PROJECT DETAILS

- **Title**: Generate knowledge and control strategies for the pollen beetle *Brassicogethes viridescens* (Coleoptera: Nitidulidae), a new invasive pest of canola
- **Funders:** Alberta Canola, SaskCanola and the Manitoba Canola Growers
- **Research program:** Canola Agronomic Research Program
- **Principal investigator:** Christine Noronha
- **Collaborators/additional investigators:** Hector Carcamo, Tyler Wist and John Gavloski
- **Year completed:** 2022

Final report

Abstract

This was the final year of the project. Over the past four years there has been significant progress in the knowledge of this invasive species. Our first objective was the rear this beetle. A method to maintain larvae in the lab was established for the first time which will help in collecting eggs and larvae from the field and rearing them to adults for laboratory studies. It was discovered that this species has an obligatory diapause which makes it difficult to have a continuous colony in the laboratory, this is a significant finding as it affects planning and timing for laboratory based research. Our next objective was to determine the efficacy of insecticides on pollen beetles that have a low impact on pollinators. Since pollen beetle are present in the canola field when it is in flower, control measures applied exposes pollinators that visit the field during the same period. Four insecticides Delegate (Spinetoram), Success (Spinosad), Sivanto Prime(Flupyradifurone), and Beleaf (Flonicamid) were tested at a high, medium, and low rate. We found that the efficacy of Beleaf was lower and more variable ranging from 60% and 20% at high and low rates respectively. The other three insecticides gave 90-100% control at all three rates indicating that the low rates should be used if needed. The third objective was to develop economic thresholds for pollen beetle in canola. We determine that between 7 – 9 beetles per plant resulted in significantly lower seed weight, lower oil content and more missing pods when compared to plants with no pollen beetles. We also found that when using sweep samples to determine population 4 beetles per sweep did not significantly increase the number of lost pods. Our fourth objective was to survey fields in Alberta, Saskatchewan and Manitoba for the presence /absence of pollen beetles and survey for naturally occurring parasitoids in Atlantic Canada. No pollen beetles were found in Alberta, Saskatchewan, Manitoba. We also surveyed fields in Ontario in 2020 and 21 and found no pollen beetles. In the Maritimes beetles numbers were highest in New Brunswick and Prince Edward Island. No parasitoids were discovered in Atlantic Canada during this four-year study.

Introduction

The bronzed blossom beetle or pollen beetle (*Brassicogethes viridescens* (Coleoptera: Nitidulidae)) is an important pest of canola and other brassica crops. It is native to Europe and was first identified in Canada in 1967 from Nova Scotia. It is now an established pest in the provinces of Nova Scotia, Prince Edward Island and Quebec (Hoebeke and Wheeler 1996). This pest is unique in that the adults can survive on pollen from any available flowers but the larvae can only feed and survive on pollen of plants from the family Brassicacae, thus it has the potential to cause serious damage and yield reduction in oilseed crops such as *B. napus*, *B. rapa, B. juncea*, and *B. carinata* (Ekbom and Borg, 1996; Noronha and Mason 2017). Adults overwinter in leaf litter along the edge of the field; they emerge in the spring and begin feeding on pollen from available flowers. When their reproductive organs are mature they seek out their larval hosts, this coincides with the bud stage of the host plant (Metspalu *et al.* 2011). The adults migrate to the host plant; females chew holes in the side of developing buds and insert 1-2 eggs. The eggs hatch into first instar larvae which feed on the pollen within the bud (Osborne, 1965; Hoebeke and Wheeler, 1996). The second instar larvae emerge as the bud opens and move from flower to flower feeding on pollen. On reaching maturity, the second instar larva drops to the ground and pupates in the soil. The new generation adults emerge and move in search of flowers and pollen of other plant families before searching for overwintering sites within hedgerows and field borders (Marczali and Nádasy, 2006). Egg laying and larvae feeding occurs at the early bud stage when the crop is the most susceptible resulting in premature flower drop; severe infestation can result in over 70% yield reduction (Nilsson 1987). Pollen beetles can also impact the evenness of crop maturity by the late formation and maturity of pods on auxiliary racemes resulting in uneven maturity of the crop and storage problems (Williams 1979, Tatchell 1983).

