PROJECT DETAILS

- **Title**: Feasibility of using *Trichomalus perfectus* for biological control of cabbage seedpod weevil in the 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:** Héctor Cárcamo
- **Collaborators/additional investigators:** Eric Lucas, Luc Belzile, Dan Johnson, Scott Meers, Meghan Vankosky, Boyd Mori, Kevin Floate, Tara Gariepy, Patrice Bouchard, Peter Mason and Tyler Wist
- **Year completed:** 2023

Final report

Introduction

The cabbage seedpod weevil (*Ceutorhynchus obstrictus* Marsham) is a serious pest of canola and related brassicaceous seed crops in North America and Europe. Adults feed on buds, flowers and immature seeds. Larvae feed on the seeds inside the pods and cause economic yield losses (Dmoch 1965, Carcamo and Brandt 2017). The weevil was first reported on the Canadian Prairies in southern Alberta in the mid 1990's (Butts and Byers 1996) and recently from Manitoba in 2018. It was reported from Quebec and Ontario in 2000 (Brodeur et al. 2001; Mason et al. 2003). Biological control with a parasitoid wasp (*Trichomalus perfectus*) is an effective strategy in Europe and the wasp has appeared as an adventive species in Quebec where it can reach high levels of pest control. It also occurs at lower densities in Ontario. Although there are no legal restrictions to relocate the parasitoid to the Prairies, it is important to determine potential non-target risks because there are related native weevils and others introduced for weed biological control in the region.

Objective 1: To assess the efficacy of *T. perfectus* for managing seedpod weevil and economic impact of parasitism and the pest on canola production (to be done in Quebec).

Data on cabbage seedpod weevil (CSW) and parasitism in canola fields of Quebec were provided to the team of Luc Belzile (IRDA) for agroeconomic analysis. Cabbage seedpod weevils have been monitored in 272 fields from 2006 to 2016. Parasitism of cabbage seedpod weevil was observed first in 2009. Before appearance of *Trichomalus perfectus*, economic threshold (2 CSW/sweep) had been reached in 17 out of 78 canola fields (22%). After 2009, 11 out of 177 fields (6%) reached 2 CSW/sweep. The team in Quebec was not able to complete this objective.

Objective 2:

Identification and initial risk assessment of potential non-target weevils and parasitoids in the Prairies.

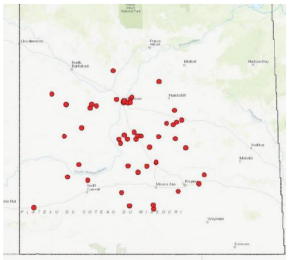
A recent analysis, by Bouchard and colleagues, using barcode datasets based on authoritatively identified specimens from Canada and Europe has led to the discovery of more than 60 species of beetles representing new records for Canada. A manuscript summarizing these results is in progress. Two species of Ceutorhynchinae will be recorded in Canada for the first time in the upcoming article. The first, *Ceutorhynchus inaffectatus* Gyllenhal, 1837, is a European species collected near Guelph Ontario in 2018. It is known to feed on *Hesperis matronalis* L. and *H. tristis* L. (Brassicaceae). The larvae of this weevil develop in the seed pods, the adults feed on leaves and various other parts of the host plants. The second species, *Ceutorhynchus mutabilis* Dietz, 1896, is native to the Nearctic region and previously found in the USA.

Although more research is needed, it appears that this species is present from Manitoba to British Columbia in Canada. Nothing is known about its ecology at this time. Additional barcode data will soon be available through ongoing collaborations, which should lead to a better understand of the diversity, distribution and ecology of Ceutorhynchinae weevils in Canada in general. Specimens of Ceutorhynchinae deposited at the CNC from this project will be mounted and used in upcoming barcoding efforts.

In 2019, over 116,000 pods of various Brassicaceae uncultivated species from Alberta were reared by Dan Johnson (University of Lethbridge). These samples yielded very few weevils and only the widespread weevil, C. neglectus emerged (see figure below)

Sampling for native weevils and parasitoids

- Sampled early flowering Brassicaceae plants (e.g. flixweed) outside fields. Also rearing pods



2021 sites where pods collected to rear weevils and parasitoids in Saskatchewan by Vankosky and Williams

common name	containers	pods
Flixweed	25	60410
White Top	18	20350
Dog Mustard	37	16940
Stinkweed	32	7225
Dame's Rocket	20	6175
Shepherd's Purse	9	2370
Desert Madwort	2	800
Tumble Mustard	3	400
Tansy Mustard	10	388
Tower Mustard	6	370
Shy Wallflower	1	300
Little Falseflax	1	150
Spreading Pod Rockcress	1	100
Ball Mustard	1	40
Wild Mustard	1	20
Nuttall's Rockcress	1	10
unknown	1	6

