



PROJECT DETAILS

- **Title:** Integrated approaches for flea beetle control II: incorporating the impacts of plant density, ground predators, and landscape- scale predictive models in the management of flea beetles in the Canadian Prairies
- **Funders:** Agriculture and Agri-Food Canada, Canola Council of Canada, Alberta Canola, SaskCanola and Manitoba Canola Growers
- **Research program:** Canadian Agricultural Partnership
- **Principal investigator:** Alejandro Costamagna
- **Collaborators/additional investigators:** Héctor Cárcamo, Jennifer Otani, Tyler Wist, Maxime Damien, Meghan Vankosky, John Gavloski, Robert Duncan, and Shayla Woodland
- **Year completed:** 2023

Final report

Introduction

Flea beetles are one of the major pests of canola in Western Canada. Preventative seed treatments are widely used to protect canola seedlings from flea beetle damage. However, the application of foliar insecticides is also required in many fields every year to control these pests. Excessive use of control measures has negative environmental consequences and increases the cost of canola production. Several gaps in our knowledge prevent efficient flea beetle management, including the effects of plant density in flea beetle management, the effects of stem feeding damage on flea beetle control, the role of natural enemies on flea beetle management, and regional predictive models for flea beetle abundance and damage. This study addresses these research gaps under four objectives.

Project Objectives

1. a- Evaluate the effects of different seeding rates and insect management options on the economic thresholds for flea beetles using current canola cultivars.
1. b- Evaluate the effects of different seeding rates of canola on flea beetle infestations under field conditions.
2. Evaluate the effect and the mechanism of stem feeding by different flea beetle species under laboratory and field conditions; determine the direct and indirect effects of predators on stem damage and defoliation.
3. Determine the effect of ground and foliar predators and landscape structure on canola defoliation, flea beetle abundance, and species composition in different regions of Western Canada.

4. Construct a predictive model for flea beetle abundance and damage to canola, incorporating predators, landscape effects, and abiotic factors.

Objective 1a: Evaluate different seeding rates and insect management options on the economic thresholds for flea beetles using current canola cultivars.

Methodology

Between 2018 and 2022, 16 trials have been conducted to determine the economic threshold of flea beetles incorporating the seeding density of canola in the prairies. The trials were conducted in Manitoba (6) and Alberta-Peace River (3), Saskatchewan (3), and Alberta-Lethbridge (4). One trial from Lethbridge in 2018 had to be removed due to late establishment and poor infestation levels.

Trials were designed in a randomized complete block design. Each treatment was replicated 4 times in small plots. The treatments were a factorial combination of seeding rates and flea beetle control methods. The seeding rates tested were: 1) a low seeding rate at 3.5 Kg/ha (3 plants per square foot), 2) an optimum seeding rate at 7 Kg/ha (6 plants per square foot), and 3) a high seeding rate at 14 Kg/ha (12 plants per square foot). At each seeding rate, there were (a) control plots with fungicide-only treated seeds (i.e. control), (b) seed treatment with insecticide and fungicide-treated seeds (i.e. seed), (c) foliar insecticide spray at 25% defoliation with fungicide-only treated seeds (i.e. foliar) and (d) weekly foliar insecticide spray with fungicide-only treated seeds (i.e. Flea beetle-free (FB-free)) (factorial design with 3 seeding rates x 4 flea beetle control treatments). The FB-free treatment (d) was added to have a control with minimal flea beetle damage.

Standard agronomic practices were followed during the plot establishment and maintenance, with adjustments to follow sanitary guidance and protocols because of the COVID-19 pandemic during the 2020-2021 seasons. In each plot, flea beetle populations were monitored using yellow sticky cards placed in the middle of each plot and changed weekly during the susceptible canola growth stage (up to 2.4 phenological stage). Plant density was assessed four times in three fixed linear 1-m rows, at the first assessment (50% of emergence), 1.0 and 2.2 stages, and once before harvest. The percentage of flea beetle damage was assessed twice a week on 20 plants per plot (Soroka et al. 2011) until the four-leaf stage. At harvest, plot yield (plot weight and 1000 kernel weight) and quality parameters (green seed percentage, oil, chlorophyll, and protein) were assessed.

Statistical analyses:

Data from all collaborators had first to be homogenized to constitute three distinct datasets combining all years and places for the three main response variables analyzed: canola defoliation (cotyledon, first leaves, and then the whole plant), flea beetle abundance on sticky cards (total flea beetles or abundances of the two main species, crucifer and striped), and yield per plot at the end of the experiment. The total dataset consisted of 137,411 samples of cotyledon damage, 72,734 samples of leaf damage, 2,208 sticky trap samples, and 695 yield measurements in plots. The two first data for defoliation and sticky cards were then aggregated at the plot level (mean canola defoliation per transect, date, and plot, and mean flea beetle abundance per sticky

cards, date, and plot) and merged with the yield dataset to obtain one final dataset with one value per plot.

