Appendix A. PROGRESS REPORT 2015-2017
INTEGRATED APPROACHES FOR FLEA BEETLE CONTROL – ECONOMIC THRESHOLDS, PREDICTION MODELS, LANDSCAPE EFFECTS, AND NATURAL ENEMIES

Objective 1: To determine economic threshold for flea beetles

Methodology:

During the three two seasons of the project, we conducted 41 economic threshold field trials in four regions. The experiments were conducted in standard agronomic small plots (1.22-4.25 x 7-12 m) arranged as four to five completely randomized blocks, with five treatments:

1) Unsprayed control
2) Neonicotinoid treated seed with no foliar insecticide spray (seed)
3) Foliar insecticide spray at 15-20% defoliation threshold (def15)
4) Foliar insecticide spray at 25% defoliation threshold (def25)
5) Foliar insecticide spray at 45% defoliation threshold (def45)

For 2016 and 2017, we secured an in-kind collaboration with Syngenta (Dr. Ted Labun and Lorne Letkeman) to obtain SY 4135 (Roundup Ready® hybrid canola) seed treated with the fungicide treatments for the Helix Vibrance technology for treatments 1, and 3-5. The seed treatment (2) received the complete Helix Vibrance treatment (fungicide + neonicotinoid insecticide). This hybrid was selected to ensure reasonable yields over a wide range of geographical conditions. These seeds were used for the trials in Lethbridge, Saskatoon, and Manitoba. In 2015, the short time we had after notification of the unofficial grant approval prevented us from obtaining fungicide only treated seeds, and therefore seed in treatments 1, and 3-5 were not treated with fungicide and seed in treatment 2 had the full Helix Vibrance treatment. Seed used in 2015 was also kindly provided by Syngenta SY 4135 (Roundup Ready® hybrid canola). For Lethbridge, a 6^{th} treatment was included: Neonicotinoid treated seed with no foliar insecticide spray at seedling (seed) but sprayed at flower or pod to quantify seedpod weevil or lygus yield damage. For the Peace River region trials, a different seed source was utilized. In Saskatchewan, a spray treatment with the seed treatment was also tested.

In each of the treatment plots, canola defoliation by flea beetles was assessed in two 1-m transects per plot, 3 times a week, following the methods by Soroka et al. (2011). This monitoring continued until plants reached the 2.2 phenological stage. Abundances of different flea beetle species and generalist natural enemies were assessed by trapping them with the double sided yellow sticky cards (18 x 14 cm^2, Alpha Scent), placed in the middle of each plot and about 3 cm above the ground level until plants reached the 2.2 stage and then to canopy level (Fig. 1). Sticky traps were replaced weekly and maintained until the 2.2 stage in treated plots (treatments 2-5), and the whole season for unsprayed control plots. In each plot, we assessed canola emergence/survivorship counts by fixing two quadrats (0.5 x 0.5 m), one in the front and one in the back of the plot and assessing the number of plants alive within the same quadrat when plants reached the 1.0 and 2.2 stages.

When the average defoliation of a sprayed treatment (treatments 3-5) reached its threshold, the plots were sprayed with Matador (lambda-cyhalothrin) at 34 ml/ac, within 24 h of the assessment. Repeated insecticide applications were performed when thresholds were reached on more than one occasion per treatment.

a

b

Fig. 1. a) Flags indicating the two quadrats (0.5m x 0.5 m) per plot used to assess plant survivorship, and b) sticky cards used to assess flea beetle and natural enemy populations in the economic threshold plots in Manitoba, 2016.

In Lethbridge, canola was desiccated and then straight cut on Sept 1 (LRDC), Sept 17 (Farming smarter – April 29), Sept 20 (Farming smarter – May 26), Aug 31 (Vauxhall – May 2) and Sept 20 (Vauxhall – May 26). In Manitoba, canola was swathed and then harvested on Aug 22 (Arb.Early), Sept 22 (Carman Early) and Sept 27 (Carman Late). Canola yield (plot weight of the seeds and 1000-seed kernel weight) and quality parameters (the number of green seeds per two hundred seed samples, oil, protein, fat, and chlorophyll) were determined in all the experiments.

Here, we report the results of the initial analysis of 22 economic threshold trials (Figures 2 - 23). A more detailed regression analysis of these results to determine economic threshold considering other variables is in progress.

2015 Field trials

In 2015, we conducted five economic threshold trials in three regions: Lethbridge (AB)-2 trials, Peace River (AB)-1 trial, and Manitoba-2 trials. In Lethbridge, the trials were conducted at the Lethbridge and Vauxhall areas. In Manitoba, the trials were conducted at the University of Manitoba campus (Bison plots) and in Carman. In the Peace River region, the trial was conducted at the AAFC Research Station near Beaverlodge.

In Carman, we only had the 15-20% foliar spray treatment, as defoliation never exceeded 25%, but in Vauxhall, both 15-20% and 25% defoliation treatments were performed. Hail damage (Bison trial) and insufficient flea beetle damage (Lethbridge trial) prevented us from having meaningful yield data for these two trials.

2016 Field trials

In 2016, we increased the number of trials performed in all the provinces to maximize the chances of having significant flea beetle damage. We also expanded our collaboration with Dr. Tyler Wist at AAFC, Saskatchewan, to include economic threshold trials near Saskatoon. This allowed us to expand economic threshold trials in a broader geographic range in the Prairies. In addition, to maximize the chances to have peak flea beetle populations at susceptible stages of canola, we performed multiple seeding date trials at multiple locations in some of our regions.

In Manitoba, 9 trials were conducted at Carman, Glenlea and at the University of Manitoba field plot at the Arboretum (Arb). There were two early planted trials in early May (Arb. and Carman), one trial in mid- May (Glenlea) and 4 late planted trials in late May or early June; two trials were planted in late June due to broken machinery. Five trials out of the total nine were sprayed for flea beetles. Among the sprayed trials, 2 trials were not harvested due to delayed crop maturity and waterlogging conditions in the field (Glenlea and Arb. trials).

In the Lethbridge region, 5 trials were conducted in total. Three trials were planted early, (end of April or early May; two near Lethbridge and one near Vauxhall), and two were planted late (near the end of May at two locations, near Lethbridge and Vauxhall). The Farming Smarter late-seeded trial (about 4 km east of Lethbridge) had gopher damage, a late herbicide application, flooding and a number of missing samples caused by inadequate storage/handling so that only about half of the plots produced meaningful data for future regression analysis.

In Saskatchewan, 4 trials were conducted in two locations (SEF, Llewellyn) and at two planting dates. The early planting date was the same at each site, and all early planted blocks were seeded on May 16^{th,} 2016 at both sites. The late seeding date for both sites was June 1^{st,} 2016.

In Peace River area, one trial was conducted at the AAFC research station at the normal planting date period matching peak flea beetle populations.

2017 Field trials

In 2017, we conducted 17 experiments; in Manitoba (7), Lethbridge (4), Saskatchewan (5), and Peace River (1).

In Manitoba, 7 trials were conducted at Carman, Bison and Fort Whyte. There were three trials planted in early May (Carman S1 early, Carman S2 early, and Kevin early), two trials were conducted in mid- May (Carman S1 mid, Carman S2 mid), and two trials were planted in late May (Kevin mid, and Bison). All seven trials were sprayed for one or more of the treatments. All the sprayed trials were harvested.

In Lethbridge, two trials were planted early (May 5 and 9; one near Lethbridge and one near Vauxhall), and two were planted late (May 26 and 27 at the same two locations). The late-seeded trial done by Farming Smarter had a very little flea beetle damage and was not sprayed.

In Saskatchewan, three sites were planted with early seeded canola, and two sites were planted with late seeded canola. One of the sites experienced heavy damage as soon as the cotyledons emerged and was well over the 45% damage threshold within two days.

In Peace River area, one trial was conducted at the AAFC research station within the normal planting period matching peak flea beetle populations.

Results, recommendations, and conclusions:

Here we present the analysis of the results of 22 economic threshold trials out of 30 harvested trials; the rest of the trials have data that is still being processed for the final regression analysis and for the publication. We also have a very large dataset of defoliation estimates and plant stand variables that we are still in the process of summarizing and will be used to relate defoliation with the abundance of flea beetles observed in the plots.

Treatments that were not sprayed due to low damage were combined with the unsprayed control for analyses. Generally, the late seeded trials analyzed (two in 2015: Fig. 2 and 3, three in 2016: Fig. 18-20, and three in 2017: Fig. 21-23) had lower flea beetle damage than in early seeded plots, and it was not possible to assess the effects of defoliation at 45% in any of these trials. Overall, the trials analyzed so far indicate no significant yield differences among treatments in the late season trials in all the three years except for the Kevin trial in Manitoba in 2017, which had a significant yield increase when sprayed at 15% defoliation (Fig. 22).

In all the three years in the 14 early seeded trials analyzed (Fig. 4-17), the damage level reached 45% defoliation in five of the trials, and we were able to test all threshold levels on them; other trials experienced moderate flea beetle damage.

In 2016, at Arb and Fairfield, the treatment sprayed with a foliar insecticide at the current nominal threshold of 25% had numerically lower yield than those that had the seed-coated insecticide, but the yield was significantly different only in Fairfield (Fig. 4 and 5). At Vauxhall, there was no significant yield benefit by the seed treatment or by foliar spray of insecticide for flea beetles, although it is not clear why controls yielded more than some of the sprayed treatments (Fig. 6). The early seeded trials at Lethbridge and Carman received lower flea beetle damage compared to the Arb. and Fairfield plots and show no difference in yield among treatments (Figs. 7 and 8). At both Saskatchewan sites, 25% defoliation treatments had numerically higher yields than the seed coated insecticide treatment (Figs. 9 and 10).

In 2017, there were 7 early seeded trials (two trials at Carman were planted in the second week of May and were included in the early seeded trials). The trial conducted at Lethbridge (FS early, Fig.11) had a numerically higher yield in the seed treatment and in the 25% defoliation treatment, which was sprayed at 30% defoliation level. At Vaxuhall (Fig.12), there was a high flea beetle pressure, and defoliation levels exceeded 34% when the 25% and the 15-20%

defoliation treatments were sprayed. There was no significant yield increase in any of the sprayed treatments. Treated seed had numerically higher yield than the control. In this trial, there was another treatment for cabbage seed pod weevil and the treatment for the cabbage seed pod weevil increased the yield significantly.

In Manitoba, none of the 45% defoliation treatments were got sprayed due to low flea beetle pressure during the susceptible stage of the crop. In most cases, we were able to spray 25% and 15% treatments. No significant yield increase was obtained in any of the Manitoba trials. Numerically higher yield was obtained in two def15 treatments (Fig. 15 and 17). In one of the def25 trials, we had numerical yield increase (Fig.15). Carman S1 (Fig.13) had less flea beetle pressure when the crop was in the phenology of 1 - 2.3 stage, but there was an increase in flea beetle numbers after the 2.4 stage. Defoliation treatments of 15-20% and 25% were sprayed, but there was no significant yield increase. The possible reasons for the non-significant results in Manitoba sites might be the lower flea beetle pressure and a buildup of weed pressure in the plots at the late season.

Among the 22 trials evaluated in this study, flea beetle pressure allowed spraying at the 45% defoliation level in 5 trials, at the 25% defoliation level in 15 trials, and at the 15-20% level on 16 trials. The seed treatment resulted in statistically higher yield than the unsprayed control in 3 trials (Fig. 4, 5 and 22) and higher yield than the 25% defoliation treatment in 1 trial (Fig. 5). The 15-20% defoliation treatment increased significantly yield than the unsprayed control in 2 trials (Fig. 10 and 22). Finally, the 25% defoliation treatment increased significantly yield over the control in 2 trials (Fig. 5 and 9). The high variability in yield prevented statistical detection of other differences, but there were numerical trends in the data that could be interpreted with caution. For example, the seed treatment increased numerically yield compared to the unsprayed control in 10 trials, compared to the 15-20% defoliation treatment in 3 trials, compared to the 25% defoliation treatment on 7 trials and compared to the 45% defoliation on 3 trials. The 15-20% defoliation treatment increased numerically yield compared to the unsprayed control in 5 trials, compared to the 25% defoliation treatment in 4 trials, and compared to the seed treatment in 4 trials. The 25% defoliation treatment increased numerically yield compared to the unsprayed control in 6 trials, compared to the 45% defoliation treatment in 3 trials, and compared to the seed treatment in 1 trial. The 45% defoliation treatment increased numerically yield compared to the unsprayed control in 3 trials. Overall, these initial analyses suggest that the seed treatment is the most frequently associated with yield increases, followed by the 15-20% and 25% defoliation treatments. Moreover, in two of the three statistically significant trials, the yield of the seed and 25% defoliation treatments did not differ (Fig. 4 and 22). This preliminary analysis supports the use of a nominal economic threshold of 25% defoliation. A more refined analysis of the data is in progress to consider other variables that affect yield, including plant density and the actual defoliation levels in plots.

2015- Economic threshold trials1

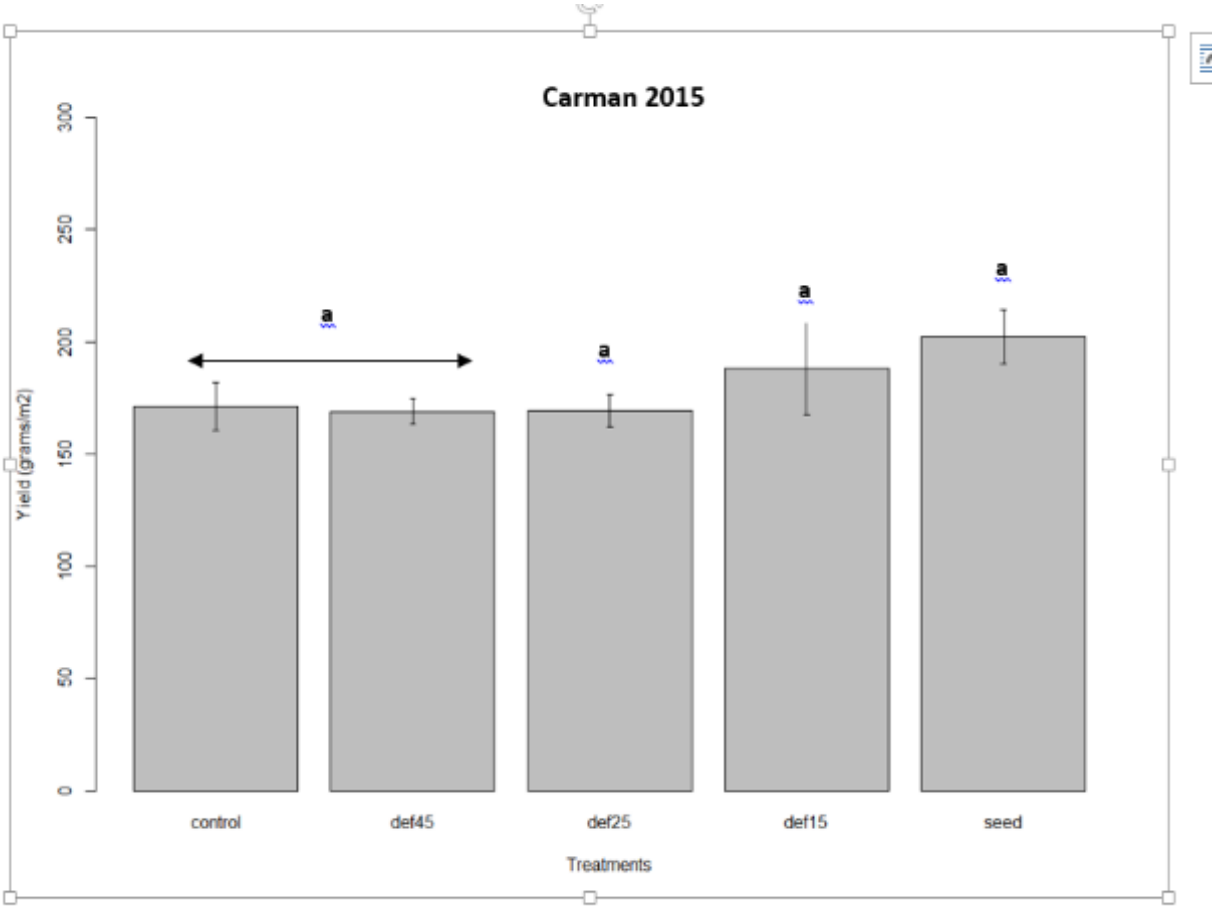


Fig.2. Yield (g/m^2) of canola from economic threshold small plot study conducted at Carman, Manitoba. Control: unsprayed control; def45: 45% defoliation; def25: 25% defoliation; def15: 15-20% defoliation; seed: seed treatment

ANOVA-Yield

Carman-2015 (Yield)	df	Sum sq	Mean sq	F value	Pr(>F)
Treatment	3	3492	1163.9	3.210	0.0585
Block	3	4560	1520.2	4.193	0.0279
Residuals	13	4713	362.5		

*Control and def45 were combined in the analysis

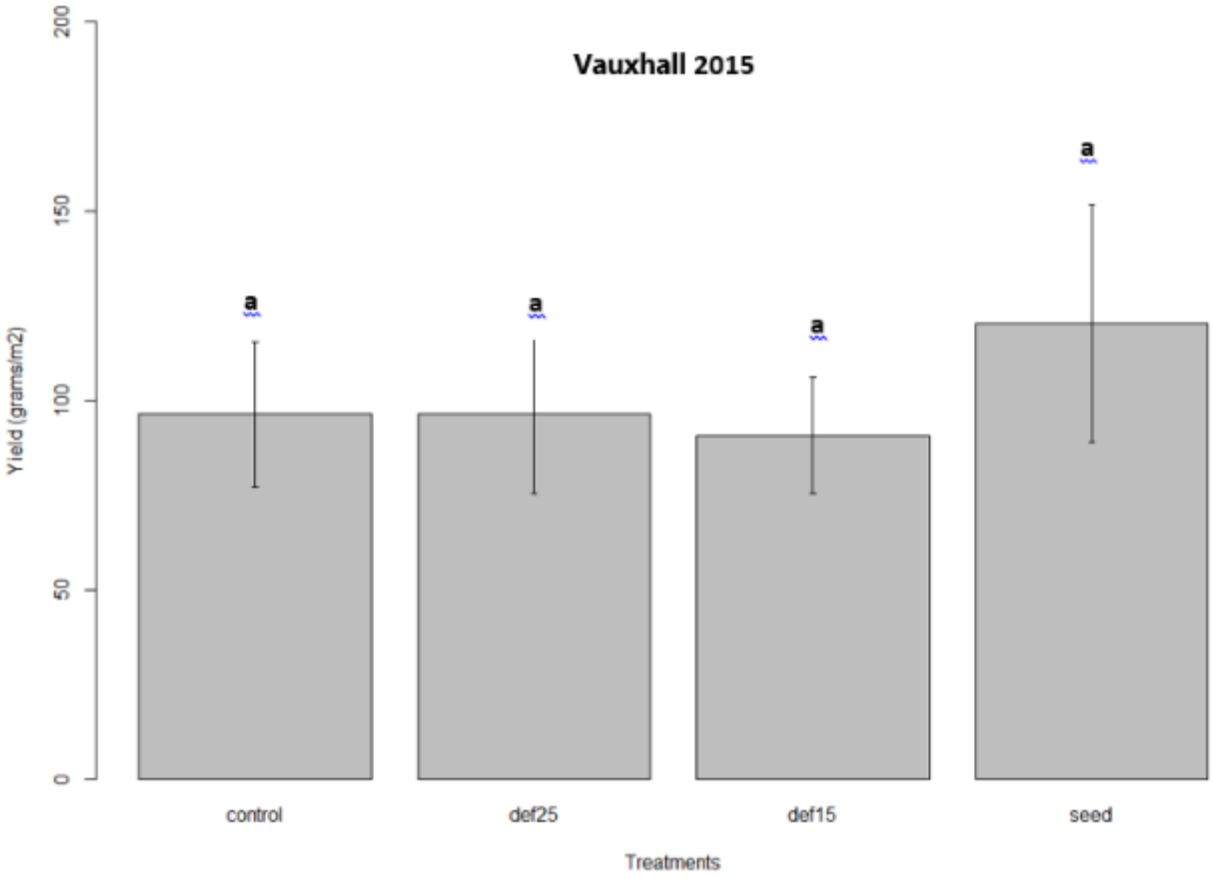


Fig. 3. Yield (g/m^2) of canola from the economic threshold small plot study conducted at Lethbridge, Alberta