Over 116,000 pods reared by Dan Johnson in 2019

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In 2021 and 2022, cruciferous weeds (e.g., shepherd's purse, pepper grass, flixweed) and volunteer crop plants (e.g., mustard) were collected from over 60 locations in Saskatchewan, starting in early August. The plant samples all had developing pods that could have supported the development of *Ceutorhynchus* weevils. Each sample was placed into a carboard emergence box that was sealed to prevent light from entering the box everywhere, except at one point where a collection vial was attached to the exterior of the box. Rearing boxes were checked daily for insect emergence and all potential *Ceutorhynchus* sp. weevils and parasitoids were collected and preserved for identification. After no insects had emerged for 30 d, the boxes were opened and examined for any remaining insect specimens. In 2022, none of the samples collected contained weevils of the genus *Ceutorhynchus*. 168 parasitoid specimens were collected from the samples, but have yet to be identified. One sample, consisting of pepper grass and brown mustard plants, had 71 parasitoid specimens emerge.

Weevils in Brassicaceae

Quebec	Ontario	Alberta	Notes
Ceutorhynchus obstrictus	***C. obstrictus***	***C. obstrictus***	**Exotic pest**
C. neglectus	*C. neglectus*	*C. neglectus*	Native minor canola pest (Dosdall 2012)
C. omissus	*C. omissus*	*C. omissus?*	Heavily parasitized by TP in Ontario; on pods of worm mustard
C. erysimi	*C. erysimi*	*C. erysimi*	Exotic on shepherd's purse
C. americanus	*C. americanus*		Non host of T. perfectus
C. typhae			Biological control agent? Negligibly attacked by TP (Haye et al 2014)
C. pallidactylus			Serious pest!
Glocianus punctiger			Biocontrol agent
Neydius apicalis			
Haroplontus litura			Biocontrol agent

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The table above shows the summary of the weevils found in the 3 regions in this study. It shows that the only native species found in the Prairies is C. neglectus. This species is attacked rarely by the parasitoid *T. perfectus* based on the study in Quebec and Ontario; furthermore it is not a species at risk because it is abundant in the west and has been considered a potential pest of canola.

Objective 3: Field assessment of the environmental impact of *T. perfectus* on non-target weevils in Quebec (Lucas/Bouchard).

In Quebec, areas with cruciferous weeds in the edge of canola fields and other cultures in regions where canola is grown have been sampled by sweep net in order to characterize the different non-target species of weevil. Five regions were visited during the summer: Montérégie, Centre du Québec, Chaudière-Appalaches, Bas-St-Laurent and Saguenay-Lac-Saint-Jean. The first three regions were visited 7 times from mid-June to the end of August. The other two regions were visited twice, once in mid-July and once at the end of August. Samples have been sorted. The different species are now being identified. So far a total of 10 species of Ceutorhynchinae have been putatively identified: Rhinoncus pericarpus, Glocianus punctiger, Ceutorhynchus obstrictus, C. erysimi, C. americanus, C. pallidactylus, C. typhae, C. omissus, C. neglectus. The occurrence of C. pallidactylus is noteworthy and should be confirmed by the taxonomist because this is a pest of Brassicaceous crops that cause considerable stem damage and may harm the canola crop. An article by Desroches et al (2023) documented the assemblages of native related weevils, here is part of the abstract: “Ceutorhynchinae were sampled in areas adjacent to canola fields or other crops in six administrative regions of Quebec during the summers of 2019 and 2020. A total of 25 Ceutorhynchinae species were collected and identified. Canonical analysis and multivariate regression tree analysis revealed that the assemblage of Ceutorhynchinae varied regionally and was either dominated by the invasive canola pest CSW or by the native weevil *Ceutorhynchus neglectus* Blatchley. Our results also highlighted new biological associations between weevils and Brassicaceae like the CSW with the yellow rocket, *Barbarea vulgaris* R. Br., native *Ceutorhynchus pauxillus* Dietz with common pepper grass, *Lepidium densiflorum*, and native *Ceutorhynchus semirufus* LeConte with Pennsylvania bittercress, *Cardamine pensylvanica* Muhl. This study also provides a useful tool to find new biological control agents against Brassicaceae weeds and to monitor the abundance and diversity of this taxon and provide baseline data to assess future impacts of exotic parasitoids of CSW on native weevils. Furthermore, they showed that *T. perfectus* is by far the dominant parasitoid in canola attacking the cabbage seedpod weevil and that it does not "spill" in a major way onto non cultivated habitats. Only two out of 112 parasitoids reared from uncultivared plants were *T. perfectus*. One emerged from the weevil Ceutorhynchus neglectus (wild mustard) and the other from C. typhae (shepherd's purse). Only the former is a native weevil and it is considered to be a potential minor pest of canola in Alberta (Dosdall 1999, Canadian Entomologist).

