2019-2020 CANOLA RESEARCH UPDATE

Northern CANOLA GROWERS Association

THE NORTHERN CANOLA GROWERS ASSOCIATION is proud to present the results of fiscal year 2019 and 2020 canola research in this region. The reports on the following pages exemplify the strong efforts made by public researchers in their efforts to improve the profitability of the canola industry by answering questions related to canola establishment, disease control, disease resistance, harvesting and other areas involved in the job of producing a canola crop that is in growing demand.

The Northern Canola Growers Association (NCGA), in partnership and with funding from the North Dakota Oilseed Council, chooses from a variety of research proposals submitted annually through its Request for Proposal process. In the last two years, the NCGA was able to provide over $600,000 towards funding of important research topics for canola in the northern region of the U.S.

North Dakota is the leading producer of canola in the nation, followed closely behind by Montana. Research in this region covers aspects of the majority of the canola crop in the U.S. As always, we welcome input and suggestions for canola research. You can reach us at 701-223-4124 or northerncanola.com.

OUR MISSION

The mission of the Northern Canola Growers Association is to promote and encourage the establishment and maintenance of conditions favorable to the production, marketing, processing, research, and use of canola; to promote efficient production through farmer education, public and private research, labeling and registration of crop protection products; to promote uniform seed and product standards; and to work to develop and implement agriculture policies that will enhance development of the industry.

2021 NCGA BOARD OF DIRECTORS

Pat Murphy
Minot, ND

Dan Marquardt
Bottineau, ND

Tim Mickelson
Rolla, ND

Matt Mongeon
Rolette, ND

Jon Wert
New England, ND

Zach Jacobson
Langdon, ND

Brian Jenks
Minot, ND

Michael Brekhus
Kenmare, ND

Kristie Sundeen
Brocket, ND

Courtney Meduna
Minot ND

Troy Romfo
Calvin, ND

Robert Haman
Devils Lake, ND

Becky Braaten
Westhope ND

Northern CANOLA GROWERS Association

125 Slate Drive Suite #4 Bismarck, ND 58503
P: 701.223.4124

WWW.NORTHERNCANOLA.COM

PRINCIPAL INVESTIGATOR
James V. Anderson, David P. Horvath, Wun S. Chao

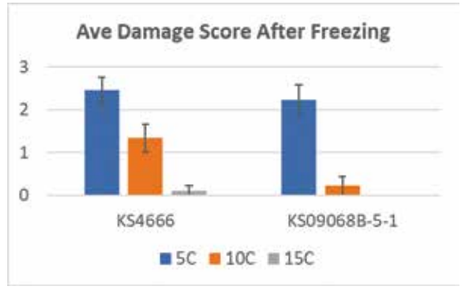
AFFILIATION
USDA/ARS, Edward T. Schafer Agricultural Research Center, Sunflower and Plant Biology Research Unit, 1616 Albrecht Blvd. N., Fargo, ND, USA 58102-2765

Increasing Freezing Tolerance in Canola Through A Better Understanding of Cold Deacclimation

The research goals of this project were to identify common regulatory elements among co-regulated gene clusters responsive to deacclimation of cold acclimated plants, functionally characterize genes associated with deacclimation using genome wide association studies, and establish transform and regeneration procedures for canola.

DEFINING CONDITIONS AND EXPERIMENTAL SYSTEMS
An experimental system developed to study cold acclimation and deacclimation in canola demonstrated that cold acclimated plants can deacclimate rapidly, losing freezing tolerance within three days at temperatures above 15C. Cold acclimated plants exposed to temperatures between 15C and 5C retained intermediate levels of freezing tolerance, even after two weeks. Interestingly, variety KS4666 showed little deacclimation at 10C after two weeks, while variety KS09068B-5-1 lost freezing tolerance at 10C within 3 days of deacclimation (Figure 1).

cont.



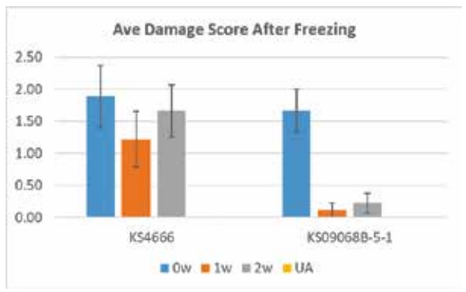


Figure 1: Freezing damage of two varieties (KS4666 and KS09068B-5-1) of winter canola after deacclimation of cold acclimated plants at the indicated temperatures (5, 10, and 15C) for 3 days (top graph) or for 0, 1, or 2 weeks at 10C (bottom graph). All unacclimated plants (UA on the bottom graph) were killed by freezing.

DIFFERENTIAL GENE EXPRESSION ANALYSIS
Gene expression analysis identified genes that were turned on during the cold acclimation process but turned off during the deacclimation process and vice versa in the two canola varieties (KS09068B-5-1 and KS4666) that show differential responses to deacclimation (Figure 2). This transcriptomics information was used to identify possible transcription factor binding sites for manipulating this process in canola, and to help identify possible candidate genes in our genome wide association study (GWAS).

IDENTIFYING AND FUNCTIONALLY CHARACTERIZING GENES
Considerable variation in freezing tolerance following deacclimation of cold acclimated plants exists in our winter canola population (Figure 3).

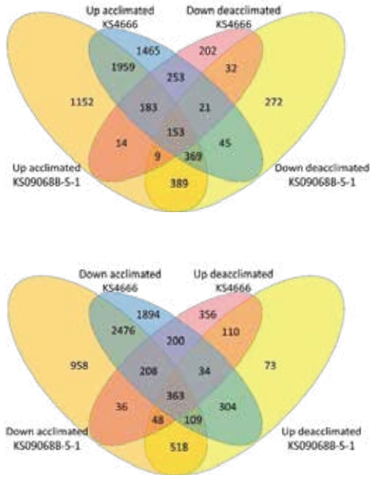


Figure 2: Venn diagrams of genes that were up-regulated during cold acclimation and down-regulated during deacclimation (top), or vice versa (bottom), in the two divergent varieties of winter canola (KS09068B-5-1 and KS4666).

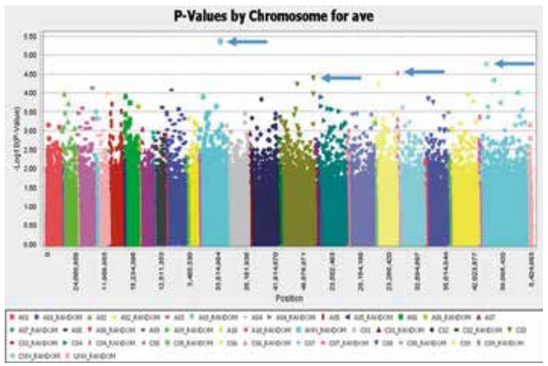


Figure 4: Manhattan plot showing probability of genetic linkage between genetic markers in the canola genome and freezing tolerance. Four markers (arrows) were chosen for further analysis.

GWAS was used to identify genetic loci associated with cold deacclimation in our winter canola diversity panel that likely contain genes controlling deacclimation processes (Figure 4).

Candidate genes among those found near the linked marker were chosen based on their putative function, and if they were differentially expressed following cold acclimation or deacclimation (Figure 5).

TRANSFORMATION OF CANOLA
Protocols and plasmids obtained from Dr. Ed Cahoon will be used in future research studies to facilitate transformation of canola to determine if reducing expression of these selected candidate genes in spring and winter canola impact freezing damage due to deacclimation of cold acclimated plants.

Figure 3: Average freezing damage scores of 397 individual varieties of canola following 4 weeks of cold acclimation at 5C and then 3 days deacclimation at 20C days and 10C nights. Error bars are standard error from 9 individuals.

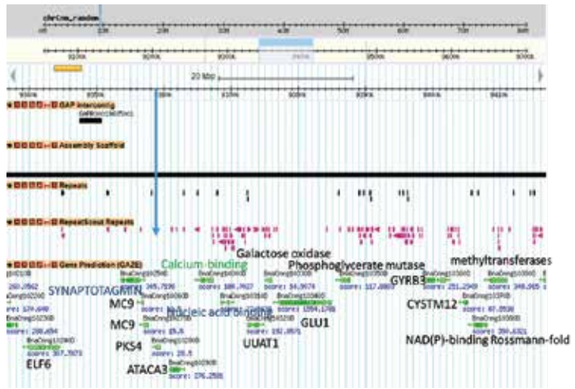


Figure 5: A map of genes near the marker on chromosome CNN_Random. The blue downward arrow shows the location of the marker, and the gene noted in green (Calcium-binding) was differentially express in our gene expression study. The two genes in purple (SYNAPTOTAGMIN and Nucleic acid binding) are known to be differentially expressed following cold treatment in Arabidopsis (a well-studied model plant closely related to canola). Based on this map, we chose the Calcium-binding gene, the PKS4 gene that contains the marker, and the cold-regulated Nucleic acid binding genes as candidates for further analysis.

PRINCIPAL INVESTIGATORS
Chris Augustin, Brian Jenks, Gary Willoughby, Tiffany Walter

Sugar Beet Waste Lime Impacts on Canola and Soil

INTRODUCTION
Soil pH is the activity of the hydrogen ion (H+) and measured by the negative log concentration of H+. That is why as pH decreases, acidity increases. North Dakota soil pH has historically been alkaline. However, several fields west of highway 83 now have a soil pH less than 5.5. The cause of the soil acidification is believed to be caused by nitrogen fertilizers. As nitrogen fertilizers convert into plant available nitrate, H+ is released and acidifies the soil. Over time, H+ accumulates and can turn the soil acidic which reduces yields. Soil acidity is rarely found throughout an entire field. Soil acidity tends to be found in depressional or summit areas. Precision agriculture can help pinpoint acidic areas.

Acidic soils reduce yields because of reduced soil microbial activity, aluminum toxicity that stunts root growth, and reduced nutrient availability such as phosphorus tie-up. Acidic soils can be managed by the application of lime. Lime is calcium-carbonate. Lime raises soil pH because carbonates react with H+. This reaction produces free calcium, carbon-dioxide, and water. Many states have developed lime recommendations based on their clay type, parent materials, and climate. Acidic soils in North Dakota are a new issue and consequently, lime recommendations have not been developed here. Commercial ag lime is not readily available in North Dakota. However, sugarbeet waste lime (beet lime) is readily available from sugarbeet processing factories. Beet lime is a by-product of the sugar refining process. Beet lime was used as the liming agent for this project.

MATERIALS AND METHODS
Experimental plots were treated with 0 (check), two, four, and eight tons of sugarbeet waste lime (beet lime) by hand application in 2018. The actual amount of calcium carbonate applied was 0, 1.5, 2.9, and 5.8 tons/ac. Beet lime was soil incorporated shortly after the application with a field cultivator. Beet lime is made up of more than just the acid neutralizing carbonates and has nitrogen, phosphorus, various micro-nutrients, and organic matter. The beet lime used for this project contained 73% calcium carbonate, 3% nitrate, 3% phosphorus, and trace amounts of zinc, copper, and manganese.

RESULTS
All beet lime treatments increased soil pH (Figure 1) and decreased extractable aluminum (Figure 2). Aluminum toxicity becomes an issue when the soil pH is less than 5.5 and extractable aluminum is greater than 25 ppm. Aluminum toxic plant root growth tends to be malformed and reduced.

Aluminum toxicity was possible with the beet lime applications of zero and two tons/ac as soil pH was less than 5 (Figure 1) and soil extractable aluminum was 25 ppm or greater (Figure 2). Excess soil aluminum can render soil phosphorus insoluble and hinder root growth. The beet lime treatment of four tons/ac canola appeared to have a more robust root system than the untreated check (Figure 3). The beet lime applications of four and eight tons reduced soil extractable levels to less than two ppm (Figure 2). Soil pH was greater at the eight tons/ac beet lime treatment than the four tons/ac beet lime (Figure 1). However, the data suggests that beet lime applications of four and eight tons/ac reduced soil extractable aluminum to similar levels.