ANOVA-Yield

Vaxuall-2015 (Yield)	df	Sum sq	Mean sq	F value	Pr(>F)
Treatment	3	2100	700	1.092	0.40134
Block	3	18433	6144	9.582	0.00366 **
Residuals	9	5771	641		

2016-Early seeded economic threshold trials

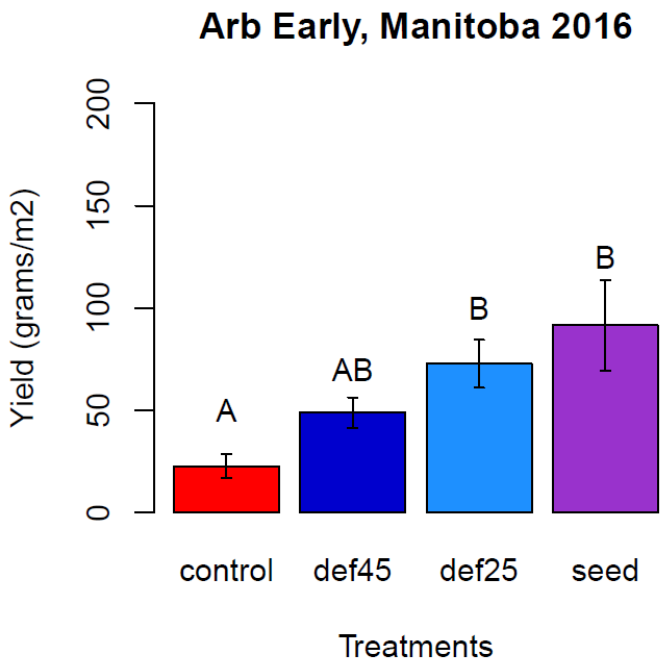


Fig.4. Yield (g/m^2) of canola from economic threshold study conducted at Arb., Manitoba, 2016

ANOVA-Yield

Arb-Early (Yield)	df	Sum sq	Mean sq	F value	Pr(>F)
Treatment	3	12137	4046	6.621	0.03419 *
Block	4	8169	2042	3.343	0.03419 *
Residuals	17	10387	611		

Note: This field has a high flea beetle pressure. There were issues with water logging.

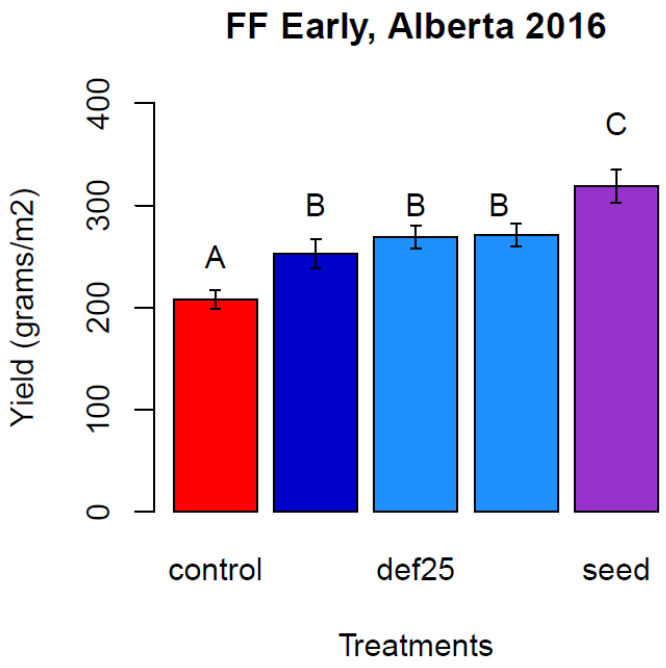


Fig.5. Yield (g/m^2) of canola from economic threshold study conducted at Fairfield, Alberta 2016

ANOVA-Yield

Fair Field-Early (Yield)	df	Sum sq	Mean sq	F value	Pr(>F)
Treatment	4	29248	7312	16.696	1.45e-05 ***
Block	4	7004	1751	3.998	0.0196 *
Residuals	16	7007	438		

Vauxhall Early Alberta 2016

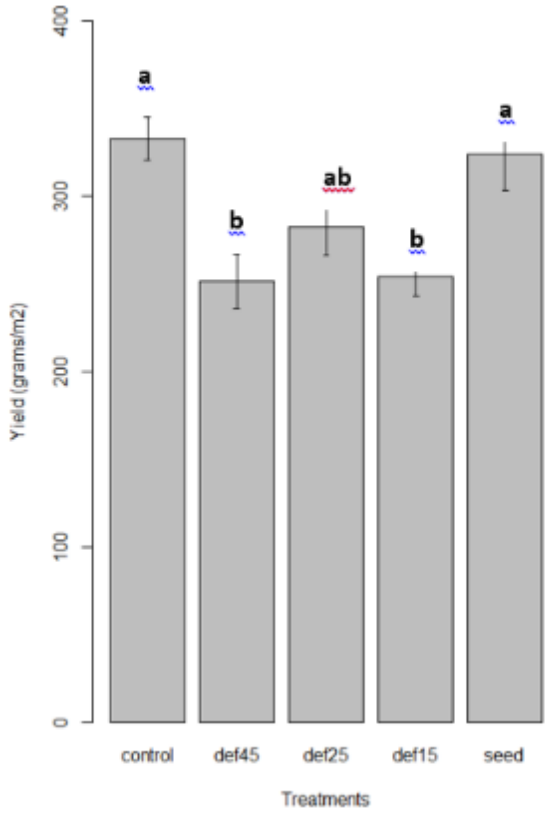


Fig.6. Yield (g/m^2) of canola from economic threshold study conducted at Vauxhall, Alberta 2016

ANOVA-Yield

Vauxhall-Early (Yield)	df	Sum sq	Mean sq	F value	Pr(>F)
Treatment	4	29077	7269	7.823	0.00108 **
Block	4	8756	2189	2.356	0.09760
Residuals	16	14868	929		

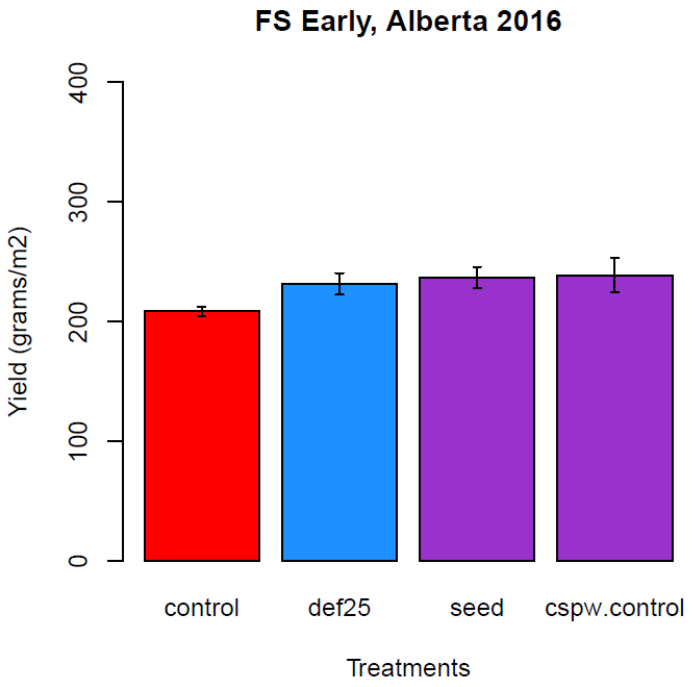


Fig.7. Yield (g/m^2) and the 1000 kernel weight (g) of canola from economic threshold study conducted at Lethbridge, Alberta 2016

ANOVA-Yield

Farming smarter –Early (Yield)	df	Sum sq	Mean sq	F value	Pr(>F)
Treatment	3	2872	957.5	3.084	0.0681
Block	4	964.6	634.7	0.0569	0.0569
Residuals	12	3725	310.4		

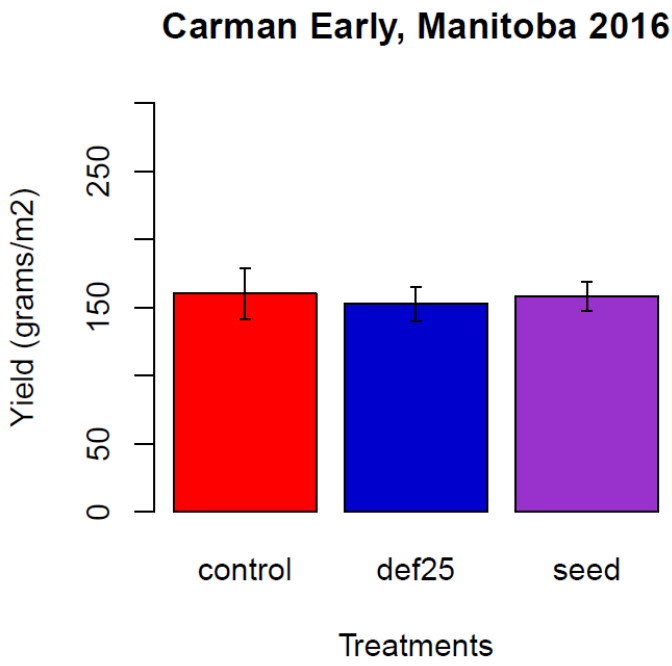


Fig.8. Yield (g/m^2) of canola from economic threshold study conducted at Carman, Manitoba 2016

ANOVA-Yield

Carman-Early (Yield)	df	Sum sq	Mean sq	F value	Pr(>F)
Treatment	2	150	74.8	0.070	0.9324
Block	4	10663	2665.7	2.502	0.0788
Residuals	18	19175	1065.3		

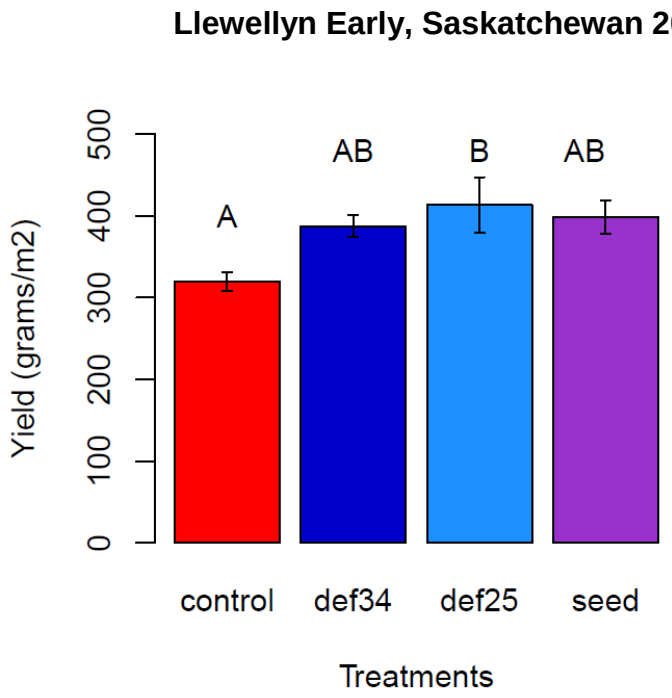


Fig.9. Yield (g/m^2) of canola from economic threshold study conducted at Saskatchewan 2016

ANOVA-Yield

Llewellyn-Early (Yield)	df	Sum sq	Mean sq	F value	Pr(>F)
Treatment	4	25405	6351	3.650	0.0361 *
Block	3	15303	5101	2.934	0.0767
Residuals	12	20865	1739		

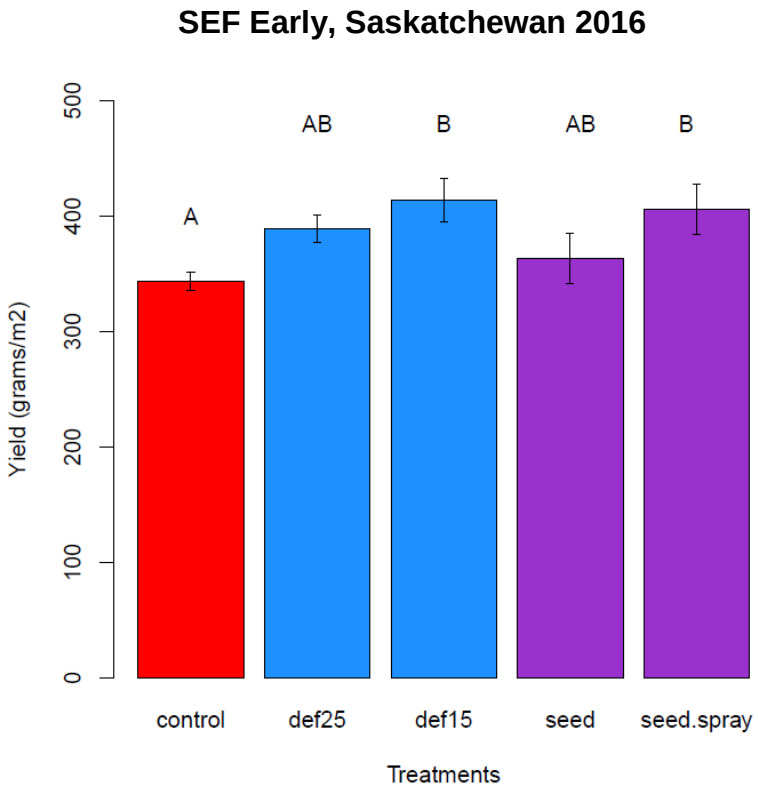


Fig.10. Yield (g/m^2) of canola from economic threshold study conducted at Saskatchewan 2016

SEF-Early (Yield)	df	Sum sq	Mean sq	F value	Pr(>F)
Treatment	4	13823	3456	4.450	0.0195*
Block	3	8652	2884	3.714	0.0424*
Residuals	12	9319	777		

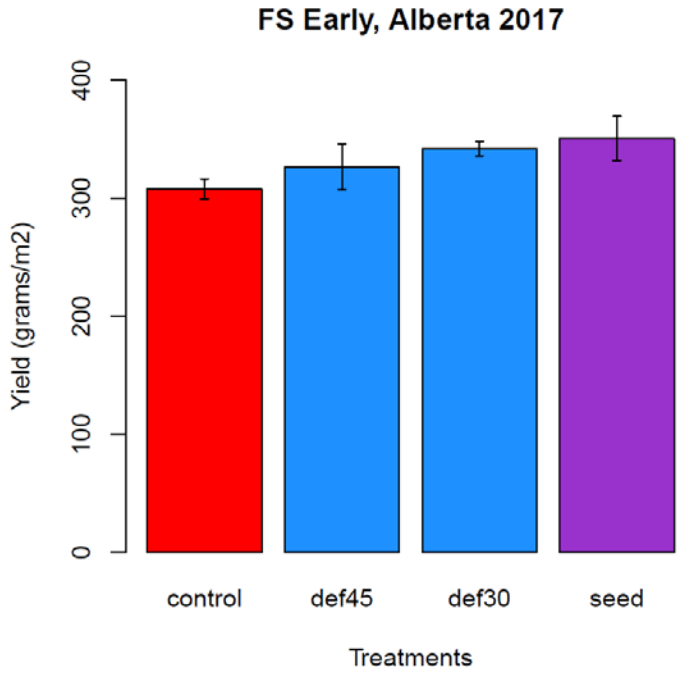


Fig.11. Yield (g/m^2) of canola from economic threshold study conducted at Lethbridge, Alberta 2017

Farming Smarter-Early (Yield)	df	Sum sq	Mean sq	F value	Pr(>F)
Treatment	3	5269	1756.3	2.027	0.169
Block	4	4229	1057.2	1.220	0.357
Residuals	18	9533	866.2		

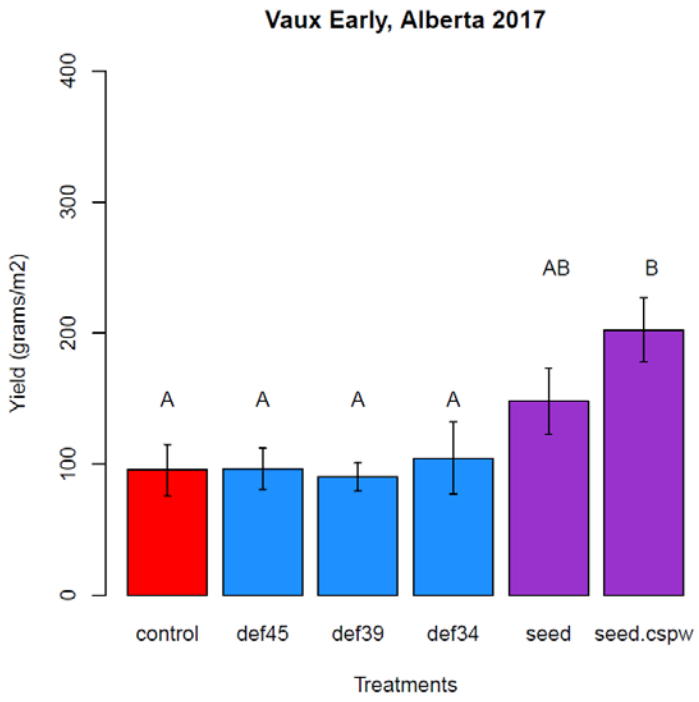


Fig.12. Yield (g/m^2) of canola from economic threshold study conducted at Lethbridge, Alberta 2017

Farming Smarter-Early (Yield)	df	Sum sq	Mean sq	F value	Pr(>F)
Treatment	5	48798	9760	5.935	0.0016 **
Block	4	21503	5376	3.269	0.0324 *
Residuals	20	32888	1644		

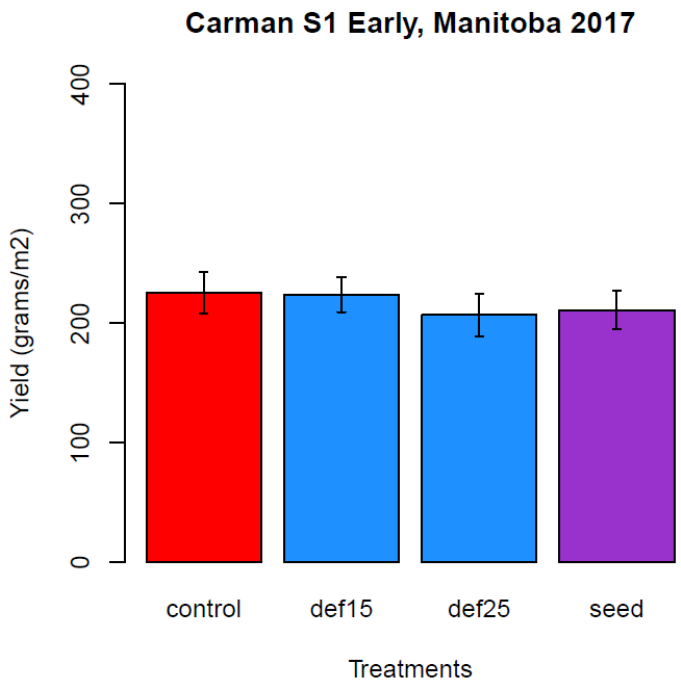


Fig.13. Yield (g/m^2) of canola from economic threshold study conducted at Carman, Manitoba 2017

Carman S1 Early (Yield)	df	Sum sq	Mean sq	F value	Pr(>F)
Treatment	3	1555	518	0.403	0.7530
Block	4	21425	5356	4.160	0.0157
Residuals	17	21888	1288		

Note: Only def15 and def25 treatments were sprayed. The defoliation in the control is also above 20%. The crop was sprayed when the stage passed 2.4.

