluated for their effectiveness in separating seed coat from cotyledon of non-conditioned and conditioned black bean seeds. The burr mill was found to be most effective. Various seed conditioning treatments were evaluated for their effectiveness in enhancing the separation of seed coat and cotyledon when milled on the burr mill. Hull and cotyledon fractions resulted from this phase of the project. The cotyledon fraction was used in baking applications. The baking part of the project included cookies, cakes and bread. Base formulas were developed with home-use in mind. Therefore, no attempt was made to add ingredients with known functional improvement properties commonly used by food manufacturers. A 10% supplementation of wheat flour with bean flour was used in the bread development based on prior experience. The products were evaluated after each change in formulation was made, Sensory evaluation was completed using a trained panel. The products were initially made using formulas designed for all-purpose flour. After the first run through of the cookie and cake production, we decided to use a trained panelist instead of an untrained panel. The sensory group was made up of individuals with multiple years of experience in food tasting. The group assessed multiple iterations of the product until the products were deemed acceptable.
Results were presented at AACC International meeting and at IFT meeting.

Goals and Outcomes Achieved

Comparison of actual accomplishments with goals established

One of the goals of the project was to "Increased knowledge linking bean flour type and application in food products." was unable to be tested due to the rearrangement of the Dry Edible Beans as Food Ingredient short course from a spring date to a September date. The Northern Crops Institute hosted a short course on September 19 and 20, 2016 that included 25 participants. Part of the course will include pulse milling. In addition a technical bulletin was not made for dry bean milling. The audience for this bulletin was small specialty milling companies. The results of this research is very applicable to small scale-product development laboratories but the processes identified here need to be scaled up to determine if they would work on a commercial level. However, knowledge was gained in the laboratory regarding the development of dry milling systems (objective 1) and the subsequent application of bean flour in baked goods, as described below during the completion of objective 2.

Objective 1. *To develop a dry milling system capable of grinding dry bean into a whole-grain flour and capable of removing and separating the seed coat from the cotyledon, which would result in a seed-coat-rich flour and a cotyledon-rich flour.*

The primary goals of this objective were to (1) identify key factors that affect dry bean milling and (2) develop a dry milling process capable of separating seed coat from cotyledon.
Factors that affect dry bean milling can be grouped into those associated with the mill and those associated with the grain. In this project, key factors that affect black bean milling were evaluated using a centrifugal mill. Centrifugal mill represents a single-stream one-pass milling

system that can be used to grind entire seed without separation of seed coat and germ from the cotyledon. Centrifugal mill generally consists of a vibratory feeder and a milling chamber that consists of a rotor and screen milling. The center of the rotor is flat open area where the seeds are first deposited by the feeder. Wedged shaped blades are located at the end of the rotor. As the rotor spins, the seeds are impacted by the blades and thrown centrifugally against a grated screen. Thus, centrifugal mill uses the particle size reduction principles of impact, abrasion, and shearing forces. Some centrifugal mills use air flow to help prevent the flour from overheating.

Mill factors.

Rotor speed, mill screen aperture, and feed rate affected black bean milling with centrifugal mill. Increased rotor speed and feed rate and decreased mill screen aperture size favored the production of fine bean flour particle size but often resulted in increased temperature of mill surface and of the bean flour. Commercially, dry beans are often milled using a one pass system. Single-stream one-pass system can generate excessive heat (80-90 °C) that can adversely affect the functional properties of milled flour. These temperatures are capable of denaturing some protein and promote lipid oxidation. A balance is needed between yield of fine particle size flour and the generation of heat.

Flour quality was affected most by screen size, intermediate by rotor speed, and least by feed rate. Screen aperture of 250 µm resulted in the greatest fine particle yield but also the greatest increase in flour temperature and loss of flour moisture content. Mill optimization for milling black beans using a centrifugal mill was best when used screen opening of 500 µm, rotor speed of 12,000 rpm, and an intermediate mill feed rate of 267 g/min.

Grain factors

Moisture content is known to affect milling properties of seeds. Moisture acts as a plasticizer which toughens the seed coat and softens the cotyledon. Seed moisture contents from 6 to 14% were evaluated. Seed moisture content had little or no effect on total starch, protein, and ash contents of black bean flour. Changes in chemical composition were not expected. Starch damage showed no significant differences for samples having 6 to 10% moisture content. However, there was a 1.2 to 2.4 percentage unit increase in starch damage for samples at 12 and 14% moisture content, respectively, which reflects the increase amount of force necessary to fracture seed with 12 and 14% moisture content. At low moisture content the seed coat and cotyledon are brittle and friable resulting in production of small particles. Experiments on black bean seeds showed that more energy was needed to fracture the seed with 14% moisture [254 Newtons (N)] than with 6% moisture (83N). These results are attributed to moisture in the seed coat causing the fiber to become more pliable and less brittle. Bean flour was coarser when bean moisture content increased.

Seed conditioning, eg. cooked or soaked black beans**,** were shown to affect milling properties of seeds. Dry beans are often cooked or soaked as a treatment to reduce anti-nutritional components such as phytic acid and protease inhibitors. Cooked and soaked beans were air-dried to 10% moisture content and then milled. Cooked-dried and soaked-dried treatments caused an increase in seed size and a decrease in seed weight which resulted in decline in test weight (weight/volume). Many mills use volumetric feeders to regulate milling rate and a decline in test weight would result in a decline in feed rate, simply because there is less beans per unit volume. Thus, feed rate for soaked-dried seed was 25% lower than for nonsoaked seed. Cooked-dried and soaked-dried treatments reduced the force needed to fracture bean seed, which indicates that these treatments increased the brittleness of the seed. Cooked-dried treatment was found to have the greatest impact on flour characteristics such as in pasting properties due to high starch

damage. Overall, soak-dried treatments had little or no effect on total starch, protein, and ash contents of the grain. Cooked-dried seed had increased total starch and starch damage but ash content was reduced significantly. Increase in total starch probably reflects the increased in accessibility of enzymes used in the starch assay due to the high level of starch gelatinization during cooking. Soaking black bean seed had little or no effect on total starch and protein content but did cause a significant decline in ash content. Fine particle yield decreased with cooked-dried or soaked-dried treatment.

Burr mill, disk mill, oat thresher, first break rolls on a roller mill, and a corn cracking roller mill were evaluated for their effectiveness in **separating seed coat from cotyledon** of non-conditioned and conditioned black bean seeds**.** Initially, conditioning involved either cooking the beans for 30 min in boiling distilled water or soaking beans in distilled water for 24 h, followed by air drying the seeds to 8% moisture content. An aspirator was used to separate seed coat particles from cotyledon. Results indicated that the burr mill was the best mill for removing seed coat and producing a coarse particle size of remaining cotyledon. Seed coat removed during burr milling was very clean – free of cotyledon. The shearing action of the burr mill is attributed to its ability to remove the seed coat from the cotyledon. The results also indicated that seed conditioning was important in the separation of the seed coat from cotyledon. Very little cotyledon was attached to the seed coat removed from cooked-dried beans, particularly when compared to non-conditioned seed. A disadvantage of cooked-dried beans was that the anthocyanin pigment in the seed coat leached into the cotyledon causing the cotyledon to appear purple. Some leaching was observed in the cotyledon from soaked-dried seed. All further experiments used the burr mill to remove seed coat from the cotyledon.

Next a second set of seed conditioning treatments were evaluated. Conditioning treatments included: 1) heat treatment (50, 70, 90°C) and time (10, 30, 50 min); 2) tempering (10, 15, 20% moisture and heat 50, 70, 90°C); 3) pressure extraction (5, 10, 15 cycles); 4) soaking (1, 3, 5 days); 5) cooking (5, 10, 20, 30 min); and 6) freezing (freeze-thaw, freeze-boil, soak-freeze-thaw, soak-freeze-boil, cook-freeze-thaw, cook-freeze-boil).

Results indicated that seed coat removal was best with cooking times of 5 to 30 min (5.5 - 6% wt/wt seed coat) and for freezing treatments that involved soaked seeds (8.1% wt/wt seed coat). Pressure washed-dried and soaked-dried treatments were better than no conditioning treatment but were less effective than cooking-drying or soaking-freezing conditioning treatments. Cooking seed in boiling water was one of the most effective treatments. For example, the seed coat fraction was 6.2 to 6.7% wt/wt for seed that were cooked in boiling water for 5 to 30 min and dried. Non-conditioned seed produced only 1.5 to 2% wt/wt seed coat. Soaking seed for 24 h, freezing the soaked seeds, thawing the frozen seeds and then air drying resulted in the highest (8% wt/wt) seed coat fraction after burr milling.

Based on these results it was concluded that a moisture treatment was needed for enhanced separation of seed coat from cotyledon. Based on this conclusion a final set of milling experiments was conducted. Two sets of seed treatments were evaluated: 1) temper-dried treatments included seed tempered from 10 to 50% moisture. Moisture was allowed to equilibrate for 24 h followed by drying at 90°C until seeds returned to their original moisture content and 2) cooked-dried treatments included black beans that were cooked in boiling distilled water for 0, 5, 10, and 20 min. Each cooked sample was divided into two sets. One set was air dried and one set was dried at 90°C in a force air oven. Seeds were dried to the original pretreated moisture content. The best boil-dry treatment consisted of boiling 20 min and drying at 90°C. This treatment resulted in 5.6% wt/wt seed coat fraction. The best temper-dry treatment

consisted of tempering to 40 or 50% and drying at 90°C. This treatment resulted in 7.4% wt/wt seed coat fraction and the seed coat remained largely intact and contained very little cotyledon. Based on these results, tempering seed to 40% moisture and drying at 90°C was selected as the best seed conditioning treatment for separating seed coat from cotyledon.

Objective 2. *To produce baked products such as cakes, cookies and bread, snack products such as crackers and chips, and extruded products such as pasta from different types of bean flours obtained during milling and assess the sensory properties of these products.*

The original intent was to develop a broad range of products such as cake, pasta and chips. However, our focus shifted to evaluating cookies and different types of cakes base on the lack of bean-fortified products commercially available in the cake and cookie market. Pasta and extruded chips have entered into the marketplace and thus a shift to cookies and cakes was made due to the lack of products on the market. Bread formulated with 10% whole bean flour or cotyledon flour were formulated. No further attempt was made for higher inclusion rates or development of a gluten free bread based on our past research on pulse fortified breads. The results of the bread followed the same observations made in previous studies. Therefore, the focus of the report will be on cookies and cakes. In addition, we elected to use experienced individuals to provide better guidance in developing products rather than untrained individuals. Observations from the sensory included taste perceptions, appearance and texture. The appearance of the cookies varied substantially among the different formulas (Figure 1). The whole black bean flour produced dark cookies with a purple hue. The cookies made from cotyledon flour had a slight grey appearance that lightened in cookies made with 50% cotyledon flour and 50% all-purpose flour. The use of molasses (as a replacement for sugar) in the formula resulted in a highly desirable cookie appearance. The sugar cookie made with all-purpose flour (i.e. the control cookie) had slight browning on the edges of the cookies. Only the cookies made with cotyledon flour and twice the egg had more browning. In this product, the eggs likely contributed to the excess browning. The cookies made with cotyledon flour had more surface cracks than other cookies. The cookies also had more air cells than other cookies as observe once the cookies were broken. In contrast, the cookies made with the whole bean flour or cotyledon flour with twice the butter had surface appearances that were similar to the all-purpose control cookie. The interior of the cookies of the made with whole bean flour appeared more dense than the control cookie. Overall, the appearance of cookies made with 100% all-purpose, 50% cotyledon flour and 50% all-purpose flour, 100% cotyledon flour with two times the butter, and the molasses cookie all had acceptable appearance.
A slight stickiness in cookies made with all-purpose flour was observed. The crunchy texture was similar across cookies; however, the addition of whole bean flour produced the greatest crunch and required the greatest force to bite into the cookie. Continued chewing of the cookies made with whole produced a slimy mouthfeel. The fiber content in the whole bean flour was likely higher and contributed to the slight slimy texture, which has been observed in other high fiber products. In contrast, the cookies made with cotyledon lacked the slimy mouthfeel. The cookies made with twice the amount of egg did not have an off-flavor as the cookies made with cotyledon alone. The cookies made with cotyledon flour with twice the amount of butter were deemed the best in the context of texture. The texture of the cookies was more like commercially produced sugar cookies and the softest but crumblier bite among the cookies. The cookies made with 50% cotyledon flour and 50% all-purpose flour had a texture that was crunchier than

cookies made from cotyledon flour with twice the amount of butter. Overall, the cookies made with 50% cotyledon flour and 50% all-purpose flour or cotyledon flour with twice the amount of butter were both rated favorably.

The flavor of the cookies made with whole bean flour was deemed to have the highest beany flavor. The cookies made with cotyledon flour had less bean flavor than the cookies made with whole bean flour. The addition of extra amounts of egg and butter to the formulas produced cookies with almost no bean flavor compared to the cookies made with cotyledon or whole bean flours. The addition of molasses to the cookie formulation completely masked the bean flavor and was deemed to be a very good cookie. Overall, the sensory data supports the use of formulas that are a combination of 50% cotyledon flour and 50% all-purpose flour, cotyledon flour with twice the butter, or replacement of some of the sugar with molasses.

In addition to sensory results, objective methods were used determined cookie quality (Figures 2-4). The cookie length and height provide information regarding the size and thickness of a cookie. Cookies made with whole bean flour had the lowest cookie length but the greatest height. This suggests that the cookies had a smaller diameter and greatest thickness among cookies tested. The control cookie had a similar diameter to the whole bean cookie, but was less thick. The cookies with molasses had the greatest length and the lowest height suggesting that the cookies spread out more during baking and resulted in thinner cookies compared to the other cookies. Cookies made with 50% cotyledon flour and 50% all-purpose flour or cotyledon flour had similar lengths and heights but greater diameters and height compared to cookies made with cotyledon flour plus twice the egg or twice the butter (Figure 2). The water activity (Figure 3) of the cookies were all less than or equal to 0.42, which indicates that the cookies produced were in the microbial safe water activity range. The force need to snap the cookie in two was also determined using a texture analyzer. The force needed to break the cookies made with whole bean flour was highest among all cookies (Figure 4). The cookies made with all-purpose flour required a force of 40 Newtons (N) to break the cookie in two. The cookies made 50% cotyledon flour and 50% all-purpose flour required similar (35 N) force to break the cookie as the control. The cookies made with cotyledon flour with twice the butter had break force values that average 24 N, which indicates that the cookies were softer than the control. The data obtained from the texture analyzer mirrored that of the sensory evaluation.

The development of cakes (Table 1) was completed using traditional butter cake and chocolate cake recipes. The butter cake formulations included the various flours (whole bean, cotyledon, all-purpose) with inclusion of different levels of butter and eggs. The cakes with whole bean flour had the darkest appearance, both crumb and surface, while the cake made with cotyledon flour had a purple crumb color and a yellow surface color (Figure 5). The cake made with all-purpose flour had a yellow color. The cake center height was highest in cakes made with all-purpose flour (i.e. control cake) followed by cake made with 50% cotyledon flour and 50% all-purpose flour (Figure 5). Although lower than the control cake, the cake made with cotyledon flour plus twice the egg as the base formula had a very good cake height, which was higher than the cake made with cotyledon flour and the same amount of eggs as the control.

The texture of the control butter cake was essentially the same as the texture in the cake made with 50% cotyledon flour and 50% all-purpose flour and cake made with the cotyledon flour (Figure 6). In contrast, cakes made with whole bean flour and cake made with cotyledon flour with no eggs had the highest firmness/hardness. The cakes made with cotyledon flour and half of the butter or twice the eggs had significantly higher adhesiveness (Figure 6). Adhesiveness indicates the stickiness of a material. Thus, the higher the adhesiveness the higher is the

stickiness. The sensory assessment of the cakes supported the physical data in that the cotyledon flour with twice the eggs had the highest stickiness. The control cake and cake made from with 50% cotyledon flour and 50% all-purpose flour had the dry but springy texture compared to cakes with whole bean flour and cotyledon flour, which were deemed to be moist and brownie-like.

Unlike butter cakes, the chocolate cakes were more difficult to formulate (Table 2). The center cake height for all cakes, including the controls, was not lower than those in the butter cakes (Figure 7). The chocolate cake made with all-purpose flour had the highest cake height followed by the cake made with 50% cotyledon flour and 50% all-purpose flour and cake made with cotyledon flour and three times the amount of egg. The addition of extra egg to the formulas of cake made with cotyledon flour was deemed necessary. The egg improved the caked height of the cotyledon flour cake and reduced the adhesiveness or stickiness of the cakes (Figure 8). Overall, the cakes made tended to be stickier than the butter cakes. The chocolate cakes were deemed to be sticky, but moist, with the addition of cotyledon and whole bean flours to the recipes by individuals testing the cakes. The chocolate also masked the beany flavor of the cakes made with whole bean and cotyledon flours. Furthermore, the cakes had a brownie-like texture. The individuals tasting the cakes recommended that the bean flours be used in brownie-type products due to the higher moistness observed in these cakes compared to the cake made with all-purpose flour.

Objective 3. *To increase the knowledge of dry bean utilization through dissemination of results at professional trade organizations and by hosting two workshops on dry bean milling and the application of bean flours in non-traditional food applications.*

A workshop was to be hosted at the Northern Crops Institute in conjunction with their Pulse Utilization short course. However, the course was postponed and was offered in September, as previously described. Other forms of information that were developed included a technical bulletin and a slide presentation. These summarize key parts of the research and provide recommendations to individuals using cotyledon flours.

Recommendations and Conclusions

The most significant lessons learned dealt with two aspects of the project, namely the milling and application. Milling results indicated that the generation of heat during mill can be a concern particularly if milling for fine particle size. High rotor speeds (above 12,000 rpm) and small aperture screen size (250 µm) can result in high temperatures. Excessive heat was not a concern when milling with a 500 µm screen. Seed moisture above 10% was found to be detrimental to dry bean milling. Moisture acts as a plasticizer and makes size reduction more difficult and can result in enhanced starch damage. Conditioning black bean seeds with a water treatment (soaking, cooking, tempering) caused seed to expand and result in decline in test weight. Many mills use volumetric feeders to regulate milling rate and a decline in test weight would result in a decline in feed rate, simply because there is less beans per unit volume. Results strongly indicated that a moisture treatment was needed for enhanced separation of seed coat from cotyledon and that the burr mill was the best machine to separate the seed coat from the cotyledon. The best moisture treatment was to temper the seeds to 40% moisture and dry to ambient moisture content at 90°C.

The application studies showed that the cotyledon flour performed best among the bean flour in both cookies and cakes. When using cotyledon flour alone, additional eggs may be required, specifically in cakes. The cotyledon flour made very good cookies and the only concern was that some of the cookies had a greyish color. However, this was further minimized upon blending with all-purpose flour at a 50:50 ratio. The whole bean flour had a more intense bean flavor compared to the cotyledon flour. Blending of the cotyledon flour with all-purpose flour resulted in a cookie that was free of beany flavor. The addition of as little as 1 tablespoon of molasses was enough to mask the beany flavor in sugar cookies. Other flavored cookies such as lemon may also be feasible. Higher butter levels in the cookies made with cotyledon flour compared to all-purpose flour was beneficial. The texture of cookies with higher butter levels was preferred over cookies with less butter added. The cake proved to be more complicated than the cookies. Unlike cookie, the cotyledon alone was not able to match the quality of the control cake, i.e. cake made with all-purpose flour, except for texture. Cakes with cotyledon flour tended to rise less than the cake with all-purpose flour. However, blends of all-purpose and cotyledon flour did improve the cake volume of the cotyledon flour alone. The chocolate cake proved to be more difficult to achieve the desire outcomes. The cocoa may have affected the starch and protein matrix and as a result prevented the cake from retaining air sufficiently long enough to allow the cake to full rise before the proteins denature. The blending of cotyledon and wheat flour also proved beneficial from a quality perspective. The blend of the different flours will improve protein quality, as beans and cereals have complimentary nutritional properties.

Beneficiaries

Small specialty milling companies that produce dry bean flour and food ingredient manufacturers in general are the beneficiaries of this research. The research completed demonstrated the feasibility of fractionating black beans (dry bean) into cotyledon and hull fractions. Ingredient companies could use the knowledge gain to create commercial products. The consumer benefits from this research because the use of the cotyledon flour in home bake good was established for cookies, cakes and bread. The producer of beans benefit because food ingredient companies can develop ingredients from beans that allow for beans to be used more wide by the population beyond as an ingredient in canned or soup products. To-date, 45 people have received information on milling and end-use of dry bean/pulse flours that was developed during the conduct of the research associated with this project.

Lessons Learned

As mentioned above, one of the goals of the project “Increased knowledge linking bean flour type and application in food products” was unable to be tested due to the rearrangement of the Dry Edible Beans as Food Ingredient short course from a spring date to a September date. In addition a technical bulletin was not made for dry bean milling. The audience for this bulletin would be small specialty milling companies. The results of this research are very applicable to small scale-product development laboratories but the processes identified here need to be scaled-up to determine if they would work on a commercial level. In addition, questions about the effect of cultivar and seed quality on the separation of seed coat and cotyledon definitely need to be addressed before a technical bulletin on milling and seed coat separation could be written.

One challenge was timely hiring of graduate research assistants to help with this project. Given the nature of the academic year, it is possible that there is no clear solution, but it is important when planning and committing to research to be conducted over the time of the grant.

Lastly, writing scientific manuscripts on bean milling and application of bean flours in food products by the end of the grant period was somewhat ambitious.

Writing quarterly reports definitely helped keep the project focused and periodically evaluated for progress.

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Additional Information

Technical Bulletin: Black Bean Cotyledon Flour Application in Bakery Items

Clifford Hall and Frank Manthey
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Introduction

Black bean (*Phaseolus vulgaris*) is an edible bean with an intense black colored hull with a milky white interior or cotyledon. The National Agricultural Statistics Service (USDA) reported that black bean production topped over 620 Million pounds from 324 thousand acres in 2015.

Black beans are traditionally consumed in soups, salads and beans and rice dishes. Identify additional applications of black beans will allow consumers to experience the nutritional benefits of these beans.

Black beans are very good sources of fiber and protein (Figure 1) and are low in lipids or fats. Black beans proteins have a high lysine content that can complement cereal proteins and make a more complete protein when combined with cereal flours. Approximately a third of the lipid is made up of the fatty acid linolenic acid, which is an omega-3 fatty acid. The fiber is composed of approximately 80% insoluble and 20% soluble fiber. Beans have also been reported to induce a feeling of fullness (i.e. satiety) during meal consumption, which could help reduce the amount of calories consumed. Both fiber and protein likely contribute to property of beans. Lastly, black beans are an excellent source of folate. Consumption of 100 g provides nearly 60% of the recommended daily intake of folate. Black beans are rich in minerals such as iron, magnesium and potassium, providing approximately 30% of the recommended daily intake per serving. The dark seed coat is high in phenolic compounds such as anthocyanins. These compounds have antioxidant properties.

Black Beans as an Ingredient

The starch, protein and fiber components contribute to the functional properties of black beans. Starch is composed of approximately 25% amylose and 75% amylopectin. Globulin and albumin make up approximately 70 and 20% of the protein with only small amounts of glutelin and prolamin. Starch and proteins have been well documented as a gelling agents in food and provide structure to the processed foods. The emulsification and foaming properties of protein for example allow for air cell stabilization in food such as cakes while the starch provided the structural network. Fiber tends to hold water making a product retain a moist texture while some fibers also provide structure to the food.

Fractionation of the bean to provide ingredients with high starch, protein and fiber benefits the food industry by making available ingredients with specific functionality and complementary nutrient profile to those of cereal for example and provide producers with an opportunity to sell beans to a different market beyond the traditional market such as canning.

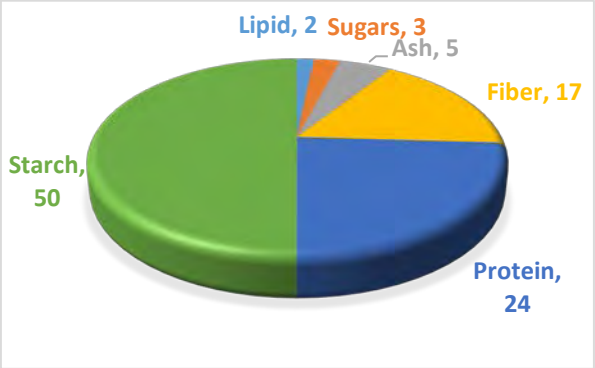


Figure 1. Proximate composition (%) of black beans.

Application of Black Bean Cotyledon Flour

Black bean cotyledon flour was obtained through a dehulling process that removed greater than 90% of the hull. The isolated cotyledon was approximately 90% pure with the remaining 10% being hull. The cotyledon was milled to completely pass a 30 mesh (600 µm) sieve.

The cotyledon flour obtained was used in a baking project that included cookies and cakes. Base formulas were developed with home-use in mind. Therefore, no attempt was made to add ingredients with known functional improvement properties commonly used by food manufacturers. The products were evaluated using sensory and the texture analyzer after each change in formulation was made. From this study, combinations of cotyledon flour produced cookies and cakes that were deemed better than the product made with whole black bean flour. Resulting cookies and cakes were compared to products made with all-purpose flours.

Cookies (Figure 2) showed that the whole produced smaller cookies while cotyledon and blends of cotyledon flours produced similar sized cookies compared to all-purpose flour (Table 1). The firmness data indicates that the cookies made with whole bean flour were significantly more firm than other cookies. The sensory data also supports the firmness of the

Table 1. Physical cookie parameters.

Cookie type	Length (cm)	Height (cm)	Firmness (N)
All Purpose	25.8	3.7	40.1
Whole Bean	25.4	4.0	100
Cotyledon	27.0	3.6	24.1
Cotyledon:All-purpose (50:50)	27.6	3.8	35.2
Cotyledon+ molasses	28.4	3.6	70.9

cookies. The interior of the cookies of the made with whole bean flour appeared more dense than the other cookies, which likely contributed to the firmness. The cookies made with cotyledon flour were deemed the best in the context of texture. The texture of the cookies was more like commercially produced sugar cookies and the softest, but crumblier bite among the cookies. The cookies made with 50% cotyledon flour and 50% all-purpose flour had a texture that was crunchier than cookies made from cotyledon flour. Overall, the cookies made with 50% cotyledon flour and 50% all-purpose flour or cotyledon flour were both rated favorably.

The flavor of the cookies made with whole bean flour was deemed to have the highest beany flavor. The cookies made with cotyledon flour had almost no bean flavor compared to the cookies made with whole bean flours. The addition of molasses to the cookies completely masked the bean flavor and were deemed to be a very good cookie. Overall, the sensory data supports the use of formulas that are a combination of 50% cotyledon flour and 50% all-purpose flour, cotyledon flour and addition of molasses as a flavoring.

The formulas (Table 2) used to prepare cookies can serve as a starting point when using cotyledon flour. Also included are the all-purpose and whole bean formulas. The formula can be adapted if eggs are unwanted. In this case, 1 to 2 teaspoons of water should be incorporated in the bean flour prior to being folding into the wet ingredients. The formulas below are ¼ the size of a normal batch of cookies (which might make 3 dozen cookies); therefore if several dozen cookies are needed, scale-up is necessary.

Table 2. Formulas of cookies made with various bean flours

Ingredients	All Purpose	WB	Cot	50% AP 50% Cot	Cot + Molasses
Sugar, granulated	1/4 c	1/4 c	1/4 c	1/4 c	1/4 c
Molasses					1 TBSP
Butter, softened	1/4 c	1/4 c	1/2 c	1/4 c	1/4 c
Vanilla	1/4 tsp	1/4 tsp	1/4 tsp	1/4 tsp	1/4 tsp
Eggs, beaten*	12g	12g	12g	12g	12g
Flour	2/3 c	2/3 c	2/3 c	2/3 c	2/3 c
Baking powder	1/8 tsp	1/8 tsp	1/8 tsp	1/8 tsp	1/8 tsp
Baking soda	1/8/tsp	1/8/tsp	1/8/tsp	1/8/tsp	1/8/tsp

*12 g = approximately 1/4 of an egg. WB= whole bean flour, Cot = cotyledon flour, AP = all-purpose flour.