The average soil calcium carbonate content was 0.29% and similar across all lime treatments (p-value 0.556). This indicates that within two years, the lime treatments reacted with the soil acidity.

cont.

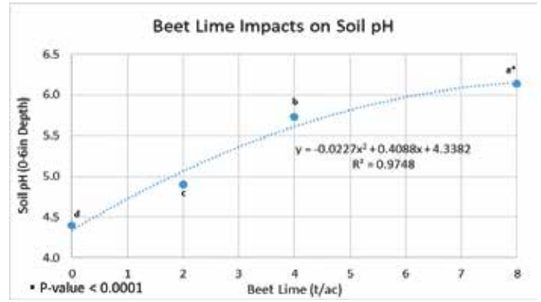


*Figure 1. The relationship of hand applied and incorporated beet lime on soil pH at the 0-6 inch depth. *Different letters indicate statistical differences at the 0.05 level.*

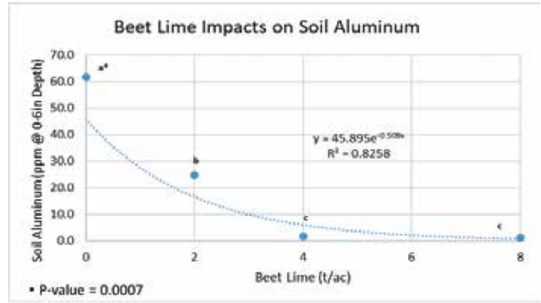


*Figure 2. The relationship of hand applied and incorporated beet lime on soil extrable aluminum at the 0-6 inch depth. *Different letters indicate statistical differences at the 0.05 level.*

Figure 3. Aluminum toxic root canola roots (right plant) systems expressed by less fibers and more frail appearance than the left plant roots.

Beet lime applications improved soil pH and soil extractable aluminum. However, lime did not impact yield or quality of canola grain. The average canola yield was 1,870 lbs/ac with a variance of 349.8 and p-value of 0.515. Canola oil content was not impacted by beet lime at the 0.05 level. The average protein content of lime was 40.2%.

Canola is susceptible to manganese toxicity when soil pH is less than 5.5. Manganese toxicity symptoms were observed early in the growing season (Figure 4), but it appeared that the canola "grew" out of the symptoms. The manganese toxic canola growth was slowed and germination was reduced. Manganese toxic leaf margins were chlorotic (indicated by red arrow). The manganese toxic canola contained 1,595 ppm of manganese. The normal canola manganese range is less than 100 ppm. However, manganese toxicity did not impact canola yield or quality.

Figure 4. Manganese toxic canola. The Red arrows are pointing to the leaf margin chlorosis that is commonly expressed by manganese toxicity.

CONCLUSIONS

All beet lime treatments increased soil pH, and decreased soil extractable aluminum. This data suggests that four tons beet lime/ac (2.9 tons calcium carbonate/ac) is an effective rate to improve soil pH and soil extractable aluminum. Yield and quality of canola was not impacted by beet lime treatments. However, young canola plant health was better from the beet lime applications.

NEXT STEPS

Soil acidity has grown to impact thousands of acres on many different soil types west of highway 83. Future research will encompass management of different western North Dakota soils as well as monitoring remediated soils. This data will be used to develop lime recommendations. Future use of nitrogen fertilizer will likely re-acidify the lime applied soil. This study site will be monitored to gather important information on the frequency of re-liming.

PRINCIPAL INVESTIGATORS

[1]Puying Zheng, [1]Mukhlesur Rahman, [2]James V. Anderson, [2]Wun S. Chao

AFFILIATION

[1]Department of Plant Science, North Dakota State University, Dept. 7670, PO Box 6050, Fargo, ND 58108-6050

[2]USDA/ARS, Edward T. Schafer Agricultural Research Center, Sunflower and Plant Biology Research Unit, 1616 Albrecht Blvd. N., Fargo, ND, USA 58102-2765

Fine Mapping to Identify Heterotic Gene(s) for Increasing Yield in Canola

The use of heterosis (as known as hybrid vigor) is one effective way to increase crop yield by enhancing superior performance in F1 hybrids generated by crossing two inbred parents. Over the last three years, progress made towards achieving the objectives of this proposal includes: 1) Testing grain yield and yield-related traits under field conditions for canola hybrids resulting from crossing between 8 introgression lines (ILs) and Westar, and 2) Confirmation of the heterotic gene(s) within a mapped 26-kilobase (Kb) region. These objectives have been completed, and the heterotic gene(s) within the 26-Kb region have been confirmed. Previously, we identified a heterotic locus in F1 hybrids from a cross between 'Westar' (canola, Brassica napus L.) and a Chromosome Segment Substitution Line (CSSL). The CSSL has the genetic background of Westar introgressed with a segment of chromosome A10 from 'Surpass 400'. To map the heterotic gene(s) within this identified locus, a set of eight ILs were developed that carry different lengths of DNA segments from chromosome A10 of 'Surpass 400'. These ILs were reciprocally crossed with Westar, and the resulting 16 F1 hybrids, along with their parents, were planted for three years (2017: Fargo and Prosper, ND; 2018: Fargo and Langdon, ND; 2019: Fargo) to estimate heterotic effects. A total of 36 entries (16 IL x Westar reciprocal hybrids, 8 ILs, 8 Westar, 2 CSSL x Westar reciprocal hybrids, 1 CSSL, and 1 Westar) were sown per replicated field plots. Grain yields were compared based on the results of eight IL groups (Table 1) from the 36 entries. Based on the three-year grain yield results (Table 1) and the known position and length of introgressed segments, the gene(s) associated with the heterotic trait have been mapped to a 500-Kb region between two molecular markers at 14.5 and 15.0 megabase in chromosome A10 (Figure 1). However, additional sequencing technology (called genotyping) helped narrow the heterotic gene(s) to within a 26-Kb region. Further sequencing of this 26-Kb region will help to identify the candidate gene(s) involved in the heterotic trait of canola hybrids. Genetic transformation of canola with these candidate heterotic gene(s) will help to confirm the molecular mechanism regulating heterosis, which should provide canola breeders with new knowledge for increasing grain yield.

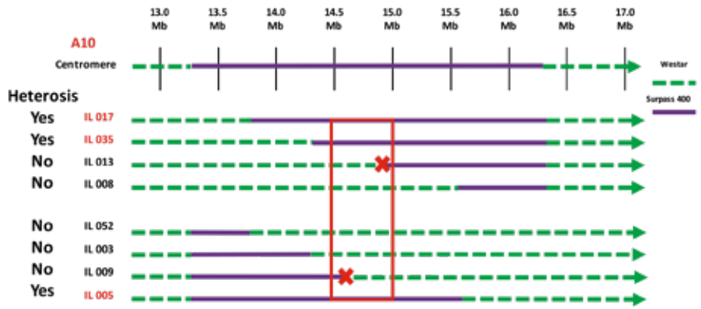


Figure 1. Confirmation of the map of heterotic locus. For the top four ILs, IL 017 and IL 035 had heterotic effect, whereas IL 013 and IL 008 did not have heterotic effect. Therefore, the heterotic gene(s) should be located at the upstream of cross over site in IL 013. Likewise, for the bottom four ILs, IL 005 had heterotic effect, whereas IL 009, IL 003 and IL 052 did not have heterotic effect. Therefore, the heterotic gene(s) should be located at the downstream of crossover site in IL 009. Based on above results, the heterotic locus was mapped between 14.5 and 15.0 Mb region in chromosome A10.

Genotypes	Seed Yield (lbs/A)			
	3 year average	MP	±MP	±BP
			%	
Westar	2128			
IL052	1447	1788		
IL052 × Westar	2199		23	3
Westar × IL052	1631		-9	-23
Westar	2098			
IL003	1463	1780		
IL003 × Westar	1457		-18	-31
Westar × IL003	1507		-15	-28
Westar	1956			
IL009	2073	2015		
IL009 × Westar	1935		-4	-7
Westar × IL009	1994		-1	-4
Westar	1672			
IL05	2372	2022		
IL05 × Westar	3402		68	43
Westar × IL05	2907		44	23
Westar	1704			
IL08	1448	1576		
IL08 × Westar	1911		26	12
Westar × IL08	1694		10	-1
Westar	1929			
IL013	1154	1542		
IL013 × Westar	1607		4	-17
Westar × IL013	1416		-8	-27
westar	1696			
IL35	2029	1863		
IL35 × Westar	2885		55	42
Westar × IL035	2715		46	34
Westar	1696			
IL017	2087	1892		
IL017 × Westar	2229		18	7
Westar × IL017	2702		43	29

Table 1. Three years grain yield results in Fargo. When combining three years' grain yield results (2017, 2018, and 2019), three IL groups (IL 005, IL 035, and IL 017) had increased grain yield in both reciprocal crosses compared with the better-parent value, suggesting that the heterotic locus is present in those introgressed chromosome segments (highlighted in yellow). The rest of the groups indicated that one of the reciprocal crosses or both had no yield increase compared with the better-parent value, suggesting that the heterotic locus is not present in those introgressed segments. MP: mid-parent value; BP: better parent-value. Formulae used for the estimation of MP% and BP%: MP = mean mid-parent value = (P1+P2)/2; Heterosis over mid-parent (MP%) = [(F1-MP)/MP X 100]; Heterosis over better-parent (BP%) = [(F1-BP/BP X 100].

PRINCIPAL INVESTIGATORS
Venkat Chapara and Amanda Arens

Evaluation of Seed Treatments to Manage Blackleg on Canola

OBJECTIVE
To evaluate seed treatments to manage blackleg on canola.

MATERIALS AND METHODS
A research trial was conducted at the Langdon Research Extension Center with an objective to evaluate the performance of seed treatments to manage blackleg on canola. The trial was planted on May 22, 2020 with treated seed of various treatments on canola cultivar "Westar" compared with non-treated seed. The design was randomized complete block with four replications. The trial followed state recommended practices for land preparation, fertilization, seeding rate and weed control. The plot size was 5 ft. wide x 16 ft. long. Data on blackleg infections were rated following the scale of 0-5. Infections obtained in the research plots were natural. Twenty five canola stubbles were rated within each plot and the incidence (number of plants had blackleg infections out of twenty five cut stems) and severity on each was recorded after swathing (August 18) on a 0-5 scale, where 0 = no disease tissue visible in the cross section; 1 = < 25% of the cross section has disease tissue; 2 = 26 to 50% of the cross section has disease tissue; 3 = 51 to 75% of the cross section has disease tissue; 4 = > 75% of the cross section has disease tissue; 5 = 100% diseased tissue/ plant dead. A blackleg mean disease severity index was calculated with weighted mean of incidence and number of plants in each severity rating. Data were subjected to analysis of variance using complete block, balanced orthogonal designs of Agrobase generation II software.

		Blackleg			
Treatments	Rate (Fl. Oz/100 lb seed)	Incidence (%)	Mean Disease Severity (0-5)	Yield (lbs/A)	Test Weight (lbs/bu)
Control	Check	69	1.78	1087	50.2
Biological	0.62	74	2.24	1258	50.8
Biological	1.23	62	1.67	1499	50.9
Biological	2.46	36	0.66	1268	50.0
Saltro	1.23	42	0.87	1529	51.1
	Mean	57	1	1328	51
	CV %	28	36	19	1
	LSD	24	1	384	1
	P-Value (0.05)	0.017	0.0052	NS	NS

Table 1: Mean blackleg disease incidence, severity and their effect on yield and test weights on application of different seed treatments on canola.

RESULTS
Canola seed treated with the biological at higher rate followed by saltro® had the lowest blackleg incidence and mean disease severity (Table 1) and were statistically significant from the other treatments tested. There were no significant difference in yield or test weight in the treatments tested when compared with the non-treated check.