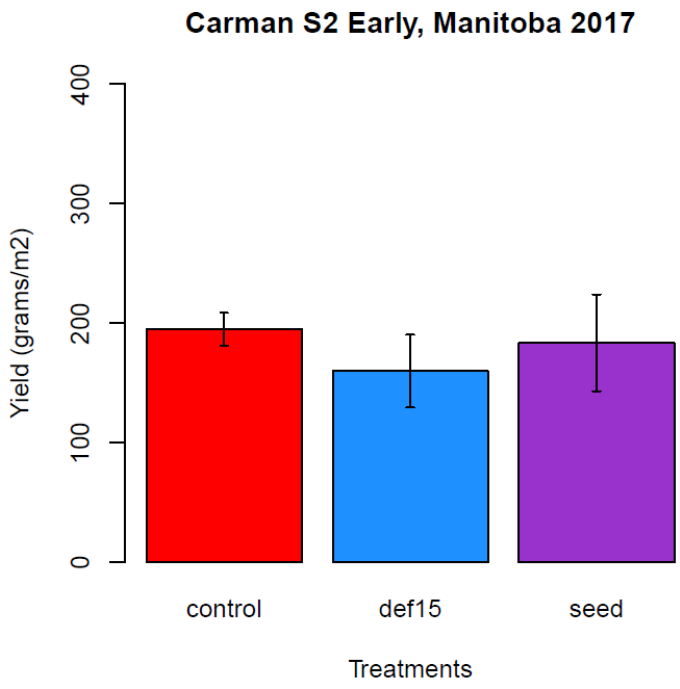


Fig.14. Yield (g/m^2) of canola from economic threshold study conducted at Carman, Manitoba 2017

Carman S2 Early (Yield)	df	Sum sq	Mean sq	F value	Pr(>F)
Treatment	2	3191	1596	0.329	0.729
Block	4	16083	4021	0.829	0.543
Residuals	8	38814	4852		

Note: Higher weed pressure was there in the plots at the late season

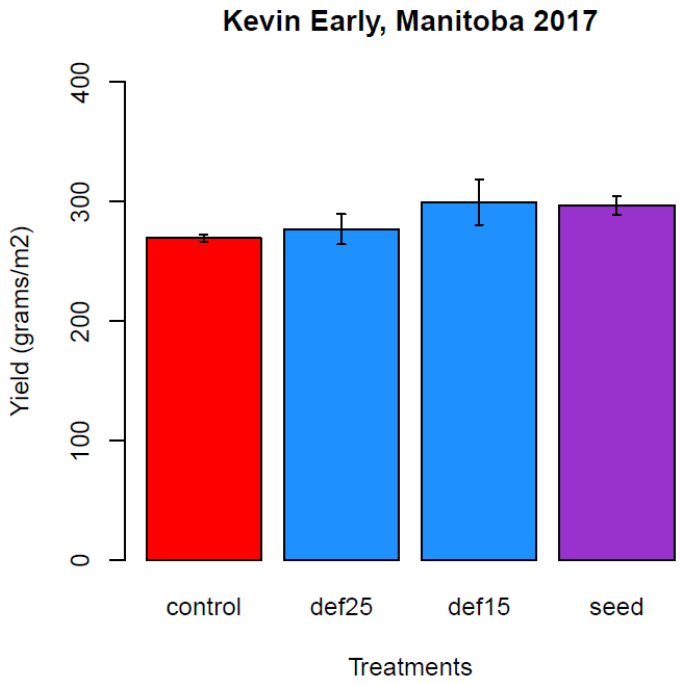


Fig.15. Yield (g/m^2) of canola from economic threshold study conducted at Fort Whyte, Manitoba 2017

Kevin Early (Yield)	df	Sum sq	Mean sq	F value	Pr(>F)
Treatment	3	3265	1088.4	1.858	0.19
Block	4	4660	1165.1	1.989	0.16
Residuals	12	7029	585.8		

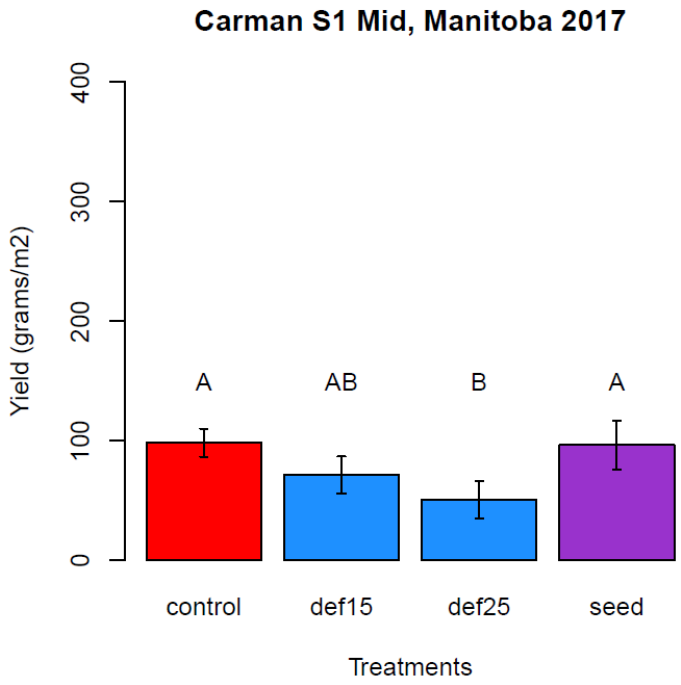


Fig.16. Yield (g/m^2) of canola from economic threshold study conducted at Carman, Manitoba 2017

Carman S1 Mid (Yield)	df	Sum sq	Mean sq	F value	Pr(>F)
Treatment	3	7387	2462.3	5.096	0.0188*
Block	4	12175	3043.9	6.300	0.0069**
Residuals	11	5311	483.1		

Note: This trial has an issue with weeds. The field was sprayed with herbicide and also hand weeded, but the problem persisted.

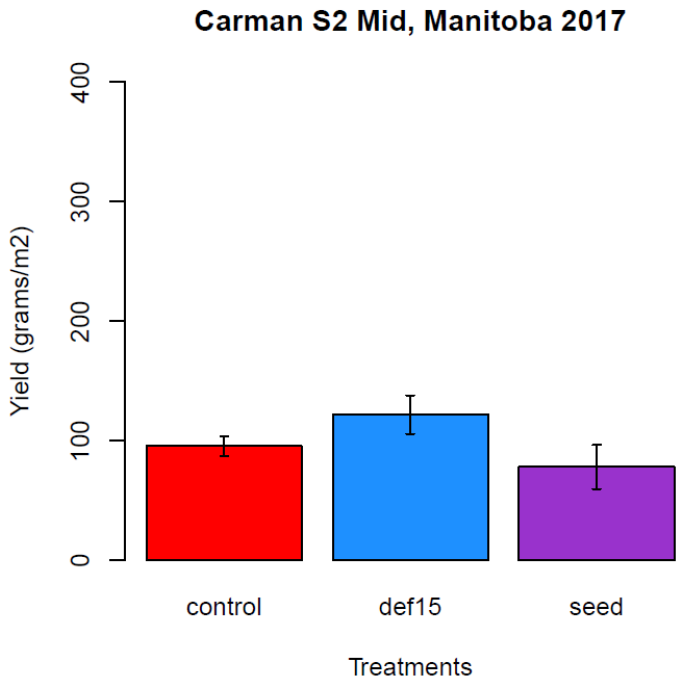


Fig.17. Yield (g/m^2) of canola from economic threshold study conducted at Carman, Manitoba 2017

Carman S2 Mid (Yield)	df	Sum sq	Mean sq	F value	Pr(>F)
Treatment	2	4864	2432	2.101	0.185
Block	4	4202	1050	0.908	0.503
Residuals	8	9261	1158		

2016-Late seeded economic threshold trials

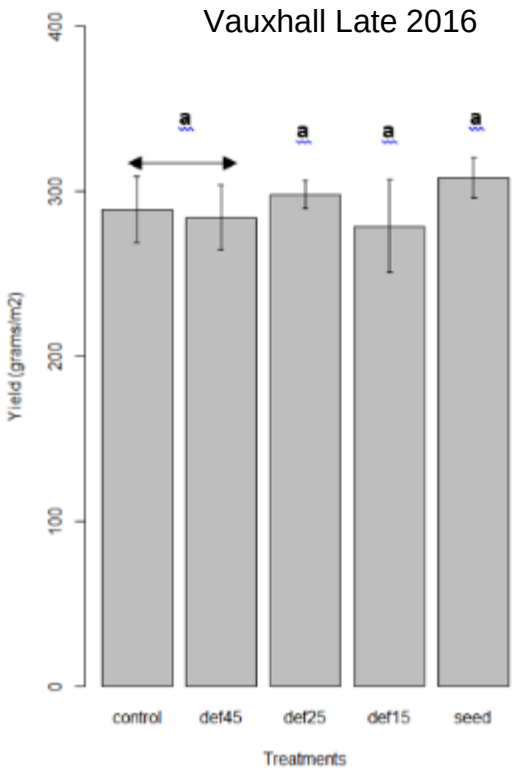


Fig.18. Yield (g/m^2) of canola from economic threshold study conducted at Vauxhall, Alberta 2016

ANOVA-Yield

Vauxhall-late (yield)	df	Sum sq	Mean sq	F value	Pr (>F)
Treatment	3	2731	910	1.089	0.3807
Block	4	21954	5489	6.562	0.0022 **
Residuals	17	14219	836		

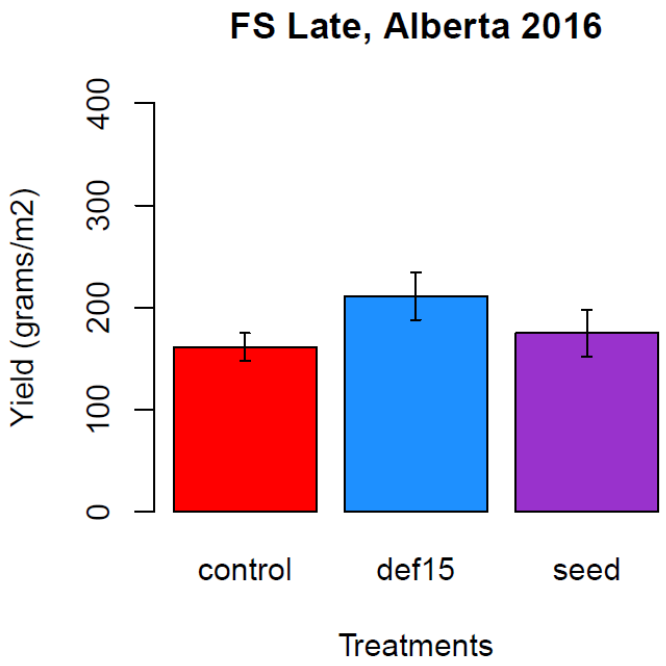


Fig.19. Yield (g/m^2) of canola from economic threshold study conducted at Lethbridge, Alberta 2016

ANOVA-Yield

	df	Sum sq	Mean sq	F value	Pr(>F)
Treatment	2	6888	3444	2.988	0.0885
Block	4	11192	2798	2.428	0.1050
Residuals	12	13831	1153		

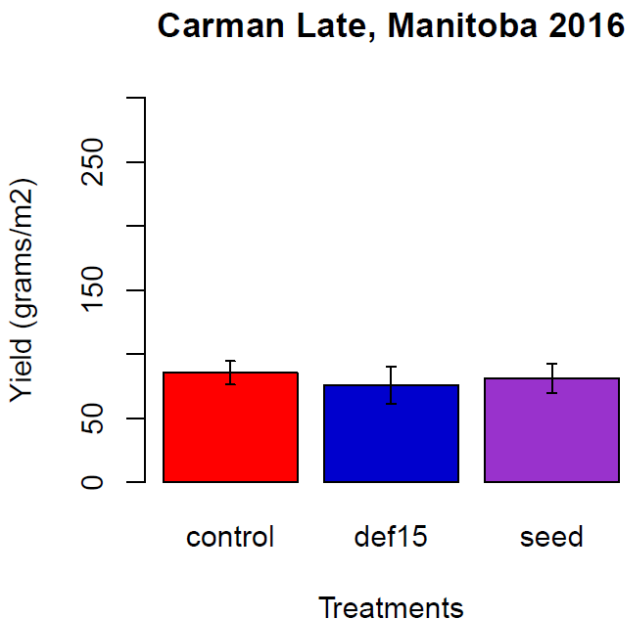


Fig.20. Yield (g/m^2) of canola from economic threshold study conducted at Carman, Manitoba 2016

ANOVA-Yield

Carman-Late (Yield)	df	Sum sq	Mean sq	F value	Pr(>F)
Treatment	2	211	105.4	0.312	0.73614
Block	4	6740	1685.1	4.987	0.00762
Residuals	17	5744	337.9		

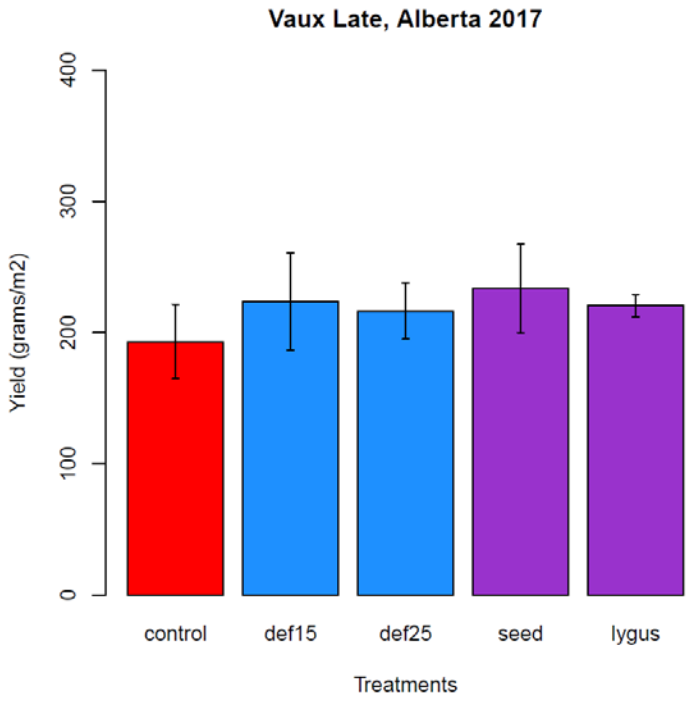


Fig.21. Yield (g/m^2) of canola from economic threshold study conducted at Vauxhall, Alberta 2017

Vaux Late (Yield)	df	Sum sq	Mean sq	F value	Pr(>F)
Treatment	4	4523	1131	0.797	0.544121
Block	4	54113	13528	9.540	0.000385
Residuals	16	22688	1418		

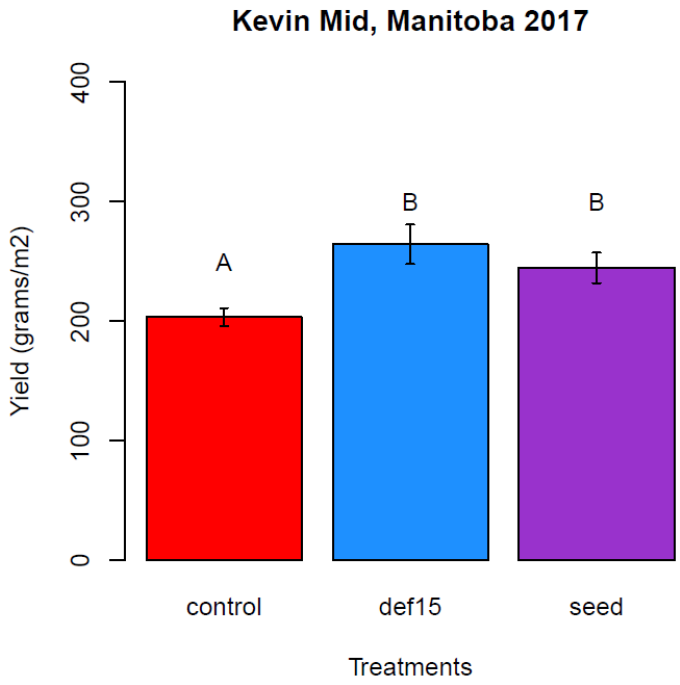


Fig.22. Yield (g/m^2) of canola from economic threshold study conducted at Fort Whyte, Manitoba 2017

Kevin Mid (Yield)	df	Sum sq	Mean sq	F value	Pr(>F)
Treatment	2	9673	4837	17.888	0.00112**
Block	4	7739	1935	7.155	0.00940**
Residuals	8	2163	270		

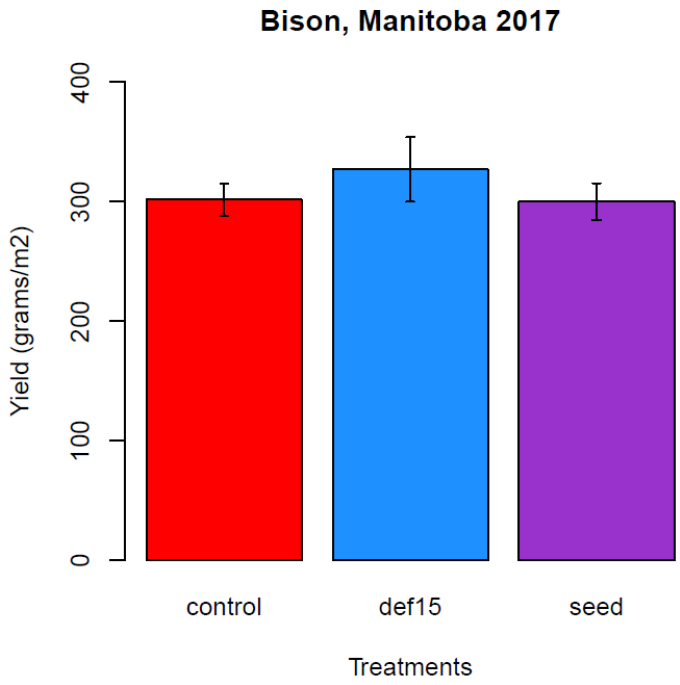


Fig.23. Yield (g/m^2) of canola from economic threshold study conducted at Bison, Manitoba 2017

Bison (Yield)	df	Sum sq	Mean sq	F value	Pr(>F)
Treatment	2	1955	977.3	0.450	0.655
Block	4	1945	486.2	0.224	0.917
Residuals	7	15208	2172.5		

Objective 2: To develop molecular tools to identify the suite of predators that impact flea beetle populations.