Defoliation of the two cotyledons was averaged and the same was done for the defoliation of the first two true leaves. Then both values were averaged to estimate the mean plant defoliation as the response variable. Flea beetle abundances were analyzed using crucifer and striped flea beetle abundances added together under total flea beetle abundances. In a second set of analyses, the mean flea beetle abundances per sticky card were weighted by the mean number of canola plants within 1m transect to obtain the flea beetle pressure per canola plant. Canola yield was estimated in Kg.Ha-1 by using the seed weight and plot size, and standardized at 8.5% moisture.

Analyses were done using linear mixed models and type 3 ANOVA (Lmer and car packages in R). In these analyses, the main effects of seeding rate, flea beetle treatment, region, and their interactions were considered explanatory variables. The year was added as an additional and individual explanatory variable and the plot area was used as a covariate. The block and plot were considered random factors. In addition to those explanatory variables and to meet the ANOVA assumptions, each response variable was transformed (when needed). The following transformations were applied: square root for mean plant defoliation and log transformation for mean flea beetle per sticky card or mean flea beetles per plant. The estimated yield did not need any transformation to meet Anova's assumptions. The data was also scaled at the trial level, to account for variability at that level and to allow the analysis of all data combined regardless of the wide variations in flea beetle abundance, damage and yield observed among trials. Scaling was done by subtracting to each observation its trial mean and dividing it by the standard deviation of the trial. Thus, positive values in figures indicate that treatments increased the value over the trial mean and negative values indicate the opposite effect. Each set of analyses started with the full model and was then manually simplified when interactions were not significant, starting with the three-way interaction among flea beetle treatment, seed rate, and province and then non-significant two-way interactions. All individual explanatory variables were left in the final models. Post-hoc comparisons within groups were performed using the *emmeans* package with "sidak" adjustment.

Results

Defoliation:

There were three significant interactions regarding mean canola defoliation (**Table 1**: region x insecticide treatment ($\chi^2 = 52.47$, $df = 9$, $p < 0.001$), region x seed rate ($\chi^2 = 32.64$, $df = 6$, $p < 0.001$) and insecticide x seed rate ($\chi^2 = 25.19$, $df = 6$, $p < 0.001$). The region and insecticide interaction is explained by differences among insecticide effects within different regions. In both Lethbridge and the Peace River lower damage occurred in seed (-40.55% and -25.62% respectively) and FB-free treatments (-18.31% and -26.32% respectively), and the maximum damage was in the control (+33.04% and +31.59% respectively). Damages in foliar treatments were at intermediate levels (+13.61% and +11.58% respectively; **Table 2, Figure 1**). There were no differences in canola defoliation among regions for control and foliar treatments. However, defoliation was significantly

lower for FB-free treatment in Saskatchewan (-46.41%) than in other regions. In Manitoba and Lethbridge, defoliation was lower in plots with treated seeds (-40.86% and -40.55% respectively; **Table 2, Figure 2**).

- Seed and FB-free treatments were more effective in reducing defoliation than the foliar spray treatment, which was intermediate compared to untreated control plots that suffered the most from defoliation.

The region and seed rate interaction is explained by differences in seed rate effects within different regions. There were no differences in canola defoliation in the Peace River according to seed rates. In Lethbridge, defoliation was lower in low-seeded plots (-10.03%) than in optimum (+6.03%) and high-seeded (+4.00%) plots. In Manitoba, defoliation was the lowest in high-seeded plots (-9.58%). In Saskatchewan, defoliation was the highest in plots with low-seeded (+14.17%) plots (**Table 2, Figure 3**). For high and low seeding rates, there were no differences in mean canola defoliation among regions, whereas mean defoliation was higher in Manitoba (+3.51%) than in Saskatchewan (-3.47%) for optimum seed rates (**Table 2; Figure 4**).

- Contrasting effects of seeding rates were found according to region. As expected, high seeding rates resulted in lower defoliation in trials in Manitoba and Saskatchewan. However, we observed lower defoliation in the low seeding rate trials in Lethbridge and no effects of seeding rates in Peace River.

The interaction between flea beetle treatment and the seeding rate is explained by the absence of differences in mean canola defoliation among seeding rates within the different flea beetle treatments, except for treated seeds. Defoliation was significantly lower in high-seeded plots (-44.33%), higher in low-seeded plots (-21.30%), and intermediate at optimum-seeded plots (-32.99%; **Table 2, Figure 5**). For high-seeded plots, defoliation is lower in treated seed plots (-44.33%), followed by FB-free plots (-20.71%), then foliar spray (1.98%), and finally the highest damage was observed in control plots (+32.71%). Under low seeding rates, defoliation was lower in FB-free plots (-25.09%), followed by plots with treated seed (-21.30%), then foliar plots (7.11%), and highest in control plots (+38.88%). Plots with optimum seeding rate had lower damages in FB-free (-17.84%) and treated seed plots (-32.99%); higher defoliation was observed on control (+44.03%) plots and intermediate levels of foliar treatment plots (+7.85%; **Table 2, Figure 6**).