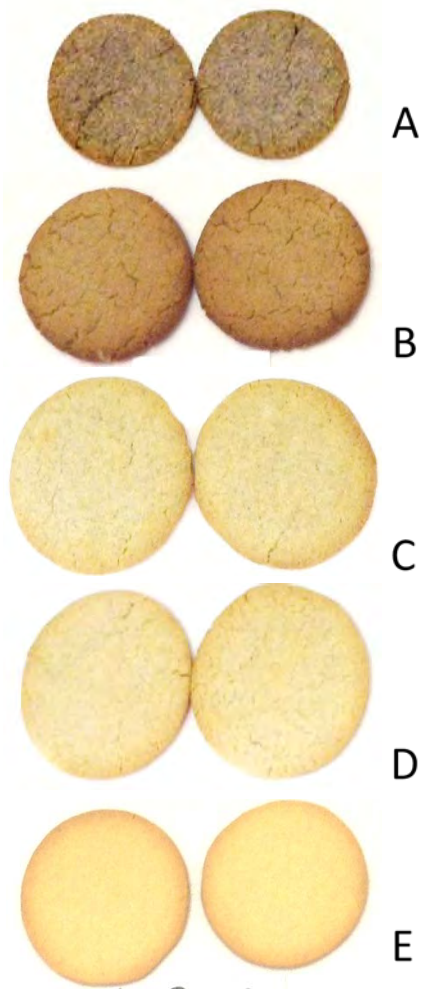


Figure 2. Sugar cookies made with whole bean flour (A), cotyledon flour with molasses (B), cotyledon flour (C), 50% blend of cotyledon flour and all-purpose (D) and all-purpose flour (E).

The application of black bean flours in cake requires careful adherence to product formulation. A number of product formulas exist on the internet where whole canned beans are used as bean ingredients. In addition, these recipes use a large number of eggs (typically 5-10). The egg provides the cake structure and thus large numbers of eggs are required. This bulletin highlights only the use of black bean flours in a basic butter cake. The resulting cakes tend to have less volume than cakes made with all-purpose flour. However, cakes made with black beans tend to have more moistness. Cakes with whole bean flour tend to have a dark appearance, both crumb and surface, while the cake made with cotyledon flour had a purple crumb color and a yellow surface color (Figure 3). The cake made with all-purpose flour had a yellow color. The cake center height was highest in cakes made with all-purpose flour (i.e. control cake) followed by cake made with 50% cotyledon flour and 50% all-purpose flour (Figure 3). Although lower than the control cake, the cake made with cotyledon flour plus twice

the egg as the base formula had a very good cake height, which was higher than the cake made with cotyledon flour and the same amount of eggs as the control. The addition of 3 times the eggs to the formulas did not improve the cake structure beyond the doubling of the eggs used in the control formulas. The whole bean flour produced a cake with a significant concave structure. This demonstrated the need to incorporate eggs into the formula.

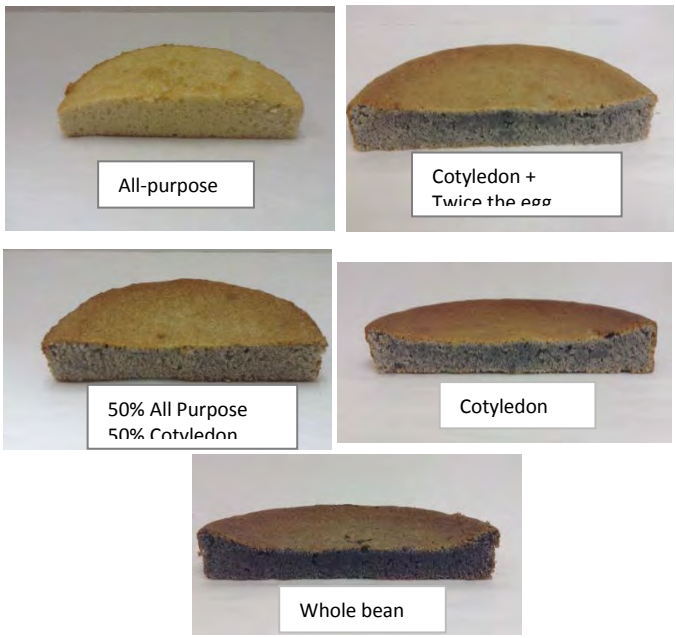


Figure 3. Butter cake prepared from various formulations of bean flour, cotyledon flour, and all-purpose flour.

The texture of the control butter cake was essentially the same as the texture in the cake made with 50% cotyledon flour and 50% all-purpose flour and cake made with the cotyledon flour (Table 3). In contrast, cakes made with whole bean flour firmness values that were higher than other cakes. However, the adhesiveness was approximately the same. The cakes made with cotyledon flour and twice the egg had significantly higher adhesiveness than the other cakes. Adhesiveness indicates the stickiness of a material. Thus, the higher the adhesiveness the higher is the stickiness. The sensory assessment of the cakes supported the physical data in that the cotyledon flour with twice the eggs had the highest stickiness. The control cake and cake made from with 50% cotyledon flour and 50% all-purpose flour had a dry but springy texture compared to cakes with whole bean flour and cotyledon flour, which were deemed to be moist and have a brownie-like texture.

Table 3. Physical cake parameters.

Base Ingredients	Center Height (cm)	Firmness (N)	Adhesiveness (mJ)
Whole Bean	19	7.82	0.7
Cotyledon	20	4.36	0.4
Cotyledon 2x egg	26	5.33	6.5
All-Purpose	39	3.58	0.5
All-Purpose:Cotyledon (50:50)	29	3.82	0.4

The formulas (Table 4) used to prepare cakes with cotyledon flour had similar sensory properties and physical parameters as the cake with all-purpose flour. However, the additional use of egg will help improve cake properties and make it more like a wheat flour-based cake. The combination of wheat flour and cotyledon flour was deemed the best among the bean-based formulas. The blending of wheat flour and bean flour in cakes is recommended. The water absorption index is quite high in the bean flours. As a result, formulas with the cotyledon flours must incorporate additional water to facilitate hydration of the flours.

Table 4. Formulas (grams) of black bean butter cake.

Yellow butter cake* **Ingredient (g)**	**All Purpose**	**WB**	**Cot**	**Cot 2x egg**	**50%Cot 50% AP**
Flour	180	180.0	180.0	180.0	180.0
Baking powder	6	6.0	6.0	6.0	6.0
Salt	1.2	1.2	1.2	1.2	1.2
Butter, unsalted	113	113.0	113.0	56.5	113.0
Granulated sugar	198	198.0	198.0	198.0	198.0
Eggs	49	49.0	49.0	98.0	49.0
Vanilla	4.4	4.4	4.4	4.4	4.4
Milk	105	105.0	105.0	105.0	105.0
Whole bean extra water		80.0			
Cotyledon extra water			60.0	60.0	30.0

*WB= whole bean flour, b.p = baking powder, Cot = cotyledon flour, AP = all-purpose flour.

Summary

The cotyledon flour performed best among the bean flour in both cookies and cakes. When using cotyledon flour alone, additional eggs may be required, specifically in cakes. The blending of cotyledon and wheat flour also proved beneficial from a quality perspective. The blend of the different flours will improve protein quality as beans and cereals have complimentary nutritional properties.

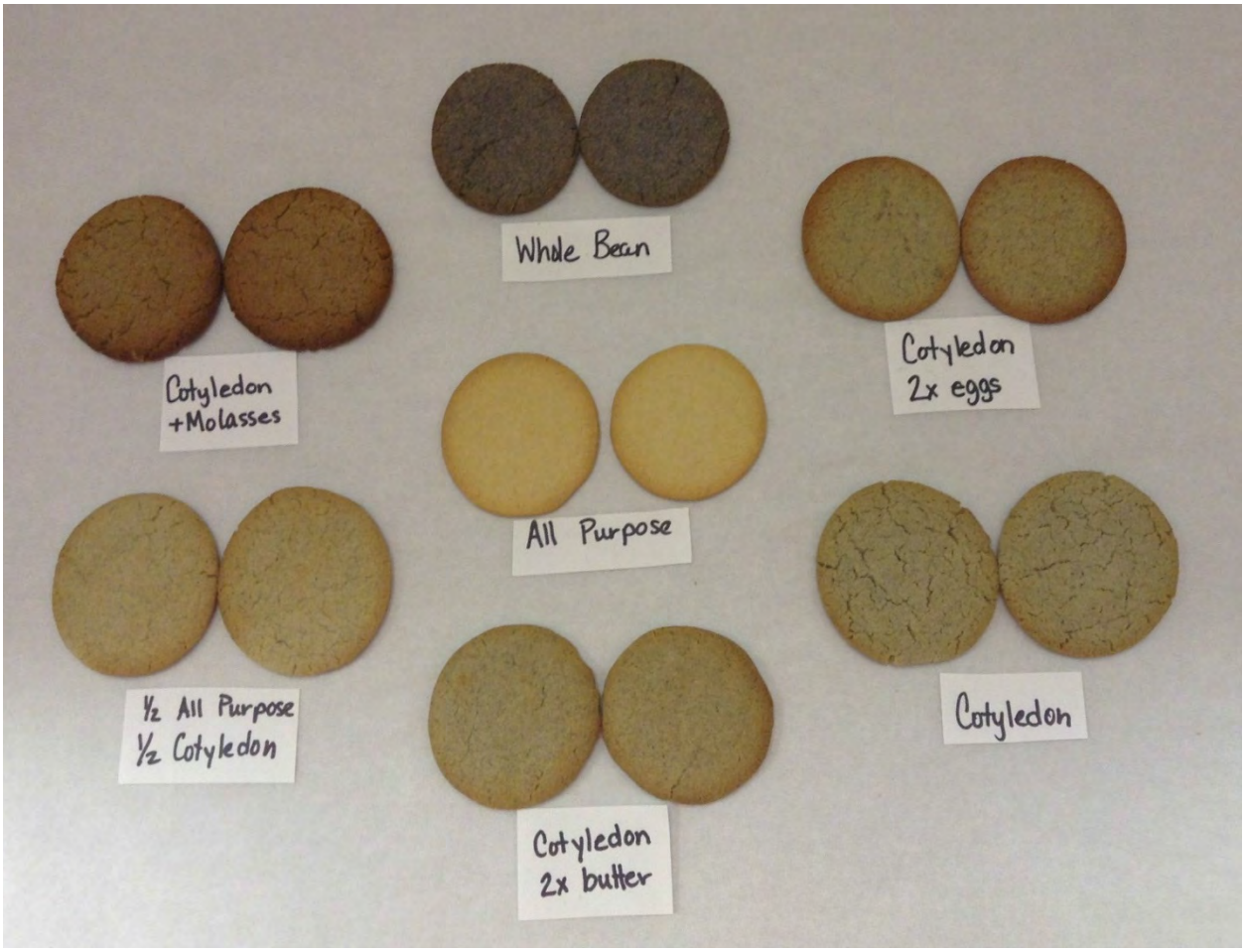
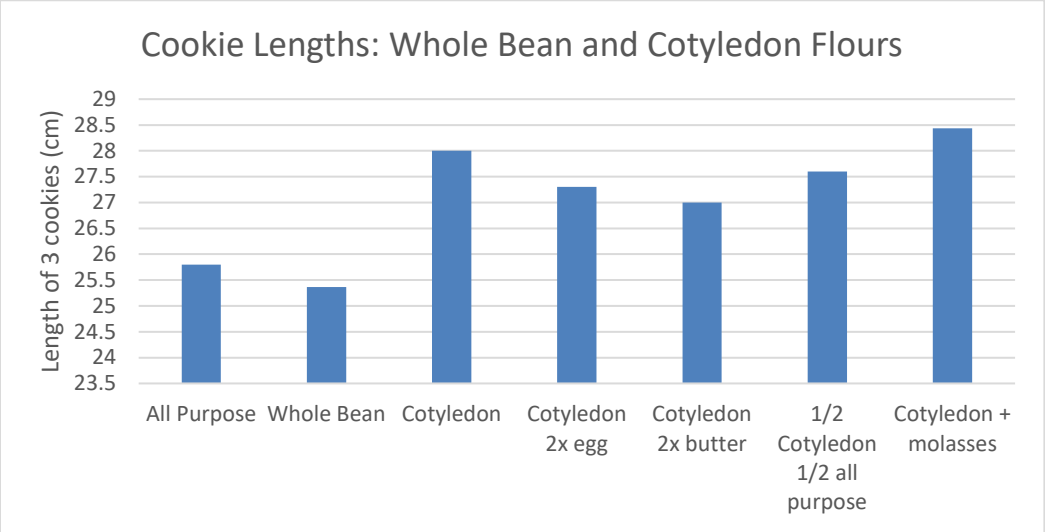


Figure 1. Cookies prepared from various formulations of bean flour, cotyledon flour, and all-purpose flour.



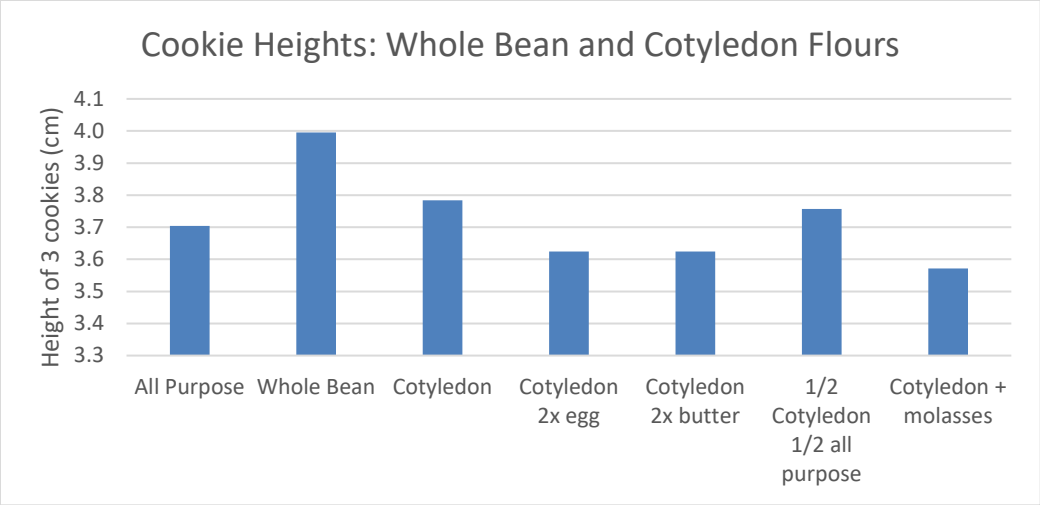


Figure 2. Quality of cookies prepared from various formulations of bean flour, cotyledon flour, and all-purpose flour.

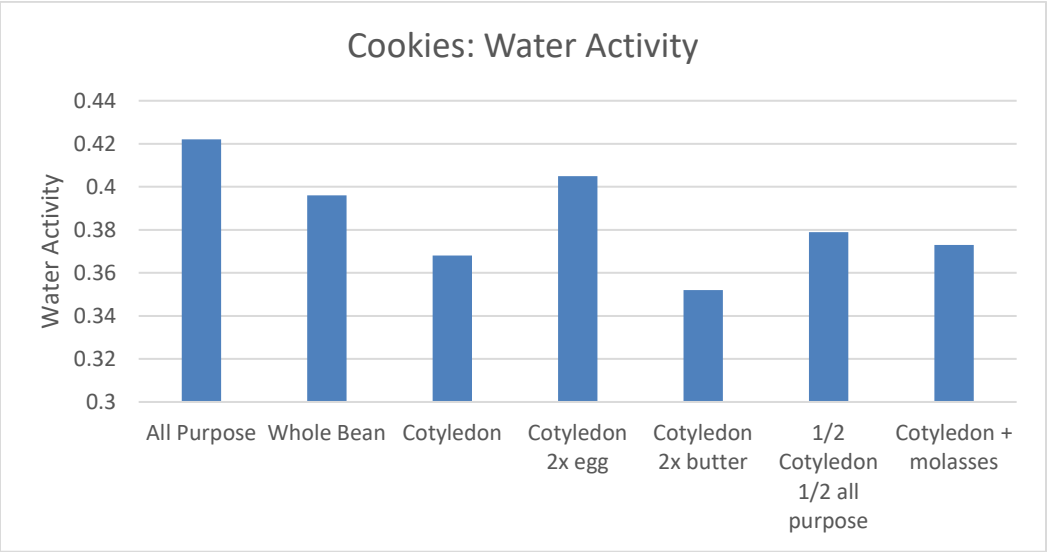


Figure 3. Water activity of cookies prepared from various formulations of bean flour, cotyledon flour, and all-purpose flour.

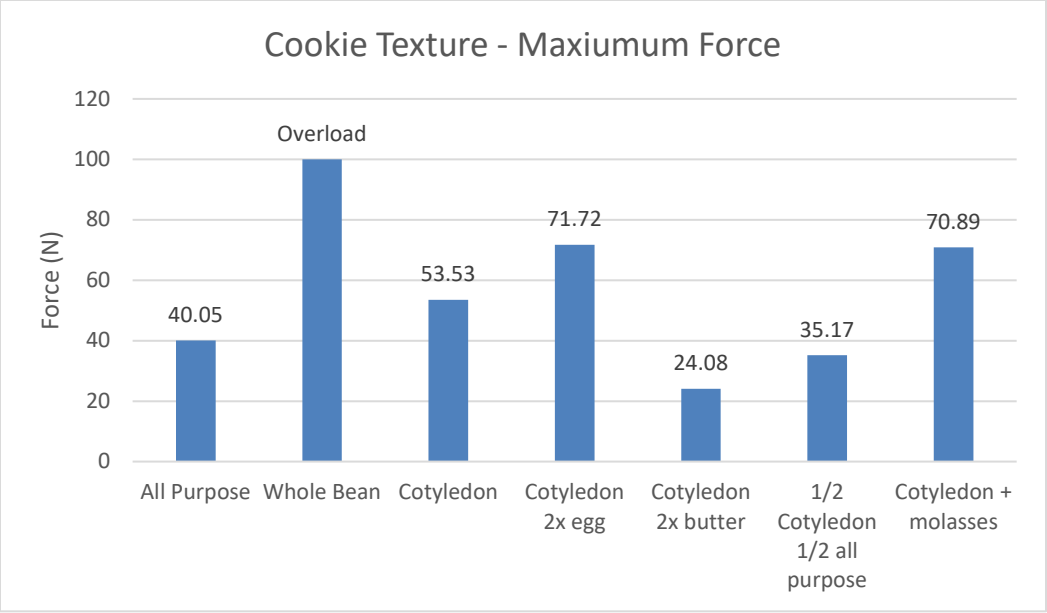


Figure 4. Texture of cookies prepared from various formulations of bean flour, cotyledon flour, and all-purpose flour.

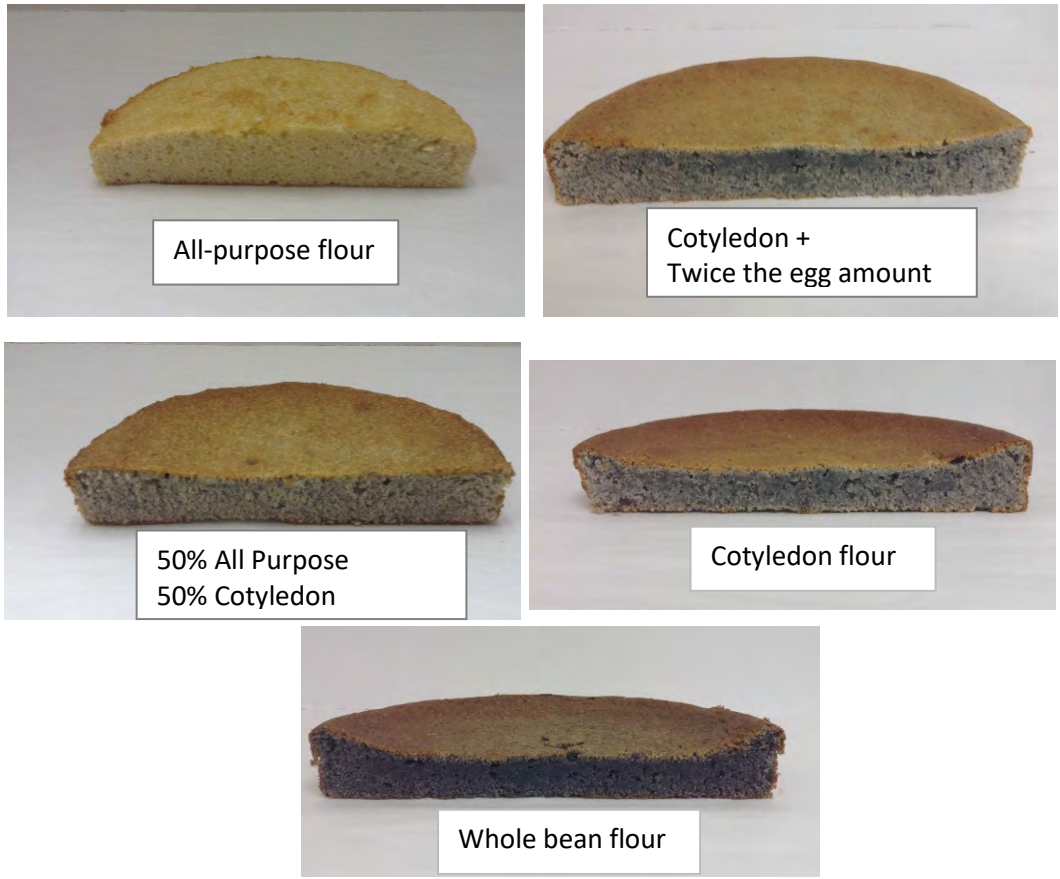


Figure 5. Butter cake prepared from various formulations of bean flour, cotyledon flour, and all-purpose flour.

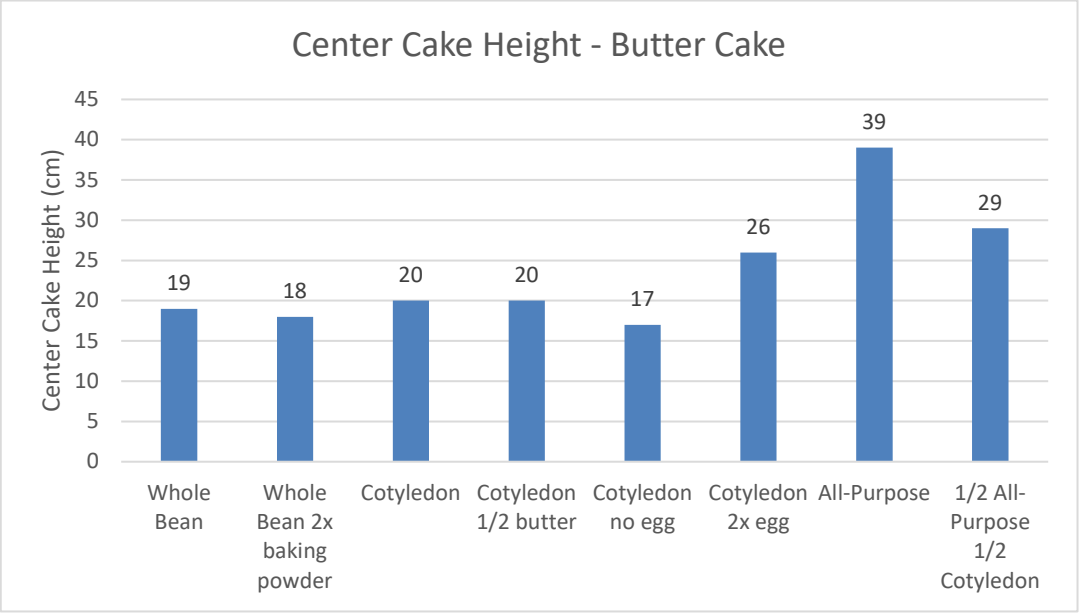


Figure 6. Height of the butter cake prepared from various formulations of bean flour, cotyledon flour, and all-purpose flour.

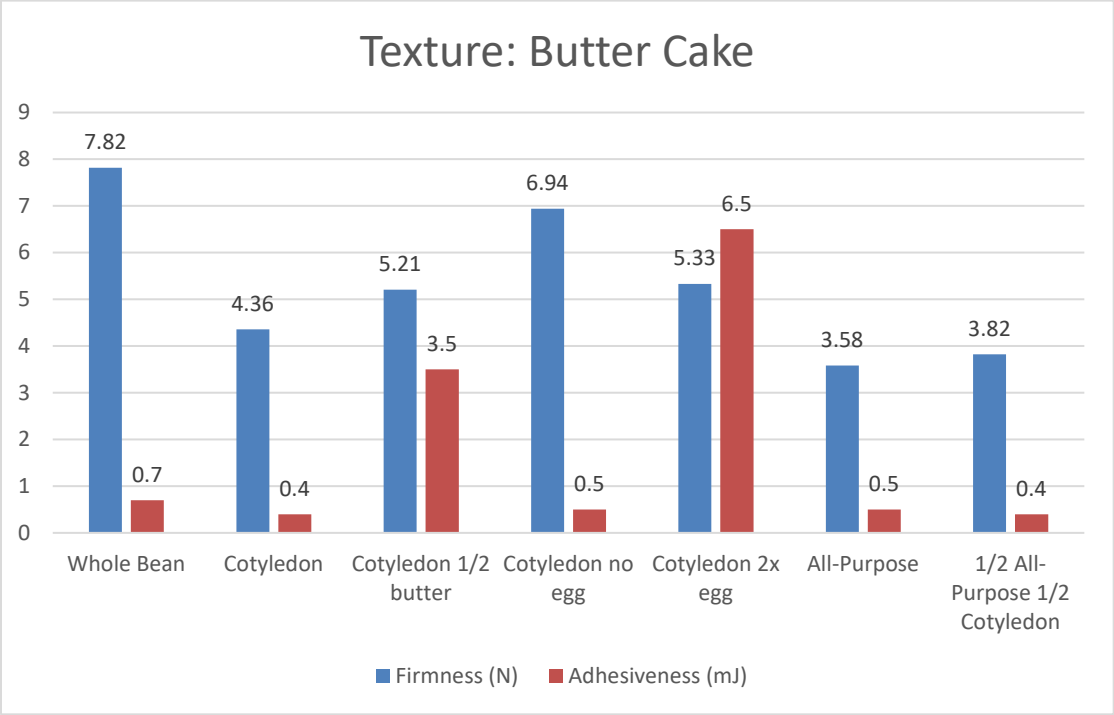


Figure 7. Firmness / hardness and adhesiveness of the butter cake prepared from various formulations of bean flour, cotyledon flour, and all-purpose flour.

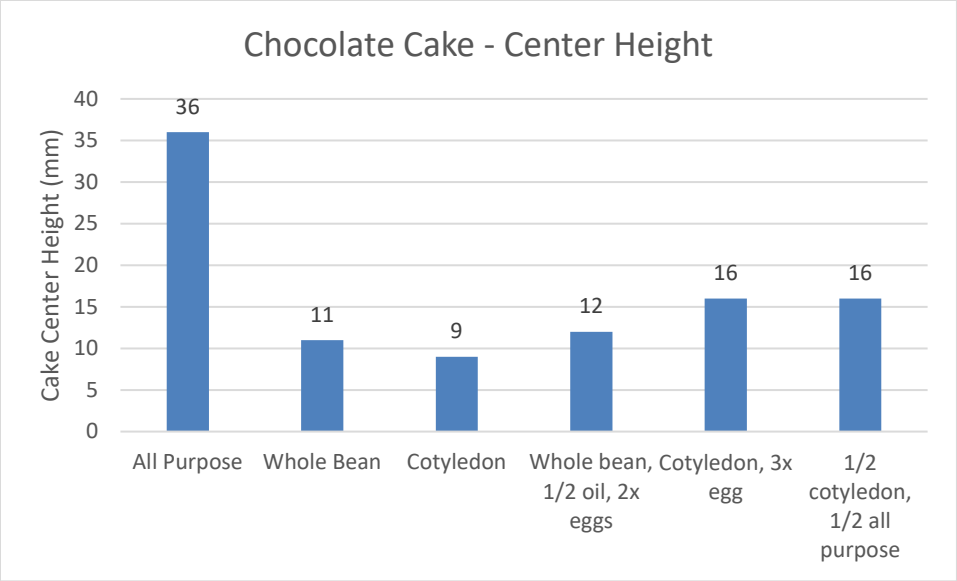


Figure 8. Height of the chocolate cake prepared from various formulations of bean flour, cotyledon flour, and all-purpose flour.

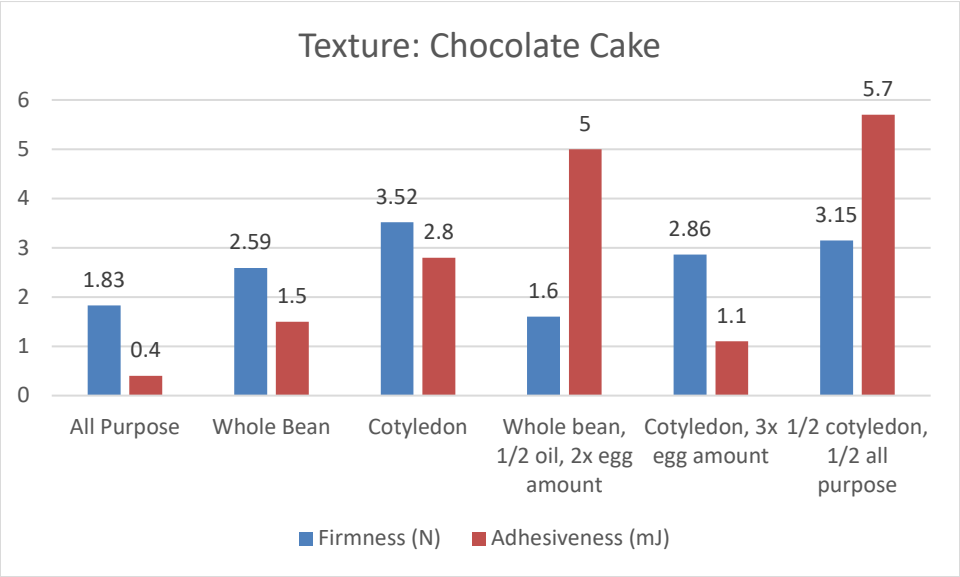


Figure 9. Firmness / hardness and adhesiveness of the chocolate cake prepared from various formulations of bean flour, cotyledon flour, and all-purpose flour.