PRINCIPAL INVESTIGATOR
Venkat Chapara (Project Investigator)

COLLABORATORS
Dr. Travis J. Prochaska, Dr. Audrey Kalil, Dr. Anitha Chirumamilla, Dr. Kishore Chittem and Dr. Luis Del Rio

Clubroot on Canola: Survey and Outreach in North Dakota

PROJECT TITLE
Survey and Creating Awareness on Identification and Management Plan of Clubroot of Canola in North Dakota

TAKE HOME MESSAGE
Ongoing clubroot survey program of over four years in various counties of North Dakota indicates threat to canola crop if proper attention is not given towards longer crop rotations (1 in 3 years) and equipment sanitation. In addition, growers should consider an available resistant canola variety to clubroot. Cleaning equipment thoroughly after done working in a clubroot infected field since the primary mechanism of spread between fields is the movement of infested soil on farm equipment. Yield losses were recorded up to 25% in severely infected canola fields in Cavalier county this year. The occurrence of clubroot in fields with acidic soils getting closer to basic pH.

SURVEY PROCEDURE
The survey involved three components 1. Visual Survey, 2. Soil sampling and 3. Molecular assays for resting spore quantification of clubroot pathogen.

1&2. VISUAL SURVEY AND SOIL SAMPLING
Clubroot scouting was done visually by inspecting canola crop roots. The disease survey was conducted in northeastern counties (Pembina, Walsh, Ramsey, Towner, Rolette and Cavalier) along with North Central and Western counties of North Dakota that are bordering Canada. County selection was done based on canola acreage and on assumption of clubroot propagules movement in all directions through equipment, soil or water to neighboring counties from infected areas. In each county, one field in every 5000 acres was targeted for scouting. Soil samples were collected from the positive and likely positive clubroot fields with an intent to know how high the pH of soil in which clubroot has been found and to determine the number of resting spores per gram of soil. In all, a minimum of 5-10 fields per county were targeted for scouting.

The survey was done in two phases.

1ST PHASE: AT FLOWERING (10% OF FLOWERING ONWARDS)
In the growing season, plants were sampled from distinct stunted patches or prematurely senescing plants in the field. Patches visible from the edge of the field were checked by digging and observing the roots for symptoms of clubroot and soil samples were collected from those spots.

2ND PHASE: AFTER SWATHING
Scouting at swathing was based on the methodology followed in Canada by the Alberta Agricultural and Rural Development (AARD) for their annual clubroot disease survey. Reports of AARD indicated that the incidence of clubroot is more in the field entrances. Hence, the survey was done from the main entrances/approaches in each field, the survey group walked along in a "W" pattern by stopping at 5 spots and uprooting 10 consecutive stems from the ground at each spot. Each sampling point was separated by 100 meters or 328 feet. In all, roots of 50 stems were evaluated for the presence of clubroot and incidence was noted. Excess soil was shacked off.

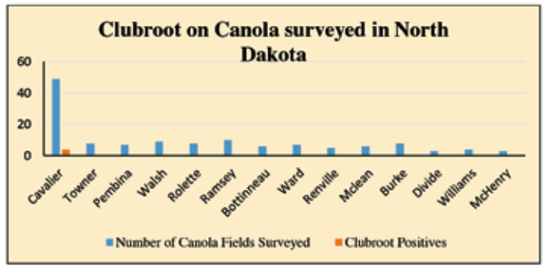


Figure 1: Fields surveyed in 2019 for prevalence of clubroot in various Counties of North Dakota.

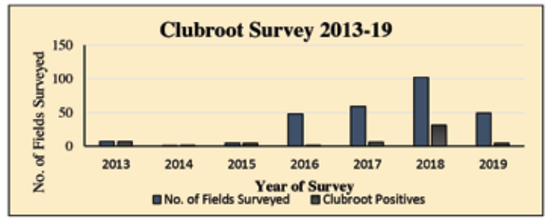


Figure 2: Fields surveyed from 2013 to 2019 for prevalence of clubroot in North Dakota.

Roots then were visually examined for presence of galls. At sample sites where infection was observed or suspected, root specimens with galls, along with soil, were double bagged and labeled with the field location. Infected roots and soil samples from all the fields surveyed were collected and a representative sample was submitted to Dr. Luis Del Rio's laboratory for molecular quantification of resting spores per gram of soil and another half-pound of soil to NDSU soil testing laboratory for pH determination.

RESULTS
In all 133 canola fields have been scouted in 2019 from various counties of Northern North Dakota, out of which only four fields have been found positive to clubroot (Figure 1) on visual symptom survey. However, 14 fields were found positive for clubroot through molecular assays. Clubroot resting spores were quantified from all of those samples ranging from 14 to 814,000 per gram of soil (detection limit of the assay 10 resting spores/gm of soil). The pH of clubroot positive field in visual symptoms ranged from (4.5 - 6.7), while molecular assays detected spores up to pH of 7.64 (Table 1) and most of them are from new counties where visible symptoms of clubroot on canola roots were not seen.

3. MOLECULAR DETECTION OF SOIL SAMPLES TO QUANTIFY PLASMODIOPHORA BRASSICAE (THE CLUBROOT PATHOGEN) RESTING SPORES

cont.

PRINCIPAL INVESTIGATOR
Venkat Chapara

The objective of this procedure to quantify resting spores of clubroot pathogen in soil and to inform growers way ahead of occurrence of on field visible gall symptoms in Canola.

RESULTS FROM MOLECULAR ASSAYS DONE ON SOIL SAMPLES IN 2019
The molecular assays indicated that the clubroot spread occurred to the neighboring counties (Table 1). However, there were no visible symptoms observed when the roots were uprooted.

No visible galls in the surveyed fields but positive in the molecular soil sample test indicate that the resting spore population may not have reached required spores per gram of soil. In general, clubroot infections express on canola plants where soil population is about 80,000 spores per gram of soil, until there will be no expression of clubroot symptoms on canola plants (Canadian Research). These results indicate that there is a need for continuous annual monitoring.

NOTICE
Growers who suspect clubroot in their field are encouraged to contact Dr. Venkat Chapara at the Langdon REC (701-256-2582), Dr. Anitha Chirumamilla at the Cavalier County Extension office (701-256-2560) or through NDSU Extension (701-231-8363).

OUT REACH ACTIVITIES CONDUCTED IN 2019-20 SEASON
I) County Wise Presentations: Cavalier (6), Walsh (1), Pembina (1) and several were cancelled due to covid-19

II) Statewide Presentations:
1. Plant Pathology Departmental Seminar
2. NDSU Extension Fall Conference
3. NDSU Spring Research Conference
3. NDSU Extension Lake Region Roundup
4. Northern Canola Growers Association Canola Expo

III) International Presentations:
1. International Rapeseed Council Conference Berlin, Germany,
2. Invited Speaker at Lacombe Field Day, Alberta, Canada,
3. Clubroot Discovery Forum Invited Panel member as clubroot expert, Winnipeg
4. Worldwide Clubroot steering committee meeting held online due to CoVid-19

Positive fields of clubroot detected through molecular assays				
Sample Id	Depth (inches)	pH	Buffer pH	Soil population/gm of soil
Cavalier County				
CCtc-38	0-3	5.3	6.73	13280
CCtc-11	0-3	7.6	7.74	184
CCtc-37	0-3	5.6	6.80	814300
Roulette County				
RLTC-3	0-3	7.6	7.42	27
Towner County				
TWC-3	0-3	7.3	7.32	17.15
TWC-7	0-3	7.5	7.39	16.56
Pembina County				
PBC-1	0-3	6.5	6.95	25.32
PBC-3	0-3	6.3	6.87	13.98
PBC-5	0-3	7.0	7.10	29.42
PBC-6	0-3	7.5	7.50	29

Table 1: Clubroot positive fields observed in neighboring counties of Cavalier County where clubroot has been identified.

Cultivar Evaluation to Manage Clubroot on Canola

OBJECTIVE
To evaluate the resistance potential of commercial canola cultivars against clubroot pathogen in field conditions.

CANOLA CULTIVARS/VARIETIES
Eleven commonly cultivated canola varieties were planted to determine the level of resistance against clubroot (Table 1).

PLANTED
First week of June (Hand planted after thorough tillage with a rototiller.)

FIELD DESIGN
Randomized complete block design (RCBD) with four replications.

PLOT SIZE
3 ft. x 5ft.

CLUBROOT EVALUATED
Early August (59 days after planting).

CLUBROOT DISEASE INDEX (CRDI)
CRDI: <30% of Susceptible Check = Resistant (R)
CRDI: 30-69% = Intermediate (I)
CRDI: >70% = Susceptible (S)
Note: To validate a clubroot research trial, the susceptible check should have > 60% of Disease Index.

RESULTS
Canola cultivars 6076CR, 4187RR, InVigor L234P, InVigor L255PC, 45H33, 45CS40 CP955RR and CP9982RR showed resistance to clubroot and were significantly different from other varieties tested.

FUTURE RESEARCH
Testing more commercial cultivars of canola will be helpful to growers and to monitor clubroot in available resistant varieties of canola.

MONITORING CLUBROOT IN RESISTANT VARIETIES
(By Canola Council of Canada)

"Growers using clubroot-resistant varieties in clubroot-infested fields may experience some infected plants, which can be attributed to susceptible volunteers and off-types. Volunteer canola seed can germinate many years after it was last grown, and if this comes from a susceptible canola crop, then the volunteers will be susceptible. Off-types are a normal part of hybrid canola production – no canola hybrid is 100% pure, so there may be a small proportion (1 to 4%) of the seed that is susceptible.

When scouting, if more than 10% of seeded plants (do not count volunteers) are infected, that may indicate that the clubroot resistance is no longer functional against the pathogen population in the field. These infected plants may be restricted to a small patch which indicates a recent pathogen change."

END NOTE
Practice crop rotation (one canola crop in three years).

Cultivar	Description
6076CR	BrettYoung Seeds
4187RR	BrettYoung Seeds
INVIGOR L255PC	BASF
INVIGOR L234P	BASF
CP9919RR	Croplan Genetics
DKL30-42	Cargill
45CS40	Pioneer (Corteva)
45H33	Pioneer (Corteva)
CP955RR	Croplan Genetics
CP9978TF	Croplan Genetics
CP9982RR	Croplan Genetics

Table 1: Commonly cultivated canola cultivars/varieties in Cavalier County.

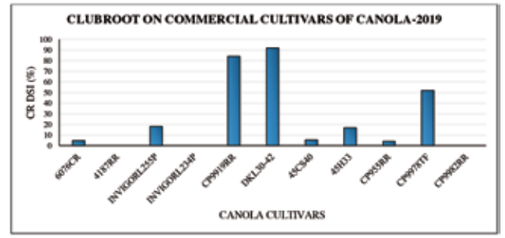


Figure 1: Mean clubroot incidence (%) on various commercial cultivars of canola tested in 2019.

Picture 1: Resistant Variety to clubroot.

Picture 2: Susceptible Variety to clubroot.

PRINCIPAL INVESTIGATOR
Luis del Rio

AFFILIATION
Department Plant Pathology, North Dakota State University

Progress Report 2020

PROJECT TITLE
Characterization and Transfer of Blackleg Resistance Present in Elite Brassica Napus Plant Introductions to Modern Canola Breeding Lines

Blackleg continues to increase in importance in North Dakota. The identification of sources of resistance against races prevalent in the state and its incorporation into modern breeding lines is imperative to keep the profitability of canola production in the region. The objectives of this project were to identify genetic markers associated with resistance in elite plant introduction materials and transfer the resistance into modern canola lines that farmers can plant.

Field and greenhouse screenings that evaluated the reaction of more than 480 Brassica napus plant introduction materials to blackleg strains of PG-4 (Figure 1) led to the identification of several lines with excellent levels of resistance against blackleg strains of PG-4. In field trials, Sumner, Aomori-1 and CRI 65/76a had average incidences < 36% and severities <10% while commercial hybrids used as controls had average incidences between 60 and 70% and severities between 27 and 37%. The objectives of this project are: (1) characterize molecular markers associated with resistance present in plant introductions Aomori-I and CR 165/76a; and 2) transfer resistance present in both lines to modern canola breeding lines.