- For control, foliar and FB-free treatments, seed rate had no effects on defoliation, whereas increasing seed rate decreased canola defoliation for treated seeds.

Total flea beetles per sticky cards:

After the backward selection procedure, there was one remaining significant interaction between insecticide and seed rate treatments on mean total flea beetles per sticky cards ($\chi^2 = 20.92$, $df = 6$, $p < 0.01$, **Table 3**). The interaction is explained by different levels of infestation among seed rates in control and foliar treatments. The mean abundance of flea beetles was significantly lower in low-seeded plots (-17.92% in control and -25.99% in foliar) and higher in high-seeded plots (+48.98% in control and +33.25% in foliar, **Table 4**). Optimum and high-seeded plots did not differ in flea beetle abundance within the FB-free (-21.49% and -9.30% respectively) and seed treatments (-3.98% and +8.59% respectively; **Table 4, Figure 7**).

For given seed rates, differences among treatments were different. The mean total abundance of flea beetles per sticky card in plots with high and optimum seeding rates was lower for FB-free plots (-9.30% and -21.49% respectively, **Table 4**) and higher for control (+48.98% and +21.91% respectively, **Table 4**). Intermediate levels were found for the foliar and seed-treated treatments. For low-seeded plots, abundance was lower in FB-free (-36.09%) plots and higher in seed-treated (-14.80%) plots (**Table 4, Figure 8**).

- For all treatments, low-seeded plots were less attractive, and high-seeded plots were the most attractive to flea beetles. The optimum-seeded had an intermediate effect only in control and foliar treatments.

Total flea beetles per plant:

After weighting the total number of flea beetles per sticky card by the mean number of canola plants within 1 m, two significant interactions remained, between region and seed rate (**Table 5**; $\chi^2 = 15.73$, $df = 6$, $p < 0.05$) and between insecticide and seed rate treatments (**Table 5**; $\chi^2 = 15.80$, $df = 6$, $p < 0.05$).

The interaction between region and seed rate is explained by different abundances of flea beetles per plant among seed rates within the different regions. In Peace River and Manitoba, the lower number of flea beetles per plant was observed in high-seeded plots (-32.05% and -37.69% respectively, **Table 6, Figure 9**), intermediate levels were observed in optimum-seeded plots (+4.60% and -1.16% respectively; **Table 6**), and higher levels were found in low-seeded plots (+27.45% and +38.85% respectively, **Table 6, Figure 9**). In Lethbridge, the mean number of flea beetles per plant was lower in high-seeded plots (-26.98%) compared to plots with low (+12.94%) and optimum (+14.04%) seeding rates (**Table 6, Figure 9**). In Saskatchewan, the number of flea beetles per plant was higher in low-seeded plots (+52.42%) than in optimum and high-seeded plots (-15.71% and -36.70% respectively; **Table 6, Figure 9**). For a given seeding rate, the number of flea beetles per plant was not different among the different regions (**Figure 10**).

- There were fewer flea beetles per plant in high seed rate plots, and more flea beetles per plant in low seed rate plots. The optimum seeding rate had intermediate levels in Manitoba and Peace River, but it was not different from low- and high-seeding rates in Lethbridge and Saskatchewan, respectively.

The interaction between flea beetle and seeding rate treatments is explained by the different numbers of flea beetles per plant within the seeding rate treatments. In foliar, FB-free, and seed treatments, the number of flea beetles per plant was lower in high-seeded plots (-31.52%, -49.52% and -41.70% respectively, **Table 6**) and higher in low-seeded plots (+17.14%, +11.47% and +49.01% respectively, **Table 9**). For plots with optimum seed rate, the number of flea beetles per plant was intermediate. In control plots, there were fewer flea beetles per plant in plots with higher (-21.61%) than optimum (+25.82%) or low (+48.42%) seeding rates (**Figure 11, table 6**). For a given seed density, there were differences among flea beetle treatments. In plots with high seeding rates, the number of flea beetles per plant was lower in FB-free and higher in control plots. The number of flea beetles per plant in plots with treated seed was not different from FB-free plots, and in foliar, it did not differ from control plots. There was no difference between foliar and seed treatments. In plots with optimum seed rate, the number of flea beetles per plant was higher in the control (+25.82%) than in the other three

treatments. In plots with low seeding rates, the number of flea beetles per plant was higher in control (+48.42%) and plots with treated seeds (+49.01%) than in the FB-free (+11.47%) and foliar (+17.14%) treatments (**Figure 12, table 6**).

- Plots with high seeding rates had fewer flea beetles per plant whereas plots with low seeding rates had the most. At a low seed rate, there were as many flea beetles per plant in seed treatments as in the control and less in foliar or FB-free treatments. In general, higher seeding rates results in a lower number of flea beetles per plant.