Table 1. Yellow butter cake formulation used in final comparisons.

Yellow butter cake* **Ingredient (grams)**	**All Purpose**	**WB**	**WB 2x b.p.**	**Cot**	**Cot 1/2 butter**	**Cot no egg**	**Cot 2x egg**	**50%Cot 50% AP**
Flour	180	180.0	180.0	180.0	180.0	180.0	180.0	180.0
baking powder	6	6.0	12.0	6.0	6.0	6.0	6.0	6.0
salt	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Butter, unsalted	113	113.0	113.0	113.0	56.5	56.5	56.5	113.0
granulated sugar	198	198.0	198.0	198.0	198.0	198.0	198.0	198.0
eggs	49	49.0	49.0	49.0	49.0	0.0	98.0	49.0
vanilla	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4
milk	105	105.0	105.0	105.0	105.0	105.0	105.0	105.0
Whole bean extra water		80.0	80.0					
Cotyledon extra water				60.0	60.0	60.0	60.0	30.0

*WB= whole bean flour, b.p = baking powder, Cot = cotyledon flour, AP = all-purpose flour.

Table 2. Chocolate cake formulation used in final comparisons.

Chocolate cake* **Ingredient (grams)**	**All Purpose**	**WB**	**Cot**	**WB 1/2 oil 2x egg**	**Cot 3x egg**	**50% Cot 50% AP**
Flour	105	105	105	105	105	105.0
baking powder	3.0	3.0	3.0	3.0	3.0	3.0
baking soda	2.3	2.3	2.3	2.3	2.3	2.3
salt	0.6	0.6	0.6	0.6	0.6	0.6
Cocoa	22.0	22.0	22.0	22.0	22.0	22.0
vegetable oil	54.6	54.6	54.6	27.3	54.6	54.6
granulated sugar	198.0	198.0	198.0	198.0	198.0	198.0
eggs	49.0	49.0	49.0	98.0	147.0	49.0
vanilla	4.3	4.3	4.3	4.3	4.3	4.3
milk	87.5	87.5	87.5	87.5	87.5	87.5
boiling water	113.5	113.5	113.5	113.5	113.5	113.5
Whole bean extra water**		130		130		
Cotyledon extra water**			90		90	45.0

*WB= whole bean flour, Cot = cotyledon flour, AP = all-purpose flour.

**added water was incorporated into the milk fraction.

Potato Yield Response and Nitrogen (N) Losses as Influenced by Fertilizer N Management and Cultivars

Final Report

Partner Organization: North Dakota State University

Project Summary

Increasing demand for food supply and concerns about environmental and soil health created a compulsion for sustainable management for the most limiting and loss prone nutrient, nitrogen (N). Enhanced efficiency fertilizers (EEFs) are believed to have a potential to reduce nutrient loss and environmental impact while maintaining or increasing yield. In 2016, a field study was conducted at Inkster, ND, to evaluate if EEFs and split application can reduce N loss with profitable yield compared to conventional fertilization practices in three irrigated potato cultivars. The potato cultivars [Russet Burbank, Dakota Trailblazer, ND8068-5 Russ] were planted with six N treatments [Growers' standard, Urea@ 200 lb N/acre; Urea, SuperU and ESN@ 250 lb N/acre in two splits and Control (no fertilizer N)] in factorial RCBD. All N treatments increased total tuber yield significantly over control, but no significant yield differences among N treatments were observed. Maximum yield was obtained with Russet Burbank followed by Dakota Trailblazer and the ND-8068-5 Russ. Grower's standard, SuperU and ESN reduce cumulative NH_3 volatilization compared to Urea@ 250 lb N/acre. When applied at same rate, cumulative N_2O emission was significantly reduced with EEFs compared to Urea and Grower's standard. Nitrate leaching was reduced with Grower's standard and Urea@ 200 lb N/acre.

Potato (*Solanum tuberosum*) is a high N demand crop that requires around 200 lb N/acre throughout the growth period for a yield goal of 450 cwt/acre. Potato N use efficiency is significantly low particularly under irrigated sandy soils. Increasing N fertilizer prices and environmental health concerns associated with N losses are forcing the growers and researchers to better manage N fertilizers and improve N use efficiency. The efficiency of fertilizer N management is primarily influenced by source, form, placement, application rate and timing of application in addition to soil environmental conditions. Enhanced efficiency N fertilizers like polymer coated urea and urea blended with urease and nitrification inhibitor showed promising results with respect to yield increase and minimization of N loss in several studies. Growers in Minnesota successfully use ESN (polymer coated urea) in potato production when applied at shoot emergence. However, not much research have been conducted in ND to evaluate the efficiency of different improved fertilizers in reducing N loss with profitable production of potatoes.

Researchers are starting to investigate alternative N formulations for efficient N management practices in specific varieties of a crop. However, the physiological and genotypic variation in N use efficiency of potato is poorly understood. Although plant breeders seldom select for nutrient use efficiency, understanding N uptake and partitioning differences may assist in the selection of genotypes that uses nutrient more efficiently.

Our experiment examined the efficiency of ESN, SuperU (Urea blended with nitrification and urease inhibitor) and split application in reducing N loss with successful production of three potato cultivars i.e. Russet Burbank, Dakota Trailblazer and ND8068-5 Russ. The main objectives of our research were

- To evaluate if EEFs (ESN and SuperU) and split application of N fertilizers can increase potato tuber yield and N uptake as well as improve quality
- To examine the efficiency of EEFs (ESN and SuperU) and split application of N fertilizers in reducing N losses through NH_3 volatilization, N_2O emission and NO_3^- leaching below root zone.
- To observe if the N losses and efficiency of EEFs differ with cultivars.

Project Approach

A field research was conducted to evaluate if enhanced efficiency fertilizers i.e. ESN, SuperU and split application of N fertilizers can reduce N loss from soil as well as increase yield over conventional practices, at the Northern Plains Potato Growers' Association (NPPGA) irrigation site near Inkster, ND in 2016. The initial basic properties of the soil of the study site are listed in Table 1.

Russet Burbank, Dakota Trailblazer and ND8068-5 Russ were planted with six N treatments in a 3 (varieties) × 6 (N treatments) factorial randomized complete block design with four replications on 6th June, 2016. The N treatments were

1. Grower's Standard (10-34-0 @ 30 gallon/acre at planting+ Urea @ 150 lb N/acre at hilling + UAN @ 70 lb N /acre at tuber initiation)
2. Urea @ 200 lb N/ acre at planting
3. Urea @ 100 lb N/ acre at planting and @ 150 lb N/ acre at hilling
4. SuperU @ 100 lb N/ acre at planting and @ 150 lb N/ acre at hilling
5. ESN @ 100 lb N/ acre at planting and @ 150 lb N/ acre at hilling
6. Control (No fertilizer N)

Important dates

- Potato planting and first fertilizer application: June 6th, 2016.
- Hilling and second fertilizer application: June 20th, 2016.
- Third split dose of fertilizer application: July 18th, 2016.
- Tubers were harvested on October 11th, 2016

Sampling and Analyses

1. *Yield and tuber quality*- Middle two rows of each plot were harvested using single row potato harvester. Potato yield and grades were determined by an optical grader.
2. *Plant N uptake*- Above ground plant samples and tuber samples were collected, dried at 60°C, grinded and total N concentration in tissue were determined through micro Kjeldahl process. Plant N uptake was calculated from tissue N concentration, tuber yield and above ground biomass.
3. *Ammonia volatilization*- NH_3 emission was measured weekly (early in the season) and biweekly (later in the season) using semi static acid trap chamber to estimate the N loss through volatilization.
4. *Nitrous oxide emission*- Headspace air samples were collected weekly (early in the season) and biweekly (later in the season) using static chamber method. Samples were analyzed for N_2O concentration using gas chromatograph fitted with a 63Ni electron capture detector (ECD).

5. *Nitrate leaching below root zone:* Soil water below root zone (2ft) was collected biweekly and analyzed with diffusion conductivity membrane apparatus (Timberline 2800) for NO_3^- concentration
6. *Soil N availability*- Soil samples were collected biweekly at 0-6" depth. Soil samples were extracted with 2.0 M KCl to estimate NO_3^- using diffusion conductivity membrane apparatus (Timberline 2800).
7. *Residual nitrate-N in soil profile (0- 3 feet)*- Profile soil samples were collected from each plot at 0-12", 12-24" and 24-36" depths after harvest to determine the residual NO_3^- leached down and deposited in the profile.

All statistical analyses were performed in R 3.2.0 using package agricolae. All parameters were subjected to analysis of variance (ANOVA) and if a significance existed, then tested with Fisher's LSD for specific treatment and interaction differences.

Additional Activities: The research outcomes have been presented several times

Event	PI	Time	Presented by	Number of attendees
1. Northern Plain Potato Grower Association Field Day Presentation	PI (Chatterjee), Co-PI (Hatterman-Valenti) and graduate student	August, 2015, 2016	Upasana Ghosh	Approximately 200 each year
2. Presentation at Annual Meeting conducted by ASA-CSSA-SSSA	PI (Chatterjee), Co-PI (Hatterman-Valenti) and graduate student	November, 2015, 2016	Upasana Ghosh	Approximately 20 each year
3. NDSU School of Natural Resource Sciences Graduate Symposium	PI (Chatterjee), Co-PI (Hatterman-Valenti) and graduate student	December, 2016	Upasana Ghosh	Approximately 50

Goals and Outcomes Achieved

All the nitrogen treatments had significantly higher tuber production compared to control (No fertilizer N). However, the N treatments didn't differ with each other in terms of total tuber production (Fig. 1.). Considering the cultivars, maximum total tuber yield was obtained with Russet Burbank followed by Dakota trailblazer and then ND8068-5 Russ (Fig. 2.). Highest total tuber yield (60.2 Mg/ ha) was obtained in Russet Burbank variety treated with ESN@ 250 lb N/acre.

In Russet Burbank and Dakota Trailblazer variety Urea@ 250 lb N/ acre increased cumulative ammonia volatilization extremely. SuperU, ESN and Grower's standard @ 250 lb N and Urea @ 200 lb N /acre reduced the ammonia volatilization compared to Urea @ 250 lb N/acre (Fig. 3.). In ND-8068-5 Russ all the N treatment except for Grower's standard equally increased cumulative ammonia volatilization (Fig. 3.). The behavior of ND8068-5 Russ was different than the other two varieties was possibly because of the early maturity and lesser vegetative growth.

Urea@ 250 lb N/ acre and Grower's standard increased cumulative N_2O emission compared to other treatments in Russet Burbank and ND-8068-5 Russ (Fig. 4.). In Dakota Trailblazer, Grower's Standard significantly increased N_2O emission in all other treatments except for SuperU (Fig. 4.). Nitrate leaching data was extremely inconsistent because of the variable soil hydrology within the field. So, no statistical analysis could be applied on the nitrate leaching data. Overall, it was observed that NO_3^- leaching highest during August. Grower's standard and Urea@ 200 lb N/ acre reduced NO_3^- leaching compared to the other treatments. ESN and SuperU increased NO_3^- leaching later in the season.

Table 1: Initial physical and chemical properties of the soil of study site

Parameters	Estimates
Texture	Sandy loam
Bulk density	1.13 g cm^{-3}
pH	6.0
Electrical conductivity	0.15 dS m^{-1}
Cation exchange capacity	10.8 cmol kg^{-1}
Available N (0-2 feet)	20 lb $acre^{-1}$
Organic matter	3.27 %

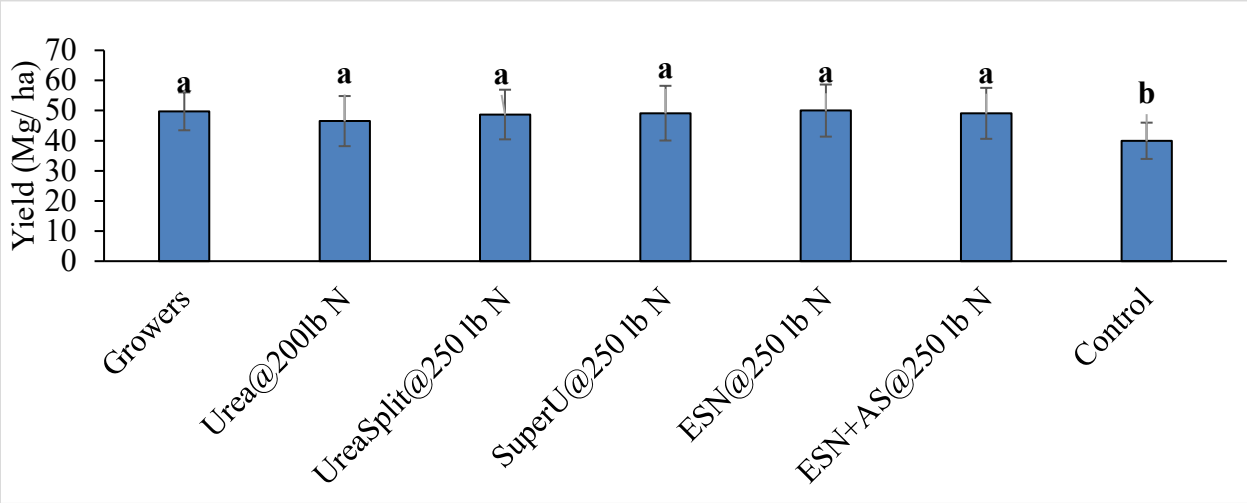


Figure 3: Total tuber yield influenced by N treatments. [Bars with same letter designation do not differ significantly at $p < 0.05$]

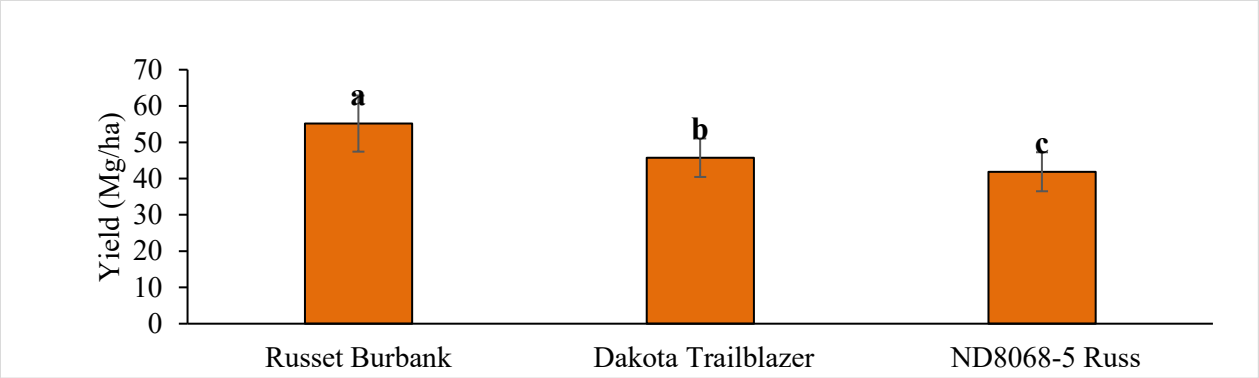
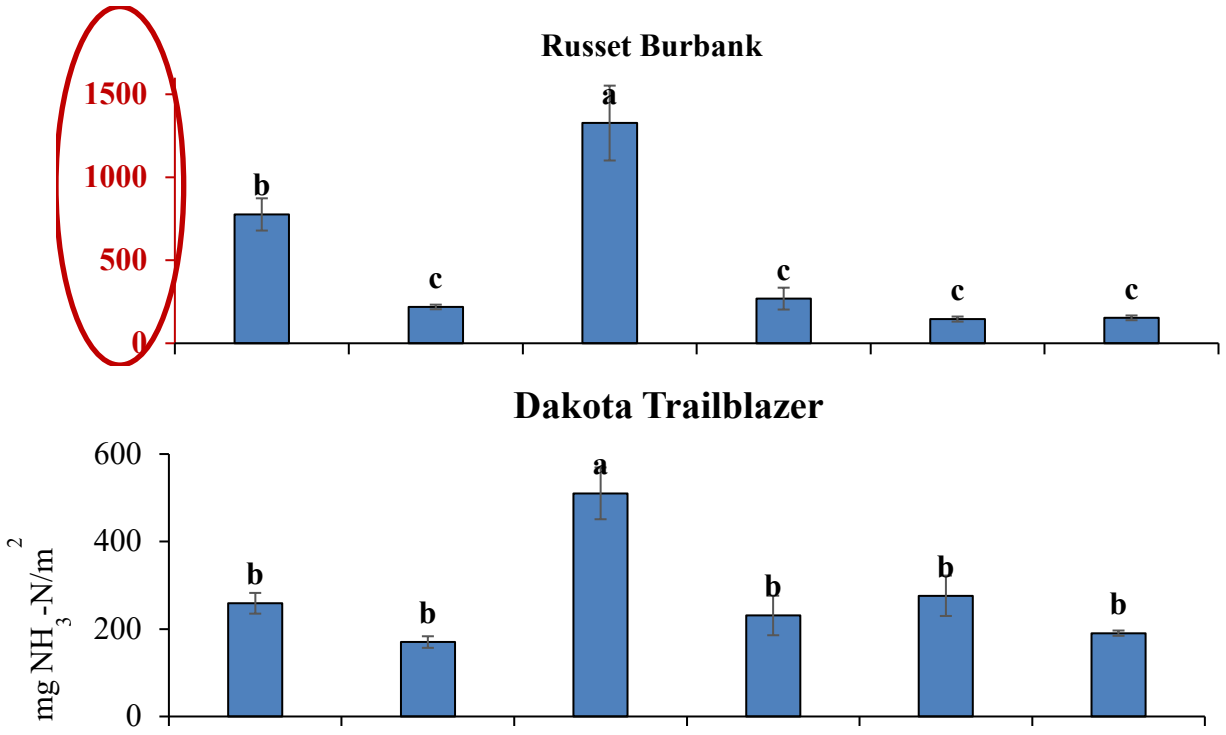


Figure 4: Total tuber yield of three potato cultivars [Bars with same letter designation do not differ significantly at $p < 0.05$]



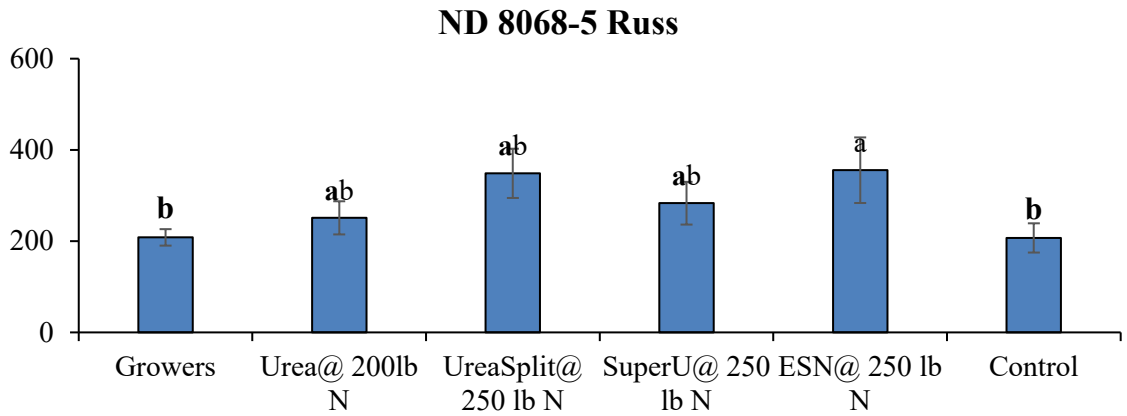


Figure 5: Cumulative ammonia volatilization in three potato cultivars. [Bars with same letter designation do not differ significantly at p< 0.05]

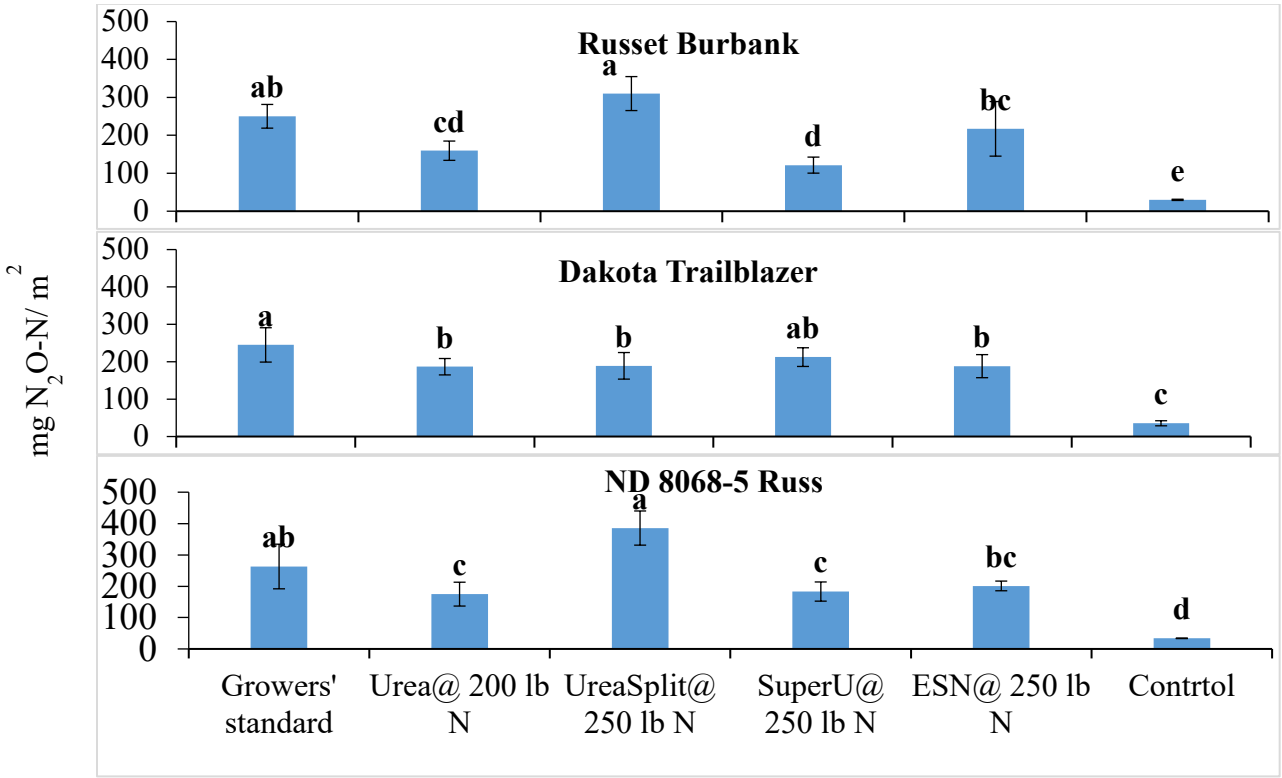


Figure 4: Cumulative N_2O-N emission in three potato cultivars. [Bars with same letter designation do not differ significantly at p< 0.05]

Conclusions

- No total tuber yield benefit was obtained over 200 lb N/acre.
- When applied at same rate (250 lb N/ acre) EEFs reduce N losses compared to Urea.
- Overall, Urea 200 lb N/ acre reduced all N losses.

- The two new russet varieties (Dakota Trailblazer and ND8068-5 Russ) could not outyield the commercial variety Russet Burbank.

Beneficiaries

Primarily the irrigated potato growers of North Dakota will benefit from the project. Currently the NPPGA currently have 250 grower members who will be directly informed and benefited. Besides that, the potential publications from this research will help numerous growers. Potato growers generally use 250-300 lb N/acre in this region. The result of this research may encourage them for judicial use of N fertilizers. Although ESN is successfully used in Minnesota, the growers of North Dakota do not generally use ESN. In this research in both the years (2015 and 2016) ESN performed promisingly in terms of yield increase and reducing N losses. This research will be helpful in convincing the growers to reconsider the fertilizer managements.
Secondly, with successful data collection and analyses, the graduate student working on the project will be enormously benefitted because the project provided a wide opportunity in learning field, laboratory and statistical methods.

Lessons Learned

The conclusions are based upon the results obtained till date. Some chemical (shoot and tuber N uptake, residual available N upto 4 ft depth) and statistical analyses (petiole N, tuber grades) are still in progress. More definite conclusions can be drawn based upon the upcoming results.
Suction cup lysimeters may not be the best option to assess nitrate leaching in sandy loam soil as it performs best at field capacity moisture content. However, the post-harvest profile nitrate analysis (upto 4 ft depth) can give an estimate of nitrate leaching. A Hydrus 1D simulation showed that the NO_3^- leaching below 4 ft is negligible.
In 2015, it was observed that in season petiole NO_3^- concentration poorly correlates with yield. So this year we collected NDVI (Normalized difference vegetation index) twice in the growing season and analyzed petiole samples for total N concentrations to see if these parameters can be a better predictor of yield.

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Soybean Cyst Nematode Outreach and Characterization of the Genetic Basis for Resistance in Dry Bean

Final Report

Partner Organization: North Dakota State University

Project Summary

Soybean cyst nematode (SCN; *Heterodera glycines*) is a soil-borne disease that threatens dry bean production in the northern tier of states in the USA. An outreach program was conducted to educate producers and others in the bean industry on the identification, biology and management of SCN. The outreach programs directly reached 4,688 persons and publications, interviews and a video on SCN will reach an even greater number. Several sources of resistance to SCN were identified in various bean classes and mapping populations in pinto and kidney bean were created by making di-hybrid crosses of resistant and susceptible parents. Screening for SCN resistance in mapping populations and advanced bean breeding lines were conducted in the greenhouse in search of resistant lines. Molecular markers linked to SCN resistance were identified on a genetic linkage map. This information is potentially useful for marker-assisted breeding and could ultimately accelerate development of resistant cultivars. The results of this research have provided the dry bean breeder with resistant inbred lines with potential use in development of SCN resistant bean varieties.

SCN is the most devastating pathogen of soybean in USA, causing economic losses in both production and resources to manage this disease. It is impossible to prevent the spread of this pathogen since it easily spreads on equipment and by anything that moves soil particles. SCN was detected in ND in 2003 and has spread to more than 20 counties. Dry bean is considered one of the most important legume crops grown in North Dakota, Minnesota, and other nearby states. Dry bean in this area has a harvest value of about 370 million dollars. Dry bean can be highly susceptible to SCN depending on the bean class, and this disease presents a major threat to the dry bean industry in this region. Recently, SCN was detected causing damage in a commercial kidney bean field in northern Minnesota and it has been found in a navy bean field in ND. As SCN expands in distribution throughout the dry bean production area, there will be a need for resistant dry bean cultivars to control this disease Crop rotation can reduce SCN populations, but research in ND has shown that populations decline slowly and populations can persist in fields for many years. Nematicides have not been shown to be economical for management of SCN in field crops and also pose environmental and human health hazards. Outreach programs can be useful for management of SCN in the field. Awareness and early detection of SCN in the field can help reduce the impact on crop yield. Developing new dry bean varieties resistant to SCN is the best long term solution and involves discovery of dry bean genotypes with significant resistance to SCN and transferring the resistance into adapted varieties. A high-density genetic linkage map is one of the most important genomic tools to accelerate marker-assisted breeding. Single nucleotide polymorphic (SNP) markers can be utilized to generate the high density linkage map of populations segregating for resistance to *SCN* with greater possibility of finding QTLs and linked markers for SCN resistance.