OBJECTIVE 1
Crosses between Aomori-1 and NDOLA-01 were made. NDOLA-01 is a canola line released by the NDSU Canola breeding program in 2019. Crosses between CR165/76a and Topas were made. Topas is an older cultivar with good agronomic traits and oil quality that also is very amenable for doubled haploid production. F 1 seeds from these crosses were produced. Production of doubled haploid lines derived from the second cross is underway in the laboratory (Figure 2). We expect to start evaluation of this mapping population in early 2021.

OBJECTIVE 2
Also, an F1 plant of the cross CR165-76a (resistant to blackleg) x NEP63 (resistant to Sclerotinia) was crossed with NDC198 (NDSU advanced breeding line). The maternal line in this cross is an NDSU breeding line that will be released in 2021. Six F 1 plants from this cross have been planted in the greenhouse. In late September, the F2 seeds will be planted and screened for blackleg at the seedling stage. Susceptible plants will be eliminated and resistant plants will be inoculated with Sclerotinia at the flowering stage. Blackleg and Sclerotinia-resistant plants will be crossed again with NDC 198. This cycle of screening and backcrossing resistant materials with NDC 198 will be repeated three more times. After that, the best line will be released as germplasm.

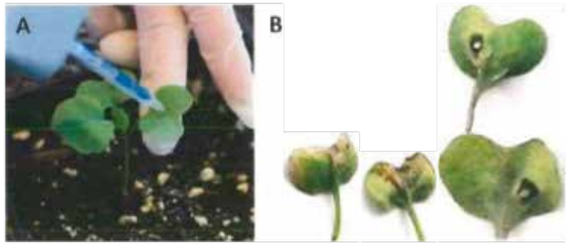


Figure 1. Greenhouse inoculation of blackleg on canola seedlings at the cotyledon stage (A) and susceptible (B) and resistant (C) reactions two weeks after inoculation. Note limited lesion development and dark ring surrounding the inoculated area in the resistant accession.

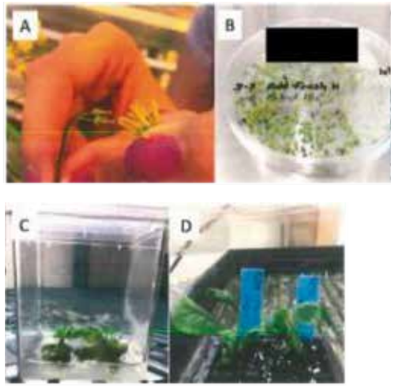


Figure 2. Production of doubled haploid (DH) plants from microspore tissue culture. A) making the cross between resistant and susceptible lines; B) microspores retrieved from unopened flowers are treated with colchicine to force doubling chromosome counts in each cell; C) callus tissues formed from each microspore are cultured in special medium to induce root and leaf formation; D) fully regenerated plantlets are transferred to greenhouse. Depending on the crosses used (not all genotypes are equally amenable for DH production), the entire process from the moment crosses are made to harvesting of DH seeds may take 12-16 months.

PRINCIPAL INVESTIGATOR
Janet Knodel

2019 Swede Midge Trap Survey in Canola in North Dakota

SOUTHEAST
***Janet Knodel**, Professor and Extension Entomologist, NDSU, Fargo, ND*
***Patrick Beauzay**, IPM Coordinator, NDSU, Fargo, ND*

NORTH CENTRAL
***Travis J. Prochaska**, Extension Specialist Crop Protection, NCREC, Minot, ND*
***Sara Clemens**, NDSU Extension Service - Bottineau County, Bottineau, ND*
***LoAyne Voigt**, NDSU Extension Service - Renville County, Mohall, ND*

NORTHEAST
***Lesley Lubenow**, Extension Specialist Cropping Systems, Langdon REC, Langdon, ND*
***Lindy Berg**, NDSU Extension Service - Towner County, Cando, ND*
***Anitha Chirumamilla**, NDSU Extension Service - Cavalier County, Langdon, ND*
***Traci Murphy**, IPM Scout and insect trapper*

NORTHWEST
***Audrey Kalil**, Plant Pathologist, Williston REC, Williston, ND*
***Nicole Stanhope**, Insect trapper*

SOUTHWEST
***Ryan Buetow**, Extension Specialist Agronomy, Dickinson REC, Dickinson, ND*
***Kia Ward**, IPM Scout and insect trapper*

INTRODUCTION
Swede midge, *Contarinia nasturtii* (Kieffer) (Diptera: Cecidomyiidae), is an invasive insect pest of canola that was introduced into Ontario, Canada in 2000 and then was first found in the United States in Niagara County, New York in 2004. In 2014, swede midge was trapped in Saskatchewan and Manitoba, Canada (pers. comm., J. Soroka, AAFC Saskatoon Research Centre). The positive detection in a pheromone trap near Winkler, Manitoba in 2014 is close to the North Dakota border (just north of Walhalla), and to one of the major canola producing areas of North Dakota. This prompted a pheromone trap survey in northeastern North Dakota in 2015. The swede midge trap survey was expanded to other canola producing areas of North Dakota in 2017, and has continued each year through 2019. In recent years, Canadian entomologists have not captured any swede midge in pheromone traps or observed any pod damage in Saskatchewan, Alberta and Manitoba. However, they cannot say that *C. nasturii* is longer present on the Canadian prairies.

Photo: Susan Ellis, www.forestryimages.org

The swede midge adult is a small brown fly »1.5-2mm long (see photo on left). It is in the same family (Cecidomyiidae) as the wheat midge. Swede midge is difficult to identify without a microscope, because of its small size and similarity to other midges. The antennae and wing venation of swede midge are two characteristics that are used in identification. Antennae of males are distinctive consisting of 12 antennal segments, each with two beadlike 'nodes' separated by more slender, stem-like connections.

Hosts of the swede midge include a wide range of species within the family Brassicaceae (formerly Cruciferae), which includes the following: canola (*Brassica napus, B. rapa*); broccoli (*B. oleracea* var. *italica*); cauliflower (*Brassica oleracea* var. *botrytis*); cabbage (*B. oleracea* var. *capitata*); and radish (*Raphanus sativus*).

Swede midge can cause significant yield loss to Brassicaceae plants. Larval feeding causes the damage to plants by changing the physiology of the plant and creating deformed crumpled leaves, shoots and/or flower galls, malformed growing points on the plant and growth of secondary shoots.

A new species, *Contarinia brassicola* Sinclair (canola flower midge, Diptera: Cecidomyiidae), was recently identified infesting canola (*B. napus and B. rapa*), and found wide spread in Saskatchewan and Alberta, Canada (2019 Mori et al. Can. Entomol. 151:131-148). Mori also observed larvae and pod damage in Swan River Valley area of Manitoba, Canada. This midge is similar in appearance to the swede midge being small (<2 mm) and brown. Larva injure the flowers by causing a swelling (or gall), that prevents flowers from opening. Damaged flowers do not produce pods or seeds. *Contarinia brassicola* does not injure leaves and shoots as does *C. nasturtii.* The shape of the flower galls is different between the two species, with *C. brassicola* having elongated, bottle-shaped, closed flower galls, where as *C. nasturtii* has caper-shaped, closed flower galls.

Canadian entomologists do not know the economic impact of this new *Contarinia* midge in canola. Early-planted canola (mid May) had more midge damaged pods compared to late-planted canola (early June) in Canada; however, the early-planted canola also had the highest yield due to more important agronomic factors (Soroka et al. 2019, Can. Entomol. 151: 219-235). Insecticide seed treatments were tested for control of *Contarinia* midges, but little or no negative effect on midge injury to pods was observed (Soroka et al. 2019). This is not surprising since *Contarinia* midges emerged about 4-6 weeks after seeding where as insecticidal seed treatment residual wanes after 3-4 weeks of seeding. Additional yield loss studies in canola are planned in Canada.

Based on phylogenetic analysis, *C. brassicola* differs genetically, morphologically and ecologically from *C. nasturtii.* Morphological characters listed in Table 1 are used to identify *C. brassicola* from *C. nasturtii* (Mori et al. 2019).

Canadian entomologists postulated that it is native to North America and may have switched hosts to canola due to the large areas planted to canola in Canada. Mori et al. (2019) described its taxonomic identification,

cont.

Contarinia brassicola (canola flower midge)	*Contarinia nasturtii* (swede midge)
Female wings mottled with dense macrotrichia	Clear wings
Deep V-shaped notch on the male genitalia	U-shaped notch on the male genitalia
Length of the female first antennal flagellomeres is 2 times as long as second flagellomere	Length of the female first antennal flagellomeres is 1.5-1.8 times as long as second flagellomere
COI sequence variation unique	DNA difference

Table 1. Morphological characters used to identify of C. brassicola from C. nasturtii.

and biology, and found two parasitoids that attacked C. brassicola with an average parasitism rate of 10% (range of 0 to 62%). In Canada, *C. brassicola* was not captured in any swede midge baited traps or attracted to the *C. nasturtii* pheromone (Mori et al. 2019). Research is underway to identify the female-produced sex pheromone of *C. brassicola*.

MATERIALS & METHODS

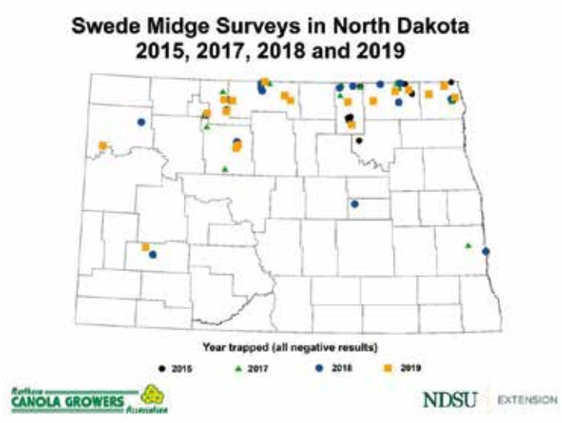
Traps were monitored at 19 field sites including 16 canola field sites and 3 garden sites with Brassicaceae vegetables in 9 counties throughout North Dakota. Most canola trap sites were concentrated in the two major canola growing areas of North Dakota, the northeast and north central areas. The three garden trap sites were located in the following counties and towns: Bottineau (Bottineau, DCB Garden), Towner (Cando Community Garden) and Ward (Minot Community Garden). One delta trap (Scentry Red LPD trap) with sex pheromone lures was placed in each field from mid-June (rosette) through mid-August (ripening crop stage). Traps were placed at 20 cm above the ground. Pheromone lures were stored in a freezer at 0°F until used. Lures were replaced in traps at 28-day intervals. Disposable gloves were used when handling lures to reduce lure-trap contamination. Sticky trap liners were removed weekly. Trap liners were kept frozen until shipment to Dr. Knodel's laboratory.

Trap liners were examined by Patrick Beauzay for swede midge using a microscope. Suspect specimens were identified using published identification keys and preserved swede midge from a laboratory colony obtained from the Saskatoon Research Centre. Any positive suspects from traps were tested to confirm its identity as swede midge: *Contarinia nasturtii*, using a sensitive and specific DNA-based method (Frey et al., 2004. The Canadian Entomologist 136: 771-780).

Data were summarized and a map was prepared using ArcMap to illustrate the presence or absence of swede midge in pheromone traps at surveyed locations.

RESULTS

All pheromone trap results were negative for *C. nasturtii* in North Dakota in 2019. Results of the 2015, 2017, 2018 and 2019 trapping are depicted in the map (right). This is good news for canola growers in North Dakota.



Future trapping and field observations will be essential for early detection of swede midge, *C. nasturtii*, and the new canola flower midge, *C. brassicola*, in North Dakota. Pheromone trapping and field scouting for midge-damaged pods will serve as an important 'pest alert' system to detect these invasive insect pests of canola. This will help the canola industry, so we will know when these midges are present or a threat to canola production in North Dakota.