By Dr. Barbara Sharanowski

Background:

Determining the predators that may impact pest populations is challenging. Gut content analysis is the major method for determining the species of predators that eat a target pest, in this case flea beetles in canola, and for quantifying the impact of pest populations. Molecular gut content analysis is the most efficient and accurate method for determining what predator species eat flea beetles and how often. One method of molecular gut content analysis involves amplifying the pest DNA from the guts of predators. This involves: (1) designing primers to amplify the target pest(s); (2) testing the sensitivity of the primers through dilution series to ensure pest DNA can be amplified in small quantities (prevent false negative results due to decay of the pest DNA in the predator gut); and (3) testing the specificity of the primers to amplify the target pest and no other prey items or the predator itself (prevent false positives). If species specific and sensitive primers can be designed, then field collected predators can be screened through DNA extraction and PCR amplification. If DNA amplifies, then it assumed the predator has consumed the target pest. When multiple target pests are being tested for, then primers are designed to be species and length specific, so PCR amplifications only need to be screened via electrophoresis and not sequencing, which is more cost efficient.

Objectives:

The overall objective of this component of the project is to develop molecular tools to identify the suite of predators that impact flea beetle populations. Our sub-objectives include:

1. Develop species and length specific primers for *Phyllotreta striolata* and *P. cruciferae*, the two most economically damaging flea beetles in canola
2. Test primers for specificity
3. Test primers for sensitivity to low DNA quantities
4. Determine the predator species that eat flea beetles in the field, and quantify their effectiveness for reducing flea beetle populations

Brief Methods:

Genomic DNA was extracted using the DNeasy™ Tissue Kit (Qiagen, Valencia, CA, U.S.A.) from eighteen specimens (*P. striolata*, n=6; *P. cruciferae*, n=11) using legs to retain specimen vouchers. DNA was amplified for the barcoding region of cytochrome oxidase I (COI) using universal primers LCO1490 and HCO2198 (Folmer *et al.*, 1994) and amplified using the cycling protocol outlined in Zhang et al., (2017). All PCRs were performed using approximately 1µg DNA extract, 1X Standard Taq Buffer (10 mm Tris-HCl, 50 mm KCl, 1.5 mm $MgCl_2$, pH 8.3, New England Biolabs, Ipswich, Massachusetts, U.S.A.), 200 µM dNTP (Invitrogen, Carlsbad, California, U.S.A.), 4 mM $MgSO_4$, 400 nM of each primer, 1 unit of Taq DNA polymerase (New England Biolabs), and purified water to a final volume of 25 µl. Concentration of DNA was

determined using Qubit fluorometric quantitation. PCR products were cleaned with Agencourt CleanSEQ magnetic beads and sequenced. Sequences were trimmed for quality and contigs assembled using Geneious version 8.18 (Kearse et al. 2012). Sequences were visually inspected for signals of NUMTs following Boring et al. (2011) and alignments performed by hand using the correct reading frame in BioEdit (Hall, 1999). Additional sequences of various Chrysomelid taxa were downloaded from NCBI and aligned with our sequences. Primers were designed using Primer3 (Rozen and Skaletsky, 2000) and visual inspections for across the alignment for species specific sequence variation. Multiple primers designed and tested for specificity.

Various predators were field collected, individually separated into petri dishes, and starved for 48 hours. After the standardized starvation period, each predator was offered a flea beetle (*Phyllotreta cruciferae*). Predators were checked every 24 hours, and each predator was killed and preserved in 95% ethanol within 24 hours of feeding. These tests provide a set of "known positives" for predation on flea beetles to ensure sensitivity of the primers.

Results:

Multiple primers were tested, but several were not species specific or resulted in excessive non-target banding. However, two primer sets did amplify the target species each at different lengths (Table 1). Each primer amplified the target species at the target length (Fig. 24). However, unspecific banding occurred, and thus annealing temperature and magnesium concentration gradient tests were performed to achieve optimal PCR cycling conditions. Optimal PCR conditions for both primers were: initial denaturation of 1 min at 95ºC, followed by 35 cycles of 95ºC for 15 s, 57ºC for 15 s and 72ºC for 45 s, and a final elongation period of 4 min at 72ºC.

Primer Specificity:

Specificity of the primers was tested by cross-amplifying the target taxa with the other primer (i.e. *P. striolata* with PcruF3/PcruR3 primers and *P. cruciferae* with PstrF3/PstrR3 primers) (Fig. 25). The cruciferae primers (PcruF3/PcruR3) were specific and did not amplify *P. striolata* (Fig. 25). Unfortunately, the PstrF3/PstrR3 primers amplified *P. cruciferae* at length 248bp, indicating a need to change the primer or perform double screens in the future (Fig. 25). Double screens means that two PCRs (or a multiplex PCR) would be performed using both primer sets. Table 2 lists which primers will amplify which taxa and at what length. Thus, different banding patterns will occur with the two primer sets depending on which species was eaten by the predator.

Table 1. Primers designed to amplify short fragment of Cytochrome Oxidase I (COI) for *P. striolata* and *P. cruciferae*. Primers sequences are listed 5' to 3'.

Primer Set Name	**Target Species**	**Amplicon Length**	**Forward Primer Sequence**	**Reverse Primer Sequence**
PcruF3/PcruR3	*P. cruciferae*	178	TTGGAGGATTTGGAAACTGACT	GCTGATAAGGGTGGGTAAACTGT
PstrF3/PstrR3	*P. striolata*	248	ATCGAAAATGGAGCTGGCACT	GGRAGGGAGAGAAGTAGGAGGA

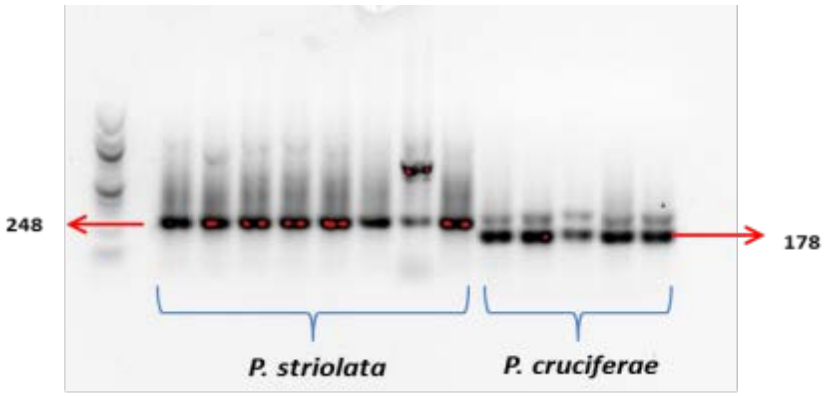


Figure 24. Amplification test of primers PstrF3/PstrR3 to amplify *Phyllotreta striolata* and PcruF3/PcruR3 to amplify *Phyllotreta cruciferae.* Both primers amplified the expected taxon at the expected length (248 bp for *P*. *striolata* and 178 bp for *P*. *cruciferae*).

Table 2. Predicted banding pattern based on specificity experiments.

	Will amplification occur?	
Target Beetle	**With PcruF3/PcruR3**	**With PstrF3/PstrR3**
Phyllotreta cruciferae	yes - 178	yes - 248
Phyllotreta striolata	no	yes - 248

Primer Sensitivity:

Sensitivity of the primers was tested using a dilution series from 1:10 to 1:100,000. Amplification occurred up until 1:1000 but not beyond, indicating the primers are sensitive but very small amounts of DNA will not amplify (Fig 25).

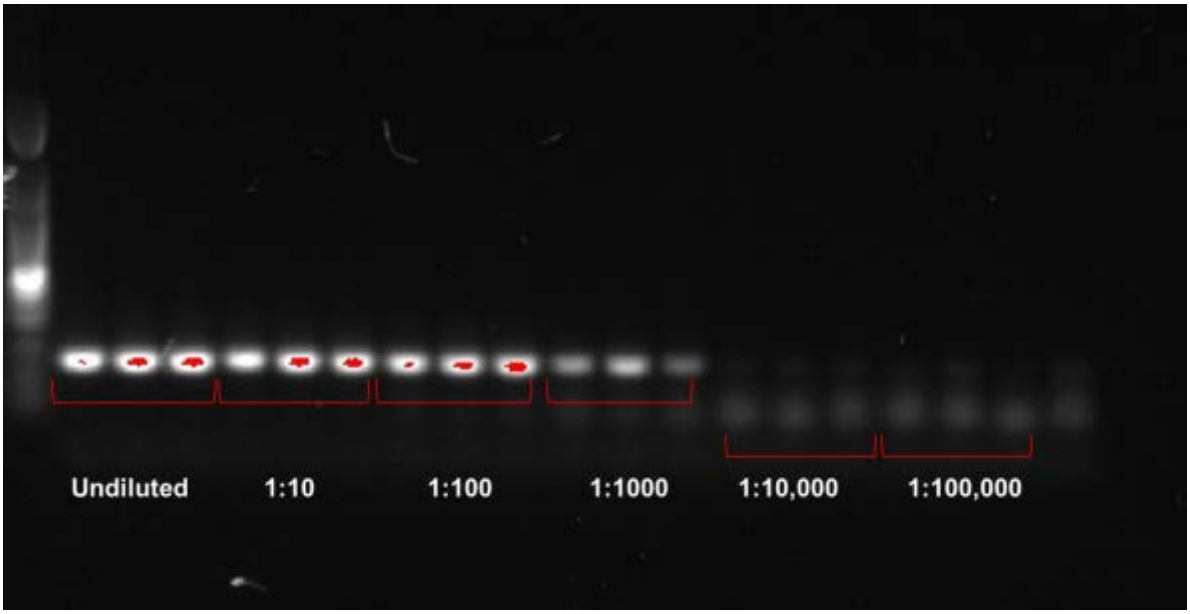


Figure 25. Dilution Series of PcruF3/PcruR3 primer set on extracted flea beetle DNA. Amplification occurred up to 1:1000 dilutions, but not beyond.

Amplification and Sequencing of Predator Gut DNA:

Amplification of predator gut DNA was largely successful, with 82.5% of samples successfully amplifying. Three of the samples did not amplify. Non-amplification may be due to species specific levels of digestion that occur. Sequencing of these samples confirmed the amplification of *P. cruciferae*, which was confirmed with comparison to our previously sequenced samples.

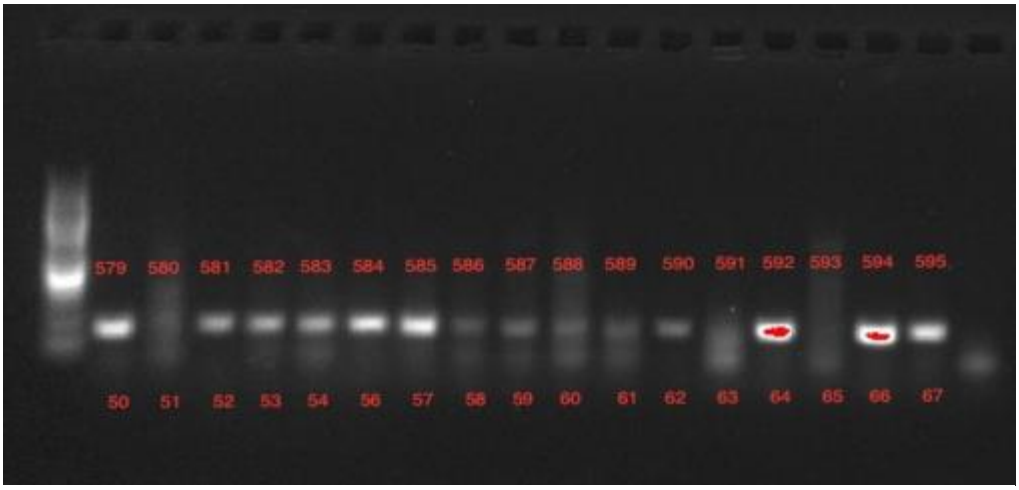


Figure 26. Amplification of known positive predators (n=17) with the PcruF3/PcruR3 primer set. Amplification occurred at 14 out of 17 samples (sample lanes 580, 591, and 593 did not amplify and are false negatives).

Discussion and Conclusions:

Two primers were developed to amplify flea beetle DNA in the gut contents of predators for two species of flea beetles, *Phyllotreta cruciferae* and *P. striolata*. The primer set PcruF3/PcruR3 amplifies *P. cruciferae* at length 178bp and does not amplify *P. striolata*. The primer set PstrF3/PstrR3 amplifies both taxa at length 248bp, and thus is not species specific. However, this primer can be used to assess predation of either flea beetle simultaneously if species level information is not required. Otherwise two PCRs need to be performed to determine the species. The first PCR would be with the PstrF3/PstrR3 and then followed by PcruF3/PcruR3. In the first PCR, *P. striolata* samples will amplify, but not in the second, whereas *P. cruciferae* will be amplified in both PCRs.

The PcruF3/PcruR3 primers are fairly sensitive, amplifying flea beetle DNA up to a 1:1000 dilution, but not beyond. However, from screening of known positive, false negatives are possible, thus the screens will likely underestimate actual predation levels.

Laboratory studies on flea beetle predation*

**Based on studies conducted by Ph.D. student Thais Silva Guimaraes, with a stipend supported by an NSERC Discovery grant to ACC and the GETS program of the University of Manitoba.*

Preliminary trials involved no-choice petri-dish assays - using generalist predators collected from field canola fields and their borders, selected based on previous literature that reports anecdotal events of predation on flea beetles. These trials were conducted to 1) determine directly potential predators of flea beetles and 2) provide known positives (i.e. predators consuming flea beetles) for molecular tests.

Insect collections

Crucifer and striped flea beetle: Allyl isothiocyanate- baited traps were installed in the border of the fields to collect flea beetles (Fig 27 a and b). Additionally, sweep nets were used when necessary.

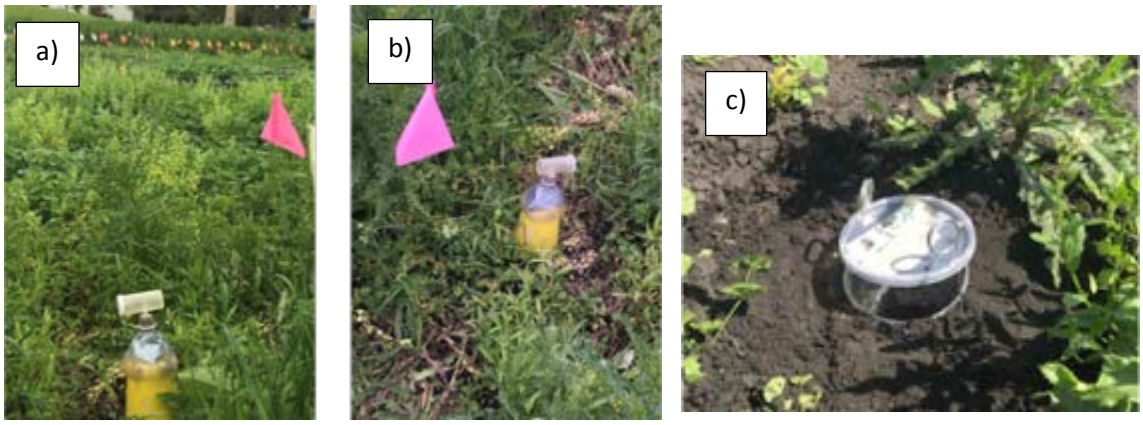


Fig.27. Allyl isothiocyanate- baited traps (a and b) and pitfall trap (c), used to capture live flea beetles and predators, respectively, for laboratory experiments.

Predators: Five pitfall traps (Fig. 27c) were installed in 2016 in the field border at the University of Manitoba experimental plots at the Arboretum (Arb) to collect ground predators. Every two days, generalist predators were collected and carried to the lab, where these predators were 24 h-starved (i.e., only water provided) before each assay.

Canola was planted twice a week in growth chambers at 24∘C, 70% relative humidity (RH) and 16L:8D photoperiod.

No-choice tests:

In each Petri dish, a wet paper was set at the bottom and a cotyledon-stage canola seedling, was placed on top and secured with wet cotton (Fig. 28). Six flea beetles (three striped flea beetles and three crucifer flea beetles), previously starved during 24 h were introduced in each Petri dish and allowed to feed on flea beetles for 72 hours.

No-choice assays had the three following treatments:

1. One control treatment with only canola leaves;

2. Another control treatment with canola leaves and flea beetles, and without predators;

3. Predator treatment

Fig. 28. Petri dish with canola seedling, flea beetles and a carabid predator, used in no-choice tests.

The Petri dishes were arranged in randomized blocks with treatments 1-3, with as many repetitions as possible, according to available predators collected in the field. Multiple predator species were tested within a block, when available. All the experiments were conducted in controlled growth chambers at 24°C, and the daily photoperiod of 15 h (Burgess and Wiens 1976).