Yields at standardized 8.5% moisture:

After the backward selection procedure, it remained only one significant interaction between region and flea beetle treatment (**Table 7**: $\chi^2 = 33.03$, $df = 9$, $p < 0.01$), and an independent effect of seed rate (**Table 7**: $\chi^2 = 66.12$, $df = 2$, $p < 0.01$) on yield.

The interaction between region and insecticide treatment is explained by different yields among flea beetle treatments within the different regions. In Peace River, there were no significant differences. In Lethbridge, the yield was lower for control plots (-14.14%) and higher for plots with treated seeds (+17.45%), with intermediate levels for foliar (-3.74%) and FB-free (+1.26%) treatments. In Manitoba, the yield was lower for control (-33.43%) and higher for FB-free (+40.15%) treatments and intermediate for treated seeds (+2.01%) and foliar (+4.19%) treatment. In Saskatchewan, the yield was higher in the plots with FB-free treatment (+23.35%), and no differences among control (-16.40%), foliar (-4.17%), and treated seeds (-2.77%) treatments (**Table 8, Figure 13**). For a given flea beetle treatment, there were no differences in estimated yields among the different regions (**Figure 14**). Independently of region and flea beetle treatments, the yield was highest in the high seeding rate treatment (+12.46%), followed by optimum (+2.07%) and low-seeded treatments (-14.60%; **Figure 15**).

- High seeding rates resulted in better yield, regardless of flea beetle control methods and across all regions. Foliar spray and seed treatments had similar yields except in Lethbridge. The FB-free treatment indicated that foliar and seed treatments did not completely prevent damage in Manitoba and Saskatchewan.

Publication in preparation: Woodland, S; Damien, M; T Nagalingam; HA Cárcamo; J Otani; T Wist; JA Bannerman, J Gavloski, R Duncan and AC Costamagna. Effects of plant density and flea beetle management treatments on canola damage and yield in western Canada.

Objective 2: Evaluate the effect and the mechanism of stem feeding by different flea beetle species under laboratory and field conditions and determine the direct and indirect effects of predators on stem damage and defoliation.

Methodology:

Effects of predators and plant density:

According to the preliminary tests, a laboratory experiment has been set up to determine the effectiveness of *Pterostichus melanarius*, *Harpalus amputatus* (Coleoptera: Carabidae) and *Pardosa* spp. (Araneae: Lycosidae) in a realistic laboratory study using microcosms. Each microcosm contained either three or six canola plants in the cotyledon stage with three *P. cruciferae* individuals, three *P. striolata* individuals and one of the predators mentioned above. The canola seedlings were planted in a single tray for 5 days and transplanted into a randomly assigned microcosm. The predators and flea beetles were randomly assigned to the microcosms in random order. A total of 12 trials took place over 12 weeks, for a total of 150 predators and 40 predator-free controls. The experiments were conducted at 16 h light at 22°C and 8 h dark at 18°C for 48 hours, using two growth chambers simultaneously. In each replicate it was quantified the proportion of live flea beetles at the end of the experiment, the prevalence (*i.e.*, number of plants damaged) and intensity (*i.e.*, area or percentage of plant tissue damaged in attacked plants) of cotyledon and stem damage. A novel method was developed to estimate stem damage per plant. First, based on preliminary observations, the length of each stem pit was categorized as either 1, 2 or 3 mm in length. This length was used to calculate the area of the pit as an oval using 0.2 mm as a constant width for all the pits. The actual calculated area of the stem pits (measured in the photo-editing software, GIMP) was highly correlated to the estimated values obtained from the novel estimation method (Linear Regression, $F_{1,45} = 424.7$, $p < 0.00001$, $R^2 = 0.90$; **Fig. 16**). This method of estimating stem damage was used for all experiments examining stem damage.

Effects of temperature and flea beetle species:

We conducted microcosm studies to determine the effects of temperature on cotyledon defoliation caused by the flea beetle species, *P. striolata* and *P. cruciferae*. Four constant temperature regimes were tested: 13°C, 18°C, 23°C and 28°C (70% RH) at a photoperiod of 16 L: 8 D. Each trial contained the following treatments at each temperature: (i) Control treatment with no flea beetles to assess plant growth in the absence of herbivores, (ii) *P. striolata* only treatments, and (iii) *P. cruciferae* only treatments to assess any feeding differences between the two species. Each microcosm contained two canola plants at the cotyledon stage with five flea beetles of the appropriate species. At the end of the 48-hour trial, the damage was digitized using the computer software GIMP (The GIMP Development Team 2019). Each cotyledon was measured across the widest point (length) and across the main vein (width) to obtain an estimation of the area, which was used to calculate estimated proportions of damaged area to total area. The defoliation was manually coloured pink on the photo editing software in order to make the damage drastically contrast the healthy tissue.

The pixels were binned using the package Colordistance (Weller 2019). The proportion of pink pixels (damaged) to green pixels (healthy) were computed to determine the area damaged in mm².

