Canadian Agri-Science Cluster for Horticulture 3

Update to Industry

Final Report – 2018 - 2023

Activity title:
Development of Regional Management Strategies and Decision Making Tools for Control of Colorado Potato Beetle

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Activity Objectives *(as per approved workplan):*

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OBJECTIVES: Our overall objective was to reduce economic losses to potato in Canadian growing regions due to the Colorado potato beetle (CPB). Specifically, we aimed to determine local susceptibility of CPB populations to several classes of insecticides through a national resistance-monitoring network, improve resistance management, better characterize the molecular basis of developing resistance, and develop novel extension tools to improve management practices. To accomplish this, we divided deliverables into three objectives as follows:

1. Determine susceptibility of Colorado potato beetle populations to multiple classes of insecticides in different potato growing regions in Canada;
2. Develop an interactive online mapping tool for growers to access results of susceptibility surveys to inform local decision making for optimal insecticide selection;
3. Identify molecular signatures of insecticide resistance that can be used to monitor the occurrence and spread of resistance in regional CPB populations and identify new pest control targets.

Research Progress & Results *(use plain language, not to exceed 1,000 words)***:**

A core strength of this project was the ongoing relationship building between the AAFC project team and our project partners, involved in extension and potato production across Canada. Between 2018 and 2023, we met virtually with our partners in Manitoba, Quebec, Ontario and Prince Edward Island several times, as well as presented at grower meetings in New Brunswick, Quebec, Ontario and Prince Edward Island. We also worked with a number of media outlets which reach growers and growers associations, such as SpudSmart, Tuber Talk, The Western Producer, Fresh Thinking Magazine, The Grower and Top Crop to ensure knowledge exchange regarding our research plans, for further engagement with our project delivery and adaptation, and to communicate project results. We remained responsive to the shifting priorities of growers in each region, and in the later three years of the project adjusted the composition of insecticides screened to ensure we were providing the most relevant results to the growers we serve.

Objective 1. Determine susceptibility of Colorado potato beetle populations to multiple classes of insecticides in different potato growing regions in Canada

In total from 2018 – 2023 we evaluated the susceptibility of 139 populations of Colorado potato beetle (CPB) collected from Alberta, Manitoba, Ontario, Quebec, and Prince Edward Island, to ten insecticides from 4 different classes including: neonicotinoids (ACTARA® 240SC, 21.6% thiamethoxam, TITAN®, 48% clothianidin), anthranilic diamides (VERIMARK®, 18.7% cyantraniliprole; CORAGEN®, 18.4% chlorantraniliprole; EXIREL®, 10.2% cyantraniliprole, HARVANTA®50SL, 5% cyclaniliprole, VAYEGO 200SC, 20% tetraniliprole), abamectins (AGRI-MEK® SC, 8.4% abamectin) and spinosyns (ENTRUST SC, 22.5% spinosad, DELEGATE WG, 25% spinetoram), were tested at the London and Fredericton Research and Development Centres (LRDC and FRDC). All populations were tested at the same larval life stage with the LC90 concentration determined previously using a lab strain of CPB that had never been exposed to insecticides at the LRDC.

Our evaluations showed resistance to the insecticides screened in our study varied both within and between provinces (Scott et al., 2023). More than one quarter (27.3% or 38 of 139) of populations we tested showed resistance to at least one insecticide (19 in MB, 8 in ON, 7 in QC, 3 in NB and 1 in PEI) with the exception of AB where no resistance was detected. A total of nearly 60% (59.7% or 83 of 139) of populations were found to have reduced susceptibility (1 in AB, 11 in MB, 16 in ON, 24 in QC, 8 in NB, and 21 in PEI). Only 18 populations (12.9%) were susceptible to all insecticides, the majority were found in AB (5 of 6) and PEI (7 of 29). Data for spinosyns are summarized in Scott et al. 2023, *Regional differences in susceptibility to spinosyn insecticides registered for Colorado potato beetle management in Canada* available open access at https://doi.org/10.1016/j.pestbp.2023.105459