Predator consumption was assessed at 24 h, 48 h, and 72 h after predator introduction. Canola leaves were scanned before and after 3-day assay to determine the percentage of defoliation the leaf tissue consumed by using the software Image J.

Results and preliminary conclusions:

In 2016, a total of 18 potential predator species were tested to assess direct or indirect effects on flea beetles and their damage to canola. From the 18 species tested, 10 predator species did consume flea beetles (Table 3). Carabid beetles in the genus *Pterostichus* have the higher consumption ratio for both flea beetle species, followed by beetles in the genus *Poecilus* and *Amara* (Table 3). Preliminary examination of cotyledon damage suggests reduced feeding in Petri dishes that had predators, even when flea beetles were alive at the end of the trials, suggesting that the presence of predators may reduce the amount of feeding by flea beetles. These experiments will be continued in more realistic arenas (i.e. potted plants at the cotyledon stage) with new predators collected at the 2017 season.

Table 3. Predation of 18 common predator species found in canola fields on striped and crucifer flea beetles

Family	Predator species	Mortality of Total FB	Mortality of Crucifer FB	Mortality of Striped FB
	Control	0.05	0.03	0.02
Carabidae	*Amara* sp.	2.56	1.00	1.56
	Carabidae 1	0.00	0.00	0.00
	Carabidae 2	0.00	0.00	0.00
	Carabidae 3	0.50	0.00	0.50
	Poecilus sp.	1.75	0.79	0.96
	Poecilus lucublandus	2.50	1.17	1.33
	Pterostichus sp.	5.50	2.75	2.75
	Pterostichus melanarius	4.58	2.17	2.42
Coccinellidae	*Coccinella septempuctata*	0.00	0.00	0.00
	C. septempunctata (larva)	0.00	0.00	0.00
Chrysopidae	*Chrysoperla carnea* (larva)	0.00	0.00	0.00
Nabidae	Nabidae 1	2.07	1.07	1.00
Phalangiidae	*Opilio* sp.	0.67	0.00	0.67
	Phalangium sp.	0.46	0.27	0.19
Lycosidae	*Pardosa* sp.	0.58	0.21	0.42
Tetragnathidae	Tetragnathidae 1	0.00	0.00	0.00

Predation Studies in microcosm trials

Several species of predators that fed on flea beetles in no-choice assays were tested in more realistic arenas in growth chambers in 2017. The microcosms cage consisted of a plastic 2-litre bottle enclosing 4 to 6 cotyledon-stage potted canola plants. Cages had two lateral windows and the top opening covered by a fine mesh to ensure ventilation (Figure 29).

Figure 29. Microcosm assay set up with randomized block design in 2017.

Three treatments were conducted: 1) control treatment with only canola plants (to estimate plant size in the absence of defoliation), 2) flea beetles only (to assess defoliation), and 3) flea beetles + predators. Ten flea beetles (either striped or crucifer) were included in each cage for treatments 2 and 3 with the addition of one adult predator in treatment 3. Trials were conducted during three days under controlled conditions ($21^{o}C \pm 2^{o}C$, 16L: 8D and RH=70%). Predators were field-collected adult individuals starved 24 hours prior to experiments (only water provided). Prior to experiments, predators were maintained in individual vials with a wet filter paper and processed dry cat food in the growth chambers, under control conditions (as above) for one to three weeks. Predator diet was replaced every two days. Experiments testing predation on crucifer flea beetles include two common Phalangiidae species (harvestmen), three Lycosidae spiders, several ground beetle predators (Coleoptera: Carabidae), including *Pterosticus melanarius* and several *Amara* spp. species. Experiments testing predation on striped flea beetles include *P. melanarius* and *A. torrida* as predators.

Results and preliminary conclusions:

The carabid beetle *P. melanarious* consumed several flea beetles of both species on separate trials, confirming previous findings in no-choice Petri-dish arenas. Similar results were found for predation of *A. torrida* on striped flea beetle, although not enough individuals were available to test predation on crucifer flea beelte. These results confirm the potential of this predators to

consume flea beetles under field conditions during early stages of canola. Other predators showed sporadic consumption, but most likely not statistically different from mortality on controls (analysis in progress). The effects of predators on a reduction of canola defoliation by flea beetles are still being quantified on digital pictures and will be analyzed in the near future.

Objective 3: Identify landscape features promoting effective natural enemies and decreasing infestation levels of flea beetles.

Seasonal pattern of occurrence and the species abundance of major flea beetle species in the prairies

Methodology:

In 2015, we sampled 29 commercial canola fields in four regions: Manitoba (6), Alberta-Peace River Area (4), Alberta Lethbridge (12), and Saskatchewan (7). In 2016, we sampled 25 fields: Manitoba (9), Alberta-Peace River Area (5), Alberta Lethbridge (6), and Saskatchewan (5) (Fig.17). In 2017, we sampled 24 commercial canola fields in four regions: Manitoba (6), Alberta-Peace River Area (7), Alberta-Lethbridge (6), and Saskatchewan (5).

The fields selected were represented in a gradient of landscape complexity, following the methods of Gardiner et al. (2009).

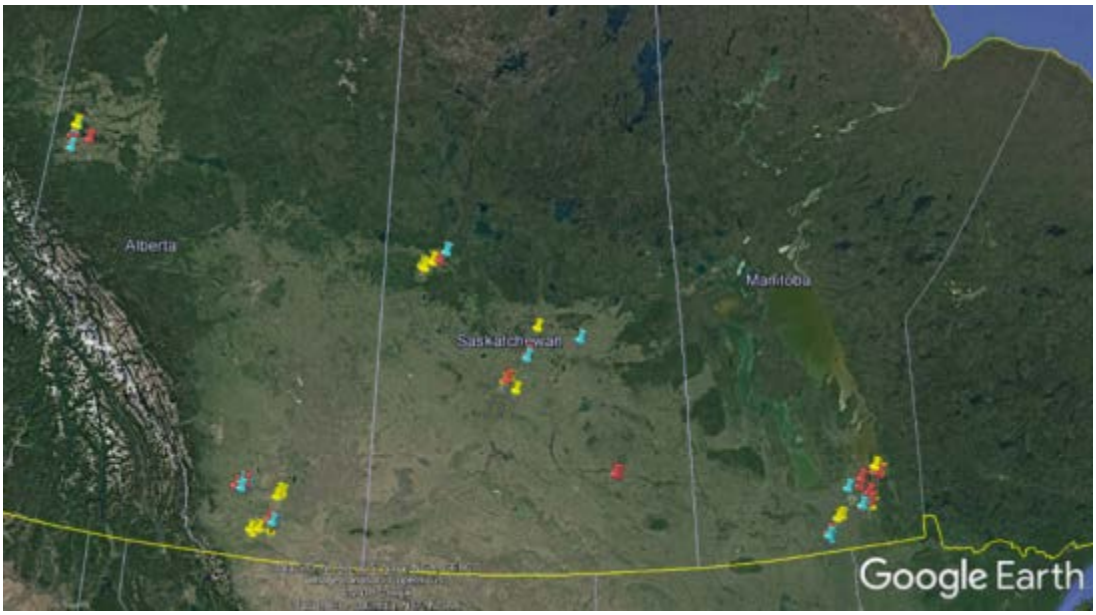


Fig. 30. Location of commercial canola fields sampled for determining flea beetle, and natural enemy abundance, species composition, and to determine landscape effects during 2015 (yellow), 2016 (orange), and 2017 (blue)

Sampling started in Mid-May when canola was in its most susceptible stage (cotyledon to the two-leaf stage) and continued throughout the season, to capture the population peaks and seasonal phenology of different flea beetle species (weeks 8-9 in 2015, weeks 7-9 weeks in 2016,

and weeks 4-11 in 2017). In 2015, in Lethbridge and in Saskatchewan, fields were sampled only up to the two-leaf stage (2-3 weeks).

At each field, we established five permanent sampling stations (Fig. 30), and sampling was performed at weekly intervals. Flea beetle and natural enemy abundance were assessed by sticky cards (Alpha Scent cards, 18 x 14 cm), following the methodology outlined in objective 1. Flea beetle damage was assessed from the emergence of the cotyledons, and up to 2-4 leaf stage in two, 1 m transects at each sampling station. Forty plants were assessed at each sampling station. Plant stand was assessed in one quadrat (0.5 m X 0.5 m) fixed closer to the sampling point growth stages 1.0 and 2.2. Further, each week crop phenology was also assessed. A total of 969 (2015), 1581 (2016), and approximately 1008 (2017) sticky card samples were collected.

In Manitoba, to assess movement of flea beetles and their natural enemies to and from the canola fields, at the sides of the canola field bidirectional malaise traps were installed. The number of malaise traps varied from 2-4 based on the field borders adjacent to canola. The contents of the malaise trap were collected weekly and stored in 95% alcohol. In the laboratory, the samples were analyzed for flea beetles and its natural enemies.

Results, recommendations, and preliminary conclusions:

The abundance of the two most common flea beetle species varied in each region sampled (Table 4). Both crucifer and striped flea beetles were abundant in Manitoba. In southern Alberta, crucifer flea beetle was the most abundant species. The striped flea beetles were abundant in the Peace River area of Alberta and in Saskatchewan. Low numbers of hop flea beetles were also found. Change in relative abundance of flea beetles is evident with striped flea beetles dominating in many areas, in comparison with historical records in the regions sampled. Further analysis of the species abundance data incorporating weather parameters, crop phenology, and flea beetle generation is in progress.

Table 4. Relative abundance (%) of flea beetle adults in 2015 to 2017

Province	Crucifer flea beetles ± SD	Striped flea beetles ± SD	Year
Alberta-Lethbridge	74.11 ± 26.72	25.38 ± 26.48	2015 to 2017
Alberta-Peace River	0.70 ± 1.14	94.40 ± 7.05	2015 to 2016
Manitoba	41.28 ± 28.41	58.24 ± 28.18	2015 to 2017
Saskatchewan	8.65 ± 15.02	89.11 ± 16.48	2015

We found highly variable patterns of seasonal abundance across and within regions for both flea beetle species (Figs. 19-24). In general, for most fields, we found two peaks of adults, but in some fields we observed a peak of adults in mid-July, suggesting that there can potentially be a second generation.

The overwintering adult emergence occurs from mid- May to Early July (weeks 4 to 9 in most cases in 2016). Within region, there are variations in species composition abundance as well. For example, in Saskatchewan 4 fields were dominated by crucifer flea beetles while one field was dominated by striped flea beetles, in 2016 (Fig. 23).

Further exploration of this data is to be done relating the abundance of flea beetles with canola phenology, weather parameters and the management practices of the farm such as foliar insecticide spray. These data were also shared to our collaborators in WIN Enterprises to develop predictions models (see objective 4).

We also determined the species composition and the abundance of natural enemies of flea beetles in sticky cards, and the data is being analyzed. The malaise traps are being processed to determine the flea beetle and natural enemy numbers, species composition and to see their pattern of movement to and from canola.

Seasonal pattern of occurrence of major flea beetle species

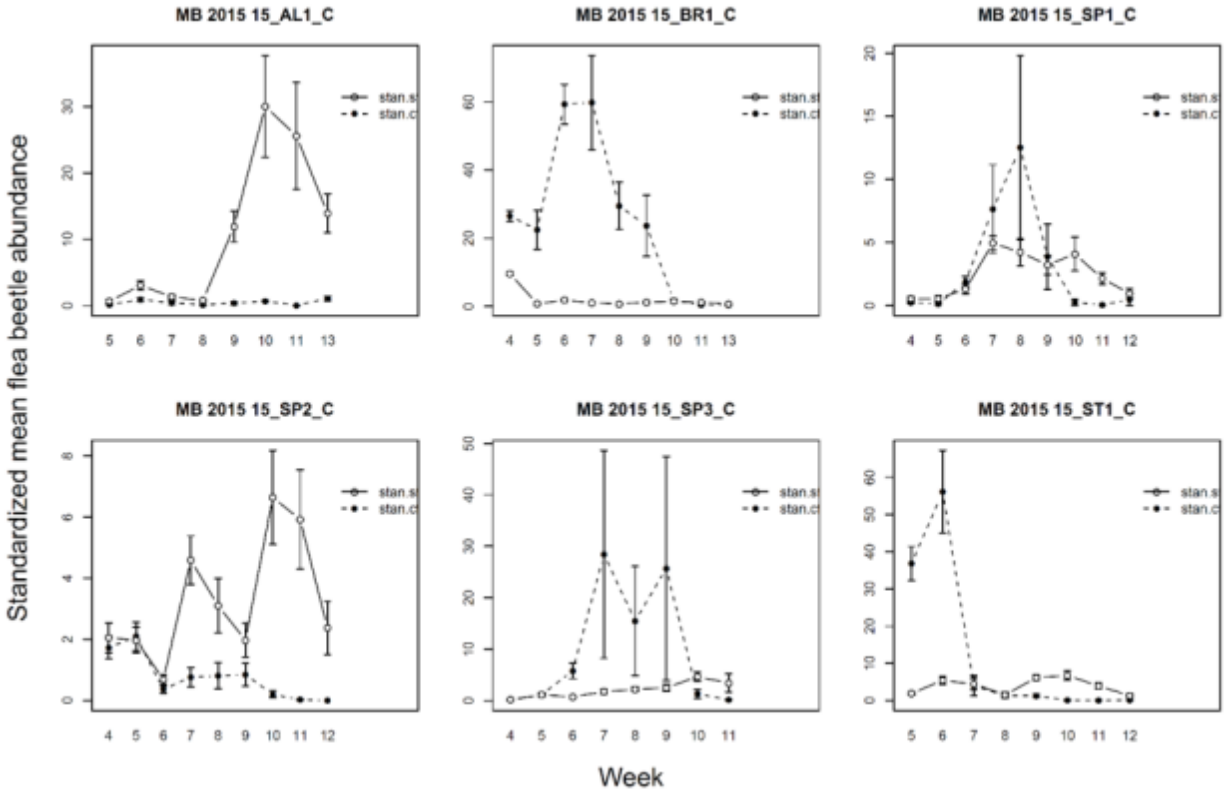


Fig. 31. Seasonal pattern of abundance of striped and crucifer flea beetles in 6 Manitoba fields in 2015. Here the flea beetle numbers are standardized 4 days and 177.6 cm^2 area of the sticky card. The week is based on the collection date of the sticky cards. Week 4 = 12 June 2015. The open symbol refers to the striped flea beetles, and the close symbol refers to the crucifer flea beetles. Each sample represents the average of 5 sticky traps / field / sampling date.

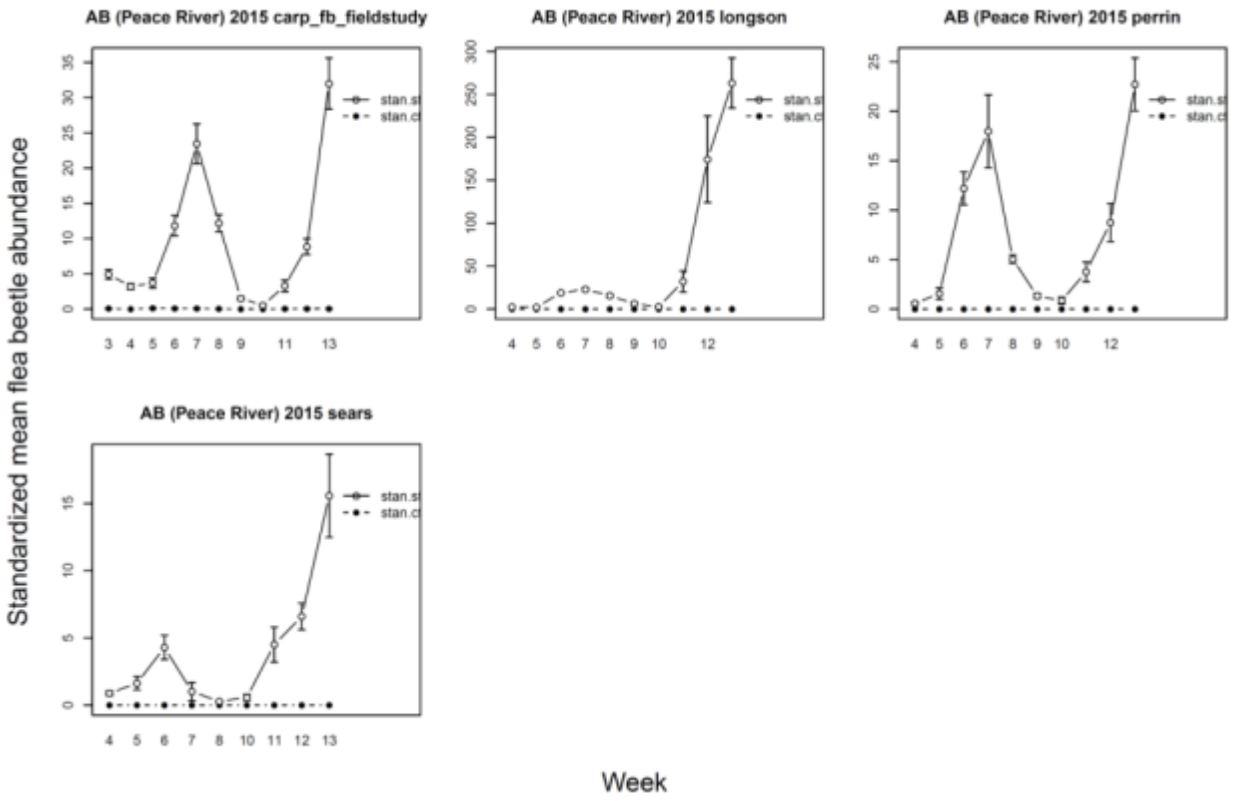


Fig. 32. Seasonal pattern of abundance of striped and crucifer flea beetles in 4 Alberta (Peace River) fields in 2015. Here the flea beetle numbers are standardized 4 days and 177.6 cm^2 area of the sticky card. The week is based on the collection date of the sticky cards. Week 3 = 04 June 2015. The open symbol refers to the striped flea beetles, and the close symbol refers to the crucifer flea beetles. Each sample represents the average of 5 sticky traps / field / sampling date.