A split-split plot ANOVA model was used to determine the effects of temperature (whole plot), flea beetle species (sub-plot) and the side of the cotyledon (sub-sub plot) on area damaged per cotyledon per flea beetle

(mm²) using trial as a random factor. Backward elimination was completed with the use of AIC scores to determine the best model. Species as a main effect was excluded from the model after no significant main or interacting effects were detected for this variable.

Effects of temperature and plant density:

Five trials took place from February 10 to March 30, 2021. Each trial contained two replicates of each of the following treatments at each plant density: (i) Control treatment with no flea beetles at 18°C, (ii) control treatment with no flea beetles at 23°C, (iii) five *P. striolata* individuals at 18°C, and (iv) five *P. striolata* individuals at 23°C. The two plant densities used were either five or ten canola plants per pot. The variables of interest were cotyledon damage and stem damage in terms of total, prevalence, and intensity. A factorial ANOVA model was used with temperature and plant density as the main factors. To test for the effects on stem and cotyledon prevalence, a type 3 *Wald chi square test* was used with a binomial distribution (attacked versus non-attacked).

Results:

Effects of predators and plant density:

The presence of *P. melanarius* (n=66) and *Pardosa spp.* (n=15) reduced the number of flea beetles after 48 hours when compared to the predator-free control group (F_{3,111.8} = 14.22, *p* < 0.00001; **Fig. 17**). *Harpalus amputatus* did not reduce the abundance of flea beetles. *P. melanarius* also reduced the mean number of flea beetles after 48 hours regardless of the planting density (F_{2,174.7} = 14.15, *p* < 0.0001; **Fig. 18**). There was no interaction found between predator species and planting density (F_{2,174.8} = 1.68, *p* = 0.19).

When placed in microcosms with *Pardosa spp.* and *P. melanarius* predators, flea beetles produced less overall cotyledon defoliation when compared to the control group when examining only the low density (F_{3,121.9} = 4.83, *p* = 0.003; **Fig. 19a**). No predators resulted in a decreased prevalence of cotyledon damage at a low density (Wald X² = 5.85, *p* = 0.119; **Fig. 19b**). At a low plant density, both *P. melanarius* and *Pardosa spp.* reduced the cotyledon damage intensity (F_{3,125.6} = 3.43, *p* = 0.019; **Fig. 19c**). With the predator *P. melanarius*, there was a decrease in mean stem damage when compared to the control (F_{3,97.2} = 5.86, *p* = 0.001; **Fig. 19d**). The presence of *Pardosa spp.* and *P. melanarius* predators resulted in flea beetles attacking fewer stems than the predator-free control group (Wald X² = 21.21, *p* < 0.001; **Fig. 19e**). The presence of *P. melanarius* as a predator resulted in less stem damage intensity per replicate when compared to the control (F_{3, 100.98} = 4.17, *p* = 0.008; **Fig. 19f**).

A higher planting density reduced the total mean cotyledon defoliation when compared to the lower planting density (F_{1,63.0} = 16.2, *p* < 0.001; **Fig. 20a**). A higher planting density reduced both the prevalence (F_{1,78.3} = 11.92, *p* = 0.001; **Fig. 9b**) and intensity of the cotyledon defoliation (F_{1,52.9} = 11.83, *p* = 0.001; **Fig. 20c**). The presence of *P. melanarius* predators reduced the total mean cotyledon defoliation when compared to the control group (F_{2,177.3} = 5.12, *p* = 0.007; **Fig. 20a**). No predator reduced the prevalence of cotyledon damage (F_{2,172} = 0.17, *p* = 0.84; **Fig. 20b**). *P. melanarius* predators resulted in less cotyledon defoliation



intensity when compared to the predator-free control group, while *Pardosa spp.* did not ($F_{2,177.5} = 4.00$, $p = 0.02$; **Fig. 20c**). A higher planting density also reduced total mean stem damage ($F_{1,52.1} = 17.5$, $p = 0.0001$; **Fig. 20d**). A higher planting reduced the intensity of stem damage ($F_{1,43.9} = 8.95$, $p = 0.005$; **Fig. 20f**). The presence of *P. melanarius* predators reduced the total stem damage when compared to the control group ($F_{2,171.6} = 11.3$, $p < 0.0001$; **Fig. 20d**). There was a significant interaction found for the prevalence of stem damage between plant density and predator (Wald $X^2 = 9.96$, $p = 0.007$; **Fig. 20e**). *P. melanarius* reduced the intensity of stem damage when compared to the predator-free control group ($F_{2,168.3} = 8.18$, $p < 0.001$; **Fig. 20f**). *Pardosa spp.* did not significantly reduce the stem damage in any aspect when compared to the control group.