ACKNOWLEDGEMENTS

This survey was supported by the Northern Canola Growers Association. We would like to thank Honggong Bu for the map using the ArcMap program. Many thanks to the hard-working IPM field scouts of 2019 who assisted with the swede midge trapping: Traci Murphy at LREC, Nicole Stanhope at DREC and Mia Ward at DREC. These IPM scouts were supported by the Crop Protection and Pest Management - Extension Implementation Program [grant no. 2017-70006-27144] from the USDA National Institute of Food and Agriculture, and the North Dakota Department of Agriculture.

PRINCIPAL INVESTIGATORS

Janet J. Knodel, Patrick Beauzay, Lesley Lubenow, T.J. Prochaska, Audrey Kalil, Scott Knoke, Ryan Buetow, Luis del Rio Mendoza and Sam Markell

2019 Canola Survey in North Dakota Flea Beetle Results

This final report summarizes the insect part of the 2019 Canola Survey results, and addresses the 2nd objective – To provide information on the population levels and distribution of different species of flea beetles in canola throughout North Dakota during swathing (August through September). The "Canola Diseases and Flea Beetle Survey" grant is lead by PI Dr. Luis del Rio Mendoza and Co-PIs Dr. Samuel Markell and Dr. Janet Knodel.

INTRODUCTION

Surveys that estimate prevalence of pests that affect canola production in North Dakota are important tools for growers, researchers, and extension agents. In North Dakota, canola disease surveys were conducted almost on an annual basis between 1991 and 2009 (Lamey et al., 1995a, 1995b, 1996, 1997, 1998, 1999, 2000, 2001a, 2001b, 2002). These surveys concluded that the two most important diseases affecting canola production in North Dakota are Sclerotinia stem rot, caused by *Sclerotinia sclerotiorum*, and blackleg, caused by *Leptosphaeria maculans*.

Flea beetles, *Phyllotreta* species, are the most important insect pest of canola and they also have been monitored for in the canola survey since 2002. Flea beetles emerge from their overwintering sites in early spring. Adult beetles feed on the cotyledons and young leaves, causing defoliation and a typical shot-hole appearance (Knodel 2017, Knodel et al., 2017). Insecticide seed treatments are the main tool used for flea beetle control, although an occasional rescue foliar insecticide is needed when cool wet springs cause seed treatment failure (Knodel 2017, Knodel and Beauzay, 2011).

The amount of flea beetle feeding injury in the spring is impacted primarily by the weather and the overwintering population levels. If the spring is warm and dry, conditions are favorable for flea beetle emergence, feeding and movements into canola fields. If the spring is cool and wet, flea beetle emergence will be slower and more erratic, reducing feeding and movements. Regular field scouting is the best strategy to ensure that insecticide seed treatments are still protecting against flea beetle feeding injury, and will determine if a foliar insecticide application is needed. The economic threshold of 25% defoliation caused by flea beetle feeding is recommended for making foliar insecticide decisions.

Flea beetles were collected and identified to species to determine if flea beetle species composition is shifting in North Dakota due to resistance to neonicotinoid insecticide seed treatments. Tansey et al. (2008) found that *Phyllotreta striolata* (striped flea beetle) had less mortality than *P. cruciferae* (crucifer flea beetle) when fed canola grown from neonicotinoid-treated seed in Canada. Consistent annual use of neonicotinoid seed treatments over millions of canola acres in North Dakota and adjacent Canada could contribute to a species shift favoring *P. striolata* as the dominant flea beetle species and lead to potential control problems. In addition, these data help indicate which flea beetle species is most common in canola, and whether any *Phyllotreta* species shift is being observed in North Dakota.

MATERIALS AND METHODS

After swathing, canola fields were swept for flea beetles using a 15-inch sweep net and 20 180-degree sweeps at 5 locations per field site for a total of 100 sweeps per field site. All collected flea beetles were bagged and labeled with county, GPS coordinates and date. Samples were kept frozen until shipment to Dr. Knodel's laboratory. Flea beetle species were identified and counted by Patrick Beauzay. Data were summarized and maps were prepared to illustrate the flea beetle species distribution using ArcMap.

RESULTS

Eighty two fields were surveyed in 18 counties of North Dakota. Three species were collected in North Dakota: *Phyllotreta cruciferae* (crucifer flea beetle), *P. striolata* (striped flea beetle), and *P. albionica* (cabbage flea beetle). Crucifer flea beetle was the dominant species collected, comprising 99.7% of all individual flea beetles collected. The other species included striped flea beetle (0.2 %), and cabbage flea beetle (0.1%).

Figure 1 illustrates the density of all species of *Phyllotreta*. The highest densities were observed throughout the major canola production areas of North Dakota. Overall, there was an increase in 2019 to 67,163 specimens, up from 46,266 specimens in 2018, but still a major decrease from the 91,470 flea beetles collected in 2017. Part of the reason for the lower total number of flea beetles collected in 2019 is probably due to the wet weather conditions and standing canola fields. However, 56% of the field surveyed were swathed, 38% of fields were standing and the remaining 6% were harvested in 2019 (Figure 2). It is difficult to sweep a standing canola field and collect flea beetles.

CRUCIFER FLEA BEETLE, *PHYLLOTRETA CRUCIFERAE* (FIGURE 3)

The most abundant flea beetle species collected was crucifer flea beetle (Fig. 3) with 66,976 specimens collected (99.7% of the total flea beetles collected). This is an increase of 32% from 2018 (45,434 crucifer flea beetles collected) and a decrease of 26% from 2017 (91,470 crucifer flea

cont.

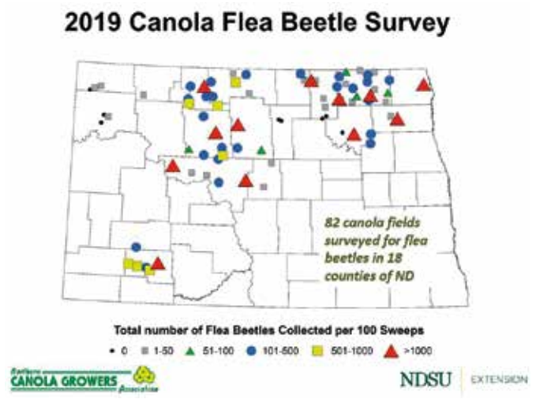


Figure 1. 2019 Canola Flea Beetle Survey showing all species of flea beetles collected.

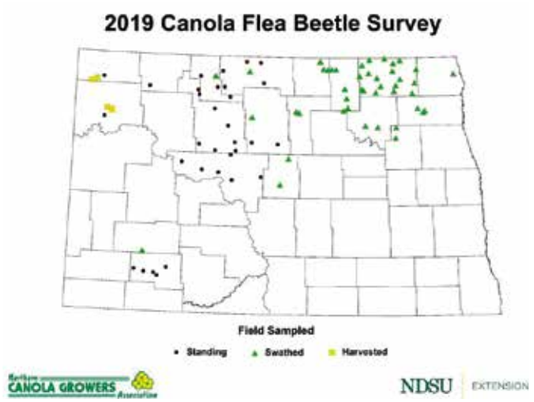


Figure 2. 2019 Canola Survey showing swathed, standing or harvested field sampled.

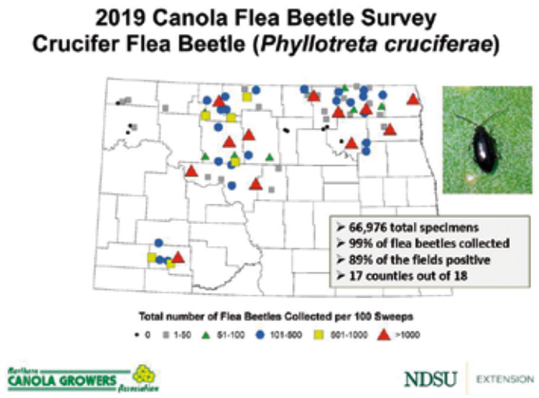


Figure 3. Crucifer flea beetle, Phyllotreta cruciferae.

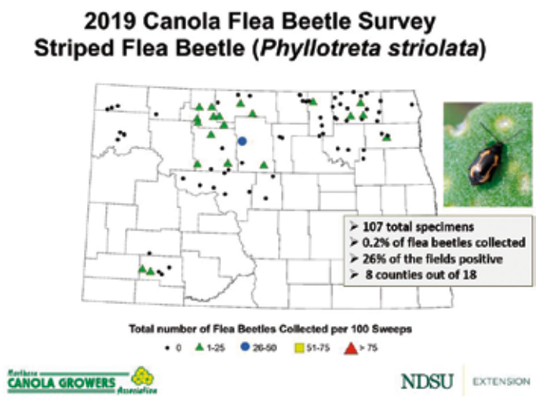


Figure 4. Striped flea beetle, Phyllotreta striolata.

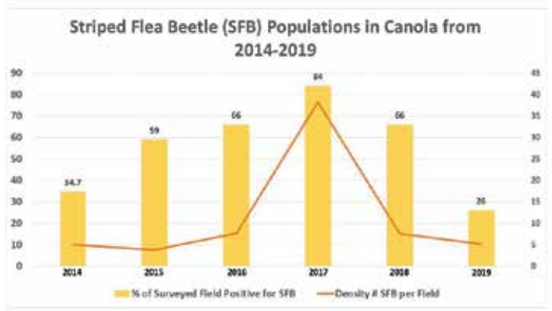


Figure 5. Percentage of surveyed fields and density of striped flea beetles from 2014-2019 Canola Surveys.

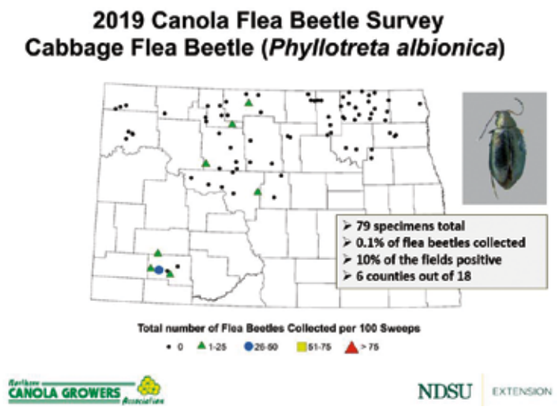


Figure 6. Cabbage flea beetle, Phyllotreta albionica.

beetles collected). Crucifer flea beetles were present in 89% of the fields surveyed and in 17 of 18 counties surveyed. Field sites with high numbers of crucifer flea beetles, >1000 flea beetles per 100 sweeps, were observed in the following 9 counties: northeast – Cavalier, Nelson; northwest – Renville and Ward; northcentral – Benson and Pierce; west central – McLean; and southwest – Hettinger and Stark.

STRIPED FLEA BEETLE, *PHYLLOTRETA STRIOLATA* (FIGURES 4 AND 5)

The striped flea beetle was the second most abundant *Phyllotreta* species with a total of 107 specimens collected (0.2% of the total flea beetles collected). This is an 84% decrease from 2018 (656 striped flea beetles collected) and a 97% decrease from 2017 (3,097 striped flea beetles collected). The striped flea beetle was present in 26% of the fields surveyed and in 8 of the 18 counties surveyed.

Striped flea beetles were collected in the Northwest (Renville, Ward Counties); North Central (Bottineau and McHenry Counties); Northeast (Cavalier, Towner and Walsh Counties); and Southwest (Hettinger County) of North Dakota. The highest density of 35 striped flea beetles per 100 sweeps was collected in McHenry County in 2019.

Data from the 2014 to 2019 Canola Surveys indicate that the percentage of canola field that were positive for striped flea beetles increased from 2014 to 2017. However, recent data from 2018-19 shows lower numbers of canola fields infested by striped flea beetles and lower densities of striped flea beetles. The percent of fields infested with striped flea beetles in decreased from 84% in 2017 to 66% in 2018 and to 26% in 2019 (Figure 5).