Project Approach

Soybean Cyst Nematode Outreach:
Between October 1, 2014 and September 30, 2016 there were 46 meetings attended by growers, seed dealers, county extension agents, crop consultants and others in the agricultural community where the biology and management of soybean cyst nematode (SCN) was presented and questions were answered by principal investigators on this project. This is basic information that is useful to dry edible bean growers. The total number of persons who attended these meetings was 4,688. The majority of these presentations were given by Dr. Sam Markell the extension plant pathologist, but other members of the team were also involved. Presentations occurred in various venues and formats, throughout the state in major dry bean producing region of the East and NE part of the state (i.e., Devils Lake, Grand Forks, Carrington, Fargo) ranging from winter meeting circuits to field day formats. Importantly, SCN was featured prominently at the Northarvest Bean Day program, which was attended by approximately 350 dry bean growers from ND, MN and MB. Scientific topics presented included; detection, impacts, management strategies. Social topics presented included; teaching to growers, dealing with stigma, and presentation strategy. Examples of these presentations were: 1) Soybean Cyst Nematode on Soybean and Dry Beans in Courtney, ND on 12/10/14; Soybean Cyst Nematode Sampling, and Seed Treatments in Carrington, ND on 12/9/14; in September 2015, three SCN field days were held in fields where there were high levels of SCN so growers could see SCN on roots of susceptible crops. In order to multiply teaching efforts in 'winter-meeting-season', an all-day training was provided to ten county Extension agents in December, 2014 by Dr. Markell. Scientific topics presented included; detection, impacts, management strategies. Social topics presented included; teaching to growers, dealing with stigma, and presentation strategy. Resources, including pre-prepared talks and constructed SCN display mounts, were distributed to each attendee. These county extension agents then give talks about SCN to their area growers.

Interviews:
Dr. Markell gave two television, ten radio and two print interviews about SCN on dry edible beans and other susceptible crops.

Popular Press Articles:
A concerted effort to publish SCN articles in popular press magazines was made during this time period. Two articles were published in the Northarvest Bean Grower magazine, the preeminent bean grower magazine for the region. The first article (mid-summer issue) included SCN on dry beans as one of the 'big four' diseases growers need to watch for, the second article (late summer issue) was a feature on SCN in the region, and detailed the first occurrence on kidneys and emphasized the need for detection.

Markell, S., Nelson, B., Pasche, J., and Yan, G. 2016. Soybean cyst nematode and the threat to dry beans. Northarvest Bean Grower Magazine. Vol. 22 (5): 16-17.

Markell. S. and Pasche, J. 2016. Big four disease guide. Northarvest Bean Grower Magazine. Vol. 22 (4): 16-17.

Extension publications:

An extension publication titled Soybean Cyst Nematode, was published by the NDSU Extension Service in the fall of 2014. This was a general treatment of SCN developed for soybean and dry bean growers. Two of the PI's (Markell and Nelson) on this project are authors on the publication. The reference is: Mathew, F., Markell, S., Jantzi, D., Yan, G., Nelson, B. and Helms, T. 2014. Soybean Cyst Nematode. Extension Bulletin PP1732, North Dakota State University, Fargo, ND.

In order to provide growers with an extension publication that could be used in the field, the Dry Bean Diagnostic Series cards were developed. This follows the lead of the Sunflower and Pea diagnostic cards of the same series, the cards are a synthetic material that are water-, tear-, crease, and glare- resistant. Currently, diagnostic card series are the most commonly requested plant pathology publications and heavily used by growers, agents and crop consultants. Prominently displayed are two SCN cards. Cards are currently in publication phase and will be distributed to growers at the January 2017 Northarvest bean growers – Bean Day. Formal citation will be available after printing, tentatively….
Markell, S., Harveson, R., Pasche, J., and Nelson, B. 2016. Dry Bean Disease Diagnostic Series. North Dakota Cooperative Extension Service Publication PPXXXX. XX Pp.

Video publication:
A professional video was created during the summer of 2016. Maps of SCN distribution, photographs, live film in the greenhouse and other visuals are used along with voiceovers recorded in a studio to develop a professional education video about the threat and management of SCN on dry beans. The video features Drs Markell and Nelson and was designed in cooperation with the NDSU Extension Service specifically to the target audience attention span and knowledge level.

Characterization of the Genetic Basis for Resistance in Dry Bean:
Dry bean genotypes with resistance to SCN type Hg 0 and type Hg 2.5.7 were identified in the *Phaseolus vulgaris* core collection. The next step was to transfer resistance into adapted bean genotypes using conventional breeding methodology. Understanding the genetic basis of host resistance to SCN is expected to lay the groundwork for a molecular screening procedure that can reduce the time for variety development. Three crosses were made between previously identified resistant and susceptible genotypes of three important classes of dry bean, i.e. black (PI 310668 X T39), pinto (PI 533561 X GTS 900) and kidney (PI 313837 X Red Hawk). The three accessions selected for crosses show desirable agronomic traits in the field in addition to the SCN resistance. Kidney bean cross between SCN susceptible parent (Red Hawk) and SCN resistant parent (PI313837) was made and kidney bean mapping population segregating for resistance to SCN was developed using the single seed descent method. The F5 generation of kidney bean population consists of 185 recombinant inbred lines (RILs). Three replications from each kidney bean RIL were screened for SCN resistance along with the susceptible check, resistant check and parents. For SCN screening, these lines were inoculated with 2000 SCN eggs per plant and cyst counting was performed after 30 days. Screening data for RILs varied from highly susceptible lines to highly resistant lines. Genomic DNA was analyzed with the BARCBean6K_3 Illumina SNP array. A total of 5398 single nucleotide polymorphism (SNP) markers were run on this population. A total of 788 markers were polymorphic between the Red Hawk and PI 313837 and also segregated among 174 RILs as well. A high density linkage map

consisting of 788 SNPs was generated. This information was integrated with SCN resistance segregation data to find the genomic regions associated with SCN resistance on the genetic map of 11 chromosomes in dry bean. We found three SNP markers:
sc02667ln32330_7503_T_C_414353090 and
sc00044ln783129_45489_C_T_52282565 on chromosome 9 and
sc00273ln341540_27479_A_G_168037303 on chromosome 11 ($p<.05$) through regression analysis. These markers can be of practical use for marker assisted selection in dry bean breeding programs while selecting for resistance to SCN. No major QTLs were detected in QTL analysis; however, several minor genes might be involved in resistance mechanism. This is the first step towards mining the vast genetic bean resources and delimits the genomic regions associated with SCN resistance. Conventional method for SCN screening in field and greenhouse can be time-consuming process and sometimes results are highly variable. In addition, tests can only be performed on adult plants and is temperature sensitive. Identification of closely linked markers will significantly reduce the time and labor invested in selection of resistant varieties.
To evaluate the agronomic characteristics of these lines in field, a large quantity of seeds was generated. This seed will be used for planting in winter nurseries. Likewise, a mapping population of 229 RILs from pinto bean cross (susceptible parent GTS 900 and resistant parent PI 533561) has been developed. The RILs for the pinto bean mapping population will be evaluated in the greenhouse for SCN resistance and genomic DNA will analyzed with the BARCBean6K_3 Illumina SNP array. This research is being continued through funding by the Northarvest Bean Growers Association. In a search of additional sources of resistance, 193 advanced breeding lines from the NDSU dry bean breeding program were also screened in the greenhouse for SCN resistance and the data indicates high susceptibility to high resistance in the breeding lines.

Goals and Outcomes Achieved

The main goals of this project were to increase awareness about soybean cyst nematode and SCN management practices and characterize resistance to SCN to facilitate the development of resistant cultivars of dry bean.

Extensive outreach activities to educate growers about SCN in dry bean were performed. This has helped growers understand the pathogen to make informed decisions about SCN damage to bean production, identification in the field, soil sampling for SCN presence, basic management and the breeding effort underway to produce SCN resistant dry bean varieties.

Kidney bean and pinto bean mapping populations have been developed and seed was increased for field evaluations. Genetic information about SCN resistance will aid the breeding efforts towards moving resistance genes into commercial bean varieties. A kidney bean mapping population has been screened for SCN resistance and resistant lines with superior agronomic traits can serve as SCN resistance source in the breeding program. Three SNP markers were identified which may play a role in the use of Marker Assisted Selection to speed up the breeding of dry bean for SCN resistance genes. Further screening of the pinto bean population will be continued with funding from the Northarvest Bean Growers Association to obtain more information about the position of genomic regions associated with SCN resistance. Field

evaluations of both pinto and kidney bean populations will be conducted in the future research to identify resistant lines with good agronomic qualities which can serve as sources of resistance.

Impact of outreach program

In the original grant proposal we proposed to survey growers and other personal associated with dry bean production to obtain a benchmark on information about SCN and then survey later again during the grant period to determine the awareness level has increased. Once we started the grant we realized that was not the best approach to measure awareness of SCN. Instead we used the normal extension outreach method of measuring the learning level of meeting participants on the topic of soybean cyst nematode on dry bean. Not all outreach meetings, especially with growers are amenable to conducting surveys, thus we used the more structured meetings where a diverse mix of growers, agricultural consultants and crop scouts, farm managers, extension agents, elevator managers, and chemical company representatives would attend and used survey questions to measure the learning level on this topic. It is also important to note that dry bean growers also grow other crops and attend meetings that are not directly focused on dry bean. The topic of SCN was of strong interest to the agricultural community and they learned new information in the outreach efforts. This is shown in Table 1 which gives the results of 9 meetings at different venues where the learning level was surveyed. In all presentations, the average response to the learning level scale was between "3 - I learned something new and useful and 4 – I gained important insight and information that I will apply in my farming operation & management". In addition, notice that "hands on" demonstrations of SCN were ranked number 1 in level of learning at all *five* meetings they were taught. This was extremely useful in showing the participants the actual pathogen problem that occurs in the field. We were unable to measure the impact of the video we produced since the grant ended as we released the video to the agricultural community. However, that video is readily available for viewing on YouTube and will be promoted by our normal outreach programs on dry bean diseases.

Beneficiaries

The dry bean industry which includes growers, crop scouts, dry bean elevators, and agricultural scientists working on dry bean will be the beneficiaries of this project. The growers have directly benefited through increased awareness and knowledge of SCN identification, biology and management, plus an understanding of the potential damage to the bean crop. Marker Information and SCN resistant lines will benefit the breeders in incorporating SCN resistance into commercial bean varieties. The outreach programs directly reached 4,688 persons and publications, interviews and a video on SCN will reach an even greater number.

Lessons Learned

SCN reproduction under controlled conditions fluctuates for unknown reasons and can cause failure of experiments. Screening of several sets of a kidney bean mapping population was not successful because of poor reproduction of SCN on RILs as well as in the susceptible check. Screening of those sets were repeated and we have complete data for 185 kidney bean RILs. Some of these lines are showing resistance to SCN and can serve as useful sources of resistant

material in the dry bean breeding program. Molecular markers identified in this study could be useful in breeding programs to eliminate the needs of cumbersome disease assays.

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Additional Information

Table 1. Impact of outreach program on knowledge of soybean cyst nematode on dry bean[a].

LOCATION in ND/MN	TALK TITLE	DATE	PARTICIPANTS	RESPONSES	LEARNING LEVEL[b]	RANK OF PRESENTATION
Minot/Newberg/[c] Streeter/Underwood	SCN Biology	January 2015	180	104	3.4	#1 of 4
Eastern Crop Scout School, Fargo	Bean and soybean hands on lab	March 2015	70	55	3.2	#1 of 19
Western Crop Scout School, Mandan	Bean and soybean hands on lab	March 2015	42	41	3.1	#1 of 10
Best of the Best in Wheat/Soybean Research, G. Forks	Soybean Cyst Nematode Hands on lab	February 2016	105	100	3.0 3.1	#3 of 9 #1 Of 9
Best of the Best in Wheat/Soybean Research, Moorhead	Soybean Cyst Nematode Hands on lab	February 2016	65	61	3.2 3.1	#4 of 9 #1 of 9
Eastern Crop Scout School, Fargo	Bean and soybean hands on lab	March 2016	59	57	3.3	#1 of 19

[a] All the talks presented in these meeting had a section on biology and or management of soybean cyst nematode.

[b] Learning level responses were: 1 – Nothing was presented that I didn't already know; 2 - Familiar with the topic but the review was useful; 3 - I learned something new and useful; and 4 – I gained important insight and information that I will apply in my farming operation & management.

[C] These were four different meetings in series titled 'Getting it Right' and the data was combined.

Evaluating Apples (Edible and Ornamental) for North Dakota Commercial Nursery and Orchard Industries

Final Report

Partner Organization: North Dakota State University

Project Summary

Apple and crabapple trees are some of the most popular trees planted by homeowners. Apples are also increasing in importance as a commercial crop commodity for North Dakota. Selection of the proper apple (edible or ornamental) cultivars is critical to success for commercial production or landscape use. Apple and crabapple recommendations for ND are outdated and recent recommendations are based on research conducted and reported by the University of Minnesota and UMN Extension Service. This project initiated a planting of new apple cultivars (edible and ornamental) including multiple different rootstocks for long-term evaluation to provide recommendations to ND nursery growers. Ground cover management and weed control was conducted to provide information for organic compared to conventional edible apple production. From this project, NDSU Extension is developing a Plant Diagnostic website focusing on edible apple and ornamental crabapple. NDSU Extension published a "From Orchard to Table: Apples!" publication that list the culinary uses and storage characteristics for ND hardy apple cultivars. This publication is available from NDSU Extension (FN1847, https://www.ag.ndsu.edu/publications/food-nutrition/from-orchard-to-table-apples)
This publication gives recommendations on apple preparation and preservation to supplement the cultivar and weed suppression trials. This project did not build on a previously funded project with the Specialty Crop Block Grant Program (SCBGP).

Project Approach

The objectives of this project were:

1. Provide best management practices based on organic vs. conventional apple orchard production.
2. Determine disease pressures on apple (ornamental and edible) across North Dakota affecting commercial and residential plantings.
3. Conduct varietal trials of edible and ornamental apple cultivars that are potentially suitable for use in North Dakota.
4. Conduct rootstock trials that are potentially suitable for orchard (commercially and residential) and landscape use.
5. To understand how to balance weed control and soil quality for optimal tree performance, fruit yield and quality in a new apple orchard in North Dakota.

Project Activity	**Who will do the work?**	**When was the activity (proposed/actually) accomplished?**
Remove old orchard.	Wood plant improvement project and high-value crops project	Wasn't proposed, but needed to be done because of the lack of room at the research location. Fall 2014/Spring 2015

Soil health assessment will be conducted by the Cornell Nutrient Analysis Laboratory. Soil samples will be collected and sent for analysis.	Dr. Harlene Hatterman-Valenti and Graduate Student	Spring 2015/Was not done because old trees were being removed from the location.
Orchard Establishment and initiation of weed control methods for conventional and organic management.	Dr. Harlene Hatterman-Valenti and Graduate Student	Spring 2015, 2016 and Summer 2015/Fall 2016
Data will be collected on weed management systems	Dr. Harlene Hatterman-Valenti and Graduate Student	Spring, Summer, Fall 2015 and Spring 2016/late spring and summer 2017
Field Day at NDSU Horticulture Research farm.	Dr. Todd P. West, Dr. Harlene Hatterman-Valenti ,Dr. Esther McGinnis, Dr. Jared LeBoldus, Dr. Julie Garden-Robinson	Summer 2015/Summer 2016/Summer 2017
Reapply weed management systems if needed	Graduate Student	Spring 2016/Did not occur
Apple Culinary Use Extension Bulletin developed	Dr. Julie Garden-Robinson	Spring 2017
Ornamental and Edible Cultivar trial – Final planting	Dr. Todd P. West, Plant Science Research Specialists, Graduate and Undergraduate Students	Spring 2017
NDSU Extension Horticulture: Plant Diagnostic website development	Dr. Esther McGinnis	Summer 2017
Initial Project Results – Printed in North Dakota Nursery and Greenhouse Association Newsletter	Dr. Todd West	Submitted June 2017

This project initiated four new apple (edible and ornamental) evaluation plantings at the NDSU Horticulture Research Farm. These four planting were; edible apple cultivar evaluation, edible apple rootstock evaluation, orchard Establishment and initiation of weed control methods for conventional and organic management edible apple planting and the ornamental crabapple evaluation planting. These evaluation planting will have a significant impact on apple (edible and ornamental) production and utilization in ND. This project is a long-term evaluation project with updates given to specialty crop beneficiaries periodically in the future outside of the funded time period of this project. NDSU Extension will assist with increased production and utilization of apple (edible and ornamental) in ND with the addition of the extension website entitled, "How About Them Apples?" and the extension publication "From Orchard to Table: Apples!" publication.

Goals and Outcomes Achieved

Goal 1: Provide cultural and evaluation information of edible and ornamental cultivars to potential apple orchard producers, North Dakota nursery plant producers/retailers and North Dakota residents.

- Performance Measure: Evaluations will be conducted on: statewide apple scab, orchard establishment and weed control methods for conventional and organic production and management, evaluation of establishment, winter and drought hardiness, soil adaptation, pest and susceptibility, aesthetic characteristics and survival of edible and ornamental apple cultivars.
- Benchmark: Apple and crabapple recommendations for North Dakota are outdated and recent recommendations are based on research conducted and reported by the University of Minnesota and UMN Extension Service.
- Target: Increased information available to apple orchard producers, North Dakota nursery plant producers/retailers and North Dakota residents.
- Performance Monitoring Plan: Evaluations will be conducted and analyzed with information being presented to specialty crop beneficiaries.
- Achievements

  <u>Conduct varietal trials of edible and ornamental apple cultivars that are potentially suitable for use in North Dakota.</u>

  Thirty cultivars of edible apples were grafted onto 3 different rootstocks (Dolgo, M7 and Bud9) with five replications planted at the NDSU Horticulture Research Farm. These thirty cultivars will be evaluated long-term for potential use in ND orchards.

  <u>Conduct rootstock trials that are potentially suitable for orchard (commercially and residential) and landscape use.</u>

  Nine different rootstocks and six different rootstock/interstock combinations were utilized with this evaluation. They are:

  1. Dolgo – standard 100% mature size
  2. Antonovka – semi-standard, 8—90%
  3. Bud9 – dwarf, 25%
  4. Bud 118 –standard, 100%
  5. M7 – semi-dwarf, 50%
  6. MM11 – semi-standard, 75-85%

7. P2 – dwarf, 35-40%
8. P22 – dwarf, 25-35%
9. M26 – dwarf, 45-55%
10. Dolgo (rootstock)/Bud9 (interstock)
11. Dolgo/M7
12. Dolgo/P22
13. Antonovka/Bud9
14. Antonovka/M7
15. Antonovka/P22

'Haralson', an edible apple that has shown great success in ND was used as a single cultivar on all fifteen different rootstock combinations for the evaluation. These grafted trees were planted at the NDSU Horticulture Research Farm for long-term evaluations to provide recommendation for ND commercial nursery producers and orchards.

Ornamental Crabapple Evaluation

Twenty-five different crabapple cultivars were planted for long-term evaluation at the NDSU Horticulture Research Farm. These cultivars include:

1. Coralburst
2. Camelot
3. Gladiator
4. Golden Raindrops
5. Harvest Gold
6. Indian Magic
7. Ivory Spear
8. Lollipop
9. Pink Princes
10. Pink Spires
11. Prairie Fire
12. Purple Prince
13. Radiant
14. Raspberry Spear
15. Red Splendor
16. Rejoice
17. Royal Beauty
18. Royal Raindrops
19. Ruby Tears
20. Show Time
21. Snowdrift
22. Sparkling Sprite
23. Spring Snow
24. Thunderchild
25. Tina

These twenty-five crabapple cultivars were planted with three replicates and will be evaluated for hardiness, disease susceptibility, flower time and duration, along with fruit

condition, color and persistence. This data will be compared to the NDSU 100 cultivar evaluation with recommendations given to ND nursery producers and retailers. NDSU Extension will also add future information to the informational apple website.

Orchard establishment and weed control methods for conventional and organic production and management

The trial map shows the layout of the weed management treatments (Figure 1). Three apple cultivars ('Haral Red', 'Honeycrisp', and 'Zestar') were grafted onto 'Dolgo' rootstock prior to transplanting into the field on 8/25/2016. Originally a living mulch treatment was included in the trial, but since a nearby orchard being established was just mowing the vegetation, we decided to use that as a treatment instead of a living mulch since growers probably would be apprehensive about the competition from the living mulch. The weed badger treatment was the mechanical control and consisted of an attachment with a circle of tines that rotate clockwise to dislodge emerged plants. One mechanical cultivation occurred late spring, prior to TDR placement. One mowing occurred mid-summer after TDR placement. All trees received supplemental water once in July during an extremely hot and dry period. The preemergence herbicide treatment was applied in the spring, prior to weed emergence.

Tree apical growth was influenced by weed management treatment. In 2017, apical growth was greatest when an herbicide was applied or the landscape fabric was installed (Table 1). Trees growing within the herbicide treated plots or the landscape fabric plots had greater apical growth compared to trees in the tillage, mowed, or untreated plots. Total tree height varied between cultivars, but was not influenced by weed management treatments. 'Zestar' trees were taller than 'Haral Red' trees (Table 2). Height differences were attributed to genetic differences in the scions since all trees had the same rootstock. The average tree canopy spread was influenced by the weed management treatments. Trees with the herbicide, wood chips, or landscape fabric plots had the greatest canopy width (Table 3). Trees growing in the herbicide treated plots had wider canopies compared to trees in the mowed, tillage, or untreated plots.

Datalogger 1 did not record in June and part of July due to a problem with the battery pack. This was corrected on 7/28. Datalogger 2 stopped recording starting 8/22 for unknown reasons. Thus, graphs for percent soil water content and soil temperature are averages from 7/28 until 8/22. Soil water content was greatest under the wood chip and landscape fabric treatments even after the rain event on 8/13 (Figure 2). The third highest soil water content occurred within the herbicide treatment. However, after the rain even the mowed treatment had the third highest soil water content. Even though more water was available in the mowed treatments compared to the tillage or the untreated after the rain event, the weed competition that occurred before the plots were mowed greatly reduced tree growth because the weeds were mowed too late in the season. In the future multiple mowing will occur in order to limit weed growth. Soil water content in the tillage treatments was similar to the untreated. This was attributed to weed regrowth after the early season tillage event. In the future multiple tillage passes will also occur to reduce weed regrowth.

Soil temperatures did not show a distinct pattern for the period between 7/28 and 8/22 (Figure 3). Soil temperatures in the herbicide, landscape fabric, and tillage treatments tended to have higher soil temperatures. These treatments also had more exposed soil compared to the other treatments during the recorded period. The untreated and wood chip plots tended to have lower soil temperatures. This was expected for the wood chip treatment as other research has shown the moderating effects of wood chip mulch. The lower soil temperatures with the untreated was attributed to the dense weed population which shaded the soil from any direct sunlight exposure.

Soil moisture (water content) data corresponded with tree growth with the exception of wood chips. Even though trees in wood chips had some of the highest soil water content readings, trees in this treatment lagged significantly in apical growth, total tree height, and canopy width suggesting that something in addition to soil moisture was influencing tree growth. Since wood chips have a high C:N ratio, soil microbes breaking down wood pieces in the soil would have to use soil N, reducing the amount of N available to the tree roots. Straw mulched has caused a N deficit in grapes due to a similar high C:N ratio. In the future, additional dataloggers will be purchased so that if one fails for some reason, fewer sensors will be affected. Multiple tillage and mowing events will occur each year in order to see if this will relieve the weed competition effect on soil moisture and reduced above ground tree growth.

Table 1. Average apical growth during 2017.

Weed Suppression Treatment	Apical Growth (cm)	
Herbicide	53.33	a
Fabric	41.22	ab
Mulch – Wood Chips	38.78	bc
Tillage	26.33	cd
Untreated	25.12	d
Mowed	20.00	d

Table 2. Average total height of trees measured 9/25/17.

	Tree Height (cm)	
Zestar	154.0	a
Honeycrisp	147.0	ab
Haralred	136.4	b

Table 3. Average canopy spread of trees measured 9/25/17.

	Canopy Width (cm)	
Herbicide	43.22	a
Mulch – Wood Chips	39.89	ab
Fabric	35.94	abc
Tillage	28.17	bcd

Untreated	24.22	cd
Mowed	22.83	d

REP 1	16	2	17	7	4	3	11	18	5	15	12	13	9	10	14	1	8	6
	Herb	Fabric	Living Mulch	Untreated	Herb	Woodchips	Living Mulch	Weed Badger	Living Mulch	Woodchips	Weed Badger	Untreated	Woodchips	Herb	Fabric	Untreated	Fabric	Weed Badger
	Haral Red	Zestar	Haral Red	Honeycrisp	Zestar	Zestar	Honeycrisp	Haral Red	Zestar	Haral Red	Honeycrisp	Haral Red	Honeycrisp	Honeycrisp	Haral Red	Zestar	Honeycrisp	Zestar
REP 2	5	11	8	14	9	13	1	17	16	6	15	3	2	18	7	12	10	4
	Living Mulch	Living Mulch	Fabric	Fabric	Woodchips	Untreated	Untreated	Living Mulch	Herb	Weed Badger	Woodchips	Woodchips	Fabric	Weed Badger	Untreated	Weed Badger	Herb	Herb
	Zestar	Honeycrisp	Honeycrisp	Haral Red	Honeycrisp	Haral Red	Zestar	Haral Red	Haral Red	Zestar	Haral Red	Zestar	Zestar	Haral Red	Honeycrisp	Honeycrisp	Honeycrisp	Zestar
REP 3	9	14	18	6	10	2	7	12	3	16	17	4	15	8	1	5	11	13
	Woodchips	Fabric	Weed Badger	Weed Badger	Herb	Fabric	Untreated	Weed Badger	Woodchips	Herb	Living Mulch	Herb	Woodchips	Fabric	Untreated	Living Mulch	Living Mulch	Untreated
	Honeycrisp	Haral Red	Haral Red	Zestar	Honeycrisp	Zestar	Honeycrisp	Honeycrisp	Zestar	Haral Red	Haral Red	Zestar	Haral Red	Honeycrisp	Zestar	Zestar	Honeycrisp	Haral Red

1	Untreated	Zestar	
2	Landscape Fabric	Zestar	
3	Woodchips	Zestar	
4	Herbicide	Zestar	
5	Mowed Vegeation	Zestar	
6	Weed Badger	Zestar	
7	Untreated	Honeycrisp	
8	Landscape Fabric	Honeycrisp	
9	Woodchips	Honeycrisp	
10	Herbicide	Honeycrisp	
11	Mowed Vegeation	Honeycrisp	
12	Weed Badger	Honeycrisp	
13	Untreated	Haral Red	
14	Landscape Fabric	Haral Red	
15	Woodchips	Haral Red	
16	Herbicide	Haral Red	
17	Mowed Vegeation	Haral Red	
18	Weed Badger	Haral Red	

15' between plants
20' between rows
4' X 15' for each treatment.
270' east and west
40' north and south
Dolga Crab rootstock
RoundUp 7/28/16

NORTH

Figure 1. Plot map of the edible apple weed management trial at the horticulture research site near Absaraka, ND.

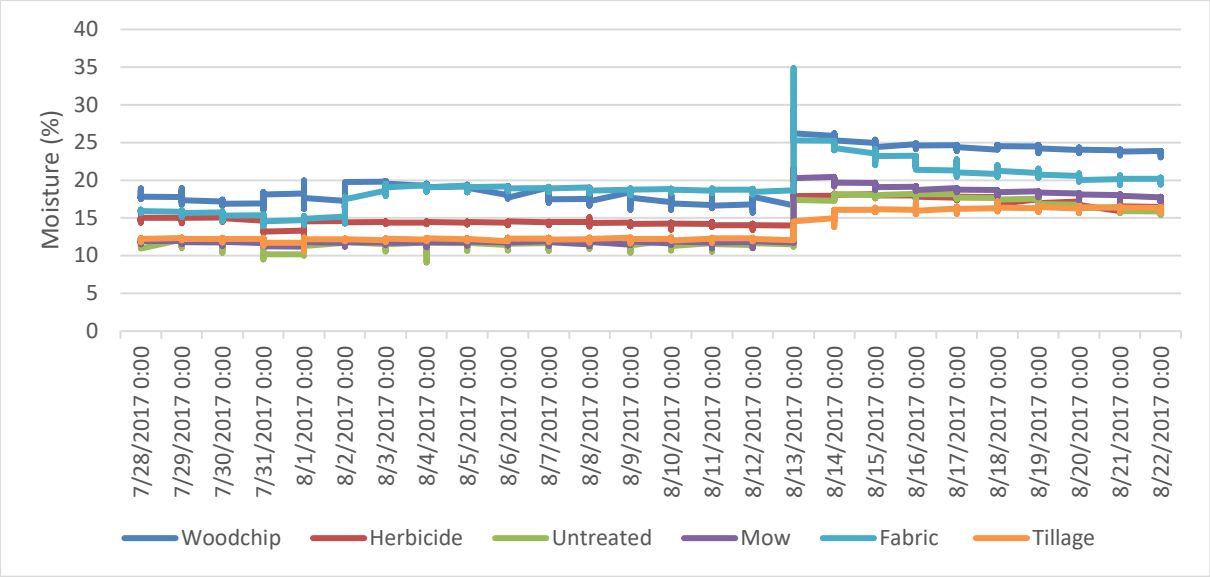

Figure 2. Average percent soil moisture under the various weed management treatments starting 7/28/17 and ending 8/22/17.