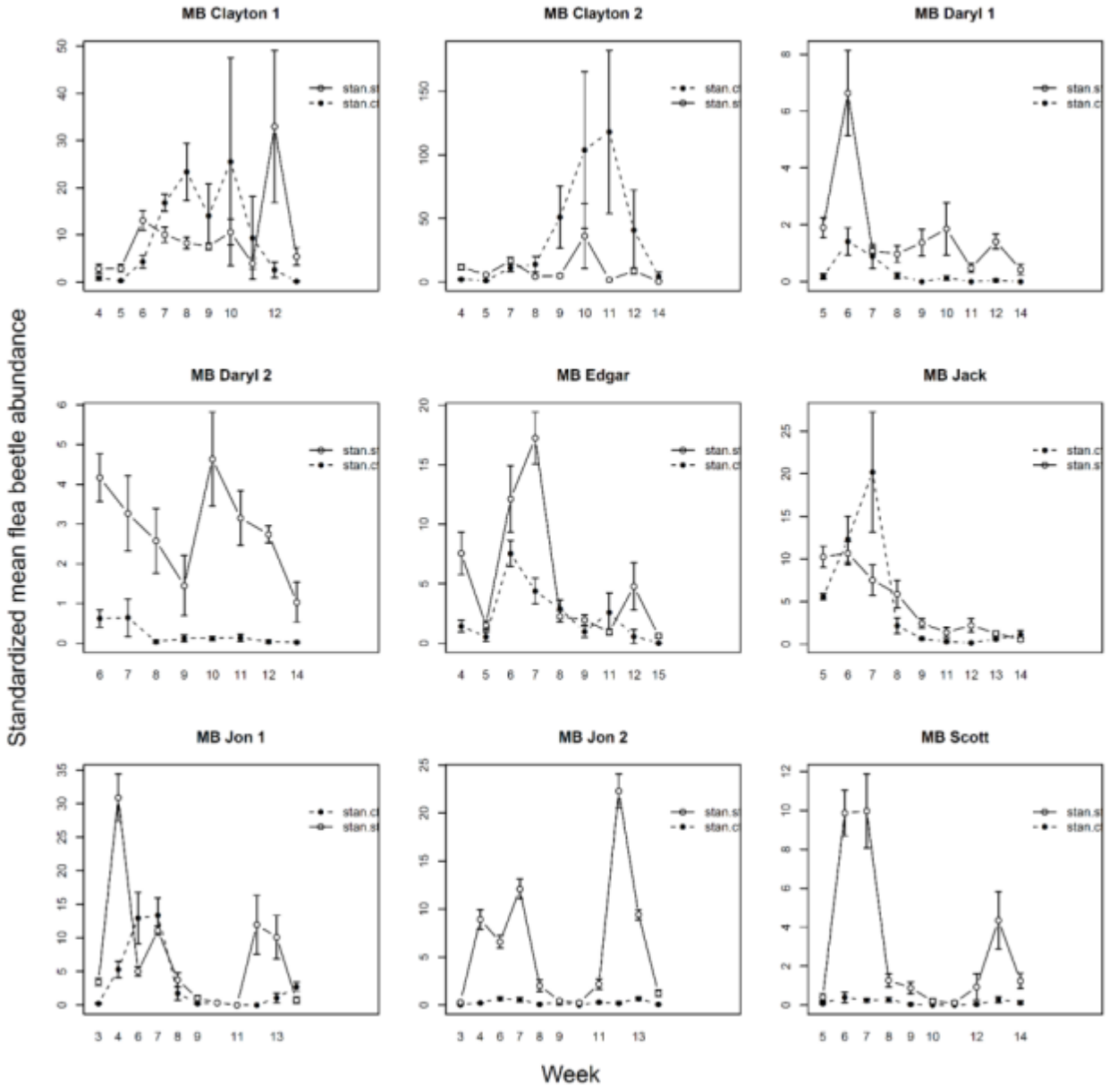


Fig. 33. Seasonal pattern of abundance of striped and crucifer flea beetles in 9 Manitoba fields in 2016. Here the flea beetle numbers are standardized 4 days and 177.6 cm^2 area of the sticky card. The week is based on the collection date of the sticky cards. Week 3 = 24 May 2016. The open symbol refers to the striped flea beetles, and the close symbol refers to the crucifer flea beetles. Each sample represents the average of 5 sticky traps / field / sampling date.

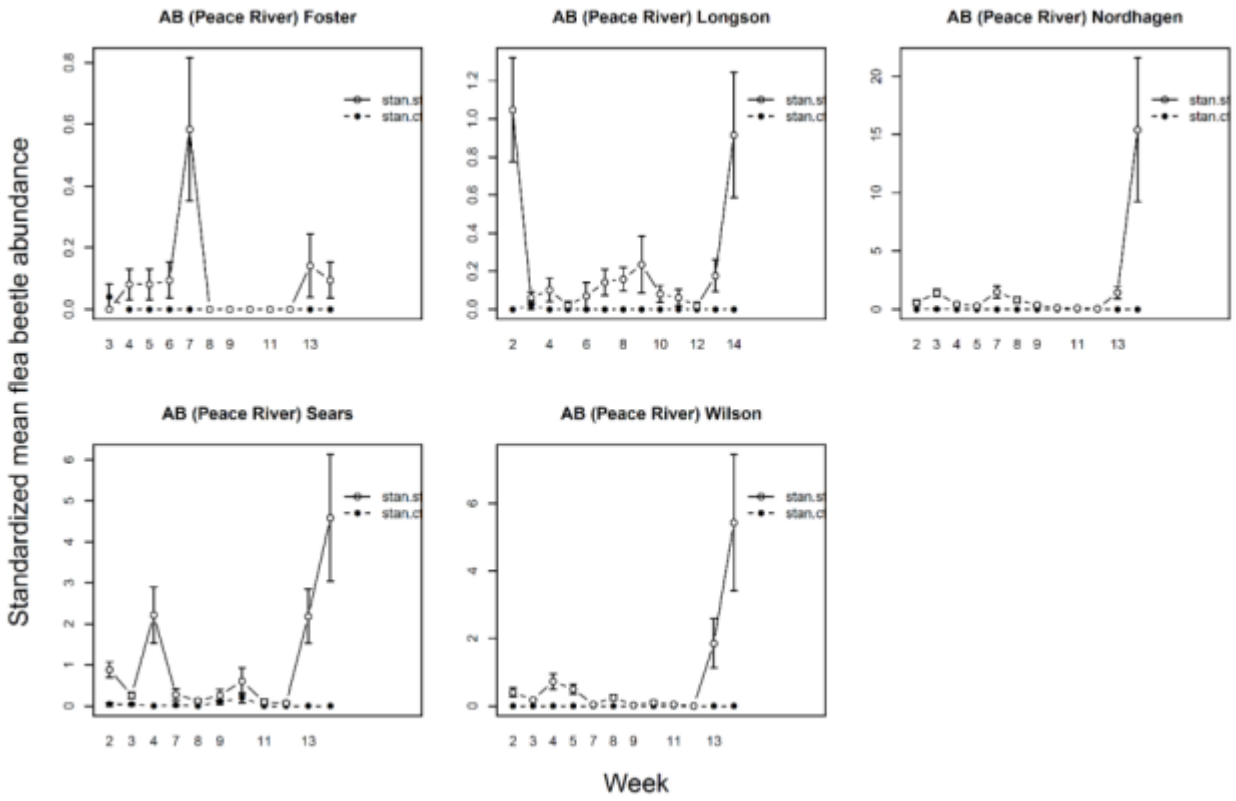


Fig. 34. Seasonal pattern of abundance of striped and crucifer flea beetles in 6 Alberta (Peace River) fields in 2016. Here the flea beetle numbers are standardized 4 days and 177.6 cm^2 area of the sticky card. The week is the collection date of the sticky cards from the field. The week is based on the collection date of the sticky cards. Week 2 = 17 May 2016. The open symbol refers to the striped flea beetles, and the close symbol refers to the crucifer flea beetles. Each sample represents the average of 5 sticky traps / field / sampling date.

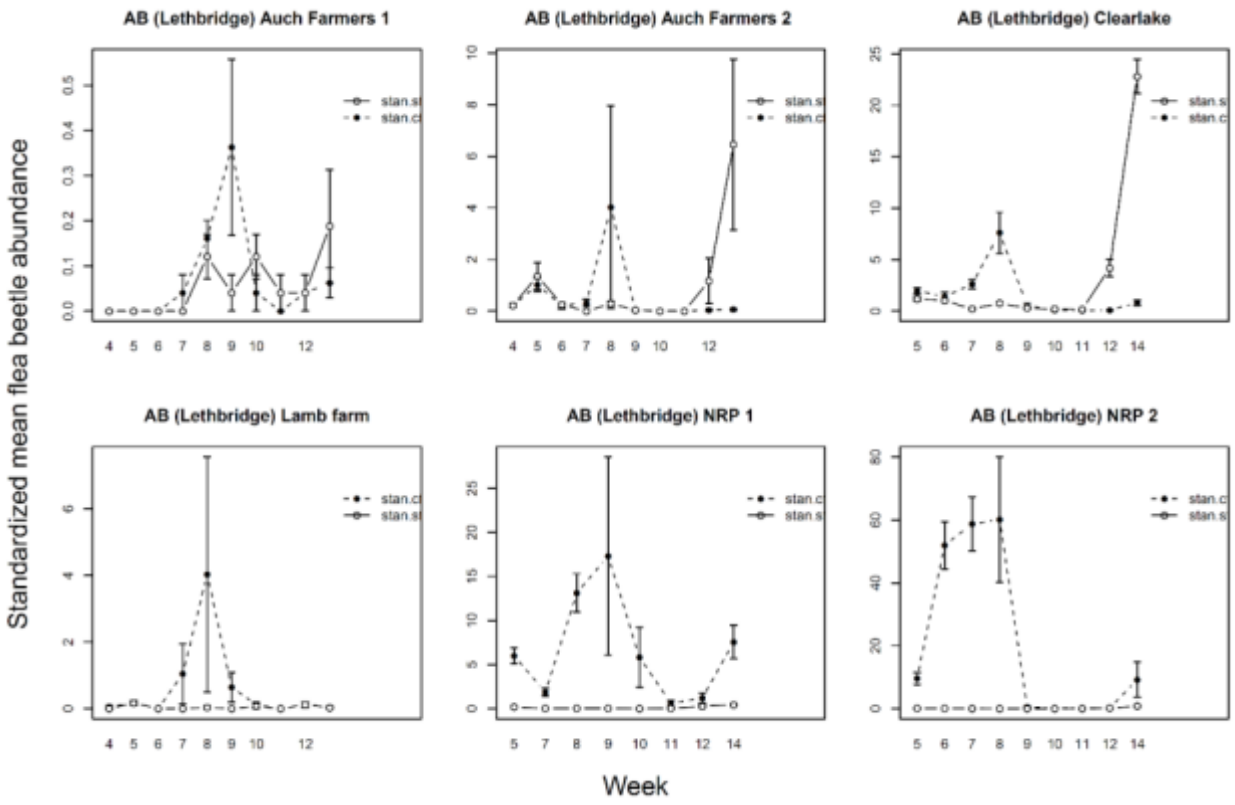


Fig. 35. Seasonal pattern of abundance of striped and crucifer flea beetles in 6 Alberta (Lethbridge) fields in 2016. Here the flea beetle numbers are standardized 4 days and 177.6 cm^2 area of the sticky card. The week is based on the collection date of the sticky cards. Week 4 = 2 June 2016. The open symbol refers to the striped flea beetles, and the close symbol refers to the crucifer flea beetles. Each sample represents the average of 5 sticky traps / field / sampling date.

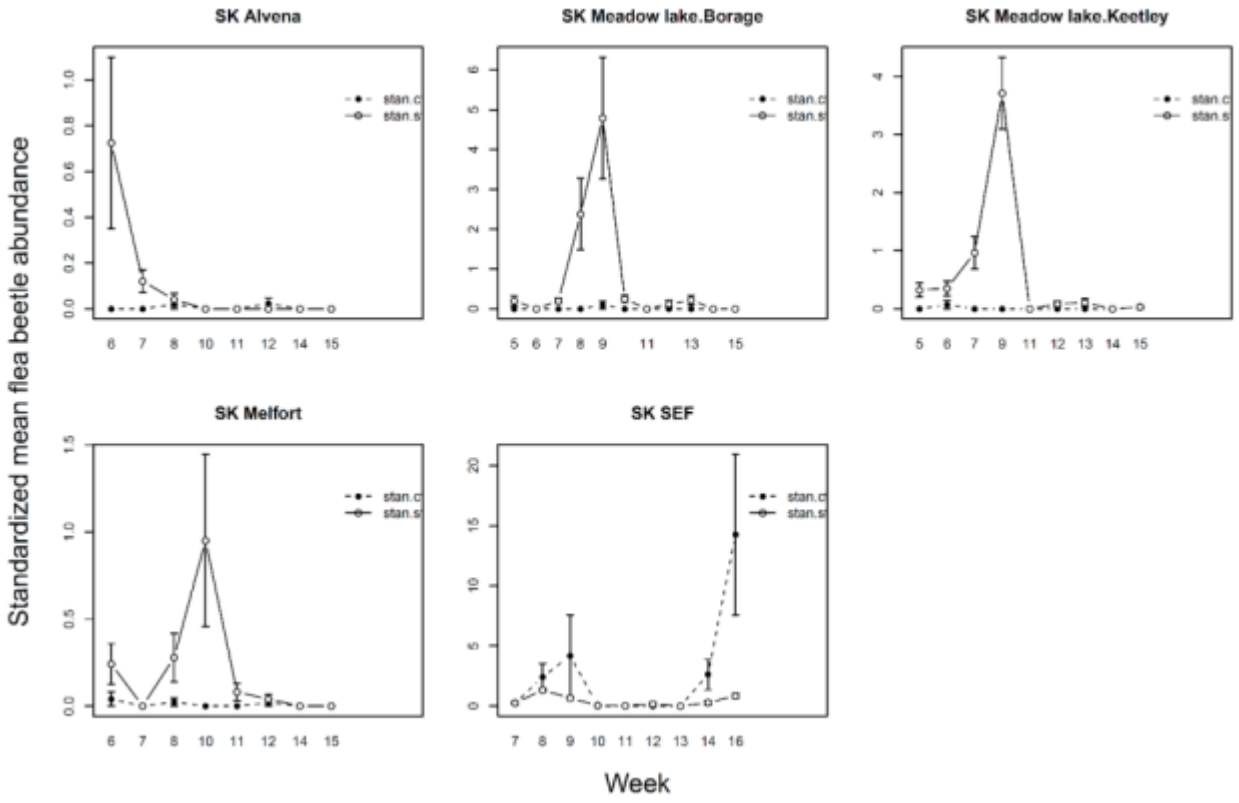


Fig. 36. Seasonal pattern of abundance of striped and crucifer flea beetles in 5 Saskatchewan fields in 2016. Here the flea beetle numbers are standardized for 4 days and 177.6 cm^2 area of the sticky card. The week is based on the collection date of the sticky cards. Week 5 = 9 June 2016. The open symbol refers to the striped flea beetles and the close symbol refers to the crucifer flea beetles. Each sample represents the average of 5 sticky traps / field / sampling date.

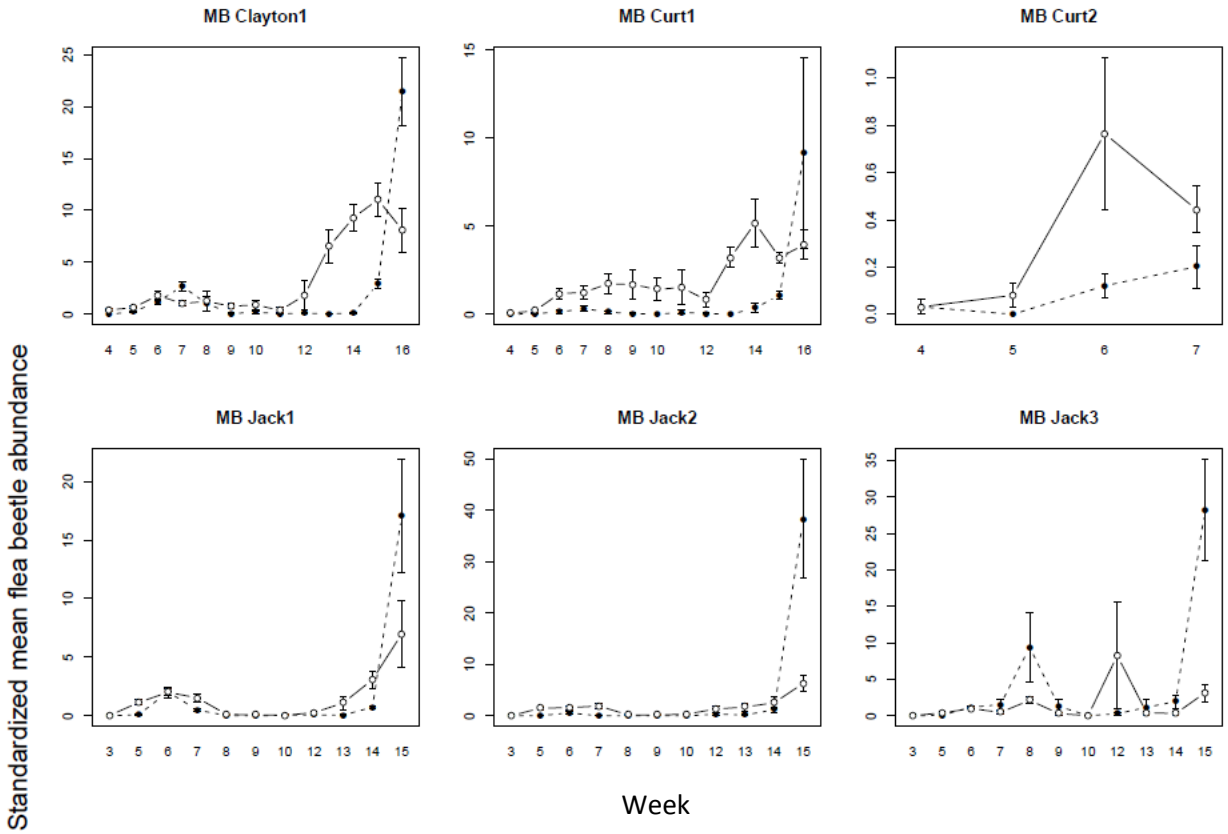


Fig. 37. Seasonal pattern of abundance of striped and crucifer flea beetles in 6 Manitoba fields in 2017. Here the flea beetle numbers are standardized for 4 days and 177.6 cm^2 area of the sticky card. The week is based on the collection date of the sticky cards. Week 3 = 24 May 2017. The open symbol refers to the striped flea beetles and the close symbol refers to the crucifer flea beetles. Each sample represents the average of 5 sticky traps / field / sampling date.