Presently *B. viridescens* is present in Atlantic Canada and Quebec, climate models predict an increase in the range and relative abundance heightening its ability to establish in new areas such as western Canada (Mason et al. 2013; Olfert and Weiss, 2006). Insecticides are the main control method used in Europe resulting in resistance being detected in the population (Hansen 2003). Not much is known about *B. viridescens* in Canada. A preliminary study in 2014 showed that showed that one spray application at 10% bloom is not sufficient to reduce pollen beetle populations because of continued migration of beetles into the field (Noronha 2014). In Europe the threshold for pollen beetles ranges from 0.5- 3 pollen beetles per plant (Larsen 2000, Nilsson 1994, Cooper and lane 1991 and Hansen 2004). There is no threshold set for *B. viridescens* in Canada. The objective of this project was to 1) Develop a laboratory rearing method for pollen beetle which would help in conducting laboratory based studied during the winter months. 2) Test the efficacies of insecticides, that have a lower impact on pollinators, against pollen beetles in order to use multiple applications, 3) Develop economic thresholds for pollen beetle in canola, and 4) Survey a) fields in Alberta,

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Saskatchewan and Manitoba to monitor the presence and potential movement of pollen beetles into the region and b) survey for naturally occurring parasitoids in Atlantic Canada.

Methods

Objective 1: Develop a laboratory rearing method for pollen beetle.

Adult rearing: Laboratory rearing of pollen beetles was undertaken from 2018-2022. First and second instar larvae (around 600) were collected from mustard and canola fields in New Brunswick and PEI in July. Larvae were transferred to a cage (W47.5 x D47.5 x H93.0 cm) with potted canola plants that was at least 20% in bloom. The canola plants were placed in a plastic box with 10cm of soil to allow the matured larvae dropping onto the soil to pupate. New flowering plants were added every 4-7 days making sure that flowers and buds were always present for the larvae. When new adults emerged, fresh flowering canola plants were provided as a food source and oviposition site for the adults. In addition 10% honey sugar solution in small vials fitted with a dental wick was provided as an additional food source for newly emerged adults. The cage were maintained, and fresh flowering canola plants were added every week and emergence of the beetles from the soil was observed until the following spring. In addition pollen beetle adults (around 400) were collected from fields in the fall and transferred to another cage and provided with budding plants and honey solution as a food source and oviposition site.

Larval rearing:

Pollen beetle larvae were collected from mustard and canola fields in New Brunswick and PEI during middle to end of July every year 2019-22. Larvae were transferred to Petri dishes (9cm) with about a half layer of moistened vermiculite and fresh canola twig with buds and flowers as a food source. The buds and flowers were checked and replace with fresh twigs every day and moisture added whenever necessary. The petri dishes were placed in an insectary maintaining 20°C with16/8h light. Within one week most of the larvae pupated. The Petri dishes with pupae were transferred into a meshed cage (Bugdorm; W47.5 x D47.5 x H93.0 cm) with two flowering plants. A new flowering plants were added in the cage every 4-7 days whenever the old plants ran out of flowers. In spring of each year, canola plants were added to all cages to observe any emerging beetles and eggs laying in the laboratory.

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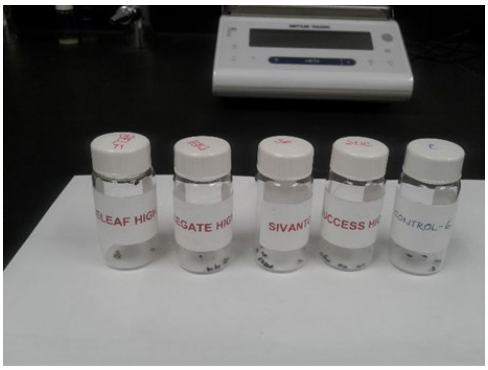
Objective 2: Test the efficacies of insecticides that have a lower impact on pollinators against pollen beetles.

Six potential insecticides (Flupyradifurone, Spinetoram, Flonicamid, Novaluron and Spinosad) were identified for efficacy testing against pollen beetles in the laboratory using the IRAC susceptibility testing method. This method is currently being used to monitor sensitivity of pollen beetle populations in oilseed rape in Europe.