Objective 4: To monitor spread and non-target effects of the exotic parasitoid *T. perfectus* in Ontario.

Due to the COVID-19 pandemic milestone achievements were compromised and outputs were fewer than anticipated in 2020 and 2021.

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4.1 Monitoring spread of *Trichomalus perfectus* in Ontario

Surveys were conducted in southwestern Ontario in 2018-2022, although these were restricted in 2020 and 2021. Cabbage seedpod weevil, *Ceutorhynchus obstrictus*, was present in some but not all winter canola fields surveyed. Although *C. obstrictus* occurs in spring canola in southwestern Ontario, populations were very low and sampling for presence of *Trichomalus perfectus* focused on mass collections in winter canola crops. Findings confirmed that *T. perfectus* is present in the canola growing areas in the southwestern parts of the province. Surveys in the Ottawa area of eastern Ontario, where much less canola is grown confirmed that *C. obstrictus* and *T. perfectus* are also present in that region, although at very low levels.

Data clearly show that *T. perfectus* has become the dominant parasitoid of *C. obstrictus* in southwestern Ontario in canola since first being reported there in 2012, having displaced several native parasitoid species that were previously documented exploiting *C. obstrictus* at low levels as a host. Data show that *T. perfectus* constituted 97% of the parasitoids reared in 2018 and 65% in 2019. Of the 149 parasitoids obtained in 2018, 145 were *T. perfectus* and the remaining 3% included *Mesopolobus moryoides*, unidentified Eulophidae, and a *Eurytoma* sp. Parasitoid samples for 2020-2022 are being processed and species identifications are pending. In 2021, based on dissection of canola pods collected in a final mass collection in June, the parasitism level was approximately 30%.

Based on the surveys conducted, *T. perfectus* occurs in canola-growing regions in the Middlesex, Perth, Huron, Bruce and Grey counties. Parasitism is highly variable depending on year, sample field location, and *C. obstrictus* infestation level. However, end of season mass collections (which may overestimate parasitism levels) from 2012 to 2019 have shown average parasitism ranging from 0 to 80% in winter canola and 0 to 64% in spring canola (T.D.G., unpublished data).

A key development was a new set of DNA barcode primers for the Pteromalidae that improve the amplification success of specimens that failed to amplify using the standard universal barcode primers. A protocol has been developed for amplification, and sequencing success is being evaluated.

4.2 Monitoring non-target effects of *Trichomalus perfectus* in Ontario

Surveys of canola and other Brassicaceae plants were conducted in southwestern Ontario and eastern Ontario in 2018-2022. Weevils reared from non-canola Brassicaceae included the seed-feeding *Ceutorhynchus omissus* and *Ceutorhynchus neglectus*, *Ceutorhynchus querceti*, and the stem boring *Ceutorhynchus americanus*, all of which are 'at-risk' of attack by *T. perfectus*. *Ceutorhynchus omissus* feeds on seeds of wormseed mustard, *Erysimum cheiranthoides*; *Ceutorhynchus querceti* feeds on seeds of yellowcress *Nasturtium officinale*; *Ceutorhynchus neglectus* feeds on seeds of yellow cress, *Rorippa palustris*, and dog mustard, *Erucastrum gallicum*. *Ceutorhynchus americanus* were found in stems of wild mustard, *Sinapis arvensis*.

Parasitism of *C. omissus* was very high (84-96%), parasitism of *C. neglectus* was low (8-14%), and parasitism of *C. americanus* was zero (Table 1). Parasitoid identifications are underway to confirm what proportion may be *T. perfectus*.

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Table 1. Number of individuals collected and parasitism levels of *Ceutorhynchus* spp. weevils collected in the Ottawa region of eastern Ontario 2019-2022 (excluding 2020).