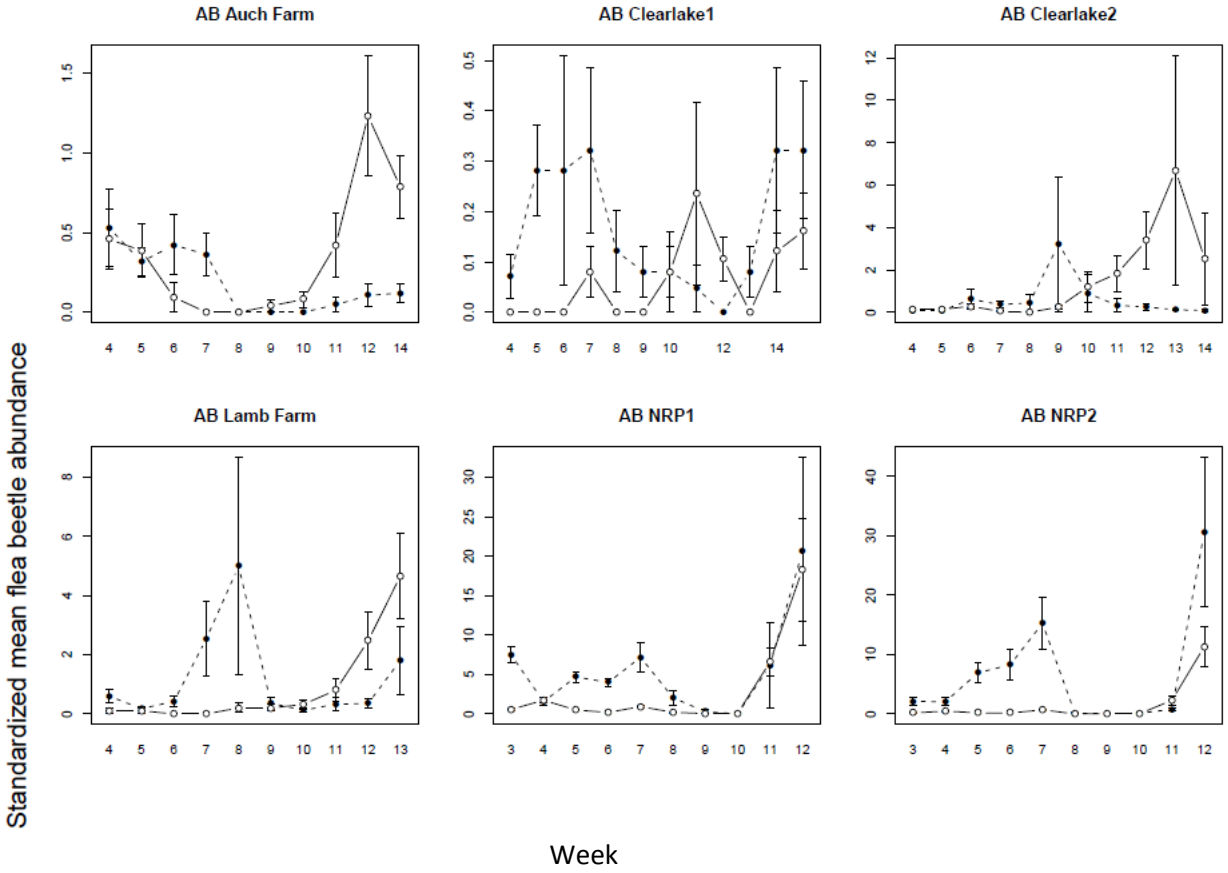


Fig. 38. Seasonal pattern of abundance of striped and crucifer flea beetles in 6 Alberta Lethbridge fields in 2017. Here the flea beetle numbers are standardized for 4 days and 177.6 cm^2 area of the sticky card. The week is based on the collection date of the sticky cards. Week 3 = 24 May 2017. The open symbol refers to the striped flea beetles and the close symbol refers to the crucifer flea beetles. Each sample represents the average of 5 sticky traps / field / sampling date.

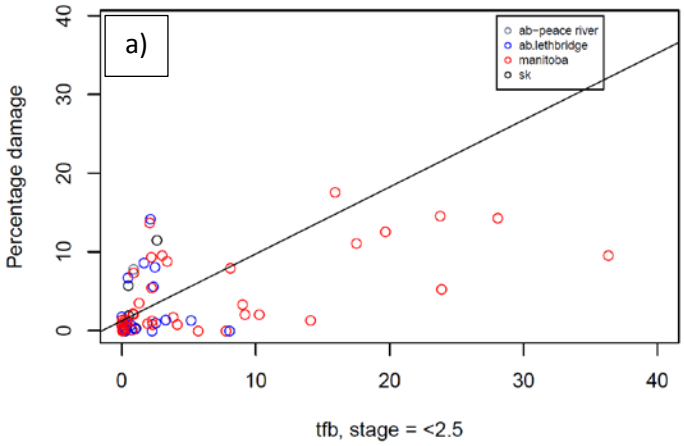
Relationship between flea beetle numbers, defoliation, and plant density

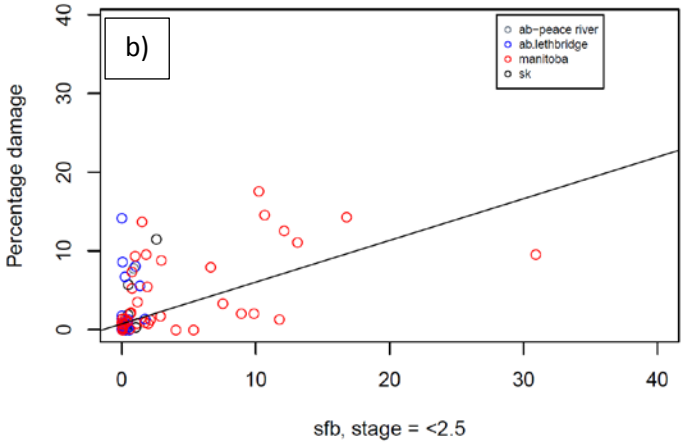
The relationship between average standardized flea beetle numbers in sticky cards per field and the average defoliation of plants was determined for the period before the 2.5 stage. Two types of analyses were conducted. In the first analysis, the average defoliation of the plant was calculated including cotyledons and first two leaves. In the second analysis, plant defoliation was calculated only for the cotyledons.

In both analyses, there is a positive linear relationship between the average standardized number of flea beetles and the corresponding average defoliation. The percentage defoliation increases with the increase in flea beetle numbers.

Furthermore, it was evident that the defoliation in these fields never exceeded the nominal threshold of 25% defoliation. This suggests that seed treatments gave adequate protection against flea beetles or that flea beetle abundance was not high enough to cause economic damage. A comparison of the associations between flea beetle levels and defoliation in the unsprayed control treatments of our economic threshold trials will be conducted to elucidate the most likely cause of reduced defoliation levels observed in growers' fields.

In some instances, there was defoliation observed when there were no flea beetles captured in the sticky cards. This might be the injury occurred before the sticky card placement, or may be due to other defoliators.





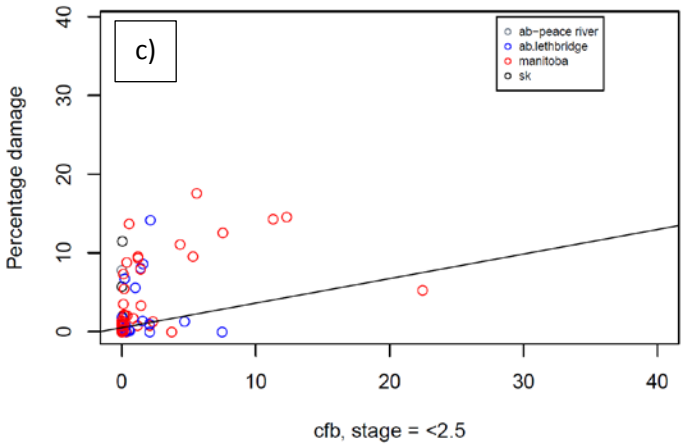


Fig. 39. Relationship between standardized flea beetle numbers and percentage defoliation when the crop stage is below 2.5 (a) tfb- total standardized flea beetle numbers (P=0.000003, Adjusted R2=0.2736), b) sfb-standardized striped flea beetle numbers (P: 0.0000037, Adjusted R2 = 0.2171), c) cfb-standardized crucifer flea beetle numbers (P: 0.0006027, Adjusted R2= 0.1518).

Abbreviations: ab-peace river: Alberta-Peace River area, ab.lethbridge: Alberta-Lethbridge area, manitoba: Manitoba, sk: Saskatchewan

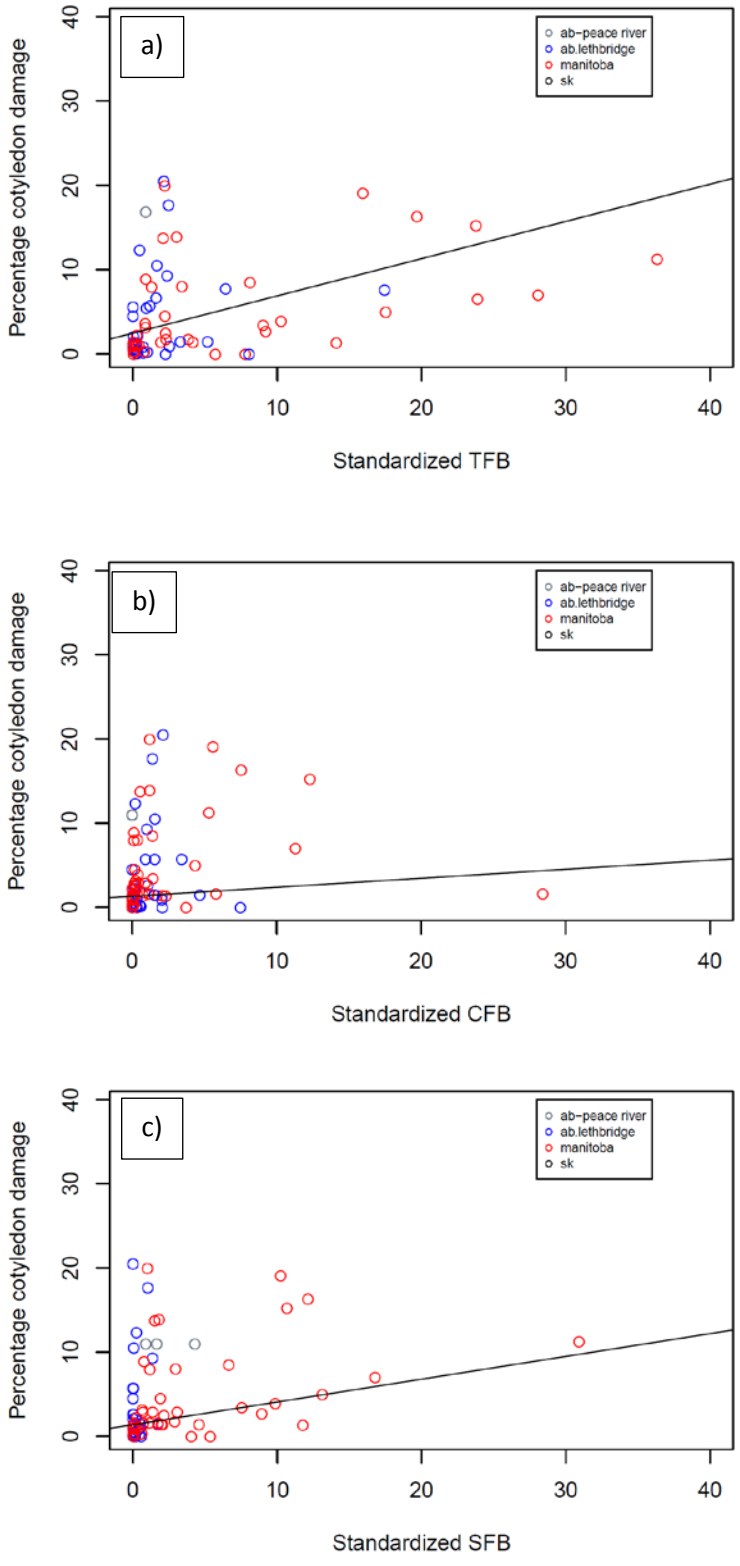


Fig. 40. Relationship between standardized flea beetle numbers and percentage cotyledon defoliation (a) TFB- total standardized flea beetle numbers (P = 0.004692 , Adjusted R2 = 0.09862), b) SFB-standardized striped flea beetle numbers (P = 0.02231 , Adjusted R2=0.06082), c) CFB-standardized crucifer flea beetle numbers (P = 0.02506 , Adjusted R2 = 0.05802).

Abbreviations: ab-peace river: Alberta-Peace River area, ab.lethbridege: Alberta-Lethbridge area, manitoba: Manitoba, sk: Saskatchewan

A multiple regression analysis was performed to assess the ability of standardized numbers of total flea beetles in the sticky cards (stan.tfb) and the number of plants in a linear meter (no.

plants) to predict the canola defoliation (defoliation). Preliminary analysis was performed to ensure that there is no violation of the assumption of normality and linearity. The standardized flea beetle numbers were log transferred in order to satisfy the assumptions better.

A significant regression equation is found (F-statistic: 11.43 on 3 and 54 DF, p-value: 6.559e-06, Multiple R-squared: 0.3883, Adjusted R-squared: 0.3543). Percentage defoliation increased with the increase in flea beetle numbers. There was no significant prediction of no. plants of the defoliation.

The model was further refined by removing the number of plants from the model. This analysis produced a significant negative interaction of no. plants and flea beetle numbers (F-statistic: 17.46 on 2 and 55 DF, p-value: 1.349e-06, Residual standard error: 3.841 on 55 degrees of freedom, Multiple R-squared: 0.3883, Adjusted R-squared: 0.3661). It implies that the varying plant densities will produce different defoliation for the same number of flea beetles and consideration of plant density is an important factor in the determination of flea beetle economic thresholds. Further analysis considering all possible variables is in progress.

Landscape effects on abundance of flea beetles*

**Based on analyses by Ph.D. student Thais Silva Guimaraes, with a stipend supported by an NSERC Discovery grant to ACC and the GETS program of the University of Manitoba.*

Methodology

We recorded all the habitats and crops present within a 3-km radius in each landscape sampled using digital images (obtained from Google Earth©) and ground observation. We created the digital maps using ARC GIS 10.2 (ESRI 2010) to quantify all cover types in the landscape. The main land cover types that we sampled were: canola, cereals, natural vegetation, other crops, semi natural vegetation, urban and water. In addition, we estimated Simpson's and Shannon's habitat diversity indexes for each landscape. Preliminary analyses were conducted using multiple regression models to identify landscape variables that best predict flea beetle abundance in canola crops. Flea beetle abundance was summarized by averaging samples collected before growth stage 3.1, and then standardized by stick trap size (i.e., by the smallest ST used in 2015 – 177.6 cm^2), days in the field (i.e., lowest period of the trap in the field (days $\geq$ 4)), and phenological date (i.e., selecting weeks with similar plant phenology across regions).

Preliminary Results

Preliminary analyses of 26 fields (12 from 2015 and 14 from 2016) indicate that crucifer flea beetle abundance decreased with an increase in the proportion of canola and other crops (i.e., minor crops combined) in the landscape at various scales. A similar pattern was found for striped flea beetles responding negatively to increases of the proportion of canola and cereals in the landscape. Further analysis, incorporating data from 2017, is needed before final conclusions can be reached in this system.

Objective 4: Develop predictive models for flea beetle populations based on weather and crop variables.

Final report: Integrated approaches for flea beetle control – economic thresholds, prediction models, landscape effects, and natural enemies

Prepared by Weather INnovations Consulting LP PO Box 23005, Chatham, ON, N7L 0B1

Final Analysis

For the years 2015, 2016 and 2017 there were 71 locations (32 in 2015, 26 in 2016, and 13 in 2017) with a total of 563 observations from May until August. These data points were then split into two categories: early season (May and June) and late season (July and August) emergence of flea beetle with temperature and precipitation parameters used for analysis. Principle component analysis (PCA) and partial least squares (PLS) analysis were done to find correlations between flea beetle populations and weather. This technique was used because it allows for many correlated weather variables to be used as a set of linearly uncorrelated variables called principle components. Linear regression was then used to fit a classification model when possible, and other statistical methods were used to verify the results. For easier analysis we broke the flea beetle populations into 4 groupings; spring and summer striped flea beetle and spring and summer crucifer beetle.

Results:

For spring beetle populations, we looked at a wide spectrum of weather data (relative humidity, temperature thresholds, etc.) and different months then narrowed it down to just the months of May, June, July, and August and the following weather variables:

- Number of days Tmax was greater than or equal to 14^{o}C
- Number of days Tmax was greater than or equal to 17 ^{o}C
- Number of days Tmax was greater than or equal to 20 ^{o}C
- GDD base 0 ^{o}C /5 ^{o}C /10 ^{o}C
- Canola Physiological Days (Pday)
- Total Precipitation
- Number of days Precipitation was greater than or equal to 1.0 mm
- Number of days Precipitation was greater than or equal to 2.0 mm

For both striped and crucifer beetles, we created an index for beetle pressure using statistical analysis on the number of sticky trap observations and standardized beetle count for each observation. The index included 4 beetle pressures: 0 – no pressure, 1 – light pressure, 2 – moderate pressure and 3 – high pressure. Using the 2015 to 2017 data set, optimal variables of weather were used to predict early and late season emergence of both crucifer and striped flea beetle.

For spring emergence of striped beetle which had an R^2 value of 77.8% (Table 5) we found that a warmer April / May period, a cooler and wetter June and accelerated plant growth (May Pday) is positively correlated with an increase in striped beetle populations. May seems to have the most impact temperature wise, as warmer temperatures (Tmax > 20, higher Pday) signal larger beetle pressure where as cooler temperatures (Tmax > 14, lower PDay) signal lower beetle pressure (see Fig.41).

For spring emergence of stripped beetle we treated 0/1 indexes (no/low pressure) as low population and 2/3 indexes (moderate/high pressure) as high populations and found that the linear regression model has a 73% accuracy rate and has 100% safety rate, which means it never predicts a low population when there is moderate/high pressure). However, the model will need more years of data for validation and to test feasibility.