IRAC Procedure: Known concentrations of the insecticide will be prepared in acetone. Glass vials (20ml) were filled with 500µl of a known concentration of the insecticide. The vial were be placed horizontally and rotated on a vial roller. Once the acetone has completely evaporated, the vials were capped and stored in a cold room at 4˚C. Beetles were be collected from the field. Ten pollen beetles were introduced into a vial, for a total of 6 replicates per treatment. After a 24 hour period of exposure at 20˚C, the beetles were be removed and checked for any signs of movement. The percentage mortality was recorded for each treatment. Two-way ANOVA was performed on the data with insecticide type and rate as two factors. The trial was conducted twice.

Objective 3. Develop economic thresholds for pollen beetle in canola.

Field trials were set up at the Harrington Research Farm in 2019, 20 and 21. Canola seeds (Liberty Link Envigor L233P) were planted in 6 × 2 m^2 plot at a seeding rate of 5kg/ha arounf the end of May each year. Before seeding, the plots were fertilized at a rate of 80kg N, 40Kg P, 40Kg K, 22Kg S, 2Kg B per ha. One third of N was broadcast at 4-leaf stage. A herbicide (Liberty) was applied at 4-leaf stage to control weeds. The experimental plots were laid out in a RCBD with four replications. A single mesh cage of 60cm × 60cm × 180cm was placed over emerging canola plants, 24 plants per cage, in each plot as soon as they emerged, to exclude other pests. Plants were monitored regularly to ascertain the exact time of budding. Sticky traps were placed in adjacent plots with no cages to determine the time of migration of the pollen beetles into the canola plots. There were four treatments T0 = non-caged plants, T1= 0 pollen beetles, T2 = 7 pollen beetles per plant, and T4= 9 pollen beetles per plant. First budding of canola plants was observed on in med July (42 days after planting). Pollen beetles were collected from nearby mustard fields and were released in the cages on as soon as budding was observed. To simulate natural migration into the fields, beetles were released in batches every tow days, 10 beetles per cage on day 1, 50 beetles per cage on day 3, another 50 beetles per

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cage on day 5 and the remaining beetles were released on July 9. In August (91 days after planting), canola plants were cut near the soil surface. A sub sample of 10 plants/cage were evaluated for the number of aborted flowers, number of total pods, and plant height. Seeds were collected and weighed and the moisture, protein and oil contents were quantified using a NIR spectrometer (SpectraStar, Unity Scientific, Milford, MA, USA). A one way ANOVA was performed to analyse the data of each parameter using the statistical package R.

In 2021, sweep net samples were collected for a canola field and the number of beetles were counted. At the end of the season, three 1x1m plant samples was collected for the field, the number of healthy pods, missing pods, yield and seed moisture, oil and protein content were analysed.

Objective 4: Survey fields in Alberta, Saskatchewan and Manitoba for the presence /absence of pollen beetles. Survey for naturally occurring parasitoids in Atlantic Canada

Sweep net samples were collected for several fields in Alberta, Saskatchewan, Manitoba and form Ontario in 2021. Each sample consisted of 25 sweeps (180 degrees with a 15 inch sweepnet). Sweep nets samples were collected from canola fields in New Brunswick, Nova Scotia and PEI in the summer in 2018-2021. The samples were brought to the laboratory the larvae were reared at room temperature until adult emergence and monitored for any parasitoid emergence.

Results

Objective 1: Develop a laboratory rearing method for pollen beetle.

Several hundreds of pollen beetle larvae and adults that were collected in the spring from fields and transferred to a cage were found feeding and damaging buds in the cage. Eggs and larvae were found in the buds. The larvae pupated in the soil in the pots and after a couple of weeks new adults emerged, they fed on the canola buds from plants placed in the cage but no egg laying was observed by these adults and after a short time they entered the soil which indicates to diapause.
Larvae brought in from the field pupated within one week. Adults emerged from these pupae and started feeding on new buds and flowers of plants placed in the cage. No mating was observed and no eggs were found in damaged buds (see figure). One week after emergence the

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adults disappeared into the soil. Fresh plants added to the cage every week showed no further feeding damage. From these studies it appeared that the pollen beetle *Brassicogethes viridescens* has an obligatory diapause in the soil making it difficult to rear in the laboratory. However, a protocol; for maintaining larvae collected from the field to obtain sufficient numbers of adult for laboratory based studies was established.