	2019		2021		2022	
	# weevils	% parasitism	# weevils	% parasitism	# weevils	% parasit
Ceutorhynchus omissus	12	83.1	4	82.5	4	97.7
Ceutorhynchus neglectus	119	4.0	206	15.9	.	
Ceutorhynchus obstrictus	30	9.1	100	31.5	106	89.0
Ceutorhynchus americanus	4	0	.	.	.	.

key finding was the recovery of *T. perfectus* from *C. obstrictus* and for the first time from *C. neglectus*. These results confirm that *T. perfectus* established in the Ottawa area and is using native seed-feeding weevils as hosts. A new plant host, dog mustard *Erucastrum gallicum* was associated with *C. omissus*.

5. CLIMEX/DYMEX model for host and parasitoid. (Vankosky)

Ross Weiss, Owen Olfert, Tim Haye and Meghan Vankosky have updated the CLIMEX model for cabbage seedpod weevil (CSW) and developed a CLIMEX model for canola (because the range of canola production is a constraint of CSW distribution). The models for CSW and canola have been validated and a manuscript describing the model is being drafted. Once the manuscript describing the canola and CSW models is published, these models will be used to update and develop models for CSW parasitoids. The models for all three trophic levels (host plant, insect pest, parasitoid) will also be used to predict the impact of climate change on this community.

Objective 6: a review of canola agronomy and abiotic comparisons in eastern and western Canada which may impact parasitoid establishment. This manuscript was started but could not be finished (abstract below).

Potential for classical biological control of cabbage seedpod weevil in the Canadian Prairies – A review.

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Abstract: Cabbage seedpod weevil is a European invasive pest of canola in North America. Growers rely on chemical insecticides to manage it, but there is potential for biological control as a more sustainable option. Two wasps, *Trichomalus perfectus* and *Mesopolobus morys*, are the main parasitoids of this weevil in Europe. Although none have been released in Canada, *T. perfectus* is established in Quebec and appears to keep weevils under threshold. Thus, it has been suggested for redistribution to western Canada. In this review we discuss the potential impact of Prairie farming practices, landscape and climate on establishment of parasitoids

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and where possible we make comparisons to eastern Canada. Early seeding of canola can maximize yield in eastern and western Canada and allows synchrony of *T. perfectus* with its weevil host. Adoption of integrated pest management strategies, including proper timing of insecticide spraying where unavoidable, will be important for the establishment of *T. perfectus*. Parasitoids will benefit from semi herbaceous habitats surrounding the fields as they provide favorable overwintering sites for adult parasitoids. Hot and dry periods may be a limiting factor for parasitoid adult emergence, although, bioclimatic models suggest that establishment of *T. perfectus* is possible in all Canadian canola growing areas of the Prairies. Ongoing environmental assessments will determine the need for future petitions to release these parasitoids in western Canada.

Objective 7: Landscape effects on cabbage seedpod weevil and its parasitoid

A manuscript has been published by the doctoral student (M. D'Ottavio) on the topic of landscape effects on cabbage seedpod weevil and its parasitoid *T. perfectus* to an international entomology journal (Insects):

"Landscape effects on the cabbage seedpod weevil, Ceutorhynchus obstrictus (Coleoptera: Curculionidae), and on its parasitoid, Trichomalus perfectus (Hymenoptera: Pteromalidae), in canola". The exotic cabbage seedpod weevil (CSW), Ceutorhynchus obstrictus, is a major pest of canola crops. This insect is mainly regulated in Europe by the parasitoid Trichomalus perfectus, a natural enemy also accidentally introduced in eastern Canada since 2009. The objective of the present study was to evaluate how the landscape influenced the CSW infestation and abundance and the parasitism of *T. perfectus*. Results from six years in eight Quebec regions show that the CSW was positively affected by roads and cereal crops. Regarding *T. perfectus*, the parasitism was variable (from about 5 to almost 25%) and positively influenced by landscape diversity, crop edge density, hay/pastures and soybean crops. These results will help determine the potential of an eventual introduction of *T. perfectus* in western Canada, where most of the canola is produced, and the optimal landscape composition and configuration needed to succeed. A similar study was conducted in southern Alberta by a doctoral student (P. Jegatheeswaran) from the University of Lethbridge but due maternity leaves the work is on hold. However, preliminary analysis suggests that landscape features may not influence in a major way, the distribution of the cabbage seedpod weevil or the parasitoids in the region. From a sample size of 61 fields, only alfalfa and canola had modest negative correlations with abundance of weevils at the flower stage (Spearman Ranked correlations Rho -.18-0.20, $p<0.10$).

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