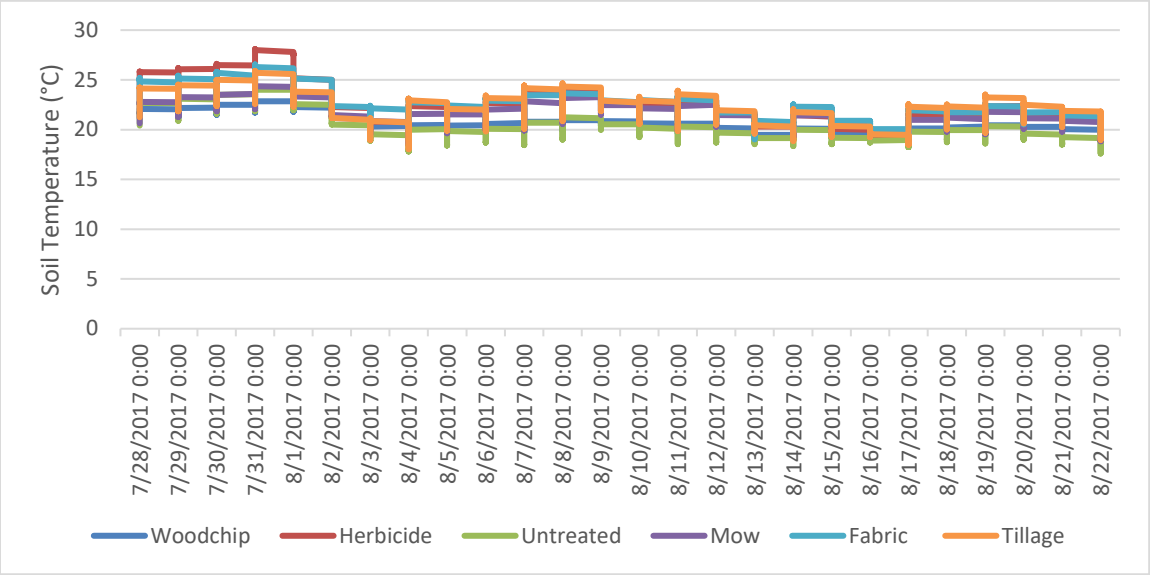

Figure 3. Average soil temperatures under the various weed management treatments starting 7/28/17 and ending 8/22/17.

Goal 2: Development of NDSU Extension Horticulture: Plant Diagnostic website/informational bulletin and bulletin on apple culinary uses and storage characteristics.

- Performance Measure: Develop plant diagnostic, apple culinary use and storage characteristics website/bulletins based on evaluation information from Goal 1.
- Benchmark: There is no information formalized for ND County Extension Agents or ND homeowners with respect to edible and ornamental apple issues in ND.
- Target: Increase awareness and abilities for ND County Extension Agents in plant diagnostics and culinary uses for apples as well as providing homeowners with information.
- Performance Monitoring Plan: Information will be gathered with respect to performance measures from Goal 1 and developed into the respective informational website and/or informational bulletin.
- Achievements

  NDSU Extension Horticulture: Plant Diagnostic Website

  The NDSU Extension Apple website entitled, "How About Them Apples?", will go live the first week in October and features a new apple diagnostic feature called "Rotten to the Core". The apple diagnostic allows people to diagnose common apple diseases, insect pests, and abiotic disorders that we see in North Dakota. The diagnostic is broken down into signs and symptoms associated with leaves, fruit, branches/trunk and blossoms. Each entry features photos, descriptions of symptoms and signs, and management recommendations. In addition, the apple website links to Extension apple publications and recipes. This website will provide essential information to ND extension agents to assist with ND nursery growers, orchard producers and homeowners with respect to apple problems both ornamental and edible.

NDSU Extension: Nutrition and Food Safety
NDSU Extension published a "From Orchard to Table: Apples!" publication that list the culinary uses and storage characteristics for ND hardy apple cultivars. This publication is available from NDSU Extension (FN1847, https://www.ag.ndsu.edu/publications/food-nutrition/from-orchard-to-table-apples/fn1847.pdf) This publication gives recommendations on apple preparation and preservation to supplement the cultivar and weed suppression trials.

Beneficiaries

The specialty crop groups that benefited from this project are apple orchard producers (current/future), ND nursery plant producers/retailers and ND residents. This project will benefit the specialty crop beneficiaries by providing them with essential information on cultivar selection, root stock selection and best management practices based on research conducted within ND and not recommendations given from research conducted in neighboring states. This information will be provided in the future based off short-term and long-term evaluations based on rootstock and cultivar evaluations. Orchard establishment with respect to weed suppression information will have an immediate effect on current and developing orchards in ND with the data that has been collected and will be collected in the future.

How many benefited from the project?
The number of direct specialty crop beneficiaries were approximately 100. These 100 are involved directly in the production and/or sale of ornamental and/or edible apple trees in ND.
How did they benefit from the project?
Cultivar and rootstock trials will increase sales for wholesale and retail nurseries in ND will the information gains and provided through this project. This will allow the nurseries to make better recommendations and have more diverse nursery stock available to customers.

Lessons Learned

Objective #1 provided information on orchard establishment and weed management for organic and conventional production as stated under the previous section, "Goals and Outcomes Achieved", under the heading of "Orchard establishment and weed control methods for conventional and organic production and management". Long-term best management practices will be conducted including fertilizers (organic vs. conventional) and disease management (organic vs. conventional). As this information is obtained, information will be given to beneficiaries utilizing field day presentations, newsletters and direct contact.

Objective #2 (Determine disease pressures on apple (ornamental and edible) across North Dakota affecting commercial and residential plantings.) was not completed. This objective was directed by Dr. Jared LeBoldus, NDSU Tree Pathologist. Dr. LeBoldus resigned from NDSU in late 2015. The NDSU Plant Pathology Dept. did not refill this vacant position and there was no one at NDSU able to take on this task.

Getting the orchard planting area ready for planting took longer than expected and should have been budgeting into the project. This by far was the largest set-back because of the lack of available planting space at the NDSU Horticulture Research Farm.

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Additional Information

2017 American Society for Horticulture Science Annual Meeting – Poster Presentation
Authors: Drs. Esther McGinnis and Julie Garden-Robinson
Title: Consumer Preference of Apple Characteristics

Abstract: In the United States, more than 2,500 apple cultivars (*Malus* x *domestica* Borkh.) are available. Apples are popular among consumers because of their ready availability in supermarkets and farmers markets, their flavor, nutrition, versatility on the menu and other attributes. Apples are naturally cholesterol free, very low in sodium and fat, a good source of fiber, vitamins and minerals and have less than 100 calories in a medium-sized fruit. In the northern Great Plains, many apple cultivars grow well and are a subject of expanding research. The NDSU Extension Service horticulture and nutrition programs conducted an online survey to determine apple preferences among consumers to help inform breeders in university research programs. The survey protocol was submitted to the university's Institutional Review Board; upon approval, the 18-question online survey was disseminated using email listservs and social media (Facebook) through the Extension network in the United States to reach educators, specialists and consumer audiences using a snowball approach to survey administration. Responding to the survey were 686 people (88% female) from 39 states, and 57% were ages 41 to 64. Most respondents (64%) were home gardeners. The participants rated several attributes of apples on a 9-point scale (1=least important; 9=most important). Flavor (8.33), crispness (7.96) and firmness (7.92) were rated as the most important attributes used to select apples, followed by cultivar (6.62), price (5.84), color (5.63) and size (5.61). Half red, half green apples were the most popular type of apple among 63% of the respondents, followed by bright red (28%). When rating their preference for sweetness (1=sweet; 9=sour), the respondents indicated an average sweetness preference of 3.94. The respondents also indicated a preference for crisp apples; the average score was 8.36 on a 9-point scale (1=mushy; 9=crisp). Respondents commented that intended use (cooking vs. fresh eating) made a difference in their apple selection, along with recommendations from family and friends, absence of "off" flavors from long storage or coatings, apple size, aroma, keeping quality, presence of blemishes, and past experience with the apple variety. Country of origin was not a consideration for buying apples according to 76% of respondents. Of those who responded with concern about country of origin, many people commented that they preferred to purchase from U.S. farmers, especially locally grown. Pesticide use was cited as an issue of concern by some respondents.

Hops Viability in Central North Dakota

Final Report

Partner: Glendon Philbrick

Project Summary

The purpose of this project was to test winter survival for five varieties of hops in central North Dakota, determine the yield and quality of the yield, and to share the data with other hops growers, breweries, and any other party interested in the project. The project successfully determine winter survivability and shared data with others. Due to hops often not producing a yield until the third season, no data could be obtained for yield or quality of yield.

There is a demand for hops due to the insurgency of micro-breweries. There is relatively little research concerning hops grown in North Dakota. This was an opportunity to experiment growing hops and evaluating how they will produce and survive the northern climate of the state. Determining viability of hops in North Dakota will assist in developing the market in the state. There is a need for hops for the existing breweries in the state of ND. The vast majority of hops are imported into the state. Another brewery has opened in ND in the past eighteen months.

The objectives are to determine what a variety will yield, how much will the variety yield, winter survival rate, and quality of the crop produced.

Project Approach

Five varieties of hops were purchased from Great Lake Hops. Five plants for each variety were planted. The varieties were chosen based on recommendations for a northern climate from Great Lake Hops. Hay was placed around each rhizome which proved to be an excellent weed barrier. Little weeding needed to be done. The plants were not covered to test winter survival rate. The survival rate for the five varieties after the first winter is as follows:
Nugget - 0
Cascade – 0
Tahoma – 3
Mt. Hood – 3
Vanguard – 2
There was an extensive dry period the first year which required a lot watering. It is recommended that future growers ensure water is easily accessible to hops when choosing a location for planting. It is recommended to develop a watering system that does not require manual watering if growing a large number of hops. The project has resulted in data for growing varieties of hops that are adaptable to the climate of central ND.

Two annual garden tours were held. The tours were promoted via The McLean County Journal, The Washburn Leader, The McLean County Extra, NPSAS, and Facebook in 2015. The tour was promoted via email, Facebook, and through connections at the BisMan Food Co-op in 2016. The first tour was August 1, 2015. The second tour was held August 2, 2016.

Goals and Outcomes Achieved

The goal and outcome of determining winter survivability was achieved. Three of the five varieties survived without ground cover. The grant was extended to ensure data could be obtained to determine the winter survival rate. The benchmark was the survival rate. The target was 100%. 100% was not achieved.

A long term goal was to obtain data concerning yield and quality of yield. Hops often do not produce until the third season. For that reason, no data could be obtained regarding yield or quality of yield. That is a goal, outcome, and target could not be achieved.

When data is obtained concerning yield of the surviving varieties, data will be shared with other growers, the local extension agent, and the Specialty Crop Block Grant administrator at the ND Department of Agriculture.

Data sharing was a goal of this project and the goal was achieved. Data was shared by hosting two garden tours, sharing data and experiences at two producer meetings hosted by the ND Department of Agriculture, and two private grower tours.

The results of this project were also shared with the McLean County extension office, NDFMGA (North Dakota Farmers and Market Growers Association), and NPSAS. These organizations provide the best outreach for project results by connecting with interested parties and also using their electronic listserv. A PowerPoint document has also been used during presentations and sent out to those requesting information. If you would like a copy of this document, please contact using the information below.

Beneficiaries

Growers in central North Dakota benefited from this project because there is data available regarding what varieties survive in this climate. This data was shared with everyone who attended the meetings hosted by the ND Department of Agriculture. An estimated twenty-five people attended each time, totaling fifty people. Beneficiaries benefited by having data available to assist them in choosing varieties of hops to grow. Information was provided regarding the experience of growing five varieties and testing the winter viability. Information was also shared regarding growing the varieties for this experiment and the challenges and opportunities of growing hops as a certified organic crop.

Seventeen people attended our first tour and twenty-one people attended the second tour. The backgrounds of those in attendance were primarily farmers, two educators, an extension agent, retired individuals, technology entrepreneurs, and people who worked with food and alcohol. Guests were provided surveys regarding all of the experimental items they viewed on the tour. Guests were asked if they were aware that hops were grown in ND. Most were not aware hops could be grown in ND.

We had a total of eighty-eight beneficiaries from this project.

Lessons Learned

Lessons learned from this project include choosing a space with easy access to water. The number grown for this project was manageable for manual watering. If grown on a larger scale, one would need to ensure watering could be done in an efficient manner. Hay has proven to be an excellent weed barrier when applied at a depth of five to six inches. This is an inexpensive method of weed control. A grower will need to be patient when growing hops. It will take a while before a crop can be obtained.

Contact Information

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Aneta Community Orchard and Gardens

Final Report

Project Partner: Aneta Specialty Crop Group

Project Summary

The Aneta Community Orchard was started on the Northwest quadrant of Aneta with the plan to plant a fenced orchard, with a sampling orchard. It has expanded to become an orchard, gardens, and arboretum. Our trees were suffering from a high water table and the area was tiled to keep the orchard viable. We have established asparagus, herb, rhubarb, and flower gardens. An arboretum is being developed to the North, which contains many specialty crop tree varieties, plus wild flowers. Two garden plots are raised each year where the public is invited to pick vegetables as they ripen. Small raised beds were built that are available for use by children and the elderly on a first come basis. Last season and again this season, we are raising a one acre sweet corn plot to provide corn for area food banks, nursing homes, schools and the community. Garden plots (20x40 feet) are available to rent each season for a nominal charge to individuals. In these private garden plots a vast array of vegetables and flowers are grown for personal use. We have provided educational opportunities for children to learn how to garden, and classes for adults to learn gardening skills, canning, pickling, jelly making, and herb use. We have held two successful rhubarb-tasting events on the third Friday in June and expect that to become an annual event.

The purpose of the project is to increase awareness and access of specialty crops in our area by growing fruits, vegetables, herbs, nuts and flowers in a fenced and well managed environment. This unique project provides specialty crop access and education to people of all ages.
In our original orchard and garden area we were able to improve tree health by tiling the area and cleaning the drainage ditches. Lon Zellmer worked tirelessly to clean up the area around the orchard and a professional was hired to clean the existing drainage ditch that carries water away. This assures our orchard trees will no longer have wet roots which were causing the leaves of the orchard trees to yellow. The project also was able to fence a shelterbelt area to the North of the orchard and expand the trees to include specialty crop tree samples. Many of the samples of trees have been developed specifically in our climate. In this new area we also were able to expand the number of garden plots we offer and place the raised beds for the elderly and children.

Project Approach

Community volunteers constructed a fence around the shelterbelt that was planted to the North of the orchard. Tall, fast growing trees are planted on the North to help block the wind and improve the microclimate of the orchard and gardens. The arboretum consists of five 100 foot blocks of like species to create mini ecosystems. There are five blocks of specialty crop tree species: the Spruce Block, Mixed Nuts, Pines, Maples and a double block of assorted species. All are labeled and mature heights and width are posted on each label. This area also contains wild flowers and allowed us space for more garden plots as needed.
The project has allowed us to replace trees that die and expand plantings. Although weather and climate is an ever-present threat to trees and gardens, the project has afforded us the opportunity to provide the best environment to produce specialty crops in our area.

Bill Miller has developed a netting system for our berries that can be moved to protect the various species as they ripen. Bill and Janice Mills developed a list of chores that volunteers could sign up to complete for the 2016 growing season.
Our educational events included the building of raised beds for children who each were assigned a mentor and given seeds to learn the joy of gardening. The NDDA food trailer was rented and classes were conducted by Extension Agents Kristy Berdal, Brenda Langley, and Caitlyn Hain on use of a pressure canner, hot water bath, pickling, jelly making, herb use, and gardening skills including planning, planting, transplanting, indoor seed starting and other facets of successful gardening. While working on the project, individuals have been instructed by Bill in the correct methods for tree planting and instructed volunteers on the benefits of seeking out healthy root stock on which to graft productive specialty crop trees.
Julie Zellmer, an ambulance volunteer, hosted games for children on the morning of the third Saturday in June, both in 2016 and 2017. These games were educational for children in the area of vegetable and fruit identification.
The project allowed us to develop a grass parking lot for the orchard. We have ordered and placed signs to direct the public and improve safety on our site. Robert and Linda Retzlaff built a locking cupboard sign in which we can post notices of events or other information. Kirsti Lukens Craig worked with Bill and Jane to create a brochure. A covered box is attached to the covered bridge that leads from the guest parking lot to the orchard. It allows people to self-tour our site. Bill has given tours during special events in Aneta.
The Aneta Orchard Garden and Arboretum host an annual potluck picnic for the public. Gardeners are especially encouraged to attend and a survey is conducted that evening. The information from the survey is used for garden improvements and to gain insight into the amount of specialty crop their plot produced and how it was used or distributed.
The Aneta Community Orchard and Gardens has a Facebook page and an Instagram account. Our Social media presence continues to be a work in progress with continued plans for increased posting. We have built an online community of over 150 fans with a majority of them residents or former residents of the Aneta area. Eight area communities as well as Fargo and Grand Forks are represented. People post their own photos and tagged our site making the photos visible on our feed.
Aneta Community Orchard Garden and Arboretum has been featured on Prairie Public Radio's Main Street USA with Ashley Thornberg. Marilyn Hagerty has published a feature article on the project and has mentioned it in her column in the Grand Forks Herald. We were received attention in the North Dakota Horizons magazine. Bill Miller spoke to the North Dakota Horticultural Society using a Power Point developed by Katie Lukens Pinke and Kirsti Lukens Craig. Janice Mills writes Orchard Updates for the Aneta Star, our local paper. Her June 17th article read in part:
"Have you ever seen a tree peony? Me neither, but there is one blooming in the arboretum. It has Peony petals on the outside that are light pink and the center is a dark pink with "eyelashes" around it. If you go in through the gate to the arboretum, turn left at the raised beds and walk just a few feet. The sign will say Tree Peony. The bloom is on the North side.
It was fun to see the beginnings of the garden plots. Personalities are already very evident. Lots of ideas.
Haskap berries are turning blue. Cedar Waxwings love them and can eat up a crop before you know they're ready, so they have to be netted to protect them. I don't know if you have ever tried to net anything, but the net is slippery, hard to handle, and catches on every twig there is. It

slides around, slides off where you want it and people say words they regret later. It ranks right up there with a root canal. So when Bill said we were going to net berries, I was not happy. He called a small brave group together and showed up with his idea for netting. He had flexible pipe that we slid onto metal rods, then pushed into the ground. We slipped the netting over the pipe, applied clasps to the net and put down metal staples along the bottom. That was it. Done. I've struggled harder getting on a bathing suit. There is a door on the North side of the net and it will look inviting for kids to run through, but please don't let them. We won't be able to mow around it, but it will be on only until the berries are picked. Birds patrol all day, so they can see any little gap. There are extra staples in the shed if you see a gap. Haskaps will color up 1 to 3 weeks before ripening. If the pulp is green they're not ready. The best way to get the tasty ones is to shake the bush. The ripe ones will fall. They look like little purple bags and are mild flavored. After the Haskaps are done we will move the net to the Juneberries, then on to the cherries, following crops as they ripen.
Thanks to all our volunteers for being such hard and willing workers. The orchard wouldn't be here without all your help."
The project has been kept neatly mowed and well maintained for the health and productivity of the specialty crops we grow.

Goals and Outcomes Achieved

Our goal for the project is to increase production of specialty crops by having over 50 individuals or families benefit from the gardens and 750 attendees tour the specialty crops while donating over 100 pounds of produce to the local food bank, nursing homes, and schools. During the project we had continued interest in garden plots. Approximately 50 pounds of tomatoes, grown in a private garden plot were shared through churches and a senior apartment complex. We planted a one acre corn plot in 2015 and 2016. The 2015 plot was very productive and we were able to provide corn to everyone who wanted it. From our gardens and corn plot we have provided in excess of 1500 pounds of garden produce to area residents. We have far exceeded our goals by providing produce to at least 1000 area residents. They include patrons of the food banks, schools, and nursing homes, as well as area churches and residents. We provide free vegetables to anyone who wants them by placing vegetables in front of the post office in Aneta. Events provided information about specialty crops to over 200 participants in events and hundreds of others who visited and toured the site. We conservatively estimated 1,000 people total entered the garden for tours in 2015 and 2016. We provide fresh flowers to churches and others who wish to pick them.
Our goal is to increase consumption of specialty crops through attendance at events and education of area residents. Rhubarb tasting events were held the third Friday in June. More than 25 types of food using rhubarb, including bread pudding, barbeque sauce, salad, slush, salsa, pizza, pickles, and more were served. At our rhubarb tasting events, 125 copies of our rhubarb recipes were distributed at each event with a survey. Janice reports that of 104 participants, all indicated positive learning experience the first year. 93 indicated they learned about rhubarb as a specialty crop in 2016. Suggestions included beginning wine making with specialty crops, as well as more dishes using rhubarb as a component in a savory dish. Fifteen cooks volunteered to prepare the food.
Classes on use of a pressure canner, jelly making, hot water bathe, pickle making were held. The classes featured lecture and demonstration followed by actual pickle and jelly making. Class size was limited. Fourteen people signed up and 11 participated. Our survey revealed that five

had attempted pickle making before, four had tried jelly making and only two had used a pressure canner. All participants stated an increase in knowledge in specialty crops after this event.
Herb use class was taught by Kristi Berdal and Caitlyn Hain USDA Extension, with ten people attending. All participants indicated they learned about herbs and how to use them more often. We offered some food and drinks featuring various herbs. Attendees were invited to pick herbs from the large variety offered at the Orchard and Garden. The 2015 berry crop allowed many children to enjoy raspberries. Nibblers and jelly makers picked from a variety of 26 different types including aronia, red and black currants, spring and fall bearing raspberries, gooseberries, and haskaps, The birds picked all the juneberries in 2015, but optimism continues for this season's crop. Guests also picked asparagus, rhubarb, herbs and flowers from the orchard and gardens.
Gardening classes led by Caitlyn Hain attracted 29 participants. All returned surveys indicated an increase in knowledge in planning, planting, starting seeds, transplanting, and choosing appropriate varieties.
Our children's gardening project began by constructing six raised beds three feet square. They were offered to children ages 4-14 free of charge. Each child was assigned a mentor. A class was held with instruction on soil and the benefit of worms. In 2016, these beds are also being offered to senior citizens.
Our goal is to increase awareness of and access to specialty crops. Two garden plots are grown and tended each year to provide food for those who need it plus a one acre plot of sweet corn has been grown for the project. We developed a process for sharing garden produce with the public by establishing an exchange in front of the post office in Aneta. The project also developed the arboretum of specialty crop trees. This area allows those who are interested to view various specialty crops possibilities such as nut crops, evergreens, or lumber trees for personal or commercial use. The trees in the arboretum are labeled and it is accessible for public viewing. Griggs County Farmers Union held an event this month at the project site and Bill was on hand for tours and information. We expect to continue to host appropriate events at the site using picnic tables and temporary seating as necessary.

Survey results are not reflective of the total number of those that came to our events, as many did not take or return the surveys. From the surveys we received back, we estimate an overall increase in awareness of specialty crops of 90% of participants.

Beneficiaries

Numerous groups have benefitted from this project, including area residents, gardeners, nursing homes, local food banks, schools, churches, and those coming into town for events. Through outreach, local events, and field tours we estimate the total number of beneficiaries is over 2,000. We expect the beneficiaries of this project to grow as the orchard does.

Lessons Learned

It took the cooperation of the City of Aneta and its residents for the project to begin. The cooperation has developed over the years. The Aneta Community Orchard, Gardens and Arboretum has improved the look of the town on its North side. The project has taught us that

with the proper expertise, in this case Bill Miller, Janice Mills, and our wonderful Extension staff, and the right resources, a dream project can be built.
We have learned that the success of specialty crops in our climate depends on many factors, some of which can be controlled by planting proper varieties and controlling the environment to the best of our ability. Installing hydrants, planting a wind break, using wood chips as a mulch, fertilizing the garden plots annually, tiling the orchard and improving the flow of water away from the project have improved the environment for specialty crop production.
We plan to continue our use of social media and hope eventually to be proficient in its use for alerting patrons to the many activities in the project.
We have learned that while fences are very effective at keeping deer and other larger animals out of the area, the gophers and other burrowers still think the area belongs to them. We fight infestation rigorously and we are always on the lookout for safe for the project, yet effective methods to control these pests.

Contact

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Additional Information

Last season was our first time growing a corn plot. Jane Lukens secured free sweet corn seed and we planted a one acre plot on land that we farm that is owned by Iris Westman. She is the oldest living North Dakota resident. The plot proved to be very productive and ripened in August for her 110th birthday. At her birthday party, we brought boxes of corn to share with the staff of the nursing home where she resides as well as her family and guests gathered to celebrate. Iris, who was raised on a farm near Aneta, was so pleased to have this bounty to share with all in attendance. Her memory is sharp and she enjoyed the corn and the many memories of gardens in her past. Her family, who traveled from the Twin Cities and beyond, were very impressed with the quality of sweet corn we could raise in this area.
The success of our project is due to many interesting components. Bill Miller holds a Master's degree in horticulture from the University of Minnesota and he is tireless in his dedication to the project. Janice Mills, a Master Gardener and a registered nurse gave her unique perspective and ambition to see the project through. Lon and Julie Zellmer have spent time cleaning up the site, clearing old trees and brush, and beautifying the project. Julie, also a registered nurse, brought her commitment to health and wellness in and around Aneta to the project. Robert and Linda Retzlaff have lent their expertise as an engineer and as gardeners. Fred and Jane Lukens gave the project a farm connection with access to farm equipment, land, and a wood chipper, as well as years of experience in marketing and public relations. From fence building to maintenance, the community has without exception, been supportive and participated as needed. We have enjoyed the support and cooperation of hundreds as we have brought the Aneta Orchard Gardens and Arboretum to reality.

The Aneta area is unique in its ability to cooperate. We do it well because we practice working together every summer when we put on the Aneta Turkey Barbeque on the third Saturday in June. We serve about 2500 people in a town of 250 people. From a Friday night talent show to a Sunday morning All Faith Service in the park, it takes cooperation from everyone to pull it off.

Aneta has done it every year for over 50 years. It is this ability to work together that has made the Aneta Orchard Gardens and Arboretum a success. Although the project does not benefit directly from the success of the turkey barbeque, it has benefitted greatly from the ability of this community to work together and the pride people take in keeping Aneta viable and beautiful. It is also a great opportunity to showcase the project with an event such as our rhubarb tasting and tours.

Please stop by and visit our project any time you pass through Aneta located on Highway 32 in North Dakota. Look West as you cross the railroad tracks on the North edge of Aneta, then follow the signs. The gates are unlocked.

Welcome to the Aneta Community Orchard, Gardens and Arboretum.

WE ARE A 0.3 ACRE MODIFIED FOREST GARDEN WHERE GUESTS CAN SAMPLE ANY OF THE 50 VARIETIES OF FRUIT THAT ARE RIPE. "READY TO PICK" SIGNS ARE POSTED BY THE FRUITS AT THEIR PEAK RIPENESS. IF YOU WANT TO PICK LARGER QUANTITIES, BRING A BUCKET TO THE ORCHARD TO THE EAST.

SAFETY IS OF THE UTMOST IMPORTANCE. PLEASE PROCEED WITH CAUTION AND WATCH THE GROUND FOR HOSES OR OTHER OBSTACLES ON THE PATH.

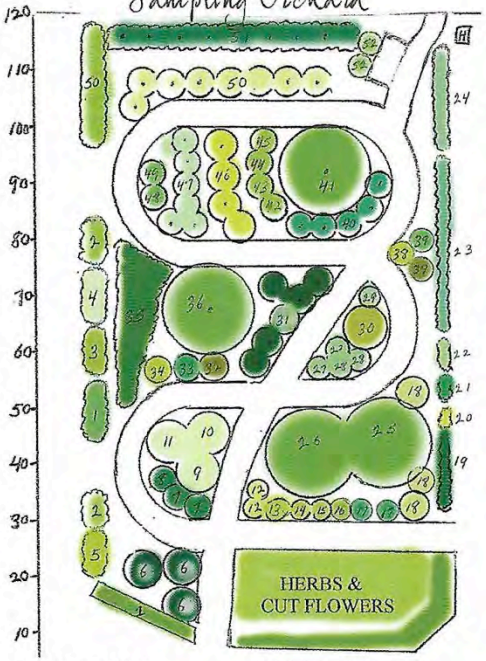
PLEASE ALSO SUPERVISE ALL CHILDREN. OUR CHILDREN'S SANDBOX AREA PROVIDES A WELCOME RETREAT FOR LITTLE ONES TO PLAY AND ENJOY.

NORTH OF THE SAMPLING ORCHARD ARE THE GARDEN PLOTS. THE FIRST TWO ON THE WEST SIDE OF THE PROPERTY ARE PUBLIC, SO FEEL FREE TO PICK ANYTHING WITH A "READY TO PICK" SIGN BY IT. THE REMAINDER OF THE PLOTS ARE RENTED ON YEARLY BASIS AND PRIVATELY HELD. ENJOY THE INDIVIDUALITY OF THE GARDENERS, BUT PLEASE DON'T PICK.