Objective 2: Test the efficacies of insecticides that have a lower impact on pollinators against pollen beetles. Pollen beetles collected from PE: There was significant interactions between insecticide types and rate (P <0.001). Hundred percent mortalities were found in all rates of two insecticides: Delegate and Success and lowest efficacy was found with the insecticide Beleaf (Figure 1). Pollen beetles collected from NB: Hundred percent mortalities were reported in all rates of the insecticides Delegate, Success and Sivanto Prime. Beleaf had the lowest toxicity on the beetles (Figure 2). Unlike populations in Europe, our results show that the population in Canada has not yet developed widespread resistance however, of the four insecticides Delegate (Spinetoram), Success (Spinosad), Sivanto Prime(Flupyradifurone), and Beleaf (Flonicamid) tested at a high, medium, and low rate, we found the efficacy of Beleaf to be the lowest and more variable ranging from 60% and 20% at high and low rates respectively. The other three insecticides gave 90-100% control at all three rates indicating that the low rates should be used if needed. Therefore control of pollen beetle using one of these insecticides can be achieved.

Figure 1: Mortality (%) of adult pollen beetles collected from Harrington, PEI, treated with four different insecticides in the laboratory on two different dates (July 31, 2019 and Aug 1, 2019). Error bars show ±SE.

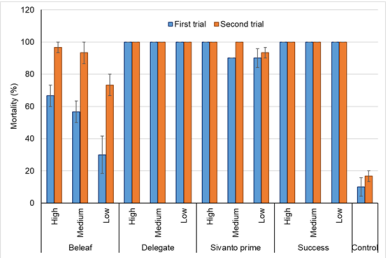
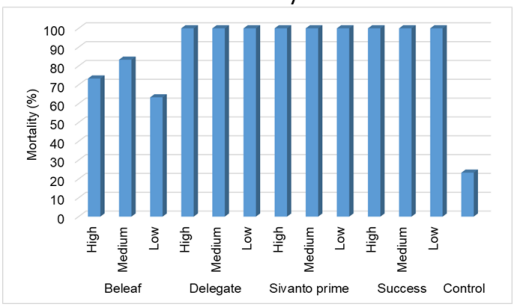


Figure 2: Mortality (%) of adult pollen beetles collected from NB, treated with four different insecticides in the laboratory



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Objective 3. Develop economic thresholds for pollen beetle in canola.

In 2019, there were lower performances of canola with higher pollen beetle infestations. Almost all of the parameters recorded were found best in the treatment with no pollen beetle in the cage. For example, there were statistically significant difference of total missing pods ($P < 0.001$) and total healthy pods ($P < 0.001$) among the treatments. Highest total healthy pods were obtained in the cages with no pollen beetle (Figure 1). Similarly, highest average plant height (Figure 2) and highest weight of seeds of ten plants ($P = 0.009$) were seen in the cages with no pollen beetle (Figure 3). There was a significant difference in protein contents in seeds among the treatments ($P < 0.001$) with the highest protein content in the seeds collected from outside of the cage. There was no variation in seed moisture contents ($P = 0.85$) and oil content ($P = 0.21$) across the treatments (Figure 4). In 2020, Overall, there was a trend of low performances of canola with pollen beetle infestations, but for unknown reason, the canola performed better with the treatment of 9 pb/plant than 7 pb/plant. Almost all of the parameters recorded were found best in the treatment with no pollen beetle in the cage. For example, highest total healthy pods were obtained in the cages with no pollen beetle (Figure 5). Similarly, highest average plant height (Figure 6) and highest weight of seeds of ten plants were seen in the cages with no pollen beetle (Figure 7). There was a difference in oil contents in seeds among the treatments with the highest oil content in the seeds collected from outside of the cage. There was no variation in seed moisture contents and protein content across the treatments (Figure 8). Results from sweeps in 2021, show that an average of 4 beetles per sweep did not reduce yield with only 18% pod loss (Table 1). These results suggest that population levels of 7-9 beetles per plant can result in lower seed weight and higher number of missing pods. Sweep samples of an average of 4 beetles or less per sweeps does not result in a high percentage of missing pods. More research on the best monitoring technique is needed to estimate the populations.