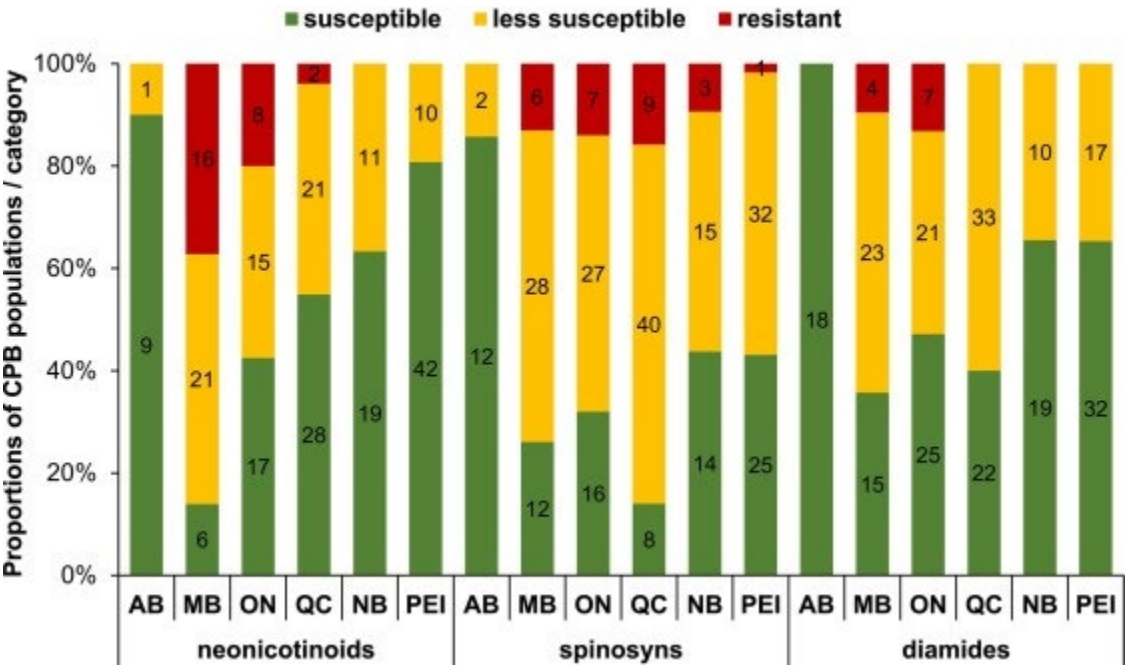

Figure 1 : The percentage of each population determined to be susceptible (green bars), less susceptible (yellow bars) or resistant (red bars) to 3 insecticide classes in CPB populations collected from Alberta (AB), Manitoba (MB), Ontario (ON), Québec (QC), New Brunswick (NB) and Prince Edward Island (PEI) between 2018 and 2022; adapted from Scott et al. 2023, *Regional differences in susceptibility to spinosyn insecticides registered for Colorado potato beetle management in Canada*

Objective 2: Develop an interactive online mapping tool for growers to access results of susceptibility surveys to inform local decision making for optimal insecticide selection

Over the course of the project we worked to incorporate our resistance datasets into an online mapping database and map projection tool in collaboration with the AAFC Agri-Geomatics team. We provided a soft release of this tool during the 2022 SpudSmart webinar. Finalization of the web-based mapping tool requires anonymization of the datasets to ensure the privacy of the sampling locations prior to open access of the mapping tool by implementing a visibility range for the dataset and generalizing the dataset to a larger geographical area such as counties/districts/ecoregions. The Web Mapping Application will provide growers/industry the ability to access and obtain detailed results from the susceptibility survey in a form that is accessible and easy-to-navigate. Upon tool finalization we plan to host an information session to growers/industry to demo the mapping tool and co-develop the end product to maximize user uptake.