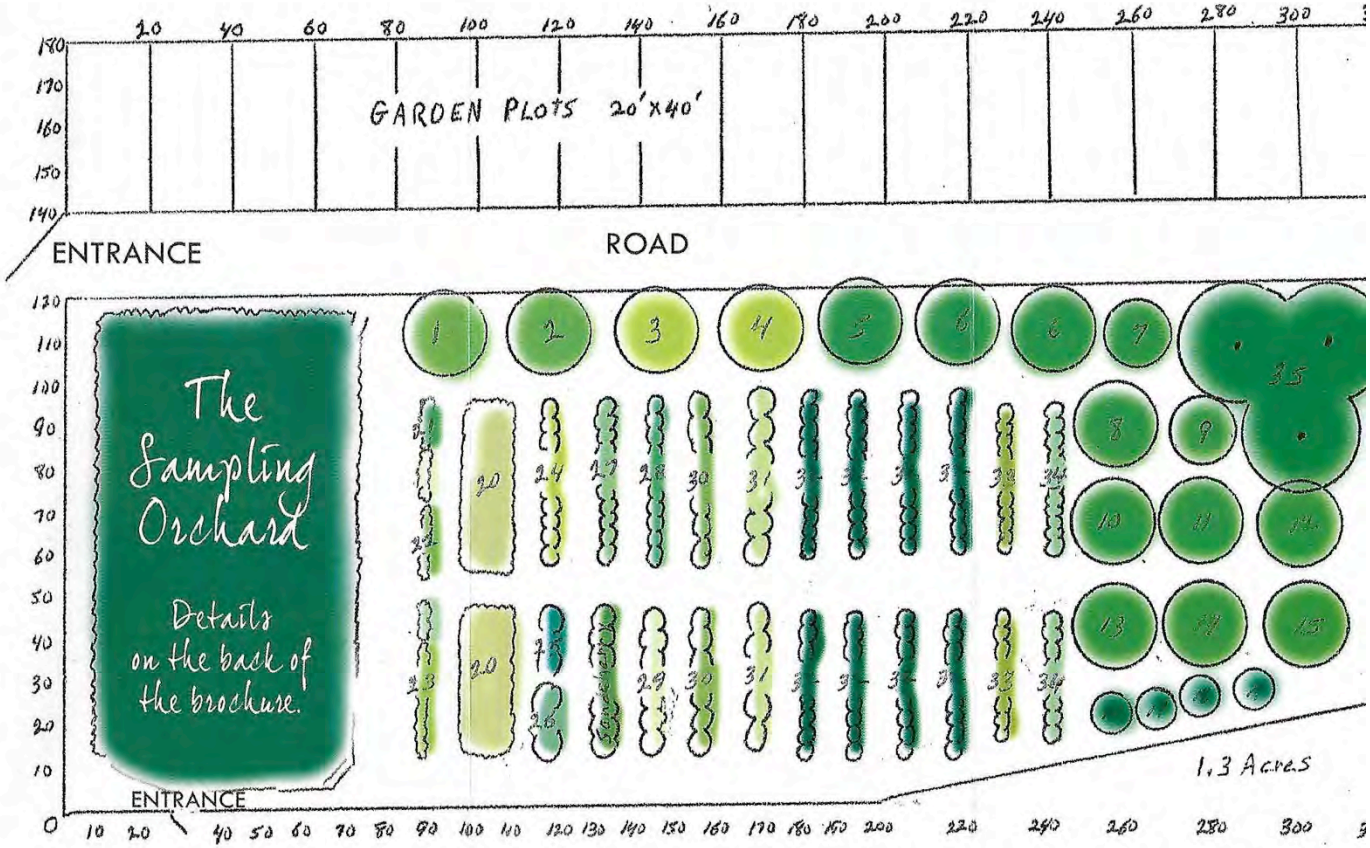
NORTH OF THE GARDEN PLOTS, BEYOND THE WALK-THROUGH GATE, IS THE ARBORETUM. IT IS IN A SHELTERBELT FORMAT WITH TALLER TREES ON THE NORTH AND SHORTER SHRUBS ON THE SOUTH, SO YOU CAN SEE EVERYTHING FROM ONE POINT. IT CONSISTS OF FIVE 100 FOOT BLOCKS OF LIKE SPECIES TO CREATE MINI ECOSYSTEMS. ON THE FAR EAST IS THE SPRUCE BLOCK, NEXT TO THE MIXED NUTS, THE PINES, MAPLES AND A DOUBLE BLOCK OF ASSORTED SPECIES. THESE WERE ALL PLANTED WITHIN A FEW YEARS OF EACH OTHER SO YOU CAN SEE THE RELATIVE SPEED OF GROWTH. MATURE HEIGHTS AND WIDTH ARE POSTED ON EACH LABEL. WHEN CROSSING FROM ONE ROW TO THE NEXT, PLEASE USE THE PLANKS WHICH ARE LOCATED BETWEEN EACH BLOCK, TO SAVE ON THE FABRIC.

ENJOY THE BEAUTY AND BOUNTY YOU FIND HERE AT THE ANETA COMMUNITY ORCHARD, GARDENS AND ARBORETUM.

Sampling Orchard



1. Grape – Valiant
2. Grape – Somerset Seedless
3. Grape – Fredonia
4. Grape – LaCrescent
5. Grape – Edelweiss
6. Pie Cherry – Bali
7. Nanking Cherry
8. Bush Cherry – Joy
9. Plum – Black Ice
10. Plum – Waneta
11. Plum – Toka
12. Black Currant – Crandall
13. Black Currant – Nancy May
14. Black Currant – Titania
15. Black Currant – Ben Connan
16. White Currant – Imperial
17. Currant – Pink Champagne
18. Jostaberry
19. Raspberry – Nova
20. Raspberry – Canby
21. Fall Raspberry – Anne
22. Raspberry – Killarney
23. Raspberry – Mammoth
24. Fall Raspberry – Caroline
25. Apple – Chestnut
26. Apple – Dakota Gold
27. Rhubarb – Crimson Red
28. Rhubarb – Canada Red
29. Rhubarb – Lee
30. White Mulberry – Northrop
31. Pie Cherry – Carmen Jewel
32. Gooseberry – Hinnomaki Red
33. Gooseberry – Captivator
34. Gooseberry - Welcome
35. Asparagus – Mary Washington
36. Apple – Sweet Sixteen
37. Red Currant – Red Lake
38. Red Currant – Rovada
39. Red Currant – Johnkheer Van Tets
40. Pie Cherry – Crimson Passion
41. Apple – Honeygold
42. Haskap – Borealis
43. Haskap - Berry blue
44. Haskap – Indigo Gem
45. Haskap - Tundra
46. Sandcherry – Seedling
47. Aronia – Viking
48. Elderberry – Johns
49. Elderberry - Adams
50. Juneberry – Seedling
51. Hazelbert - Seedling
52. Woodbine



1. APRICOT - GOLDCOT
2. APRICOT
3. PEAR - GOURMET
4. PEAR - WISCONSIN
5. APPLE - DAKOTA GOLD
6. APPLE - HONEYCRISP
7. APPLE - CHESTNUT
8. APPLE - ZESTAR
9. APPLE
10. APPLE - HAZEN
11. APPLE - WODARZ
12. APPLE - HARALRED
13. APPLE - SWEET SIXTEEN
14. APPLE
15. APPLE - HONEYGOLD
16. PLUM - ALDERMAN
17. PLUM - LECRESENT
18. PLUM - SUPERIOR
19. PLUM - PEMBINA
20. ASPARAGUS
21. GOOSEBERRY
22. RED CURRANT
23. BLACK CURRANT
24. ARONIA
25. RHUBARB
26. ELDERBERRY
27. NANKING CHERRY
28. HASKAP (HONEYBERRY)
29. CARMEN JEWEL CH
30. CRIMSON PASSION
31. BALL CHERRY
32. JUNEBERRY
33. CHOKECHERRY
34. HAZELNUT
35. BUTTERNUT

Improving arthropod pest management on grapes in North Dakota

Final Report

Partner Organization: North Dakota State University

Project Summary

The ND grape industry continues to expand, and producers have increasing needs for information that will help enhance their competitiveness and profitability. Arthropod pests (insects and their relatives) can negatively impact crop production. However, there is a lack of basic information on pest and beneficial arthropods associated with grapevines in ND. We sampled insects associated with managed vineyards, and wild grapevines in riparian areas from mid-June through early September 2015. Our results indicate that spotted wing Drosophila (SWD, *Drosophila suzukii*) is the primary arthropod pest of concern, and adults were found across the state in 13 of 17 managed vineyards and 3 of 5 wild grape sites. In 2016 we collected and reared SWD adults from grape clusters collected at 4 managed vineyards. Most, but not all, of the grape berries with SWD larvae were dark purple with damaged skin. One Japanese beetle was trapped in the Fargo area. Increased awareness of pest and beneficial arthropods associated with grapes can help growers improve production and profits by enhancing monitoring and early pest detection, effective use of insecticides, conservation of beneficial natural enemies, and the development of integrated pest management programs.

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

Project Approach

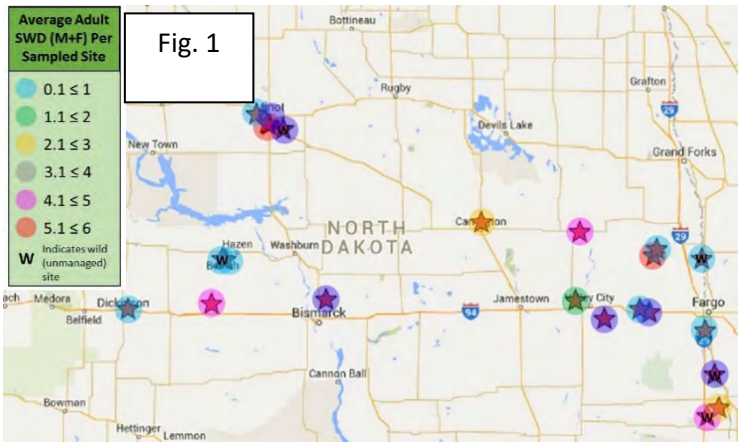
Arthropod pests are detrimental to grape production and profitability in several ways. They can damage vines, reduce fruit quantity and/or quality, and vector plant diseases. Understanding what insects are present, including those that represent economic threats or are beneficial, is the first step towards implementing efficient, cost-effective, and sustainable insect pest management (IPM) programs. Early detection of invasive species is especially important, since they can decimate production and fruit marketability or increase costs associated with pest control. It's also important to understand how crop management, such as chemical use and plant diversity in the form of weeds or cover crops, impacts arthropod pest problems and biological control by natural enemies (i.e. predators and parasitoids). Broad-spectrum chemicals can kill beneficial and lead to higher pest densities. Non-crop plants can serve as refuges and/or food sources that support beneficials and contribute to pest control. Conversely, these plants could enhance densities of pests. Selecting the most appropriate plants (i.e. having the right kind of plant diversity) might be an important consideration when managing weeds or diversifying the landscape (e.g. planting cover crops, having flowering plants surrounding your field, etc.). Although a solid foundation of knowledge is required for effective pest management, the information needed to support the ND grape industry is lacking. Grape production in ND is an emerging industry, and acres devoted to this specialty crop will likely continue to expand. Growers have indicated they want information relevant to grape vine cultivation and land management, which encompasses arthropod pest management. In 2013, an invasive fly (i.e. SWD, spotted wing Drosophila, *Drosophila suzukii*) that attacks several types of fruit, including

grapes, was discovered in ND for the first time, and it is important to obtain more information on the distribution and phenology of this pest as it relates to grape production. All field sampling was associated with grapes. Greenhouse experiments will focus on plants and arthropod pests that are relevant to the grape industry.

A primary project activity was to document the arthropod fauna associated with grapes, including traditional pests, invasive pests, and beneficials. We contacted grape growers in the state and identified and visited several potential field sites. In 2015, we sampled arthropods on grapevines bi-monthly (6 sampling periods total) at 19 managed and 5 wild (non-managed) sites by collecting leaf, vacuum suction, and sweep net samples. We used traps to monitor for the following invasive species (SWD = spotted wing Drosophila, *D. suzukii*; Japanese beetle; Egyptian cotton leafworm; false codling moth; European grape berry moth; European grapevine moth; and light brown apple moth).

In 2016, we revisited select sites ($n = 9$) to determine if *D. suzukii* (SWD) maggots were present in grape berries. We trapped adult SWD and reared SWD adults from infested grape clusters and positive controls (raspberries and blackberries from a site with known SWD infestation). Adult SWD were reared out of 6 of 7 positive control cups (raspberries and blackberries). Adult SWD were reared out of grapes from 4 sites (Carrington, Cooperstown, Hillsboro, and Minot REC). Most, but not all, of these grapes were varieties with dark purple skin that had been compromised (by hail, etc.), and included: Marquette, St. Croix, and Valiant.

Our results indicate that *D. suzukii* (SWD) is the primary invasive arthropod pest of concern, and adults were found across the state in 13 of 17 managed vineyards and 3 of 5 wild grape sites (Fig. 1 and 2; Table 1). One Japanese beetle was collected in 2015 in the Fargo area. None of the other invasive insect pests were detected.



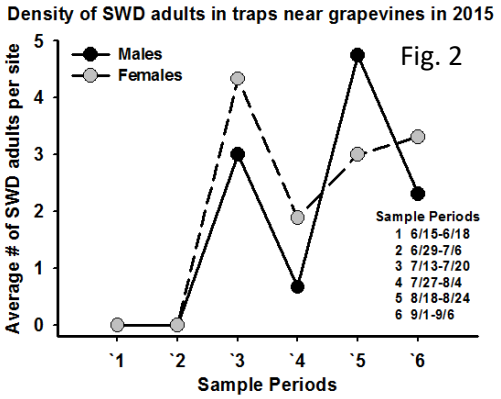


Table 1

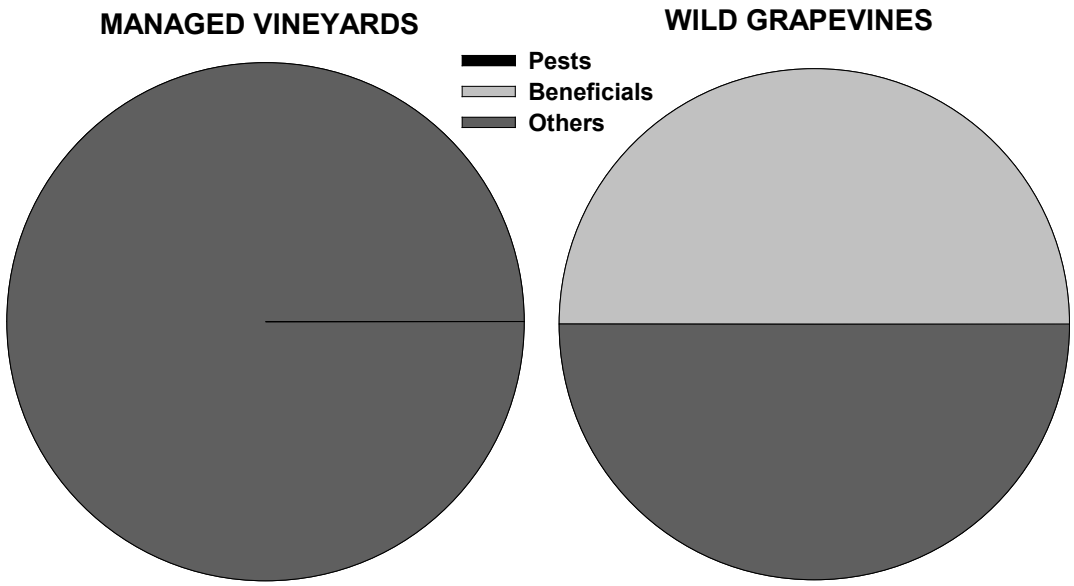
2015 MANAGED VINEYARDS – adult SWD from traps						
2015 Sample period	# sites sampled	# sites with SWD	ALL MANAGED SITES			
			Total # SWD		Ave. # SWD per infested site	
			F	M	F	M
1, mid-June	14	0	0	0	0	0
2, late June	10	0	0	0	0	0
3, mid-July	16	3	13	9	4.3	3

4, late July	16	7	16	5	2.3	0.7
5, mid-Aug	15	7	23	38	3.3	5.4
6, early Sept	17	11	28	18	2.6	1.6
2015 WILD GRAPEVINES – adult SWD from traps						
2015 Sample period	**# sites sampled**	**# sites with SWD**	**ALL WILD SITES**			
			Total SWD		**Ave. # SWD per infested site**	
			F	**M**	**F**	**M**
1, mid-June	2	0	0	0	0	0
2, late June	2	0	0	0	0	0
3, mid-July	4	0	0	0	0	0
4, late July	4	2	1	1	0.5	0.5
5, mid-Aug	4	1	1	0	0.25	0
6, early Sept	5	2	15	12	7.5	6
2016 GRAPE SITES – adult SWD from traps						
2016 Sample period	**2016 # sites sampled**	**2016 # sites with SWD**	**ALL MANAGED SITES**			
			Total # SWD		**Ave. # SWD per infested site**	
			F	**M**	**F**	**M**
1, mid-June	ns	.	.	.	.	.
2, late June	ns	.	.	.	.	.
3, mid-July	ns	.	.	.	.	.
4, late July	ns	.	.	.	.	.
5, mid-Aug	ns	.	.	.	.	.
6, early Sept	9*	8*	58	66	7.25	8.25
SWD = Spotted wing Drosophila, *Drosophila suzukii;* F = female SWD flies, M = male SWD flies; ns = not sampled * includes one wild site						

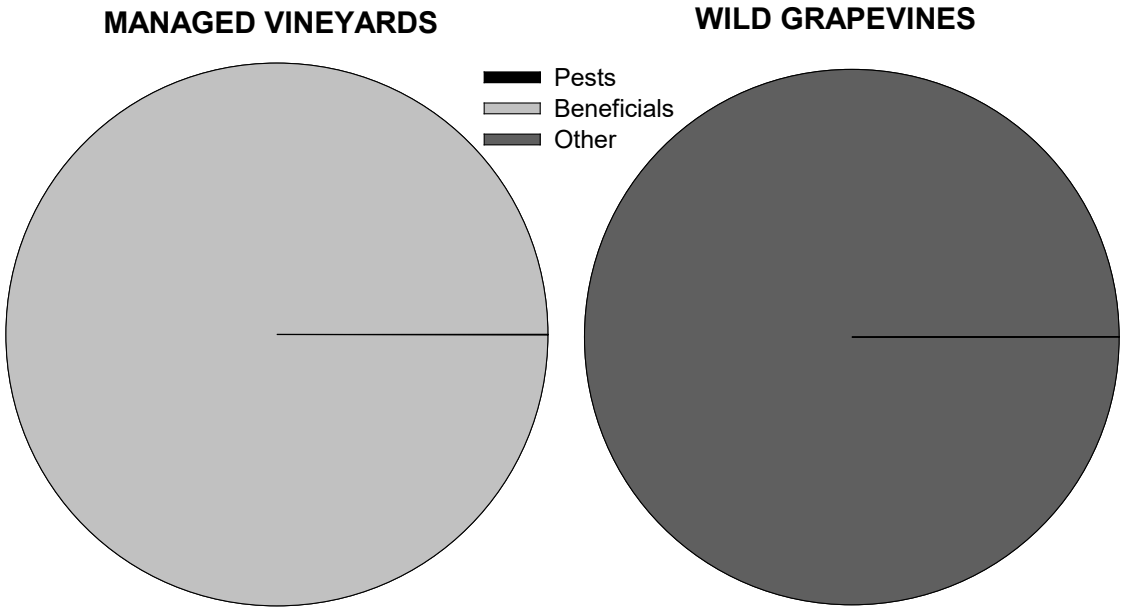
There appear to be few problems with arthropods considered to be major grape pests in other grape-growing regions (e.g., spider mites, leafhoppers) (Fig. 3a-d). For leaf samples the primary grape pests were phylloxera (galls), leafhoppers, and some thrips, while beneficials were mostly predatory mites, spiders, and brown lacewings and other abundant arthropods included mites that were likely fungivorous. For vacuum samples pests were mostly leafhoppers and some flea beetles, while beneficials were mostly spiders, beneficial Hymenoptera (bees, wasps), and green lacewings, and other abundant arthropods were mostly flies. Arthropods sampled in sweep nets primarily consisted of flies, non-grape leafhoppers, and grasshoppers.

Fig. 3a-d

GRAPE LEAF SAMPLES



CANOPY VACUUM SAMPLES



A secondary objective was to investigate how management, including tactics that impact plant diversity, might impact arthropods associated with grapes. We primarily focused on aspects associated with plant diversity, as this can be altered by management and can impact arthropod

populations. In addition, grape growers in our study indicated that they do not use insecticides and thus there was no variation in this factor to investigate.

Greenhouse and lab experiments showed that herbivorous spider mites (known pests of grape) can reproduce on two weed species often found in proximity to grapes (common lambsquarters, wild mustard), although no pest reproduction occurred on Eastern black nightshade and redroot pigweed (Fig. 4). We also found that when spider mites reared on soybean were moved to grape their reproduction was low and that adult females moved off the grape plants, potentially indicating that the presence of more suitable vegetation around vineyards would lessen potential impacts of this pest on grapes.

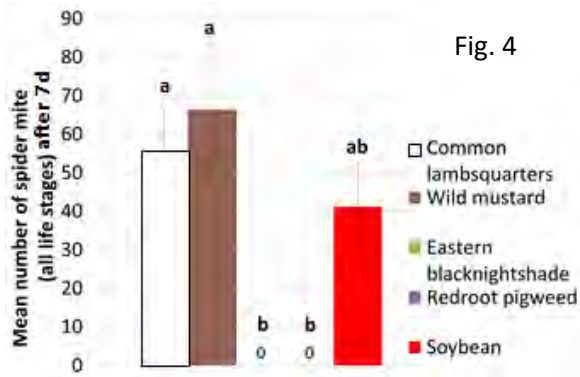


Fig. 4

We also investigated how aspects of our field sites impacted adult SWD densities in 2015. We used an online mapping program (ND Weed Mapper) and notes made at grape sites to quantify aspects of each site (area, groundcover, type of vegetation border grapes and within 1 km of grape sites, region site was located in). Our analysis indicated a strong correlation between SWD densities and non-SWD *Drosophila* spp. (Pearson Correlation Coefficient = 0.743, $P < 0.001$). Additionally, percent agriculture within 1 km of grape sites was negatively correlated to percent natural vegetation (Pearson Correlation Coefficient = -0.884, $P < 0.001$), and so the former variable was not used in the best subset regression model. Percent riparian vegetation within 1 km of grape sites and the presence of non-grass groundcover were the factors that explained the most variation in density of adult SWD (mean per sampling date), at sites where SWD was found in traps ($R^2 = 0.492$, % riparian vegetation, $t = -2.788$, $P = 0.016$; groundcover, $t = 2.546$, $P = 0.026$). Adding in information on the presence of alternative SWD hosts would likely improve the model.

Goals and Outcomes Achieved

There were two goals: 1) increase knowledge of pest and beneficial arthropods associated with grapes in ND, and 2) increase awareness of how management practices can impact grape arthropods.

We sent out pre- and post-project surveys (see additional info section) to grape growers. In 2015, 11 surveys were returned and in 2017, 12 surveys were returned. All of the returned surveys were from growers from cooperating sites. In 2015, the majority of survey respondents (45.5%) were concerned about both native and invasive insects pests, although in 2017 an equal percentage were concerned about both (33.3%) and invasives (33.3%). And although in 2015, 27.3% of respondents had not heard of SWD, this dropped to zero in 2017 (i.e., all respondents had heard of this insect pest). People expressed greater concern about SWD in 2017, although concern about Japanese beetles dropped, perhaps because this project showed SWD was widespread but only detected one Japanese beetle.

When it came to the impact of management practices on grape insects, both years the majority of respondents (27.3-72.7%) corrected indicated that chemicals and plant diversity could impact both pest and beneficial insects. While in 2015 of respondents 54.6% did not scout or monitor insects, this dropped to 8.3% in 2017. In addition, from 2015 to 2017, the % of respondents who

had heard of Integrated Pest Management (IPM) increased 22.0% and the % using IPM increased 14.4%.

In general, over the course of the study, recognition and knowledge about pest and beneficial arthropods increased (Table 2; actual survey in appendix). By the end of the study, 91% of respondents correctly identified adult SWD and knew that they were pests. Questions that focused on arthropods that weren't emphasized during the study (e.g. lacewing egg), tended to have low % of respondents that answered correctly and no or low degree of improvement between years. The goal of increasing grower knowledge about pest and beneficial arthropods associated with grapes in ND to 80% was met for SWD, which was the most relevant pest arthropod and the focus of the information dissemination efforts.

Table 2	**% of respondents that answered question correctly in 2015**	**% of respondents that answered question correctly in 2017**	**Change from 2015 to 2017**
Ladybeetle adult, *Harmonia* ID	27.3	75.0	+47.7
Ladybeetle adult, *Harmonia* pest status	Question not assessed, as *Harmonia* is generally beneficial, but can be a pest when grapes are made into wine		
Ladybeetle larva, *Harmonia* ID	27.3	33.3	+6.1
Ladybeetle larva, *Harmonia* pest status	Question not assessed, as *Harmonia* is generally beneficial, but can be a pest when grapes are made into wine		
Leafhopper ID	45.5	66.7	+21.2
Leafhopper pest status	45.5	66.7	+21.2
Leafhopper leaf damage	9.1	25.0	+15.9
SWD ID	54.5	**91.7**	+37.1
SWD pest status	54.5	**91.7**	+37.1
Lacewing egg ID	45.5	33.3	-12.1
Lacewing egg pest status	27.3	33.3	+6.1
Flea beetle ID	54.5	41.7	-12.9
Flea beetle pest status	45.5	66.7	+21.2
Japanese beetle leaf damage	9.1	41.7	+32.6

Beneficiaries

The North Dakota Grape and Wine Association (NDGWA) is the primary entity that represents grape growers and wine makers in ND, and the people associated with this group and growers that were participating locations were the primary beneficiaries of the project. At the time the project started, there were 48 vineyards registered with the ND Department of Agriculture (http://www.ndgga.org/), although a publication from 2010 indicates that there are 125 vineyards in the state. Producers benefitted by hearing / seeing presentations at meetings, interactions with

project personnel, and receiving project results via the mail. The meetings were: the National Entomological Society of America Meeting (Minneapolis MN, Nov. 2015), the 2016 North Dakota Grape and Wine Association (NDGWA) conference (Bismarck ND, Feb. 2016, estimate of 50-70 people), and the 2017 NDGWA annual conference (Carrington ND, Feb. 2017, estimate of 50-70 people). At the latter, approximately half of the attendees took a hardcopy of the poster we presented (approx. 25 distributed). In 2017, we also sent personalized information to growers focusing on SWD found at their site and project methods, and provided a topical NDSU Extension publication (McGinnis et al. 2014. https://www.ag.ndsu.edu/publications/crops/integrated-pest-management-of-spotted-wing-drosophila-in-north-dakota). Data relevant to invasive species of concern to the ND grape industry will be entered into the NAPIS database. If additional relevant information / publications are developed after the end of the grant, they will be shared with the NDGWA.

Lessons Learned

The timing of data collection versus information dissemination was challenging, especially since we were collecting baseline data. The samples needed to be processed and data summarized in order to determine what arthropod were most relevant for growers and to have data to present to growers. However, those tasks were more time consuming than anticipated. For future projects, I think it would be best if more people were hired to process samples early-on.

Another challenge was what arthropods to focus on for information dissemination. Opportunities / time for the latter were limited, and we chose to focus on the most relevant pest arthropod we found – i.e., SWD. Knowledge about this arthropod increased to target levels based on the grower surveys. However, because there are many other arthropods associated with grape and due to the constraints of the project, we did not reach our target level of knowledge for some of the other arthropods in grapes, although in general knowledge did increase.

It was challenging to communicate effectively with growers, whether that was obtaining information from them or disseminating information to them. The only surveys we received in the first year were from growers at a participating locations. The best way of information sharing seemed to be via the NDGWA annual meetings, and this would be a good venue to from which to obtain survey-time information, perhaps using clickers or another interactive methods during an actual talk. Guest posts on the NDGWA Facebook site or a pest-focused workshop might also be useful.

Contact Information

Dr. Deirdre Prischmann-Voldseth, NDSU Entomology Dept. 7650, PO Box 6050, Fargo ND 58108-6050, Deirdre.Prischmann@ndsu.edu, Phone: 1-701-231-9805, Fax: 1-701-231-8557

Additional Information

- Survey sent to growers
- Info about sampling methods sent to growers

SURVEY OF NORTH DAKOTA GRAPE GROWERS - SPRING 2017

Please circle the letter next to your answer choice(s) and answer all of the questions to the best of your ability. The word 'insect' also includes insect relatives, like spider mites.