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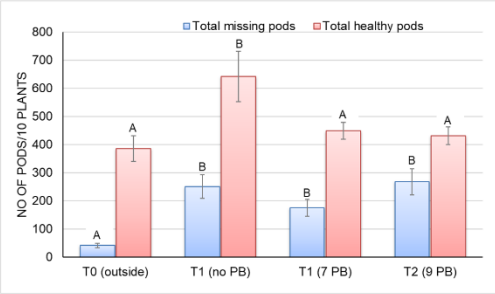

Figure 1. The numbers of total missing pods (±SE) and total healthy pods found in 10 canola plants infested with pollen beetles of different densities (0 or 7 or 9 beetles per plant) compared with natural infestation (outside). Same letters above the bars of the same parameter indicate statistically not significant ($P < 0.05$).

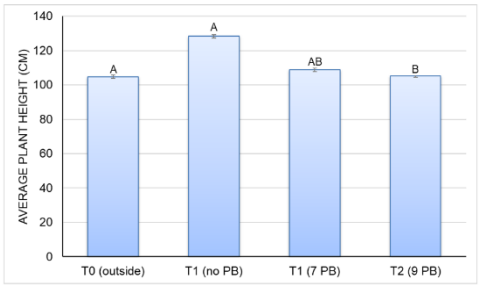

Figure 2. Average plant height (cm ± SE) of 10 canola plants infested with pollen beetles of different densities (0 or 7 or 9 beetles per plant) compared with natural infestation (outside). Same letters above the bars indicate statistically not significant ($P < 0.05$).

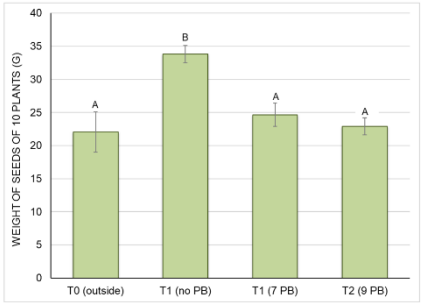

Figure 3. Average seed weight (g ± SE) of 10 canola plants infested with pollen beetles of different densities (0 or 7 or 9 beetles per plant) compared with natural infestation (outside). Same letters above the bars indicate statistically not significant ($P < 0.05$).

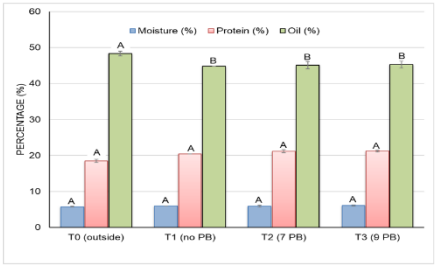

Figure 4. Percentages of moisture, protein and oil contents of canola seeds collected from the canola plants infested with pollen beetles of different densities (0 or 7 or 9 beetles per plant) compared with natural infestation (outside). Same letters above the bars of the same parameter indicate statistically not significant ($P < 0.05$).

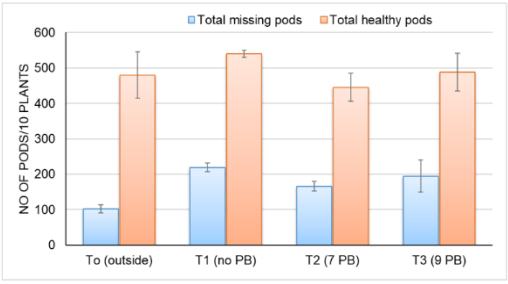

Figure 5. The numbers of total missing pods (±SE) and total healthy pods found in 10 canola plants infested with pollen beetles of different densities (0 or 7 or 9 beetles per plant) compared with natural infestation (outside) in 2020.

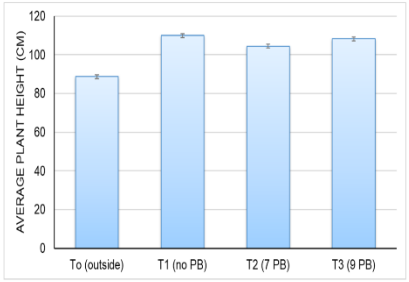

Figure 6. Average plant height (cm ± SE) of 10 canola plants infested with pollen beetles of different densities (0 or 7 or 9 beetles per plant) compared with natural infestation (outside) in 2020.