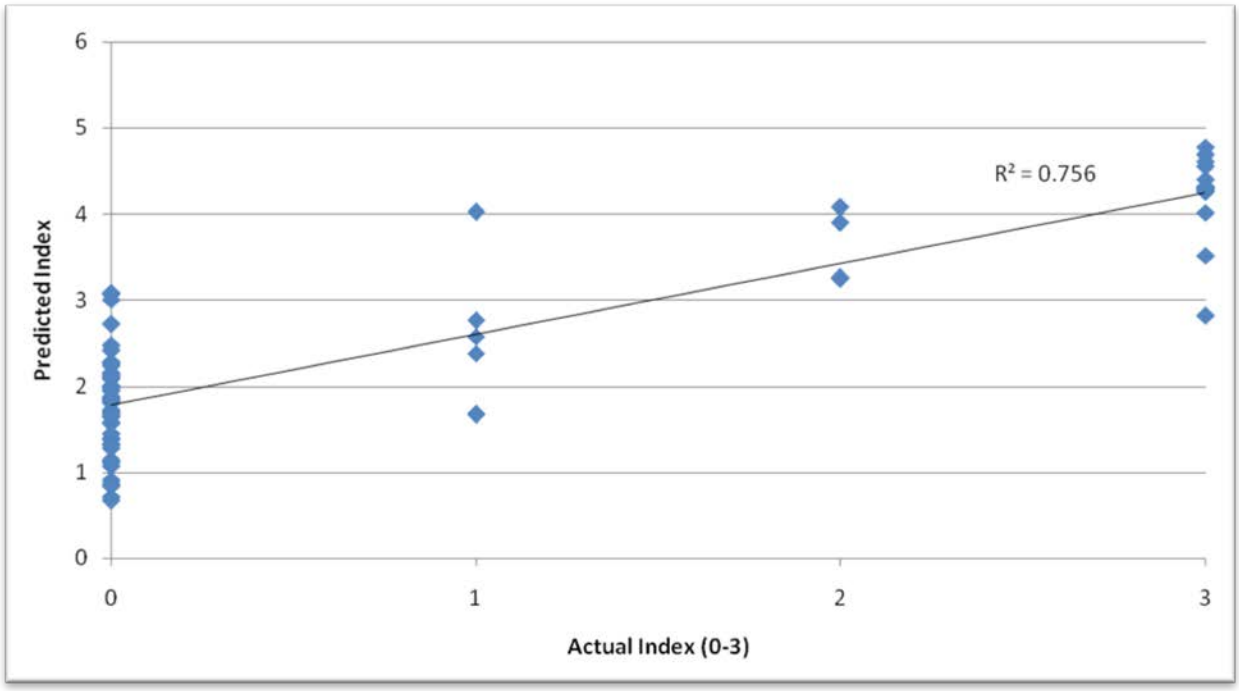


Fig.41. Striped flea beetle linear regression: predicted index vs. actual index

For spring crucifer beetle we used the same techniques that were used for the spring striped beetle however with a linear regression of 44.2% (Table 6) we did not get conclusive regression with just weather variables as we did with striped flea beetle. However, we can infer that more rainy days in May and June are positively correlated with crucifer beetle population but too much volume of rain in June shows a negative correlation. Also, a warmer May is positively correlated with spring crucifer beetle population numbers.

Regarding the summer population of striped beetle, which had an R^2 of 33.9% (Table 7), there were 55 trials over 3 years we found that:

- 20 trials had no to low pressure during both spring and summer seasons,
- 15 trials had moderate to high pressure in spring and summer,

- 4 trials had moderate to high pressure in spring, with no to low pressure in summer and;
- 16 trials had no to low pressure in spring, with moderate to high pressure in summer.

Overall, we found that 79% of trials had high pressure in spring and summer and 44% of trials had low pressure in spring, with high pressure in summer. However, striped beetle pressure in the spring does not mean they will not be active in the summer.

For summer crucifer beetle there were again 55 trials over 3 years (which had an R^2 of 44.2% (Table 8) we found that:

- 33 trials had no to low pressure in spring and summer,
- 12 trials had moderate to high pressure in both spring and summer,
- 6 trials had moderate to high pressure in spring, with no to low pressure in summer and;
- 4 trials had no to low pressure in spring, with moderate to high pressure in summer.

Overall we found that 67% of trials had high pressure in spring and summer and 11% of trials had low pressure in the spring, with high pressure in the summer. We also found that low crucifer beetle pressure in spring means low probability of crucifer population in summer.

Other Findings: Comparing Locations

When we compared locations that were close to each other and had similar weather patterns we found that sometimes they had different flea beetle patterns, which makes modeling using only weather data difficult. Two examples of this occurrence are Manitoba locations 15_AL1_C versus 15_BR1_C and Alberta location Old Elm 1 versus Old Elm 2 location.

Locations 15_AL1_C and 15_BR1_C are approximately 17 km apart and both show different flea beetle patterns despite having similar weather. Location 15_AL1_C shows little to no crucifer activity and shows striped flea beetle activity between mid-July and mid-August. While location 15_BR1_C shows high crucifer activity between mid-June and mid-July and only a modest amount of striped flea beetle activity on June 12th and minimal activity for the rest of the season. For the Alberta fields, Old Elm 1 and Old Elm 2, which are approximately 9 km apart, Old Elm 1 had crucifer flea beetle activity in June while Old Elm 2 did not have any crucifer flea beetle activity in June even though both locations have similar weather patterns and are located very close to one another.

There are a number of factors outside of weather that could contribute to these differences such as the number of overwintering sites for the flea beetle, previous years prevalence of flea beetle, tillage practices, field data (seeding date, spaying data, etc.), sticky traps uniformity in each plot (height, direction, type, etc.) among many other factors.

Conclusions

For spring striped beetles, a warmer April and May, a cooler and wetter June and accelerated plant growth is positively correlated with an increase in striped beetle populations. May has the most impact temperature wise, as warmer temperatures signal larger beetle pressure whereas cooler temperatures signal lower beetle pressure. For spring crucifer beetle we can infer that more rainy days in May and June are positively correlated with crucifer beetle population, but

too much volume of rain in June shows a negative correlation. Also, a warmer May is positively correlated with an increase in the population of spring crucifer beetles.

For summer striped beetle we found that 79% of the trials had high pressure in spring and summer and 44% of trials had low pressure in spring, with high pressure in the summer. For summer crucifer beetle we found that 67% of trials had high pressure in spring and summer and 11% of trials had low pressure in the spring, with high pressure in the summer. We also found that low crucifer beetle pressure in spring means a low probability of crucifer population in the summer.

Lastly, we concluded that modeling using only weather data is difficult because in some instances where locations were less than 20 km apart and had the same weather we found that there were different flea beetle patterns. This could be because we have not taken into account such outside factors as; the number of overwintering sites for the flea beetle, previous year's prevalence of flea beetle, tillage practices, field data (seeding date, spaying data) among many other factors.

Table 5. Spring striped beetle linear regression (R^2: 77.8%)

Weather Parameter	Correlation	P Value
April Pday Accumulation	-	0.000
April GDD_0 Accumulation	+	0.000
May Pday Accumulation	+	0.001
May: Days Tmax >= 14 ^oC	-	0.000
May: Days Tmax >= 20 ^oC	+	0.000
June GDD_0	-	0.005
June Precipitation	+	0.000

Table 6. Spring crucifer beetle linear regression (R^2: 44.2%)

Weather Parameter	Correlation	P Value
April Precipitation	-	0.002
May Days >= 1mm	+	0.001
May GDD_0	+	0.000
June Precipitation	-	0.002
June Days >= 2mm	+	0.000

Table 7. Summer striped beetle linear regression (R^2: 33.9%)

Weather Parameter	Correlation	P Value
April Precipitation	-	0.001
June GDD	+	0.001
June Precipitation	+	0.008

Table 8. Crucifer beetle linear regression (R^2: 44.2%)

Weather Parameter		Correlation	P Value
May Precipitation		+	0.000
July Days > 2mm		-	0.026
August PDays		+	0.000
August Precipitation	-	0.000	

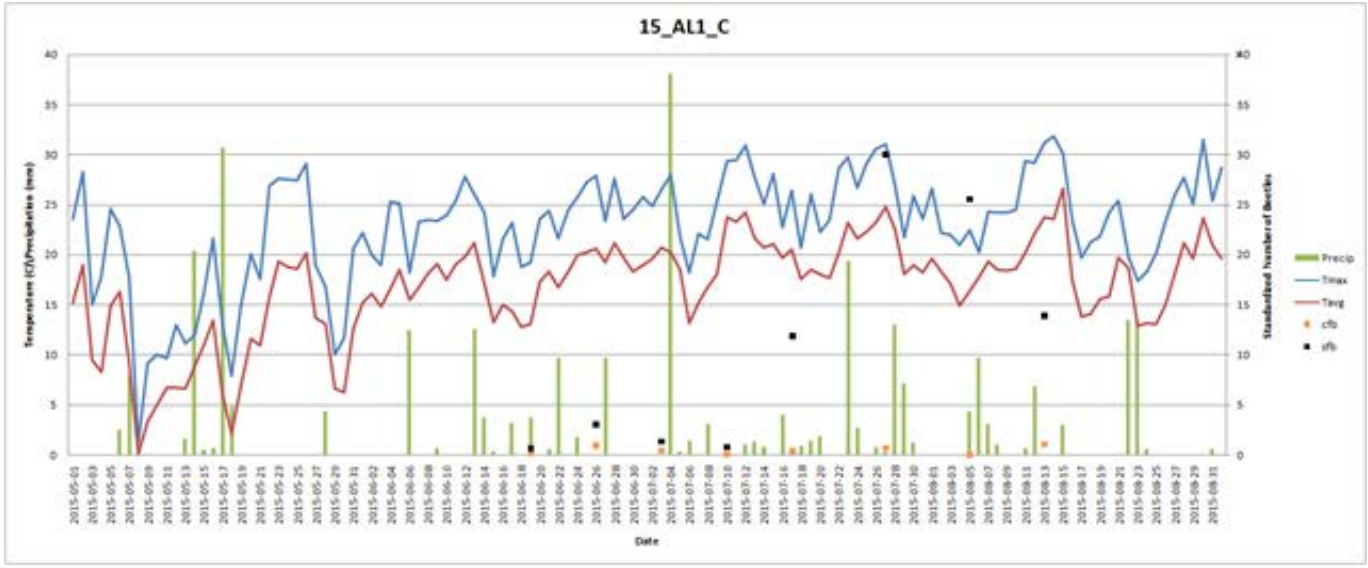


Fig.42. Location 15_AL1_C shows little to no crucifer activity and low striped flea beetle activity between mid-July and mid-August.

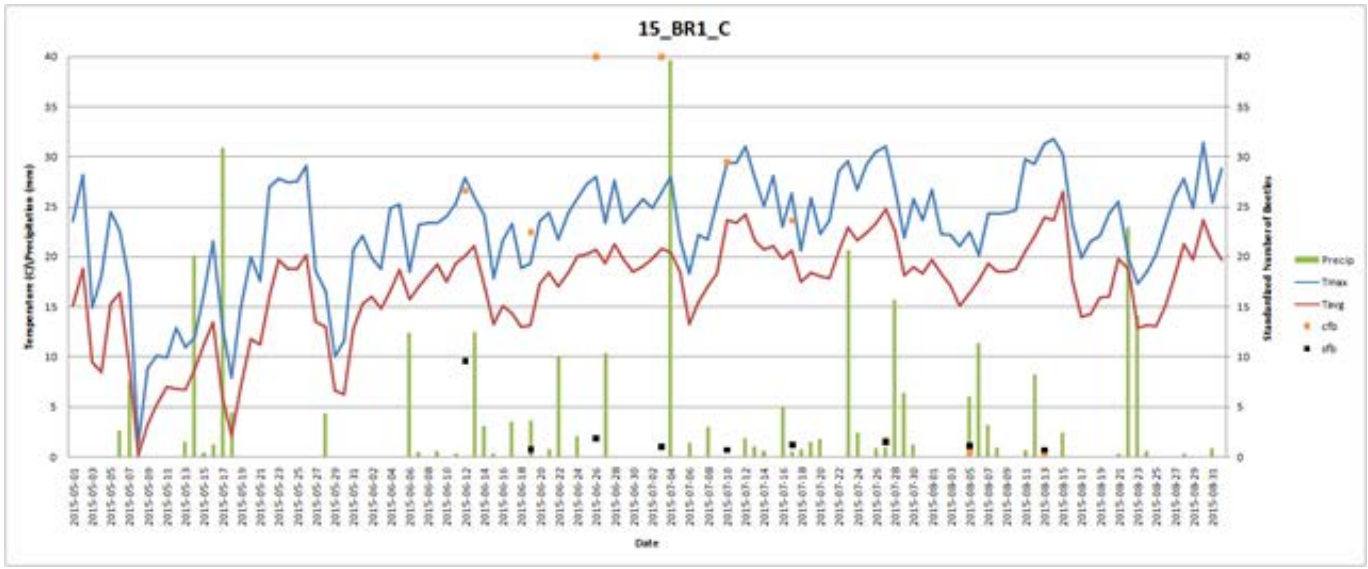


Fig.43. Location 15_BR1_C shows high crucifer activity between mid-June and mid-July and only a modest amount of striped flea beetle activity on June 12th and minimal activity for the rest of the season.

An improved method for rearing striped flea beetles Phyllotreta striolata (Coleoptera: Chrysomelidae) in the laboratory*

**This is a manuscript draft prepared by Tharshi Nagalingam and Alejandro Costamagna to be submitted soon for publication.*

Background:

Crucifer flea beetles (*Phyllotreta cruciferae* (Goeze)) and striped flea beetles (*Phyllotreta striolata* (Fabricius)) are the two dominant flea beetle species (Coleoptera: Chrysomelidae) damaging canola (oil seed rapae) (Brassica Linnaeus, Brassicaceae) in the Canadian Prairies (Burgess 1977 and 1981). Flea beetles overwinter as adults in the soil and under the leaf litter (Westdal and Romanow 1972). Adults emerge from hibernation in spring and cause economic loss by defoliating the canola seedlings (Westdal and Romanow 1972). Canola plants are most susceptible to flea beetle damage during the cotyledon and early true leaf stages (Lamb 1984). The damage after fourth true leaf stage does not have a significant impact on yield (Westdal *et al*. 1979). Flea beetle damage causes an average loss of 8–10% of crop yield (Lamb and Turnock 1982).

Flea beetles lay eggs in the wet soil and close to canola plants; emerging larvae feed on canola roots. Bracken and Bucher (1986) estimated yield loss of 5% when the larval densities exceeded $0.16/cm^2$ in soil core samples. As there was no easy-to-use technique to rear flea beetle immature stages in the laboratory, they indirectly assessed the yield difference by drenching carbofuran to kill the larval stages and then compared the results with the untreated plots.

Previous studies describe rearing procedures for flea beetles. Kinoshita et al. (1979) developed a method to rear crucifer flea beetles in the laboratory and studied its biology. Burges and Wiens (1976) raised striped flea beetles in greenhouse on Horseradish (*Armoracza rusticma* (Lam.)) plants, but this method produced only a small amount of insects. Wang et al. (2008) and Xian et al. (2009) rear striped flea beetle stages through a single generation using Chinese flowering cabbage (*Brassica parachinensis* L.) and Raddish (*Raphanus sativus* L.). Even though these methods produce immature stages of flea beetles, the method is labor intensive and lack detailed description of the procedures.

Mass rearing of flea beetle in the laboratory will facilitate research during the times when flea beetles are not abundant in the field. We developed two improved methods to rear striped flea beetles continuously in the laboratory to produce immature stages and adult flea beetles. The rearing method is described in detail.

Methodology

Source of flea beetles:

The colony was established from three different sources of flea beetles. The first source (source 1)was the flea beetle adults collected with sweep nets from the University of Manitoba Research facility at Winnipeg in May 2017. These beetles were reared through five generations in the

laboratory without hibernation. The second source (source two) of beetles was the first generation adults emerged from the above method (source one). The third source (source three) of beetles were field collected adults from the University of Manitoba Research facility in Winnipeg in late August 2017. Source three beetles were hibernated in the laboratory.

Hibernation of adults:

For hibernating the beetles, we adapted a method developed by Kinoshita *et al*. (1979). The hibernation was performed in two steps. Beetles were pre-hibernated in a 4L glass jar with 5 cm of wet peat moss in the bottom of the jar, and 5 cm of dry peat moss covered the wet peat moss. Air-dried Napa cabbage leaves were provided as food, and the jar was then closed with a lid covered by a fine mesh. Jars were checked periodically, and food added was added as needed. Jars were incubated at 19 °C for 8 hours of light and 7 °C for 16 hours of dark, during one month (pre-hibernation). After the pre-hibernation month, the cabbage leaves from the jar were removed, and the jar was covered with black cloth or with Aluminium foil and placed at 5 °C in the dark for a month (hibernation).

Method one:

Oviposition cages:

Flea beetles were transported to the laboratory in plastic bags and in the laboratory they were released in perforated plastic tubs (12 cm diameter, 15 cm height; Bug Tub® (Royal Oak Point NW, Calgary, Alberta, Canada). The bottom part of these tubs was cut open, and it was covered with cheesecloth (mesh size 0.1 mm) and on the top, it was covered with a fabric screen lid. These cages were then placed on top of a small plastic cup (10.5 cm diameter, 7 cm height) containing wet muck, sand, the potting mixture in equal proportion and covered with two brown paper towels cut into a circular piece to fit the circumference of the cup (10.5 cm diameter). In each cage, 25–50 unsexed beetles were caged and provided with a single air-dried Napa cabbage leaf. The insects were reared in the growth chambers at 24 °C, 60-70% RH, and 16:8 (L:D) h cycle under fluorescent lighting. The observation for eggs on paper towel was done three times a week. The number of eggs or larvae found on paper towel was recorded. The Napa cabbage was checked for flea beetle stages once in a week, and the number of eggs or larvae found was recorded.

Larval rearing cages:

The eggs laid on paper towel and on cabbage leaf was removed carefully with a camel hair paint brush and transferred to canola plants in cotyledon stage and grown in pots (14 cm diameter and 10.5 cm height) at 24 °C and 60-70% RH with the planting material containing muck, sand, and potting mixture in 1:1:1: ratio. These containers were then placed in a BugDorm cage (width 47.5 cm, length 47.5 cm, height 47.5 cm, BugDorm, Taichung, Taiwan). In each cage, one to four pots with eggs were kept. The number of eggs in each pot did not exceed 50. The cages were placed in a walk-in chamber at 24 °C, 60-70% RH, and 16:8 (L:D) h cycle under fluorescent lighting. The cages were checked three times a week and watered adequately. Too much watering was avoided to prevent larval mortality. Close observation of cages was done

after a week of placement of eggs in the bug dorm cages. Once the beetles emerge from the larval cages, the beetles were collected using an aspirator, and the number of beetles emerging from each bug dorm cage was counted. These beetles were then used to start a new generation of the colony in the method described as above.

Method two

Method two was developed as an easier methodology to maintain the flea beetle colony with minimal work, when only adult beetles are needed. For this method, the beetles used were fourth generation adults from the source one, second-generation adults from the source two, and the second generation of adults from the source three, all produced using rearing method one. For method two, 100–150 unsexed flea beetles were placed in bug dorm cages (width 47.5 cm, length 47.5 cm, height 47.5 cm, BugDorm, Taichung, Taiwan) with two to four pots (14 cm diameter and 10.5 cm height) of canola in a fully expanded cotyledon stage. These plants were planted with an equal proportion of muck: sand: potting mix. Every week, the pots were replaced with new canola pots, and the old pots were transferred to new bug dorm cage for the adult emergence from the eggs laid. The plants were watered three times a week adequately to ensure that the plants are not dry and over watering was avoided. The number of adults emerged from the eggs laid were monitored three times a week, and the cages were continued until we get no adult emergence from the cages.

Our methods allowed continuous rearing of striped flea beetles in large numbers from May 2017 to May 2018 (i.e., over eight generations) and thus provides a good methodology to maintain a striped flea beetle colony for research purposes. Striped flea beetles underwent several generations without hibernation, indicating that striped flea beetles collected in Manitoba do not need an obligatory diapause in their life cycle. We are currently working on adjusting this methodology to rear crucifer flea beetle.