CABBAGE FLEA BEETLE, *PHYLLOTRETA ALBIONICA* (FIGURE 6)

The cabbage flea beetle was present in 10% of the field surveyed, and 79 were collected in 6 of the 18 counties surveyed (Fig. 6). The cabbage flea beetle was most common in the Northwest (Renville, Ward Counties); North Central (Bottineau County); and Southwest (Hettinger and Stark Counties) of North Dakota. The highest density recorded was 28 cabbage flea beetle per 100 sweeps in Hettinger County. Hettinger County also had the highest populations of cabbage flea beetles from 2016 through 2018.

SUMMARY

Crucifer flea beetle continues to be the most common and widely distributed flea beetle species in North Dakota. Striped flea beetle was the second most common flea beetle species. It is interesting to note that populations of striped flea beetles have declined in the survey since 2017. The 2019 survey provides important data on striped flea beetle population levels and distribution in canola in North Dakota. Future survey efforts will help document any changes in flea beetle species diversity and distribution in North Dakota.

ACKNOWLEDGEMENTS

This survey was supported by the Northern Canola Growers Association. We would like to thank Honggong Bu for mapping using the ArcMap program. Many thanks to the hard-working IPM field scouts of 2019 who assisted with the canola survey. The IPM scouts were supported by the Crop Protection and Pest Management - Extension Implementation Program [grant no. 2017-70006-27144] from the USDA National Institute of Food and Agriculture, and the North Dakota Department of Agriculture.

PRINCIPAL INVESTIGATORS
Janet Knodel, Patrick Beauzay, Lesley Lubenow, Vero Calles-Torrez

2020 Progress Report: Best Pest Management of Flea Beetles in Canola

OBJECTIVE 1
Bioassays for Insecticide Resistance in Flea Beetles

MATERIALS AND METHODS
Mortality RCBD factorial bioassay experiments were completed at the NDSU Research Greenhouse, Fargo ND, using *Phyllotreta cruciferae*. The factors were days after planting timing (7 or 14 DAP) for flea beetle infestation and four insecticide treatments, thiamethoxam (Helix Vibrance, Sygenta, Greensboro, NC, 400 g/100 kg seed), clothianidin (Prosper EverGlo, Bayer CropScience, Research Triangle Park, NC, 200.8 g/100 kg seed), cyantraniliprole (Fortenza, Sygenta, Greensboro, NC, 1000 g/100 kg seed), and an untreated control. Separate experiments were run with Langdon, Minot and Dickinson collected flea beetles. Additionally, experiments were performed with spring generation and summer generation flea beetles. We planned to run spring generation experiments on *P. striolata*, striped flea beetles, however the year was poor to collect enough needed to run the experiments.

Ten flea beetles (*Phyllotreta cruciferae*) were introduced to a deli cup containing five canola plants (Fig 1.). Spring generation flea beetles were added on May 26 (Langdon), June 2 (Dickinson), June 9 (Minot) and June 16 (Minot – repeated run). Summer generation flea beetles were added on August 11 for all locations. Living flea beetles were counted at one, three, seven, and 10 days after *Phyllotreta spp*. infestation. Additionally, feeding injury by flea beetles was recorded using a cotyledon scoring system where number of feeding-pits were scored on a zero to six scale (Table 1).

For each observation date, living flea beetle counts and feeding score data was compared were using PROC GLIMMEX by using SAS analysis systems (SAS Institute 2012). Block was considered random and all other factors were considered fixed effects. Means separation was performed using LSMEANS with Tukey's HSD test (α = 0.05).

RESULTS
Spring generation: impacts of location and seed treatment. Each observation date had a significant location by seed treatment interaction (df = 3; Day 1, P = 0.0069, Day 3, P = 0.0089, Day 7, P = 0.0001, Day 10, P < 0.0001). Generally, within a location, all insecticide-laden seed treatments tended to have similar efficacy (Fig. 2). Flea beetle mortality can be generally summarized as Dickinson ≥ Langdon ≥ Minot.

Fig. 1. Bioassay deli cups.

Feeding scores were significant for location by seed treatment, as well (df = 3; all Day 1, P = 0.0006, Day 3, P = 0.0004, Day 7 and Day 10, P < 0.0001). Cyantraniliprole tended to have lower feeding scores than thiamethoxam and clothianidin (Table 2).

Spring generation: impact of days after planting (DAP). Across all observation dates, 14 DAP plots had between six to 10 percent greater mortality than 7 DAP plots (data not shown).

Summer generation: impacts of location and seed treatment. Unlike the spring generation, there was no location by seed treatment interaction. However, the main factors of location (alone) and seed treatment (alone) were significant for all dates (Location: df = 2; Day 1, P = 0.0018, Days 3, 7 and 10, P < 0.0001; seed treatment: df = 3; all dates, P < 0.0001.)

Generally, across all observation dates, mortality can be summarized as Minot > Dickinson = Langdon (Fig. 3). Cyantraniliprole tended to be lower than thiamethoxam and clothianidin on Days 1 and 3, but cyantraniliprole was equal on Days 7 and 10 (Fig. 4).

Feeding score observations showed a significance with the main effect of seed treatment (df=3; all dates, P < 0.0001). Generally, feeding scores were untreated controls > thiamethoxam = clothiandin > cyantraniliprole (Table 3).

Summer generation: impact of days after planting. No differences were seen on summer generation mortality with the main factor days after planting (data not shown) (df = 1; all dates, P > 0.05).

DISCUSSION
For the spring generation of *Phyllotreta cruciferae*, the Minot-originating flea beetles mortality percentiles were lower than Langdon and Dickinson. This is the third year of this study, previous reports (Knodel et al. 2018, 2019) to the NCGA have shown that the Minot-originating flea beetles are not dying at the same rate as the Cyantraniliprole showed a lower mortality rate and less feeding activity. Cyantraniliprole acts

Number of Pits	Score
0	0
1-3	1
4-9	2
10-15	3
16-25	4
>25	5
Plant death	6

Table 1. Cotyledon Feeding Injury System

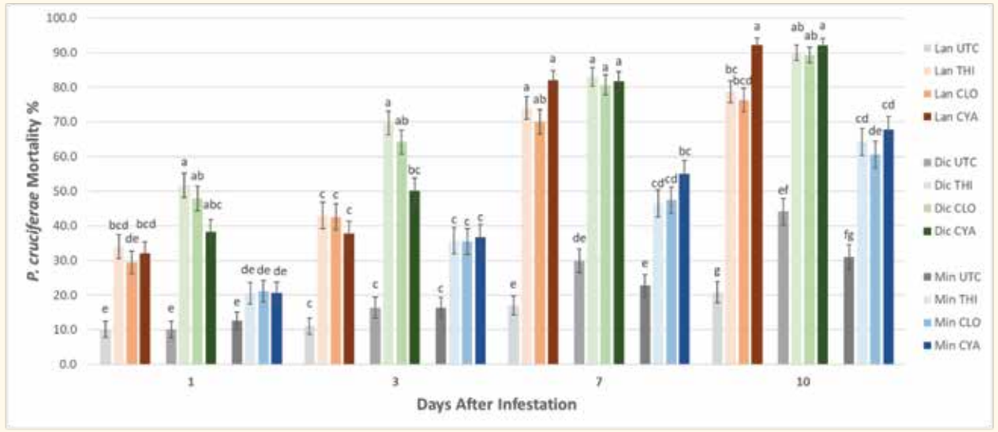


Fig. 2. Spring generation Phyllotreta cruciferae mean mortality percentage to three seed treatments (thiamethoxam 400 g/100 kg seed (THI), clothianidin 200.8 g/100 kg seed (CLO) and cyantraniliprole 1000 g/100 kg seed (CYA)) and an untreated control (UTC) to wild flea beetles originating from Langdon (LAN), Dickinson (DIC) and Minot (MIN), North Dakota, captured in 2020. Means are averaged across two days after planting (7 and 14). Letters represent differences from each day, separately, after initial infestation (Tukey's HSD, P < 0.05). Error bars represent standard error.

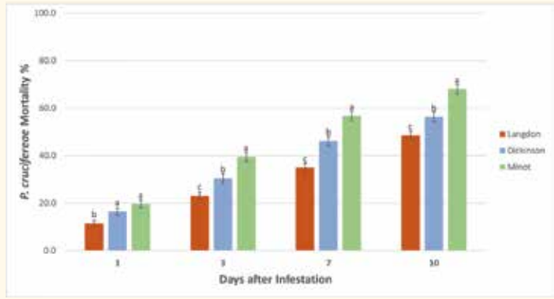


Fig. 3. Summer generation Phyllotreta cruciferae mean mortality percentage with flea beetles originating from three locations averaged across four seed treatments (thiamethoxam 400 g/100 kg seed, clothianidin 200.8 g/100 kg seed, cyantraniliprole 1000 g/100 kg seed and an untreated control) and two days after planting infestations (7 and 14 DAP). Letters represent differences from each day, separately, after initial infestation (Tukey's HSD, P < 0.05). Error bars represent standard error.

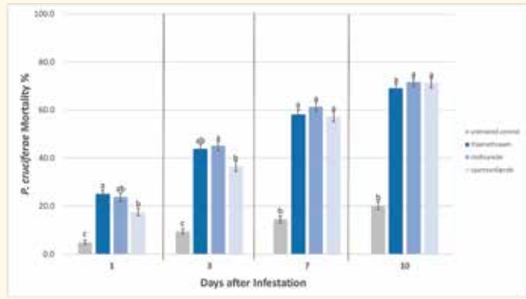


Fig 4. Summer generation Phyllotreta cruciferae mean mortality percentage of four seed treatments (thiamethoxam 400 g/100 kg seed, clothianidin 200.8 g/100 kg seed, cyantraniliprole 1000 g/100 kg seed and an untreated control) average across three locations (Langdon, Dickinson and Minot) and two days after planting infestations (7 and 14 DAP). Letters represent differences from each day, separately, after initial infestation (Tukey's HSD, P < 0.05). Error bars represent standard error.

Insecticide Treatment	1 DAI			3 DAI			7 DAI			10 DAI		
	LAN	DIC	MIN	LAN	DIC	MIN	LAN	DIC	MIN	LAN	DIC	MIN
	Flea beetle injury rating[a]											
Thiamethoxam (400 g/100 kg seed)	1.0de	1.1cd	1.5bc	2.1bcd	1.6def	2.3b	2.2de	2.0de	2.9c	2.5d	2.1de	3.1c
Clothianidin (200.8 g/100 kg seed)	1.0d	1.2cd	1.3cd	2.2bc	1.7cde	1.9bcd	2.3cd	2.2de	2.5cd	2.5d	2.3de	2.7cd
Cyantraniliprole (1000 g/100 kg seed)	0.7e	1.0de	1.0de	1.2ef	1.1f	1.2ef	1.4f	1.3f	1.6ef	1.4f	1.3f	1.7ef
Untreated control	1.8b	2.5a	2.5a	3.4a	3.8a	3.8a	3.5b	4.5a	4.4a	3.9b	4.9a	4.7a

Means across columns and rows followed by the same letter at each DAI are not significantly different at P < 0.005.

DAI = days after infestation, LAN= Langdon, DIC= Dickinson, and MIN= Minot.

[a] Flea beetle feeding injury rating scale of 1 = 1-3 pits per seedling; 2 = 4-9 pits; 3 = 10-15 pits; 4 = 16-25 pits; 5 > 25 pits, and 6 = dead plants.

Table 2. Loaction-based spring generation P. cruciferae feeding score injury ratings upon canola plants. Means are averaged across days after planting (7 and 14 DAP).

Insecticide Treatment	Days after Infestation			
	1	3	7	10
	Flea beetle injury rating[a]			
Thiamethoxam (400 g/100 kg seed)	1.2b	2.8b	3.2b	3.5b
Clothianidin (200.8 g/100 kg seed)	1.1b	2.1b	3.0b	3.1c
Cyantraniliprole (1000 g/100 kg seed)	0.7c	1.1c	1.5c	1.7d
Untreated control	2.5a	4.1a	5.1a	5.4a

Means in columns followed by the same letter are not significantly different at P<0.005.

[a] Flea beetle feeding injury rating scale of 1 = 1-3 pits per seedling; 2 = 4-9 pits; 3 =10-15 pits; 4 = 16-25 pits; 5 > 25 pits, and 6 = dead plants.