- P. melanarius and Pardosa spp. consume flea beetles resulting in a direct reduction in canola defoliation. Predators tend to decrease overall stem damage, but not cotyledon damage prevalence. This suggests a potential behavioral change of the flea beetles in the presence of predators, resulting in fewer stems being attacked as the flea beetles move up the plant to avoid predation. Predators result in an overall reduction in whole-plant damage compared to controls without predators.

Effects of temperature and flea beetle species:

Higher temperatures significantly increased cotyledon defoliation ($F_{3,26.6} = 31.64$, $p < 0.0001$), with the most defoliation observed at 28°C, the least defoliation at 13°C, and intermediate levels at 18°C and 23°C (**Fig. 21c**). More defoliation was observed on the abaxial than on the adaxial side of the cotyledons ($F_{1,118.3} = 24.55$, $p < 0.0001$; **Fig. 21d**). There was no significant difference in cotyledon defoliation between flea beetle species ($F_{1,26.6} = 0.38$, $p = 0.542$; **Fig. 21e**) and no significant interaction with the other treatments (flea beetle species x temperature x side: $F_{3,112.2} = 0.16$, $p = 0.924$; flea beetle species x temperature: $F_{3,23.7} = 0.52$, $p = 0.671$; flea beetle species x side: $F_{1,115.2} = 0.30$, $p = 0.586$), and thus the flea beetle species treatment was removed from the final model.

- P. striolata and P. cruciferae damage more cotyledon tissue at 28°C, indicating an increase in activity with warmer temperatures when compared to cooler temperatures. Both species produce more damage on the underside of the cotyledon when compared to the upper side at all temperatures. There is no difference in amounts of damage produced between the species P. striolata and P. cruciferae.

Effects of temperature and plant density:

There was a significant interaction found between the temperature and planting density on overall cotyledon defoliation ($F_{1,32} = 5.48$, $p = 0.03$). There was more defoliation found at a lower plant density at 28°C but not at 18°C (**Fig 22a**). There were more cotyledons attacked (higher prevalence) at a higher temperature and a lower planting density (temperature: Wald $X^2 = 29.1$, $p < 0.00001$; plant density: Wald $X^2 = 6.12$, $p = 0.01$; **Fig 22b**). There was a significant interaction between temperature and planting density when examining their effects on intensity of cotyledon defoliation ($F_{1,31} = 6.72$, $p = 0.014$), meaning there was a higher intensity of damage at a lower plant density at 28°C but not at 18°C (**Fig. 22c**).



There was a significant interaction found between the temperature and planting density on the overall stem damage, resulting in more stem damage at a low plant density at 28°C but not at 18°C ($F_{1,32} = 4.47$, $p = 0.042$; **Fig. 22d**). Higher temperatures significantly increased the prevalence of stem damage ($\text{Wald } X^2 = 33.1$, $p < 0.00001$), resulting in more attacked plants at a higher temperature (**Fig. 22e**). There was no significant difference in prevalence of stem damage between different plant densities ($\text{Wald } X^2 = 2.86$, $p = 0.091$) and no significant interaction between temperature and plant density ($\text{Wald } X^2 = 1.52$, $p = 0.217$; **Fig. 22e**). A higher temperature results in more damage per attacked plant (intensity) ($F_{1,28} = 30.32$, $p < 0.00001$; **Fig. 22f**).

There was no correlation found between the cotyledon defoliation and stem damage at a high planting density or low planting density at 18°C ($p > 0.05$; **Fig. 23a, c, d**). There was a significant correlation found between cotyledon defoliation and stem damage at a low planting density at 28°C ($F_{1,8} = 9.00$, $p = 0.017$, $R^2 = 0.47$; **Fig. 23b**).

- Cotyledon damage prevalence increased with warmer temperatures, while cotyledon damage intensity increased with warmer temperatures only at a low density. High temperatures increased stem damage regardless of the plant density. stem damage is correlated with cotyledon damage at a low seeding rate and high temperature, but not the other 3 treatments.

Publication in preparation: Woodland, S; Damien, M; and AC Costamagna. Effects biotic and abiotic factors on the interaction between flea beetles and their host plant, canola.

Objective 3: Determine the effect of ground and foliar predators and landscape structure on canola defoliation, flea beetle abundance, and species composition in different regions of Western Canada.

Methodology:

Refined analyses have been focused on data collected from 2015 to 2017 within grower's fields in four ecoregions (i.e., Lethbridge, Beaverlodge, Saskatoon, and Manitoba). During these three years, 75 canola fields have been monitored, and their surrounding landscape mapped (27 fields in 2015; 25 in 2016, and 23 in 2017). A total of 103,651 flea beetles have been counted, with a dominance of striped flea beetles (*Phyllotreta striolata* Fab., 57,126 individuals) and crucifer flea beetles (*Phyllotreta cruciferae* Goeze., 46,022 individuals). Among the remaining samples, the most important species was the hop flea beetle, *Psylliodes punctulata* Melsh. Identification of all sampled predators is still ongoing.