Please return in the self-address, stamped envelope we have provided - **Thank you for participating!**

(Dr. Deirdre Prischmann-Voldseth, NDSU Entomology Dept. 7650, PO Box 6050, Fargo ND 58108-6050,
Deirdre.Prischmann@ndsu.edu, Phone: 1-701-231-9805, Fax: 1-701-231-8557)

Did you cooperate in our study of grape insects in summer 2015?

a. Yes
b. No
c. I don't remember

In the past 1-3 years, have you had to apply an insecticide to control an insect pest on your grapes?

a. Yes
b. No
c. I don't know

If you answered YES, what insect pest(s) were you trying to control? (circle all that apply)

a. Phylloxera
b. Grape berry moths
c. Grape flea beetles
d. Multicolored Asian ladybird beetles
e. Spider mites
f. Leafhoppers
g. Other _____

Currently, what insect(s) are you most concerned about when it comes to grape management?

a. I'm not concerned about any grape insect pests
b. Native (resident) grape insect pests
c. Non-native (invasive) grape insect pests
d. b and c
e. I'm concerned about any insect that is on my grapes even if it's not a pest

How concerned are you about Spotted Wing Drosophila?

a. I'm not concerned
b. Somewhat concerned
c. Very concerned
d. I've never heard of Spotted Wing Drosophila

How concerned are you about Japanese beetles?

a. I'm not concerned
b. Somewhat concerned
c. Very concerned
d. I've never heard of Japanese beetles

Within your vineyard, does anyone scout (aka monitor) for: (circle all that apply)

a. We don't scout / monitor for insects
b. Insect pests
c. Beneficial insects
d. b and c

Have you heard of IPM (Integrated Pest Management)?

a. Yes
b. No

Are you interested in using insect pest management tactics other than conventional insecticides?

a. Yes
b. No

Do you currently use any insect pest management tactics other than conventional insecticides?

a. Yes (if yes, please explain briefly):
b. No

Using a non-selective (aka a broad spectrum) INSECTICIDE on grape plants can impact:

a. Pest insects on grape plants
b. Beneficial insects on grape plants
c. Pests AND beneficial insects on grape plants
d. NEITHER pests or beneficial insects on grape plants

Using a non-selective (aka a broad spectrum) FUNGICIDE on grape plants can impact:

a. Pest insects on grape plants
b. Beneficial insects on grape plants
c. Pests AND beneficial insects on grape plants
d. NEITHER pests or beneficial insects on grape plants

Weeds within vineyards can impact:

a. Pest insects on grape plants
b. Beneficial insects on grape plants
c. Pests AND beneficial insects on grape plants
d. NEITHER pests or beneficial insects on grape plants

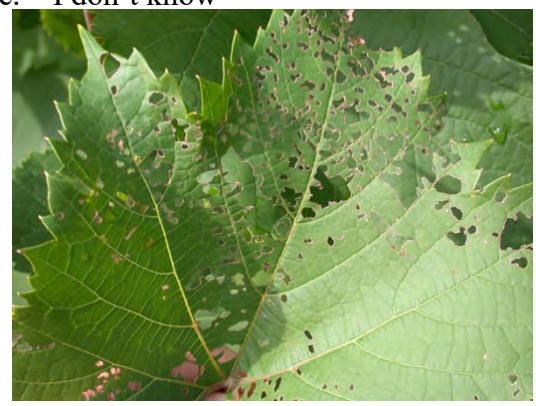
NON-FLOWERING cover crops within vineyards can impact:

a. Pest insects on grape plants
b. Beneficial insects on grape plants
c. Pests AND beneficial insects on grape plants
d. NEITHER pests or beneficial insects on grape plants

FLOWERING cover crops within vineyards can impact:

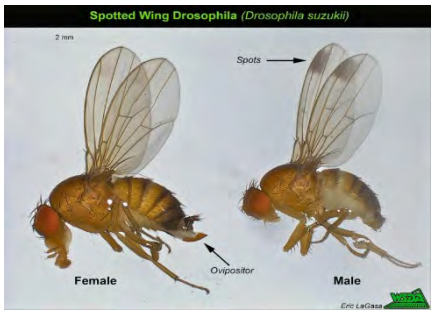
a. Pest insects on grape plants
b. Beneficial insects on grape plants
c. Pests AND beneficial insects on grape plants
d. NEITHER pests or beneficial insects on grape plants

What is this insect? a. Lacewing b. Leafhopper c. Ladybeetle d. Japanese beetle e. I don't know Is it a pest of grapes? a. No b. Yes c. I don't know	What is this insect? a. Lacewing b. Leafhopper c. Ladybeetle d. Japanese beetle e. I don't know Is it a pest of grapes? a. No b. Yes c. I don't know
What is this insect? a. Spotted wing Drosophila b. Leafhopper c. Ladybeetle d. Flea beetle e. I don't know Is it a pest of grapes? a. No b. Yes c. I don't know	What is this insect? a. Leafhopper b. Flea beetle c. Ladybeetle d. Japanese beetle e. I don't know Is it a pest of grapes? a. No b. Yes c. I don't know
What is this insect? a. Spotted wing Drosophila b. Hover fly c. Leafhopper d. Lacewing e. I don't know Is it a pest of grapes? a. No b. Yes c. I don't know	What is this insect? a. Hover fly b. Leafhopper c. Ladybeetle d. Japanese beetle e. I don't know Is it a pest of grapes? a. No b. Yes c. I don't know

What insect caused this damage? a. Spider mites b. Leafhoppers c. Flea beetles d. Japanese beetles e. I don't know 	What insect caused this damage? a. Spider mites b. Leafhoppers c. Flea beetles d. Japanese beetles e. I don't know 

BASIC INFO ABOUT SPOTTED WING DROSOPHILA (SWD)

NDSU Extension article on this invasive insect pest (included in this letter): https://www.ag.ndsu.edu/publications/crops/integrated-pest-management-of-spotted-wing-drosophila-in-north-dakota

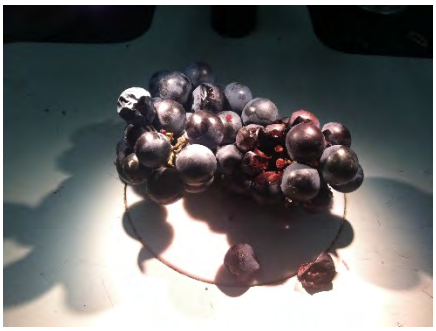


SAMPLING METHOD: Trap for SWD adults

- For each 2-wk sampling period, we hung one trap per site. The trap was a red cup with a lid and a few holes poked in the side. It contained some apple cider vinegar and yellow sticky card.
- Back in the lab, we removed any suspected SWD flies from the yellow sticky card (and vinegar if any remained).
- Then we looked for key characteristics to confirm that the fly was SWD.

SAMPLING METHOD: Rearing SWD adults from grape berries

- We did this in 2016 at a subset of sites to see SWD larvae were inside grape berries.
- Grape clusters were collected in late August / mid-Sept.
- Grapes were separated by site and variety. For most sites, we separated berries based on their appearance (undamaged, scarring, split skin).
- Berries were placed in plastic cups covered with mesh. Each cup had sand in the bottom and a yellow sticky card. Cups were kept on shelves in the lab.
- Raspberries and blackberries were used as positive controls.



Japanese Beetle Project

Final Report

Partner Organization: North Dakota Department of Agriculture

Project Summary

Japanese beetles (*Popillia japonica*) have the potential to cause damage to nursery crops in North Dakota. The establishment of the beetle could affect all nursery shipments from North Dakota to Canada and to protected Category 1 states.

This project would help supplement an ongoing trapping survey to any established populations of Japanese beetle. It would also address a great need for education and outreach of the potential effects of establishment of Japanese beetle in North Dakota. This product would benefit the entire nursery industry in North Dakota and allow shipment of nursery crops without restriction to states with exterior quarantines for Japanese beetle. North Dakota grape production is developing and grape growers will benefit from this project. If Japanese beetles were to become established in North Dakota, vineyards could be damaged by the beetle. Trapping will help to quickly determine if the insect is in the vineyard, and treatment can begin more promptly if necessary.

The success of the project will be measured by number and location of traps placed, number and location of beetles captured and and how much educational material is distributed.

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

Project Approach

North Dakota remained free of Japanese beetles (other than one beetle found in 2001) until the summer of 2012 when nursery shipments coming from an infested area in Minnesota delivered adult beetles along with some nursery stock. Since 2012, Japanese beetles have been caught each year in ND. The occurrence of Japanese beetle in the state has been isolated and was not introduced by natural movement of the beetles.

Japanese beetles pose a threat to nursery stock. Each year North Dakota Department of Agriculture licenses nearly 300 nurseries. In 2013, the economic impact of the "green" industry, including nurseries, florists, greenhouses and landscapers, totaled over $695 million in North Dakota. Other than the potential cost of damage caused by Japanese beetles, it could also halt shipments of nursery stock to Canada and western states. ND nurseries ship more than 150,000 trees to Canada annually. The requested funding would aid in determining the extent of the regulatory incidence, determining if the beetles overwintered and thus establishing the status of Japanese beetle in ND. Researching the full extent of the beetle will aid in determining where beetles are becoming establish and at the same time determine if beetles are coming into the state from out-of-state suppliers and to maintain negative status for many counties in ND.

In summary, the work accomplished during the grant period during the growing seasons of 2015-2017, NDDA could maintain a large trapping survey for Japanese beetle across the entire state, placing between 500 – 1700 traps annually, identified sites where Japanese beetles were found, and printed and distributed thousands of outreach publications at outreach events across the state. The list of outreach events can be found under the additional information section below. Beetles were caught in each year from 50+ beetles in 2015, nearly 400 beetles in 2016, and nearly 1500 beetles in 2017. An unfavorable incident also occurred in 2017 that affected the overall catches in traps as an out-of-state wholesale nursery sent infested nursery stock to North Dakota. This can help to explain the dramatic increase in beetles caught. The project utilized additional master gardener volunteers to assist with the trapping. By the end of the survey season, trappers could easily identify and educate others about Japanese beetles. For more quantitative data, see annual state report on the NDDA website at https://www.nd.gov/ndda/plant-industries/pest-survey-and-outreach/japanese-beetle. We printed outreach and promotional information and these attached as supplemental information. While the project could potential benefit corn and soybean growers, we did not place traps in corn or soybean fields. All traps were placed in nurseries, landscapes, or vineyards/fruit growing operations.

Goals and Outcomes Achieved

Objectives of the project were to determine the extent of interceptions of Japanese beetle in North Dakota, locate possible sources of the beetles, determine if there is an overwintering population of Japanese beetles in North Dakota, and use the outcomes to best establish a pest response and/or quarantine to protect the nursery industry of North Dakota. More education and outreach to the nursery/landscape industry to make them especially aware of Japanese beetle and the threat it poses was also an objective of the project. Initially delimiting and eradication was included in the project, however shortly after approval of project, it was determined that portion was unattainable (see lessons learned) therefore amending the project and focus was on education and outreach and survey work. Goals were to trap nurseries, grape vineyards and statewide as well as print and distribute outreach material statewide. As part of the project, NDDA printed an integrated pest management for Japanese beetle in North Dakota publication twice, printed outreach posters that were distributed to nearly 300 nurseries, and printed Japanese beetle identification cards that were distributed to hundreds of stakeholders at nurseries and outreach events. Traps were placed at 1,703 locations in 2015, 536 locations in 2016, and 1,203 locations in 2017, exceeding our target goal of 1936 traps. Goals were attained as almost all counties of the state were trapped as well as nurseries and grape vineyards. Beetles were caught each year in various counties and indications of where beetles may become established have been identified for future survey and outreach work. NDDA plans to continue survey work especially in counties where Japanese beetle has not been identified as well as locations where low numbers of beetles were identified to determine if overwintering populations develop. Outreach will continue if internal funding is available to do so. Over the period of three trapping seasons covered by this grant, 3,442 traps were placed across the state. 83 nurseries were surveyed in the first year, 42 nurseries in the second year, and 67 in the third year, bypassing our goal of 50 nurseries. Indicated greater numbers of both nurseries and traps places as compared to target. 22 vineyard traps were placed in the first year, surpassing the 20 vineyard goal, however in subsequent years, the traps were focused in areas surrounding nurseries due to vineyards being

in low-risk, rural areas. As for the awareness target, NDDA surveyed trapping volunteers at the beginning and end of the first trapping season that they participated in, and greater than 90% were more aware of the insect at the end of the season than they were at initial survey. NDDA also participated in 5 outreach events each year of the survey to present information to the general public. 100% awareness is a lofty goal, and NDDA will continue to participate in outreach events around the state to continue to make people more aware of not only Japanese beetle but other invasive pests of specialty crops.

We had three main goals for our project: (1) to hang enough traps to perform an eradication program for Japanese Beetle in North Dakota, (2) to have a zero-catch result with the survey, and (3) to increase 100% awareness of Japanese Beetle preventative measures among specialty crop stakeholders. We succeeded with goals 1 and got close with 3, but ended up not receiving the zero-catch result we were hoping for.

Beneficiaries

Stakeholders include the nursery industry, specialty fruit crop producers (specifically grape growers) as well as the public. Estimates of stakeholders benefitting include approximately 300 nurseries, 20 grape growers, and thousands of citizen gardeners. The nurseries and grape growers benefitted from the trapping survey to determine if beetles are in their nursery or vineyard as well as becoming more informed of the implications of establishment of Japanese beetle. Citizen gardeners benefitted by becoming more aware of the implications of establishment of Japanese beetle.

Lessons Learned

The original goal of eradication and delimiting was determined to be too expensive to be attainable as the Japanese beetle becomes established further west. High-level monitoring of high risk sites is much more attainable especially when paired with an education and outreach program. Utilizing a well-established master gardener program to assist with the monitoring and education was very beneficial to all stakeholders. Supplies ordered in bulk was very cost effective at the beginning of the survey, allowing us to utilize the funds for three years rather than the initial one year funding anticipated.

Contact Information

Contact person for the project is Charles Elhard (701) 220-0485, celhard@nd.gov

Additional Information

Please see attached documents. The table below shows outreach events and activities we completed Japanese beetle outreach at.

Event	Location	Month	Year	Stakeholders	Information
NDNGA	Fargo	Jan	15-17	Nursery and Greenhouse	Japanese beetle publications,

					cards, presentations, outreach
NDUCFA	Fargo	Jan	15-17	Forestry	Japanese beetle publications, cards, presentations, outreach
NDSU Gardening Saturday	Grand Forks	Apr	15-17	Master Gardeners	Japanese beetle publications, cards, presentations, outreach
Nursery Outreach	Statewide	Apr-July	15-17	Nursery owners/employees	Japanese beetle trapping and information presented
Master Gardeners Outreach	Statewide	May/October	15-17	Volunteer trappers	Japanese beetle trapping and information mailed
Big Iron	Fargo	Sept	15-17	Citizens of ND	Japanese beetle publications, cards, outreach
NDSU Classes	Fargo	April	15, 17	Horticulture and Nursery students	Japanese beetle publications, cards, presentations, outreach
Press Releases	Statewide	July	15-17	Citizens of ND	Press release educating about Japanese beetle

WATCH OUT

FOR JAPANESE BEETLES

Japanese beetles are a relatively new pest in North Dakota. They have slowly spread west over the past 100 years and made their first appearance in North Dakota in 2012. They have been found in the state every year since.

As members of the public, you can be proactive by checking nursery stock and bedding plants for the insect and reporting any sighting of Japanese beetle to your local extension agent or to the North Dakota Department of Agriculture (NDDA). For more information visit: **http://www.nd.gov/ndda/pest/japanese-beetle.**

NORTH DAKOTA DEPARTMENT of AGRICULTURE

Japanese Beetle
Identification Guide

Japanese Beetles are highly destructive to plants. They feed on more than 300 host plants, including: field crops, ornamental trees and shrubs, flowers, vegetables and turf.

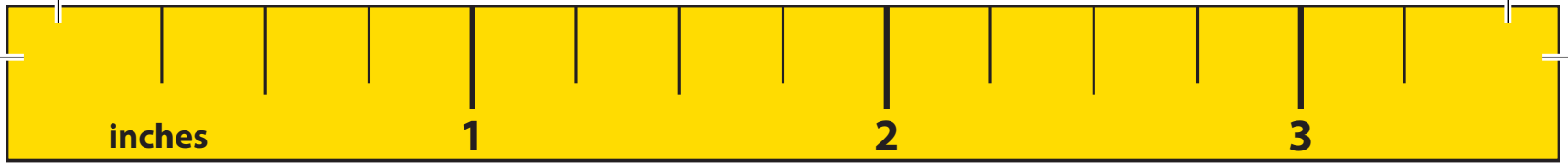
Traps like the one seen below can be used to monitor infestation and control the Japanese Beetle population.

Report any sighting of Japanese Beetle to your local extension agent or to the North Dakota Department of Agriculture.

You can learn more at:
www.nd.gov/ndda/pest/japanesebeetle.

Agriculture Commisioner
Doug Goehring

701-328-2231
ndda@nd.gov



Adult Japanese Beetle

(actual size)

Look for metallic green head and thorax, and coppery wings.

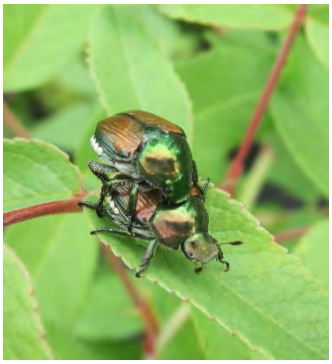
Japanese Beetle Larvae

(actual size)

Look for creamy white color, brown head capsule and V-shaped hair pattern.

Japanese Beetle Survey 2015

All photos by Charles Elhard, NDDA.



Charles Elhard, Plant Protection Specialist

December 31, 2015

Background

Japanese beetle (*Popillia japonica*) was first discovered in the United States in New Jersey in 1916. A native of Japan, the beetle occurs in all states east of the Mississippi River and in some counties and partial states west of the Mississippi River. Nearest to us, South Dakota, Minnesota, and Montana have established populations in some areas. Japanese beetles attack a broad range of host material including nearly 300 species of plants. The adult beetle will feed on field crops, ornamentals, trees, shrubs and garden plants, severely defoliating and skeletonizing the host plants. The larvae will feed on the roots of turf grasses and field crops (especially corn and soybeans) as well as organic matter in the soil, severely damaging golf courses, lawns and pastures. Japanese beetle prefer irrigated turf sites such as golf courses and lawns for reproduction. The Japanese beetle is a highly destructive plant pest that can be very difficult and expensive to control. Japanese beetle is regulated by USDA-APHIS-PPQ only at airports to prevent artificial spread by aircraft. All other regulatory arrangements are decided state-to-state in cooperation with the Japanese Beetle Harmonization Plan.

Japanese beetle adults are about ½ inch long with the male slightly smaller than the female. The insects are metallic green in color with bronze wing covers called elytra. Adults begin to emerge mid-June with peak emergence occurring approximately 3 to 4 weeks after initial emergence. The female beetle will burrow into the soil during the day to lay eggs, laying up to 60 eggs in her 4-6 week lifespan. Eggs will hatch in about two weeks. Larvae, which are about 1 inch long and cream colored with a brown head, will live in the soil, feeding on plant roots. The insect will overwinter as third instar larvae below the frost line and will pupate and emerge the following spring. Flight period for Japanese beetles is recognized as June through October.

Japanese beetle trapping has been ongoing in North Dakota since the early 1960s. Traps are typically deployed in June and removed in October. Two transient beetles were first intercepted in North Dakota in 2001 in Bismarck, but not again until 2012. Since 2012, beetles have been caught every year. Beetles were first caught in nurseries that were importing nursery stock from infested areas of Minnesota.

In 2012, 139 beetles were caught in traps in Stark, Grand Forks, Cass, Burleigh, Ward, Dickey, and Griggs counties. All of these were at or near nurseries that imported nursery stock from Minnesota suppliers. In 2013, over 400 beetles were caught, but only Burleigh, Cass, and Grand Forks counties had positive traps. In 2014, after a large supplier of nursery stock to North Dakota implemented safeguarding protocols, numbers of beetles caught decreased to about 50 beetles also in Burleigh, Cass and Grand Forks counties. The difference with 2014 positives were in locations caught. Along with nurseries, several parks and golf courses had positive traps in Burleigh and Cass counties.

2015 Survey

With support from the North Dakota Nursery and Greenhouse association, NDDA applied for a USDA Specialty Crop Block Grant initially to perform delimiting and eradication activities around

the infestation areas. After it was determined that ND would be better served by a wide scale state-wide survey, with assistance from NDSU-Extension, NDDA sought out volunteers across the state to help with the survey to place 1700 traps in all counties. The initial goal was to place approximately 25 traps in every county, traps in several nurseries and grape vineyards across the state, as well as a higher concentration of traps in the cities where Japanese beetle traps had been positive in 2014 (Fargo, West Fargo, Grand Forks, and Bismarck). 130 people across the state volunteered to assist with the survey and 112 completed the survey. Each of them were asked to complete a survey at the beginning of the trapping season, and upon completion of this report, will be asked to do it again. This is a requirement of the grant to show that the volunteers gained awareness of Japanese beetle while participating in the survey. North Dakota Forest Service and NDSU-Extension also helped hang traps, covering 8 counties between the two groups.

All traps were baited with a lure to attract both male and female beetles. The lure is very effective and has been shown to attract Japanese beetles that are within 500 feet of the trap. Each trap was individually labeled with a 15-**** number. Traps were placed beginning in May and most traps were placed by June 30, 2015. The traps were collected starting in late August with the majority removed in October. All traps were removed by November 15, 2015. Traps were in place for an average of 110 days. Table 1 below shows the number of traps hung in various locations across the state. The map in Figure 1 shows the number of traps placed in each county.

Table 1.

Site Type	Number of Traps Placed
Cemetery	82
Campground/Park	811
Golf Course	89
Nursery	182
Residential	212
Rural	107
Vineyard	22
Other/Unknown	198
Total Traps Placed	**1703**

Figure 1.

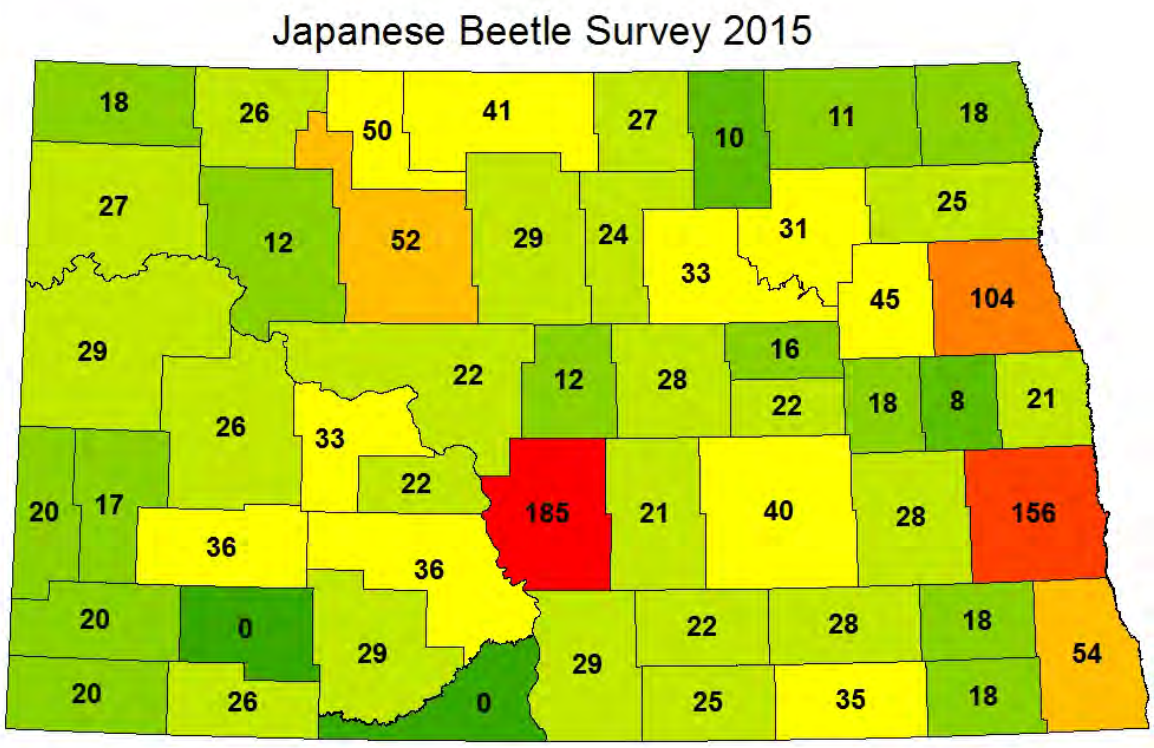


Figure 1: Map shows number of traps placed in each county.

Results

After all traps were removed, the data was collected from all trappers. In all traps, 56 beetles were caught across the state. Ten counties had positive traps. These were Barnes (1 positive trap, 1 beetle), Burleigh (12, 15), Cass (10, 14), Grand Forks (2, 2), McKenzie (1, 1), Richland (1, 1), Stark (2, 3), Stutsman (1, 1), Ward (6, 10), and Williams (4, 8).

The map in Figure 2 below shows the results for each county.

Figure 2.

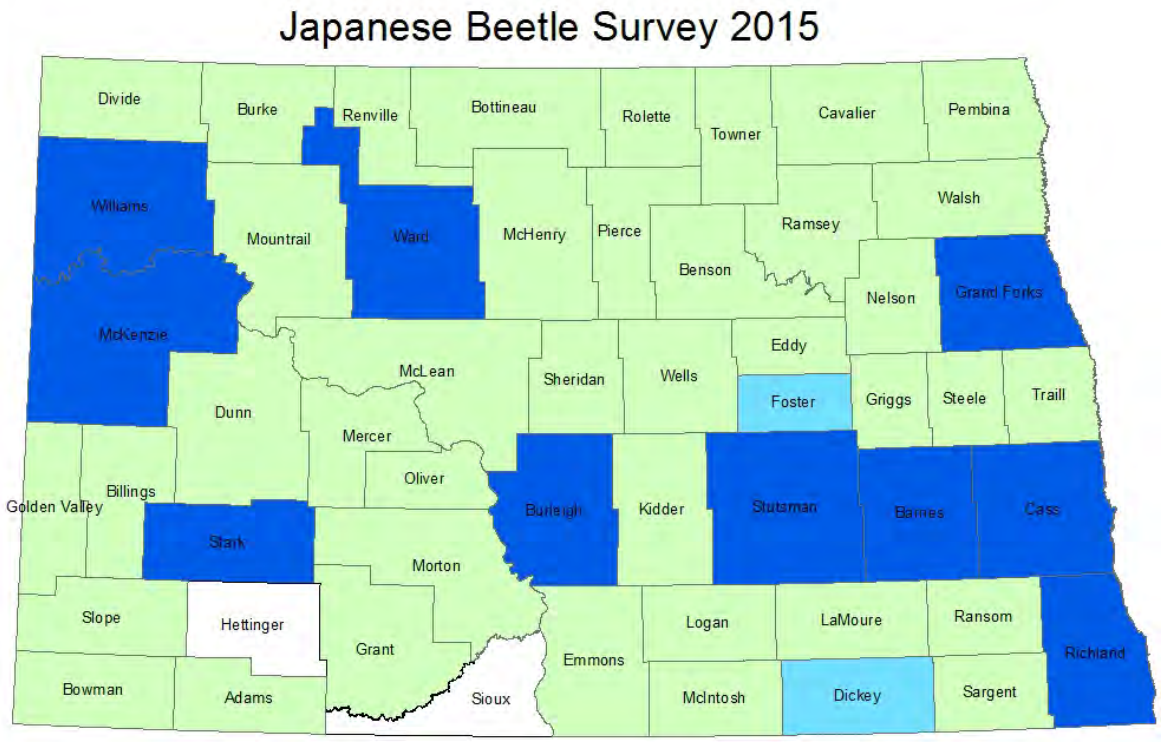


Figure 2: Map shows positive and negative counties for 2015. White counties did not have any traps placed; green counties were negative in 2015; light blue counties were positive previously but not in 2015; and dark blue counties were positive in 2015.

Future Plans

Many discussions have been held on what kind of Japanese beetle activities NDDA would continue to participate in coming years. With the spread of Japanese beetle appearing imminent, and eradication efforts unfeasible due to cost, limitations of regulatory authority, and extent of suspect infestations, NDDA will likely be backing off survey work and focusing more on outreach activities to make the public more aware of Japanese beetle. We were able to extend the grant through 2016, and with the remaining funds we intend to do a smaller scale survey, using a few of the 2015 volunteers and high risk trap sites as well as sites that require trapping for shipment of nursery stock into un-infested Japanese beetle areas. The 2016 survey will include approximate 300-500 trap sites. We are also printing some outreach material to be handed out at outreach events. We will continue to work with our partners and stakeholders to

answer questions that remain in regards to North Dakota's Japanese beetle status and move forward with protection and outreach of North Dakota in regards to Japanese beetle.