Table 3. Feeding scores by seed treatment for canola plants fed upon by summer generation P. cruciferae averaged across days after planting (7 and 14 DAP) and three locations (Langdon, Dickinson, and Minot) in 2020.

a feeding suppression agent. In both spring and summer generations, cyantraniliprole mortality becomes equal to thiamethoxam and clothianidin by Day 7 (Figs. 2 and 4).

Days after planting was significant for flea beetle mortality in the spring generation. Older plants (14 DAP) showed greater mortality impacts, approximately six to 10 percent more death, on flea beetle populations than 7 DAP treated plants. The summer generation was not impacted by days after planting likely due to their youth and higher fat reserves than the overwintering spring generation which has gone through fat-dwindling dormancy period.

OBJECTIVE 2
Economic Threshold – Correlating Flea Beetle Population Densities to Defoliation and Yield Loss

MATERIALS AND METHODS
A two-factor RCBD field experiment was conducted to determine economic injury levels of *Phyllotreta cruciferae*, crucifer flea beetle, on canola at the Langdon REC in 2020. Flea beetle populations of 0, 6, 12, 18, 24 and 30 flea beetles per sq. foot were added at three different crop stages of seedling, 2-lf, and 6-lf for six days. Both factors were considered fixed effects in the experiment. Five replications were used. Untreated InVigor L234P shatter-resistant canola (Bayer CropScience, Leverkusen, Germany) was seeded. Wild flea beetle pressure was high around caged plots, so much so that the original border plants died. We had to re-seed by hand due to loss of seedlings. Later in the season, grasshopper feeding was present in plots to a low degree.

CAPTURE & CARE OF WILD FLEA BEETLES
Overwintering adult *Phyllotreta cruciferae* beetles were collected from the wild using a flea beetle trap using 95% allyl isothiocyanate solution (Fig. 5).

TIMELINE
Canola was seeded on May 21, 2020. Emergence cages were placed on May 22 to May 24 (Fig. 6). Flea beetles were placed cotyledon-stage canola on May 30, 2-lf stage canola on June 4, and 5 to 6-lf stage canola on June 14. After the six day feeding window, flea beetles were removed via hand application of bifenthrin at a rate of 2.6 fl. oz/acre (2 lb. a.i. per gallon). Plant observations of plant death, visual estimate of cotyledon defoliation percent, a plant injury feeding score on number of pits and plant vigor (Table 1) were taken at this timing. Plots were hand-harvested on September 1 and 3. After hand-threshing, crop yield and oil content was measured.

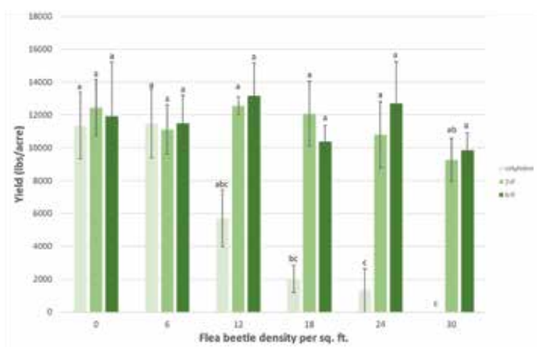


Fig. 7. Yield means (lbs/acre) by crop stage P. cruciferae infestation timing and flea beetle density per sq. ft for field grown canola at LREC, Langdon ND, in 2020. Letters with different letters are significantly different at P < 0.05 and standard error bars are presented.

Fig. 5. Traps set out along shelterbelt.

Fig. 6. Setting down cages at LREC.

DATA ANALYSIS
Statistical analysis of crop yield, pit count, pit score, vigor, flowering date, flowering period and oil percent was performed using PROC GLIMMEX by using SAS analysis systems (SAS Institute 2012). Block was considered random and all other factors were considered fixed effects. Means separation was performed using LSMEANS with Tukey's HSD test (α = 0.05). Linear regression statistics using EXCEL were done with the cotyledon, 2-lf and 6-lf data sets with significance level of P < 0.05.

RESULTS
Yield. Yield was impacted by flea beetle density and crop stage (df = 10; P = 0.008). At the cotyledon-stage infestation, canola yields were similar in low densities of 0, 6 and 12 flea beetles per sq. ft, but by 18 flea beetles per sq. ft. and higher, canola yields dropped (Fig. 7). No differences were seen in canola yields when plants were infested at the 2-lf and 6-lf stages.

Yield Regression for EIL. Only the cotyledon stage data set had significance (df = 1; P < 0.001; R2=0.6376). As flea beetle density increased, the canola yield decreased (Fig. 8). The 2-lf and 6-lf regressions lines were not significant (df = 1; 2-lf, P = 0.233; 6-lf, P = 0.609).

Calculating an Economic Injury Level – Cotyledon Stage. Using Pedigo's economic injury level equation, an cotyledon EIL was calculated > 1 flea beetle per plant using factors of market price of $11.30 per lb, a POST-foliar insecticide cost $2 per acre and an additional $7 per acre for custom application.

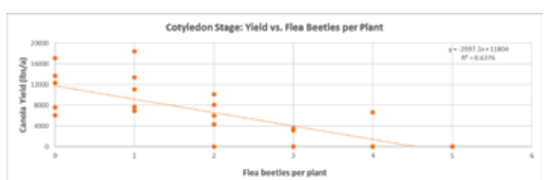


Fig. 8. Canola yield scattergram with linear regression line, linear equation and R2 value for cotyledon stage canola infested with P. cruciferae at six different flea beetle densities in a field trial at LREC, Langdon ND, 2020.

Other agronomic factors. All agronomic observations of plant death, defoliation percentage, pit count, plant injury feeding score, plant vigor, first flower, and flowering period had a significant interaction of crop stage introduction by flea beetle density (df = 10; plant death, defoliation percentage, pit count, plant vigor, first flower P <0.0001; plant injury feeding score and flowering period, P = 0.0001). At the cotyledon stage, higher densities of flea beetles had increased feeding activity, lower plant vigor and delayed flowering (Tables 4 and 5).

DISCUSSION
The cotyledon stage infested plants showed the predicted negative yield line (Fig. 8) with an economic injury level of less than one flea beetle per plant. Due to wild flea beetles killing border plants and subsequent hand-reseeding around the cage-protected plots, canola plants in this study were able to thrive by branching out into open unoccupied space. Canola yields were very high – though we expect high yields when the plot size is one sq. ft. We observed quite of number of plots with plant death in 2020 and it more than in other years.

Flea beetles were hungry. The no rainfall weather period during infestation may have slowed development of plants which is advantageous to flea beetle injury. This perfect environment pushed the economic injury level regression line to steep slope (Fig. 8). This year after 6 days of feeding, a rate of 1 flea beetle per plant (6 flea beetles per sq. ft) had a mean 25% defoliation (Table 6) which is more than current IPM recommendations.

Flea beetle injury to canola at the 2-lf and 6-lf stages was not severe enough to show a yield response (Fig. 6). No significant regression line at the 2-lf stage is somewhat of surprise, however we did see the same in the 2018 field study.

Other agronomic observations like plant vigor, flowering date and oil percentage were expected results. Severe flea beetle damage will delay flowering and flowering period may be shortened due the canola's programmed flowering response to shortening daylight. Flea beetle injury is expected to reduce plant vigor. Oil yield has not been impacted by flea beetle feeding in previous years studies.

Plant Stage	*P. cruciferae*	Plant Death	Defoliation	Pit Count	Flea Beetle Injury Rating[a]	Plant Vigor
	per sq. ft.	No. of dead plants	Percent Loss	No. of pits		0 - 100 (L to H)
Cotyledon	0	0.0 d	0.0 f	0.0 h	0.0 h	99.0 a
	6	0.2 d	25.7 cde	23.6 efg	4.2 abcdef	73.0 abc
	12	2.6 c	68.7 b	49.3 bc	5.3 abc	25.0 de
	18	4.0 bc	90.6 a	58.0 abc	5.3 abc	12.0 e
	24	5.2 ab	98.0 a	64.8 ab	5.7 a	3.0 e
	30	6.0 a	100.0 a	70.0 a	6.0 a	0.0 e
2-lf	0	0.0 d	0.0 f	0.0 h	0.0 h	91.0 ab
	6	0.0 d	9.1 fe	14.9 fgh	3.3 bcdefg	81.0 abc
	12	0.0 d	11.8 def	18.2 fgh	4.5 abcde	78.0 abc
	18	0.0 d	17.6 cdef	27.2 def	5.1 abcd	66.0 bc
	24	0.2 d	34.9 c	41.5 cde	5.4 ab	55.0 cd
	30	0.0 d	28.9 cd	43.4 cd	5.5 a	66.0 bc
6-lf	0	0.0 d	0.0 f	0.0 h	1.2 gh	84.0 abc
	6	0.0 d	6.1 f	7.5 gh	2.2 fg	93.0 ab
	12	0.0 d	6.1 f	7.7 gh	2.3 fg	97.0 ab
	18	0.0 d	6.1 f	7.8 gh	2.9 efg	97.0 ab
	24	0.0 d	9.8 fe	13.0 fgh	3.2 cdefg	93.0 ab
	30	0.0 d	7.4 fe	11.4 fgh	3.0 defg	94.0 ab

[a] Flea beetle feeding injury rating scale of 1 = 1-3 pits per seedling; 2 = 4-9 pits; 3 =10-15 pits; 4 = 16-25 pits; 5 > 25 pits, and 6 = dead plants.
Columns with different letters are significant at the P < 0.05.

Table 4. Plant death, defoliation percentage, pit counts, flea beetle injury rating and plant vigor rating to field grown canola where five densities of flea beetles were added at three crop stages at LREC, Langdon ND, 2020.

Plant Stage	*P. cruciferae*	First Flower	Flowering Period	Canola Oil %[NS]
	per sq. ft.	Julian Date	Days	adjusted to 8.5%
Cotyledon	0	183.2 c	25.8 a	40.5
	6	184.2 bc	31.4 a	41.2
	12	186.2 b	24.76 a	41.7
	18	186 b	24.32 a	40.0
	24	199 a	14 b	42.3
	30	n/a	n/a	n/a
2-lf	0	184.6 bc	24 a	41.6
	6	183.2 c	23.8 a	41.6
	12	183 c	26.8 a	41.2
	18	183.6 bc	23.2 a	41.4
	24	184.4 bc	24.6 a	41.1
	30	183.6 bc	24.6 a	41.4
6-lf	0	183 c	24 a	41.6
	6	183.6 bc	23.6 a	41.1
	12	182.8 c	24.2 a	40.0
	18	183 c	28.6 a	41.0
	24	183.2 c	23.8 a	41.5
	30	183.8 bc	24.8 a	39.8

NS = not significant; N/A = not available due to dead plants.
Columns with different letters are significant at the P < 0.05.

Table 5. First flower date, flowering period length, and canola oil means to field grown canola where five densities of flea beetles were added at three crop stages in Langdone, ND, 2020.

Insecticide Treatment	Days after Infestation			
	1	3	7	10
	Flea beetle injury rating[a]			
Thiamethoxam (400 g/100 kg seed)	1.2b	2.3b	3.2b	3.5b
Clothianidin (200.8 g/100 kg seed)	1.1b	2.1b	3.0b	3.1c
Cyantraniliprole (1000 g/100 kg seed)	0.7c	1.1c	1.5c	1.7d
Untreated control	2.5a	4.1a	5.1a	5.4a

Means in columns followed by the same letter are not significantly different at P < 0.05.
[a] Flea beetle feeding injury rating scale of 1 = 1-3 pits per seedling; 2 = 4-9 pits; 3 =10-15 pits; 4 = 16-25 pits; 5 > 25 pits, and 6 = dead plants.

Table 6. Feeding scores by seed treatment for canola plants fed upon by summer generation P. cruciferae averaged across days after planting (7 and 14 DAP) and three locations (Langdon, Dickinson and Minot) in 2020.

NDSU canola breeder, Dr. Mukhlesur Rahman.