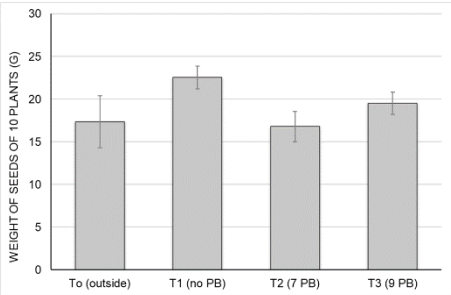

Figure 7. Average seed weight (g ± SE) of 10 canola plants infested with pollen beetles of different densities (0 or 7 or 9 beetles per plant) compared with natural infestation (outside) in 2020.

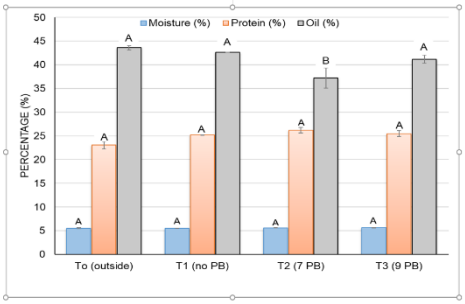

Figure 8. Percentages of moisture, protein and oil contents of canola seeds collected from the canola plants infested with pollen beetles of different densities (0 or 7 or 9 beetles per plant) compared with natural infestation (outside) in 2020. Same letters above the bars of the same parameter indicate statistically not significant ($P < 0.05$). Error bars show ±S.E.

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Average no. of beetles per sweep	No. of healthy pods/10plants	No. of missing pods/10plants	Yield/ha (mt/ha)	Percent moisture	Percent Protein	Percent oil
4	459	183	2.55	5.48	15.15	49.43

Table 1. The impact of average number of beetle per sweep over three weeks on number of healthy and missing pods per 10 plants, yield and seed moisture, protein and oil content in a canola crop in 2021.

Objective 4: Survey fields in Alberta, Saskatchewan and Manitoba for the presence /absence of pollen beetles. Survey for naturally occurring parasitoids in Atlantic Canada

Results from the sweeps conducted in several field in Alberta (12(2018),12(2019),10(2020)), Saskatchewan (20(2018),36(2019,16(2020)) and Manitoba (16(2018),17(2019),26(2020)) were monitored for pollen bbetles, no pollen beetles were found. In Atlantic Canada 15 fields from various location were monitored each year and no parasitoids were found.

Our results show that pollen beetles have not yet moved into western Canada but continued monitoring is prudent. The lack of parasitoids in its existing range of Atlantic Canada shows that the native population of parasitoids are not controlling this pest and an introduction of a parasitoid from Europe may be the solution.

Conclusions and Recommendations

Over the 4 years of this study we have increased our knowledge of this pest, we know that it has an obligatory diapause, and developed a protocol for maintaining larvae to the adult stage in the laboratory. We also learned that the population in Canada does not have wide spread resistance and can be controlled by certain insecticides that have a reduce impact on bees, the low rate of the insecticides are effective in controlling the pest. We also found that a threshold of 7-9 beetles per plant can significantly reduce yield and when using the sweep net method of estimation we found that and average of 4 beetles per 10 sweeps does not significantly impact pod loss. Thus, insecticide applications may not be necessary at this population level. Further research is needed to determine the best method to evaluate pollen beetle population in canola fields. Our research has shown that pollen beetle has not yet been found in ON, MB, SK, and AB. Monitoring for this beetle should continue so that populations can be kept in check as soon as infestations begin. Canola growers in Atlantic Canada and Quebec should monitor their field for pollen beetle and apply control measures to prevent population increase in fields which can enhance pollen beetles ability to move to new locations.

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Acknowledgements

We would like to acknowledge SaskCanola, Alberta Canola and Manitoba Canola for providing the funds to do this research, the growers who allowed us to enter and sweep their fields and Agriculture and Agri-Food Canada for providing some in-kind assistance.

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