For more information, check out the following links:

http://nationalplantboard.org/wp-content/uploads/docs/Japanese beetlecolumn.pdf

http://www.nd.gov/ndda/pest/japanese-beetle

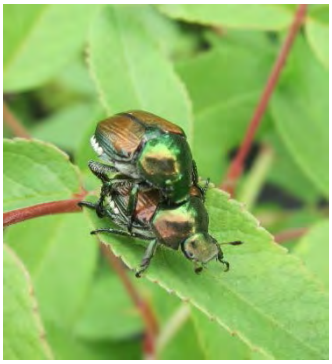
https://www.ag.ndsu.edu/pubs/plantsci/pests/e1631.pdf

Acknowledgements

I would like to thanks many people and organizations that have assisted with the 2015 survey. Hopefully I do not miss anyone. North Dakota State University Extension (Thomas Kalb, Esther McGuiness, Janet Knodel, Patrick Beauzay, Deirdre Prischmann-Voldseth, Robert Brenner, students, and county agents), North Dakota Forest Service (Aaron Bergdahl, Dylan Roberts and Anthony Pappas, interns), North Dakota Department of Agriculture Plant Industries Staff (Carrie Larson, Lane Kozel, Rachel Siefert-Spilde, Samantha Brunner, Chelsey Penuel, Darold Walls, Richard Weisz, and Allan Aufforth), North Dakota Department of Agriculture Specialty Crop Grant Administrator Emily Edlund, North Dakota Nursery and Greenhouse Association and participating nurseries, city and park districts across the state, and the many volunteers (Agnes Vetter, Amy Sand, Andrew Moe, Barb Anton, August Rahlf, Barb Garceau, Beth Binkowski, Beth Didier, Beverly Paluh, Wayne Beyer, Penny Seifert, Bill Miller, Bob and Edythe Cull, Bonnie Gillett, Jackie Buckley, Renae Tokach, Cari Flores, Carleen Soule, Carol Helgren, Char McLaughlin, Erin Markestad, Cheryl Planert, Chuck Weiser, Colleen Cousins, Dan Johnson, Dawn Alderin, Deanna Donnelly, Deb Ott, Deb Syvertson, Deb Zillich, Debbie Alber, Dennis Schott, Diana Tviet, Diane Byrum, Diane Randle, Don Bauer, Don Reimer, Donna Maston, Dwight Dockter, Eileen Stokkeland, Garland Sagen, Geraldine Geiger, Gloria Maragos, Jackie Hope, Jamie Good, Janell Quinlan, Janice Mills, Jean Madsen, Jeanne Ahlers, Jeannie Jacobs-Kopp, Jessie Marshall, Jill Donahue, Jodi Peterson, John Eide, Julie Dostal, Karen Bonnet, Karen Jacobus, Karen Morrison, Karen Ust-Grothmann, Kathleen Wiese, Kathy Nash, Katie and Levi Reese, Ken Eraas, Kristin Moynihan, Laura Munski, Laurie Duncan, Laurie Podoll, Lela Grim, Lillian Volochenko, Lindy Berg, Lois Fleck, Lois Pearson, Lori Keller, Lori Martin, Lorraine Manz, Lynette Baumiller, Maggie Belle Herr, Marcia Hellandsaas, Marie Halvorson, Marlys Albright, Martin Hochhalter, Merita Dablow-Aldayel, Michelle Azure, Michelle Willyard, Mike Rose, Nelson Rosit, Paige Brummond, Sister Pamela Pranke, Pat Gates, Patty Anderson, Rachel Brag, Ray Utter, Rena Mehlhoff, Rhoda Lund, Robin Shoberg, Rose Heiser, Roxie Hawley, Sally Brovold, Sally Niessen, Sandra Van Eck, Sandy Birst, Sara Mittelsteadt, Sharon Leinen, Sharon Sand, Sheila Ostrem, Sherwood Gibbs, Susan Nelson, Susan Wefald, Sylvia Hansen, Thom Sand, Tim Becker, Tish Skarloken, Tony Aamont, Trudy Kalstad, Vicki Griffin, Valley City Community Gardens, Virginia Becker, and Zach Peterson). This project was supported by the U.S. Department of Agriculture's (USDA) Agricultural Marketing Service through grant [14-SCBGP-ND-0038; NDDA Japanese Beetle]. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the USDA. Sources used for data in this document include North Dakota Department of Agriculture Facts Sheets; NDSU Extension Japanese Beetle IPM Publication and the National Plant Board Japanese Beetle Harmonization Plan.

Japanese Beetle Survey 2016

All photos by Charles Elhard, NDDA.



Charles Elhard, Plant Protection Specialist

December 1, 2016

Background

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Japanese beetle adults are about ½ inch long with the male slightly smaller than the female. The insects are metallic green in color with bronze wing covers called elytra. Adults begin to emerge mid-June with peak emergence occurring approximately 3 to 4 weeks after initial emergence. The female beetle will burrow into the soil during the day to lay eggs, laying up to 60 eggs in her 4-6 week lifespan. Eggs will hatch in about two weeks. Larvae, which are about 1 inch long and cream colored with a brown head, will live in the soil, feeding on plant roots. The insect will overwinter as third instar larvae below the frost line and will pupate and emerge the following spring. Flight period for Japanese beetles is recognized as June through October.

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In 2012, 139 beetles were caught in traps in Stark, Grand Forks, Cass, Burleigh, Ward, Dickey, and Griggs counties. All of these were at or near nurseries that imported nursery stock from Minnesota suppliers. In 2013, over 400 beetles were caught, but only Burleigh, Cass, and Grand Forks counties had positive traps. In 2014, after a large supplier of nursery stock to North Dakota implemented safeguarding protocols, numbers of beetles caught decreased to about 50 beetles also in Burleigh, Cass and Grand Forks counties. The difference with 2014 positives were in locations caught. Along with nurseries, several parks and golf courses had positive traps in Burleigh and Cass counties. In 2015, numbers of total beetles was greatly reduced, but more counties became positive. Traps in 10 counties had beetle catches.

2016 Survey

With support from the North Dakota Nursery and Greenhouse association, NDDA applied for a USDA Specialty Crop Block Grant initially to perform delimiting and eradication activities around the infestation areas. After it was determined that ND would be better served by a wide scale state-wide survey, with assistance from NDSU-Extension, NDDA sought out volunteers across the state to help with the survey to place 1700 traps in all counties in 2015. Funds remained after 2015 survey, so it was decided that the survey would continue until the funds were exhausted. The final survey will take place in 2017. In 2016, 69 people across the state assisted with the survey.

All traps were baited with a lure to attract both male and female beetles. The lure is very effective and has been shown to attract Japanese beetles that are within 500 feet of the trap. Each trap was individually labeled with a 16-**** number. Traps were placed beginning in May and most traps were placed by June 30, 2016. The traps were collected starting in late August with the majority removed in October. All traps were removed by November 15, 2016. Traps were in place for an average of 110 days. A total of 530 traps were placed across the state. The map in Figure 1 below shows the number of traps placed in each county.

Results

After all traps were removed, the data was collected from all trappers. In all traps, 386 beetles were caught across the state. Twelve counties had positive traps. These were Barnes (3 positive trap, 4 beetles), Burleigh (13, 33), Cass (31, 191), Grand Forks (10, 24), McClean (1, 1), Morton (1, 3), Richland (1, 1), Rolette (1, 2), Stark (3, 20), Stutsman (1, 1), Ward (11, 87), and Williams (3, 19).

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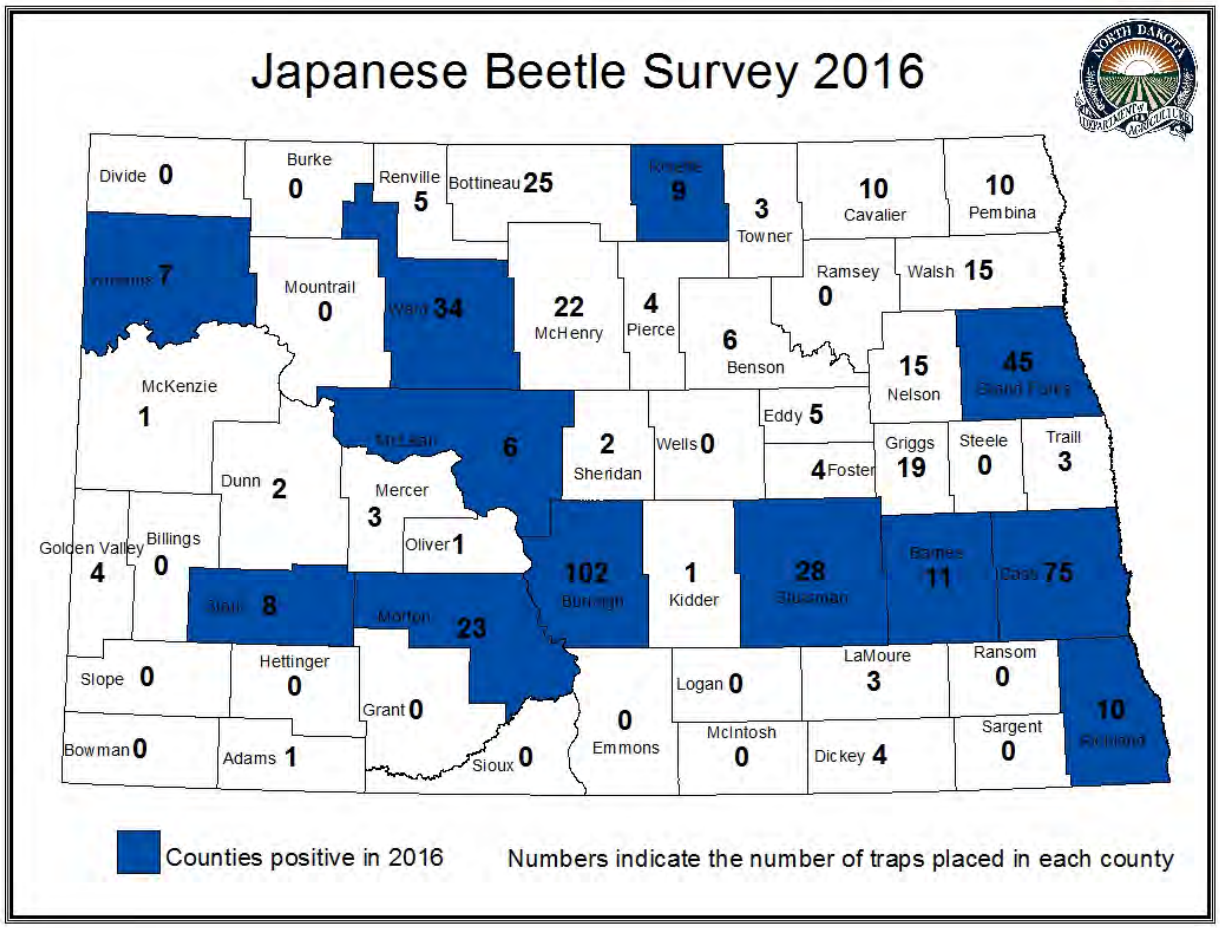
Figure 1.



Future Plans

Many discussions have been held on what kind of Japanese beetle activities NDDA would continue to participate in coming years. With the spread of Japanese beetle appearing imminent, NDDA will focus survey work on unifested areas as well as areas that are picking up small numbers of beetles to determine if the are just transient beetles or if populations are establishing. We also will focusing more on outreach activities to make the public more aware of Japanese beetle. We were able to extend the grant through 2017, and with the remaining funds we intend to do a large scale survey, using a few of the 2015/2016 volunteers and high risk trap sites as well as sites that require trapping for shipment of nursery stock into un-infested Japanese beetle areas. We also anticipate applying for continued funding for future surveys. The 2017 survey will include approximate 1500 trap sites with intention to include all 53 counties. We are also printing some outreach material to be handed out at outreach events. We will continue to work with our partners and stakeholders to answer questions that remain in regards to North Dakota's Japanese beetle status as a Category 2 state and move forward with protection and outreach of North Dakota in regards to Japanese beetle.

For more information, check out the following links:

http://nationalplantboard.org/wp-content/uploads/docs/Japanese beetlecolumn.pdf

http://www.nd.gov/ndda/pest/japanese-beetle

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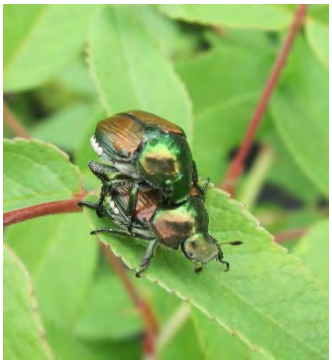
Japanese Beetle Survey 2017

All photos by Charles Elhard, NDDA.



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November 1, 2017

Background

Japanese beetle (*Popillia japonica*) was first discovered in the United States in New Jersey in 1916. A native of Japan, the beetle occurs in all states east of the Mississippi River and in some counties and partial states west of the Mississippi River. Nearest to us, South Dakota, Minnesota, and Montana have established populations in some areas. Japanese beetles attack a broad range of host material including nearly 300 species of plants. The adult beetle will feed on field crops, ornamentals, trees, shrubs and garden plants, severely defoliating and skeletonizing the host plants. The larvae will feed on the roots of turf grasses and field crops (especially corn and soybeans) as well as organic matter in the soil, severely damaging golf courses, lawns and pastures. Japanese beetle prefer irrigated turf sites such as golf courses and lawns for reproduction. The Japanese beetle is a highly destructive plant pest that can be very difficult and expensive to control. Japanese beetle is regulated by USDA-APHIS-PPQ only at airports to prevent artificial spread by aircraft. All other regulatory arrangements are decided state-to-state in cooperation with the Japanese Beetle Harmonization Plan.

Japanese beetle adults are about ½ inch long with the male slightly smaller than the female. The insects are metallic green in color with bronze wing covers called elytra. Adults begin to emerge mid-June with peak emergence occurring approximately 3 to 4 weeks after initial emergence. The female beetle will burrow into the soil during the day to lay eggs, laying up to 60 eggs in her 4-6 week lifespan. Eggs will hatch in about two weeks. Larvae, which are about 1 inch long and cream colored with a brown head, will live in the soil, feeding on plant roots. The insect will overwinter as third instar larvae below the frost line and will pupate and emerge the following spring. Flight period for Japanese beetles is recognized as June through October.

Japanese beetle trapping has been ongoing in North Dakota since the early 1960s. Traps are typically deployed in June and removed in October. Two transient beetles were first intercepted in North Dakota in 2001 in Bismarck, but not again until 2012. Since 2012, beetles have been caught every year. Beetles were first caught in nurseries that were importing nursery stock from infested areas of Minnesota.

In 2012, 139 beetles were caught in traps in Stark, Grand Forks, Cass, Burleigh, Ward, Dickey, and Griggs counties. All of these were at or near nurseries that imported nursery stock from Minnesota suppliers. In 2013, over 400 beetles were caught, but only Burleigh, Cass, and Grand Forks counties had positive traps. In 2014, after a large supplier of nursery stock to North Dakota implemented safeguarding protocols, numbers of beetles caught decreased to about 50 beetles also in Burleigh, Cass and Grand Forks counties. The difference with 2014 positives were in locations caught. Along with nurseries, several parks and golf courses had positive traps in Burleigh and Cass counties. In 2015, numbers of total beetles was greatly reduced, but more counties became positive. Traps in 10 counties had beetle catches. In 2016, beetle numbers increased. In all, 530 traps were placed with 386 beetles caught in 79 traps across 12 counties.

2017 Survey

With support from the North Dakota Nursery, Greenhouse and Landscape Association (NDNGLA), North Dakota Department of Agriculture (NDDA) applied for a United States Department of Agriculture (USDA) Specialty Crop Block Grant initially to perform delimiting and eradication activities around the infestation areas in 2015. After it was determined that ND would be better served by a wide scale state-wide survey, with assistance from North Dakota State University (NDSU)-Extension, NDDA sought out volunteers across the state to help with the survey to place 1700 traps in all counties in 2015. Funds remained after 2015 survey, so it was decided that the survey would continue until the funds were exhausted. This year, 2017, is the last year of the Specialty Crop Block Grant funded survey. In 2017, 87 people across the state assisted with the survey. More than 80 nurseries also participated.

All traps were baited with a lure to attract both male and female beetles. The lure is very effective and has been shown to attract Japanese beetles that are within 500 feet of the trap. Each trap was individually labeled with a 17-**** number. Traps were placed beginning in May and most traps were placed by June 1, 2017. Additional traps were placed June-July after the discovery of Japanese beetle larva in nursery stock containers. The traps were collected starting in late September with the majority removed by September 30. All traps were removed by October 15, 2017. Traps were in place for an average of 84 days. A total of 1,203 traps were placed across the state. The map in Figure 1 shows the number of traps placed in each county with positive counties highlighted in blue.

Japanese Beetle Larva Introduction

As mentioned above, many additional traps were placed around the state in response to the discovery of Japanese beetle larva in container nursery stock in late June. NDDA nursery inspectors were alerted by a neighboring state that larva was found in container stock from a nursery supplier in Minnesota. Upon further inspection, NDDA staff also found larva and pupating beetles in nursery containers. Potentially infested stock was shipped to nearly 80 nurseries and landscapers from March through June. NDDA worked with the supplier to treat and/or remove infested nursery stock that remained at the nurseries. NDDA staff placed traps at all the nurseries, hoping to catch as many remaining beetles as possible. 80% of the beetles caught statewide were at these nurseries. NDDA will continue to monitor these nurseries.

Results

After all traps were removed, the data was collected from all trappers. In all traps, 1,467 beetles were caught across the state, 1,181 in nurseries. Twenty-two counties had positive traps. These were Adams (1 positive trap, 1 beetle), Barnes (6, 14), Bowman (1,1), Burleigh (32, 278), Cass (51, 515), Dickey (3, 4), Dunn (1, 8), Eddy (1, 1), Foster (5, 20), Grand Forks (13, 37), LaMoure (2,2), McKenzie (1, 1), Morton (10,24), Mountrail (1, 1), Ramsey (4, 56), Ransom (3, 3), Richland (14, 83), Stark (7, 47), Stutsman (7, 55), Walsh (1, 4), Ward (27, 180), and Williams (5, 132). Two previously positive counties were negative in 2017 (McLean and Rolette).

The map in Figure 1 below shows the number of traps positive compared to the total number traps placed in each county.

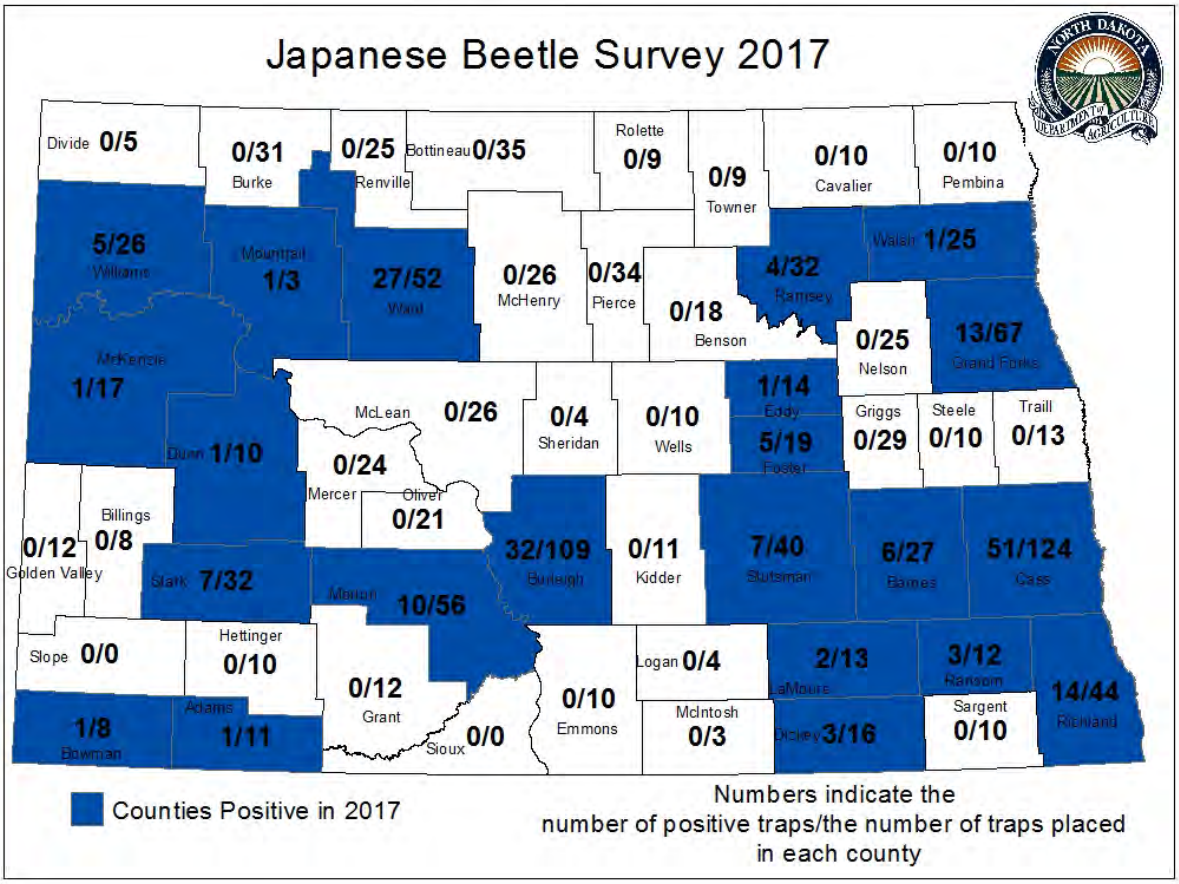

Figure 1.

The map in Figure 2 on the next page shows the total number of beetles caught in each county.

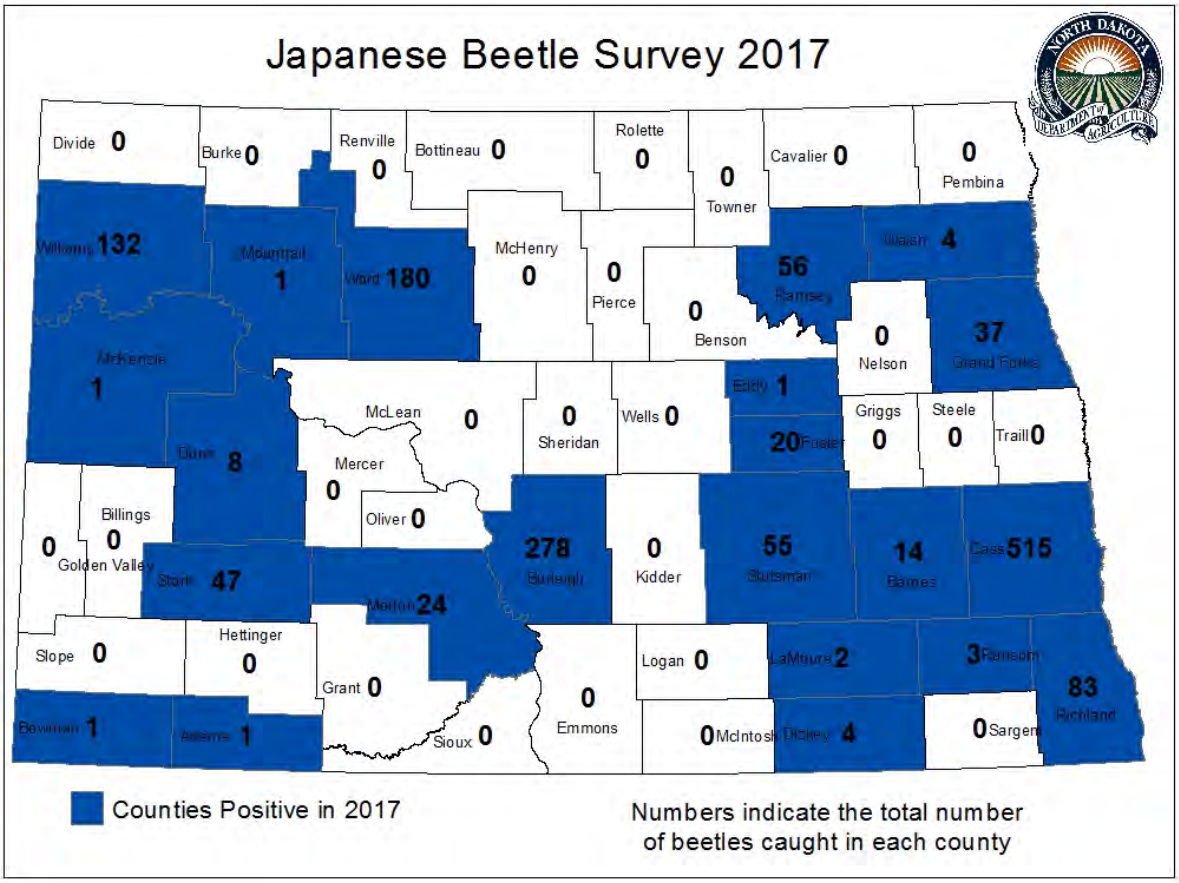

Figure 2.

Future Plans

NDDA will continue to focus survey work on uninfested areas as well as areas that are picking up low numbers of beetles to determine if they are hitchhiking beetles or if populations are becoming established. We also continue outreach efforts to make the public more aware of Japanese beetles and the damage they can cause. The NDSU Extension Integrated Pest Management (IPM) publication was updated in 2017 and reprinted. Tentative plans for a 2018 survey will include additional trapping at sites that were positive in 2017, to determine if the intercepted beetles become established. All nurseries receiving nursery stock from infested states, especially Minnesota, will be included in the survey. We will also continue to monitor uninfested counties to maintain their negative status. NDDA may seek more Master Gardener volunteers to assist with the survey in 2018 in more rural areas to help reduce costs.

For more information, visit these websites.

http://nationalplantboard.org/wp-content/uploads/docs/Japanese beetlecolumn.pdf

http://www.nd.gov/ndda/pest/japanese-beetle

https://www.ag.ndsu.edu/pubs/plantsci/pests/e1631.pdf

Acknowledgements

I would like to thank the following people and organizations that have assisted with the 2017 survey. Hopefully I do not miss anyone. North Dakota State University Extension (Thomas Kalb, Esther McGinnis, Janet Knodel, Patrick Beauzay, Deirdre Prischmann-Voldseth, and county agents), North Dakota Department of Agriculture Staff (Scott Hochhalter, Jeannie Jacobs-Kopp, Lynette Baumiller, Melanie Gaebe, Bonnie Sundby, Dr. Susan Keller, Jody Reinke, Lane Kozel, Rachel Siefert-Spilde, Samantha Brunner, Chelsey Penuel, Darold Walls, Richard Weisz, Greg Machart, Jeanne David and Allan Aufforth), North Dakota Department of Agriculture Specialty Crop Grant Administrator Emily Edlund, North Dakota Nursery and Greenhouse Association and participating nurseries, city and park districts across the state, and the many volunteers (Carol Helgren, Charlotte McLaughlin, Dawn Alderin, Deanna Donnelly, Deb Ott, Diana Tveit, Diane Bingeman, Donna Maston, Janell Quinlan, Jeanne Ahlers, Jessica Marshall, Julie Dostal, Laurie Podoll, Lillian Volochenko, Lorraine Manz, Maggie belle Herr, Marie Fragodt, Mark & Lily Hoffman, Paige Brummund, Penny Seifert, Rachel Brag, Sally Brovold, Sally Niessen, Sandra Birst, Sharon Leinen, Thom Sand, Tish Skarloken, Barbara Anton, Beverly Paluh, Bill Miller, Carleen Soule, Cameron Young, colleen cousins, Deb Syvertson, Debbie Alber, Don J Reiner, Eileen Stokkeland, Jodi Peterson, Joletta Schmidt, John Eide, Karen Jacobus, Lindy Berg, John Gaebe, Lori Keller, Marissa Leier, Martin Hochhalter, Michelle Willyard, Pat Gates, Robert/ Edythe Cull, susan nelson, Sylvia Hansen, Barbara Garceau, Ken Eraas, Chuck Weiser, Gail Kartes, Karen Bonnet, Amy Sand, Andrew Moe, Beth Didier, Charlie Kopp, Cari Flores, Dennis & Lois Schott, Diane Randle, Dwight Dockter, Karen Morrison, Lois Pearson, Marlys Albright, Mary Kay Tokach, Mike Rose, Rena Mehlhoff, Sara Mittelsteadt, Sheila Ostrem, Tim Becker, Trudy Kulstad, and anyone I mistaken forgot). This project was supported by the U.S. Department of Agriculture's (USDA) Agricultural Marketing Service through grant [14-SCBGP-ND-0038; NDDA Japanese Beetle]. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the USDA. Sources used for data in this document include North Dakota Department of Agriculture Facts Sheets; NDSU Extension Japanese Beetle IPM Publication and the National Plant Board Japanese Beetle Harmonization Plan.