In each region, monitored canola fields were selected to represent a maximum range of complexity in the surrounding landscape. Complex landscapes were considered as those with a high proportion of natural and semi-natural habitats (e.g., native vegetation, forests, hedgerows, shelterbelts, riparian habitats, grasslands, and windbreaks). By contrast, more simplified landscapes were those dominated by cultivated lands, more or less dominated by crucifer crops as well. To be considered independent, each sampled field was at least distant to 4 km from the other. Ground predators were sampled using pitfall traps during the weeks when canola crops are most vulnerable to damage by flea beetles (emergence to 2.4 stage). Predators were also quantified using the same sticky traps deployed to monitor flea beetle populations during the season. Foliar

predators and flea beetles were monitored as well using 10 sweep net samples at each sampling point. At each trapping point, defoliation and phenology were assessed in 20 canola plants.

Within a 5m radius of each flea beetle sampling point (i.e., sticky cards), two 1m transects were also randomly established, and the number of plants was counted. Ten consecutive plants were assessed for defoliation and phenology. Landscape maps were drawn for 2-3 km buffer areas from the focal field. To determine landscape effects on flea beetle abundance and canola defoliation, all habitat and land cover types present in the surrounding landscape of each field were mapped within a 3-km radius circle from the sampled field. Polygon maps were built for each field using ArcGIS 10 analysis, based on Google Maps images and on-site assessments of all different landscape components within 500, 1000, 1500, and 2000m around sampling fields.

For all analyses, we restricted the datasets considering only the susceptible period of canola seedlings (i.e., the cotyledons and the first two true leaves - 2.4). To do that, canola median stages were calculated from the 20 canola seedlings around each sticky trap from each field and then averaged for a given field and a given sample week. Similarly, the total flea beetle abundances, as well as the abundances of the two main species from the five sticky cards were averaged for a given field and a given week. Finally, the different mean flea beetle abundances were averaged among sampling weeks. These restrictions resulted in a subset of data from 53 fields across the three years and four regions, yielding a total of 7,375 flea beetles (4,973 striped flea beetles and 2,284 crucifer flea beetles) analyzed for landscape effects on flea beetle abundance and canola defoliation during the susceptible period.

Among all potential landscape predictors, the first step was to select those expected to be most influential on flea beetle biology. The second step was to select landscape factors that were uncorrelated to avoid any bias in statistical analyses. To do so, after a first selection of all potential predictors, correlation matrices were built between all selected predictors at the four different spatial scales. The landscape predictors kept after this procedure were those related to 1) landscape composition (proportion of surrounding canola crops, grassland, woodland, grass border, and other crops than canola), 2) landscape configuration (Mean canola crop size and the field edge density), and 3) the experimental design (number of sampling week prior canola reached 2.4 stage, region, and year). For canola defoliation, the mean abundances of striped and crucifer flea beetles were added as potential and complementary predictors.

The information theory approach was used to identify the significance of those landscape predictors on the different tested response variables. This method aims to select the best competitive models through the Akaike information criteria to identify predictors that fit best the data. The method allows to provide of a 'weight' for all of the tested predictors through their occurrence within all best competitive models (from 0 when a predictor is never present in none of the best competitive models, to 1 when a predictor is present in all models) and to compare them to the weight that could be expected from random models after a permutation procedure of the response variables (200 iterations). When the weight of a predictor is superior to this hypothetical weight, it can be confidently assumed that the predictor significantly contributes to explaining the tested response variable. Finally, the positive or negative contribution of a landscape predictor can be

identified through the value of their estimated coefficient within the best competitive models (**Figures 24, 25 & 26**).

Results:

Striped flea beetles

The abundance of striped flea beetles varied significantly due to eco-region, year, and the number of sampling weeks (**Figure 24**). Considering landscape composition, the proportion of woodland within 1500m and the proportion of canola crop within 2000m appeared to contribute to reducing striped flea beetle abundance (**Figures 24 and 27**). Across scales, the landscape configuration components such as mean canola field size and edge density were significantly contributing to striped flea beetle abundances (**Figure 24**). Mean canola field size had a positive association, whereas edge density had a negative association (**Figure 28**).

Crucifer flea beetles

Several landscape predictors significantly affected crucifer flea beetle abundance at different spatial scales, whereas years contributed significantly to abundance in models at 500 m, and regions showed significant effects across all spatial scales. Within 1500 m, similar to models for striped flea beetles, only the proportion of woodland appeared to contribute negatively to the abundance of crucifer flea beetles (**Figures 25 and 27**). Within 1000 m 1500 and 2000 m, we observed a negative contribution of the surrounding proportion of canola crops to crucifer flea beetle abundance (**Figure 27**). Finally, from 1000 to 2000 m, there were negative associations of edge density and positive associations of the proportion of grass border with crucifer flea beetle abundances (**Figure 27**). Across scales, the most predictive scale to explain crucifer flea beetle abundance was 1500 m (**Figure 25**). The most important predictors related to the composition were the proportion of canola crops, the proportion of woody vegetation and the proportion of grass borders. Regarding the landscape configuration, mean canola crop size and field edge density were the most relevant landscape predictors.