Annual Report 2019-20: Increasing Genetic Diversity in Spring Canola *(Brassica napus L.)*

Rapeseed/canola (*Brassica napus L.*) was evolved in 13th century through spontaneous interspecific hybridization between its two diploid species *B. rapa* L. and *B. oleracea* L. Because of recent cultivation history and intensive breeding within different types, this species has a relatively narrow genetic diversity. Thus, introgression of subgenomic component from the two-progenitor species or related species can broaden the genetic diversity in oilseed *B. napus*. Increasing genetic diversity in spring canola is needed for increased productivity and to improve agronomy to withstand both in biotic and abiotic stresses. Interspecific crosses may bring many undesirable agronomic and seed quality traits, which require intensive or repeated breeding cycles and long-term commitment to develop elite breeding lines for use in practical breeding programs. Currently, canola cultivar development is led by commercial seed companies, where repeated breeding cycles and long-term commitments using methods such as interspecific crosses are not a preferred method for cultivar development. The NDSU canola breeding program is especially well set up to introgress new traits into canola using interspecific crosses and then license the improved inbred lines for use by commercial seed companies. Development of high yielding hybrid canola cultivars requires the presence of adequate genetic diversity among the parental inbred lines, which is possible through this type of research. The NDSU canola breeding program has in place a joint-research program with DL Seeds Inc. and Cibus to provide inbred lines under a Material Transfer Agreement (MTA) to determine their utility as an inbred in their respective hybrids.

We have screened 400 *B. rapa*, 120 *B. juncea*, 70 *B. carinata*, and 100 *B. oleracea* in the greenhouse. Data on days to flowering, days to maturity, breeder's score were taken. Finally, we have selected 40 *B. rapa*, 20 *B. juncea*, 10 *B. carinata*, and 4 *B. oleracea* germplasm accessions. We planted the selected lines of the three different species of *Brassica* in the field at Prosper in summer 2019. All we know that 2019 was a difficult year of heavy rainfall, which causes prolonged flooding in the breeding nursery at Prosper, resulted mortality of all accessions in the field. Therefore, our field evaluation was unsuccessful in 2019. However, we will evaluate them again in the greenhouse. In 2019-2020, we will made crosses between *Brassica napus* (5 breeding lines) and *B. rapa* (9 accessions), *B. napus* (5 breeding lines) and *B. juncea* (20 accessions), and *B. napus* (5 breeding lines) and *B. carinata* (2 accessions). All the F1s will be advanced to F2 in greenhouse in spring 2020 season. The F2 populations will be planted in the field in summer 2020 at Prosper for primary selection.

PRINCIPAL INVESTIGATOR
Dr. Mukhlesur Rahman

Annual Report 2019-20: Canola Breeding for Increasing Oil Yield Per Acre

2019 canola planting for Sclerotinia stem rot disease screening under misting irrigation system.

Genetic diversity is very important for increasing the variability in crop for improvement of various traits. The NDSU canola breeding program is devoted to create new germplasm by utilizing genetically diverse *Brassica napus* accessions and is testing in different regions of North Dakota to identify the best cultivars with desired traits. The breeding program works closely with the collaborative researchers. The program also continue its existing breeding collaboration with DL Seeds Inc., and Cibus for hybrid canola development program, and Caldbeck Consulting (Kentucky) for non-GMO canola development.

This project will allow broadening the genetic base of canola lines to develop new high seed yield, high oil content, disease resistant, and desired agronomy cultivars for North Dakota. The new germplasm will be released as open-pollinated non-GMO cultivars for canola growers or as inbred lines (A lines and B lines) for hybrid cultivar development, or as parents in other breeding programs or to license to commercial company to make it available for North Dakota. The breeding lines with valuable traits can also be shared with other public breeding program to expand the production of U.S. canola. The ultimate goal of the project is to develop and release elite canola varieties adapted to North Dakota that will bring benefit to canola growers through increased yields, targeted traits and premiums, and net farm income.

The 2019 summer testing program included 1,842 genotypes (Breeding lines + F2), and evaluated in 2,741 plots at 6 locations in North Dakota. The canola testing locations were distributed across the state, including, Fargo, Prosper, Carrington, Minot, Williston, and Hettinger of North Dakota. The experimental trials include (1) wide-area trial of 36 germplasm with three replications at five locations, (2) advanced yield trial of 81 germplasm with two replications at three locations, (3) early generation trial of 270 germplasm with replicated checks at two locations, (4) joint hybrids with DL seeds of 30 hybrids with three replications in two locations, (5) blackleg disease screening of 36 advanced lines in field at Fargo, (6) sclerotinia stem rot disease screening of 36 advanced lines in field at Fargo, (7) performance yield trial of three new with 4 commercial hybrids on 10ft x 360ft each plots (8) 1,842 single rows of *B. napus, B. juncea, B. carinata* were planted at Prosper breeding nursery. The data on seed yield, seed moisture at harvest, early vigor, days to flowering, relative maturity, standability, lodging, breeder's impression were taken from each trial plots. We have been using four greenhouse rooms at NDSU new greenhouse complex for canola breeding program. Crossing, backcrossing and selfing are conducting in the greenhouse. We are growing three crop cycles in the greenhouse per year. A total of about 6,000 plants were planted and harvested in three growing cycles of the year. Seed quality (oil content and fatty acids) analysis is conducted at Northern Crops Institute (NCI), Fargo, ND.

In 2019 North Dakota Canola Variety Trial, six advanced open pollinated non-GMO canola breeding lines from NDSU canola breeding program were evaluated at five locations (Langdon, Minot, Carrington, Williston, Hettinger) in North Dakota. Among them, three advanced breeding lines were out-performed over commercial hybrids from BASF, Croplan, Cargill, Canterra Seeds, Dyna Gro, BrettYoung, Photosyntech. These lines were identified from a segregating population derived from winter- and spring-type canola crosses.

PRINCIPAL INVESTIGATOR
Dr. Mukhlesur Rahman

Brassica oleracea flowering in the greenhouse.

Annual Report 2020-21: Increasing Genetic Diversity in Spring Canola *(Brassica napus L.)*

Rapeseed/canola (*Brassica napus L.*) was evolved in 13th century through spontaneous interspecific hybridization between its two diploid species *B. rapa* L. and *B. oleracea* L. Because of recent cultivation history and intensive breeding within different types, this species has a relatively narrow genetic diversity. Thus, introgression of subgenomic component from the two-progenitor species or related species can broaden the genetic diversity in oilseed *B. napus*. Increasing genetic diversity in spring canola is needed for increased productivity and to improve agronomy to withstand both in biotic and abiotic stresses. Interspecific crosses may bring many undesirable agronomic and seed quality traits, which require intensive or repeated breeding cycles and long-term commitment to develop elite breeding lines for use in practical breeding programs. Currently, canola cultivar development is led by commercial seed companies, where repeated breeding cycles and long-term commitments using methods such as interspecific crosses are not a preferred method for cultivar development. The NDSU canola breeding program is especially well set up to introgress new traits into canola using interspecific crosses and then license the improved inbred lines for use by commercial seed companies. Development of high yielding hybrid canola cultivars requires the presence of adequate genetic diversity among the parental inbred lines, which is possible through this type of research. The NDSU canola breeding program has in place a joint-research program with Cibus to provide inbred lines under a Material Transfer Agreement (MTA) to determine their utility as an inbred in their respective hybrids.

We have screened 400 *B. rapa*, 120 *B. juncea*, 70 *B. carinata*, and 100 *B. oleracea* in the greenhouse. Data on days to flowering, days to maturity, breeder's score were taken. In 2019-2020, we will made crosses between *Brassica napus* (5 breeding lines) and *B. rapa* (9 accessions), *B. napus* (5 breeding lines) and *B. juncea* (20 accessions), and *B. napus* (5 breeding lines) and *B. carinata* (2 accessions). All the F1s have been advanced to F2 in greenhouse in spring and summer 2020 season. The F1s were also backcrossed to advanced canola breeding lines (NDOLA-01, NDC-E16198) to introgress better agronomic traits into newly generated lines. The F2 and BC1 populations have been planted in the 2020 fall greenhouse program to develop F3 and BC2 populations. We will continue the selfing and backcrossing program in the greenhouse. We will also plant F3 and BC2 populations in the field in summer 2021 in single row at Fargo under proper irrigation system.

PRINCIPAL INVESTIGATOR
Dr. Mukhlesur Rahman

Annual Report 2020-21: Canola Breeding for Increasing Oil Yield Per Acre

Genetic diversity is very important for increasing the variability in crop for improvement of various traits. The NDSU canola breeding program is devoted to create new germplasm by utilizing genetically diverse *Brassica napus* accessions and is testing in different regions of North Dakota to identify the best cultivars with desired traits. The breeding program works closely with the collaborative researchers. The program also continue its existing breeding collaboration with DL Seeds Inc., and Cibus for hybrid canola development program, and Caldbeck Consulting (Kentucky) for non-GMO canola development.

This project will allow broadening the genetic base of canola lines to develop new high seed yield, high oil content, disease resistant, and desired agronomy cultivars for North Dakota. The new germplasm will be released as open-pollinated non-GMO cultivars for canola growers or as inbred lines (A lines and B lines) for hybrid cultivar development, or as parents in other breeding programs or to license to commercial company to make it available for North Dakota. The breeding lines with valuable traits can also be shared with other public breeding program to expand the production of U.S. canola. The ultimate goal of the project is to develop and release elite canola varieties adapted to North Dakota that will bring benefit to canola growers through increased yields, targeted traits and premiums, and net farm income.

The 2020 summer testing program included 1,842 genotypes (Breeding lines + F2), and evaluated in 2,741 plots at 6 locations in North Dakota. The canola testing locations were distributed across the state, including, Fargo, Prosper, Carrington, Minot, Williston, and Hettinger of North Dakota. The experimental trials include (1) wide-area trial of 36 germplasm with three replications at six locations, (2) advanced yield trial of 64 germplasm with three replications at three locations, (3) early generation trial of 324 germplasm with replicated checks at three locations, (4) joint hybrids with CIBUS of 12 hybrids with three replications in three locations, (5) sclerotinia stem rot disease screening of 152 lines in the field of Carrington and Osnabrock, (6) shattering tolerant study of 150 lines in the field of Fargo, Carrington and Osnabrock, (7) a total of 1,092 single rows of *B. napus*, were planted in a single-row breeding nursery at Prosper and 500 F3 breeding lines were planted at Fargo – all the lines were self-pollinated using micro-perforated selfing bags and bamboo sticks in the nursery. The data on seed yield, seed moisture at harvest, early vigor, days to flowering, relative maturity, standability, lodging, breeder's impression were taken from each trial plots. We have been using four greenhouse rooms at NDSU new greenhouse complex for canola breeding program. Crossing, backcrossing and selfing are conducting in the greenhouse. We are growing three crop cycles in the greenhouse per year. A total of about 10,000 plants were planted and harvested in three growing cycles of the year. Seed quality (oil content and fatty acids) analysis is conducted at Northern Crops Institute (NCI), Fargo, ND.

In 2020 North Dakota Canola Variety Trial, six advanced open pollinated non-GMO canola breeding lines from NDSU canola breeding program were evaluated at five locations (Langdon, Minot, Carrington, Williston, Hettinger) in North Dakota. Due to unavaoidable environmental condition, the experiments at Minot and Hettinger were abandoned. Among the three locations, two advanced breeding lines (NDC-E12079 and NDC-E16257) were out-performed over trial means where commercial hybrids from BASF, Croplan, Cargill, Canterra Seeds, Dyna Gro, BrettYoung, Photosyntech were included. These lines were identified from a segregating population derived from winter- and spring-type canola crosses.

2019 canola planting at Carrington, ND.

Northern CANOLA GROWERS Association

WWW.NORTHERNCANOLA.COM

Northern CANOLA GROWERS Association

WWW.NORTHERNCANOLA.COM

125 Slate Drive Suite #4
Bismarck, ND 58503
P: 701.223.4124