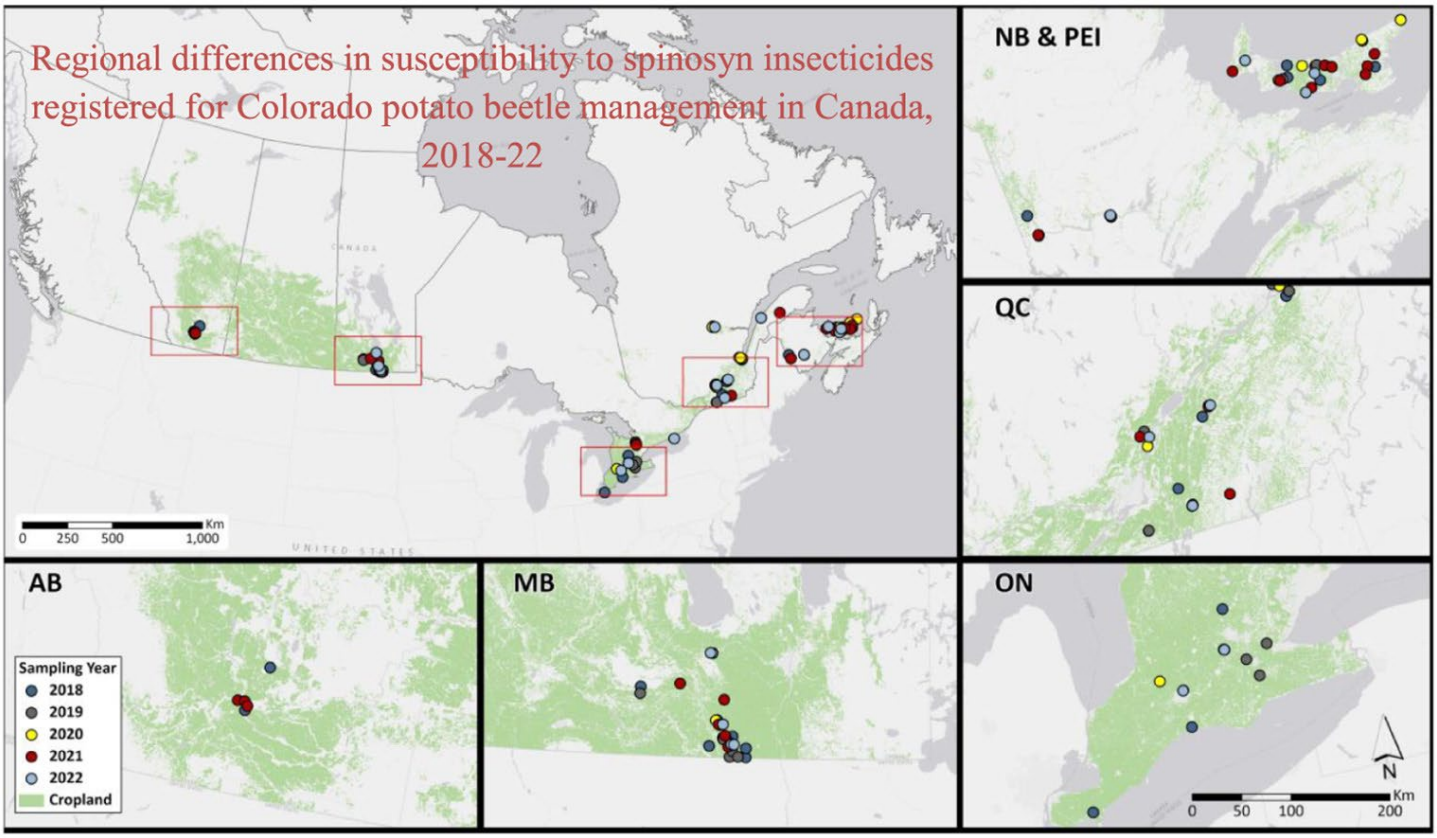

Figure 2: Visualization of maps produced by year and susceptibility to selected insecticides (i.e, spinosyns) that will be viewable upon release of the mapping tool. Adapted ~~from adapted~~ from Scott et al. 2023, *Regional differences in susceptibility to spinosyn insecticides registered for Colorado potato beetle management in Canada*

Objective 3: Identify molecular signatures of insecticide resistance that can be used to monitor the occurrence and spread of resistance in regional CPB populations and identify new pest control targets

Substantial advancements were made to generate new knowledge of potential molecular signatures of insecticide resistance in Colorado potato beetle. A number of novel gene targets were identified in Colorado potato beetle, with further potential to develop RNA-interference based pest management tools should additional funding opportunities be identified by the project team. Several candidate genes potentially involved in diamide and spinosyn insecticide resistance have been identified in samples obtained from surveying. Over the last few years, we focused our efforts on determining relative mRNA levels of the seven target genes identified, developing tools for RNA interference testing to show effects of the target genes on resistance, bioassays to determine lethal doses of Entrust insecticide in an Entrust resistant population from QC, and RNA interference testing on the Entrust resistant QC population of beetles.