From these results, we assume that the positive effect of canola field size is consistent with the resource concentration effect, which predicts a higher density of a specialist pest in areas where their resource is abundant and distributed in a large “patch” size (Root 1973). The negative contribution of canola crop proportion at large scale is most likely a particular case of this theoretical framework, resulting from the well-known “dilution effect”. It can be explained by a decrease of the striped and the crucifer flea beetle densities at the field level due to their dispersion from overwintering sites into larger areas with canola. In landscapes with smaller canola area, flea beetles will tend to aggregate on those smaller areas. The positive effect observed for grass borders could be related to the use of such habitats as overwintering sites and acting as potential sources of flea beetles. The negative effect of the proportion of woodland on crucifer and striped mean abundances could be explained by a potential role as physical barriers that limit flea beetle movements. Indeed, those insect displacements consist of typical small jumps (25 cm) or in short flies eventually. Then woody patches could have limited movement, but also may have limited cues dispersal from canola crops, decreasing canola crop attractiveness as the surrounding woodland proportion increased. The negative effect of edge density,

which represents the fragmentation of the agricultural landscape, could also result in more difficulties in flea beetle movement. Alternatively, both woodland and edge density have been associated with increased populations of predators in crop fields, which can reduce pest populations.

- The composition of the landscape affected flea beetle abundance. We observed dilution of flea beetles in landscapes with a proportion of canola. Grassy field borders were associated with higher crucifer flea beetle abundance at large spatial scales, whereas the proportion of woodland was associated with a reduction in the abundance of both striped and crucifer flea beetles. There were effects of landscape configuration as well, with large canola crops that increased pest abundances, and the fragmentation of the agricultural landscape that decreased flea beetle abundances.

Defoliation (Figure 23)

Defoliation was significantly affected by sampling week at all scales and region at the 2000 m scale (**Figure 26**). At the 1500 m spatial scale, we found a significant contribution of crucifer flea beetle abundance on canola seedling defoliation (**Figure 26**). Among the landscape predictors, no landscape components related to configuration significantly contributed to canola defoliation. Among landscape composition predictors, the proportion of other crops at 500 m and woodland at 1500 and 2000 m were negatively associated with defoliation (**Figure 26**). Within 1500m, the proportion of canola crops contributed negatively to canola defoliation. Finally, the proportion of grass border from within 500 m had a negative contribution to canola defoliation, but a positive one within 1000 and 1500 m. Across scales, the most predictive scale to explain seedling defoliation was by 1500 m. Among predictors that significantly contributed to canola defoliation across scales, striped flea beetle abundance was weakly significant, whereas crucifer flea beetle abundance was much more above the significant threshold. Among the landscape predictors, the proportion of grass border had a positive contribution, and both woodland and canola crop proportion were negatively associated with canola defoliation (**Figure 29**).

Cotyledon defoliation of canola plants was related to the abundances of both flea beetle species across scales, suggesting some robustness on the method applied to identify significant predictors on tested response variables through the information theory approach. This result suggests that across all spatial scales the abundances of the two flea beetle species were significant contributors to explain defoliation rates in several of the best competitive models, with increasing flea beetle abundance associated with increased damage.

Cotyledon defoliation rates were only driven by landscape components related to the composition across or within scales suggesting that landscape configuration across spatial scales acted on flea beetle abundances but not on plant damage. Locally (500 m) an increasing proportion of other crops may provide associational resistance to canola against flea beetle damage. At a large spatial scale, the negative contribution of host crop proportion supports our interpretation of dilution effects on flea beetle abundance, with less individual and less damages at the field scale with increasing abundances of the host plant within the surrounding landscape. It is worth mentioning that this benefit of surrounding host crops may occur only early in the growing season. Finally, as observed on flea beetles, benefits at large scale of woodland are confirmed with a decrease in

damages as increases in their proportion within the surrounding landscape, most likely by providing associational resistance such as described above.

- Landscape effects on crucifer and striped flea beetle populations are consistent with expected process between plant diversity and specialist herbivores demonstrated previously at much smaller spatial scales. Large canola crops favor flea beetle abundances at the field scale, whereas a fragmented agricultural landscape with higher proportion of crops other than canola helps to decrease infestation levels. Despite being beneficial locally by reducing defoliation, grass borders at large scale appeared to favor flea beetle abundances most likely by providing overwintering/dispersing sites for flea beetles. Edge density and woody habitats seem beneficial against flea beetle infestations by acting most likely as physical barriers against flea beetle movement and dispersal to canola.

Publication in preparation: Damien, M; T Nagalingam; HA Cárcamo; J Otani; T Wist; JA Bannerman, J Gavloski, and AC Costamagna. Landscape composition and configuration affects flea beetle abundance and damage to canola in western Canada.