RNA interference (RNAi) testing on beetles exposed to Entrust insecticide was investigated. Resistant beetles were fed dsRNA specific for the two genes most highly overexpressed in this resistant population, and expression of the genes was analyzed to demonstrate silencing of the genes. Treated beetles were then challenged with a dose of insecticide. Mortality of the insecticide-challenged insects was measured and compared to mock treated controls to assess the contribution of the tested genes to insecticide resistance. Silencing of each gene produced a measurable, but statistically insignificant, increase in mortality to both Entrust and Delegate insecticides. Then, silencing of both genes at once was tested, and in this case, a significant increase was noticed in the mortality with Entrust treatment, but not with Delegate treatment. Similar experiments were conducted using a population resistant to the insecticide Coragen and dsRNA for the three genes most highly overexpressed in the resistant population, but no effect on mortality resulting from insecticide exposure was observed.

In summary, two genes identified as being over-expressed in an Entrust-resistant QC population of CPB were shown using RNA interference to significantly contribute to the resistance to toxicity of the insecticide Entrust in this population of CPB. These genes could form the basis for a potential molecular signature for spinosyn insecticide resistance. Since the effects of double knockdown of the genes on mortality produced by Entrust exposure was additive, the results provide evidence of multi-gene resistance to Entrust in this population.

The characterization of molecular signatures with relevance to small non-coding RNAs and associated with insecticide resistance in Colorado potato beetles is ongoing. During the past year, identification of differentially expressed miRNAs was notably performed using high-throughput sequencing in Colorado potato beetles that displayed susceptibility or resistance to the insecticide Titan (clothianidine). Successful optimization and dsRNA-based knockdown of transcripts associated with small non-coding RNAs synthesis or function, such as Ago2, was performed in Colorado potato beetles and assessment of the impact of such modulation on insecticide response is envisioned. In parallel, measurement of several key transcripts coding for proteins associated with insecticide response, such as cytochrome P450s, was conducted in multiple populations of Colorado potato beetles with confirmed resistance to insecticides including Entrust (spinosad) and Verimark (cyantraniliprole), to name a few. This approach revealed numerous transcripts that exhibited varied expression in insects resistant to insecticides warranting a thorough exploration of cytochrome P450s expression in response to various classes of insecticides.

Key Message(s):

The key statement(s) from the project highlighting the benefit to industry.

This project generated substantial new knowledge on the regional patterns of established and developing resistance to insecticides in the Colorado potato beetle, the most economically important pest of potato. Substantial resistance had been previously detected in this insect to the neonicotinoid insecticides in select regions of North America, and increasing resistance in Canada previously shown. In this project, we were able to evaluate resistance to ten insecticides over the course of the project. Our data showed that the highest levels of resistance are found in organic potato production, to the spinosyn insecticides, but that spinosyns remain generally effective despite developing resistance. Regionally, Manitoba showed the highest amount of resistance to the neonicotinoids, with nearly 40% of sampled populations showing resistance to this group and in total nearly 85% showing reduced susceptibility or resistance. Ontario beetles showed the next highest amount of resistance to the neonicotinoids, with nearly 60% of beetles showing reduced susceptibility or resistance. For the spinosyns, reduced susceptibility was detected in high percentages again in Manitoba and Ontario, but the highest (86%) was in Quebec. Resistance or reduced susceptibility to the diamides showed similar patterns in Manitoba, Ontario and Quebec, with higher susceptibility shown in the Maritimes and full susceptibility in Alberta.

Evidence of cross resistance was found among the spinosyns and conventional insecticides tested. Growers are encouraged to continue to rotate chemistry groups whenever possible to avoid the development of resistance to one group of insecticides and the potential for development of cross resistance to other chemical groups.

Substantial new knowledge on the molecular basis of resistance, particularly to the spinosyns, was generated during this study, with several candidate gene targets for the development of RNA interference control methods identified. Future work on developing RNAi control methods is an active and promising area of further investigation for extending the protection provided by current chemistries.

Overall benefit to industry:

Expand the key message(s) and provide all grower-relevant details in a form easy to assimilate and compare.

This project generated substantial new knowledge on regional patterns of insecticide resistance in the most economically devastating insect pest of potato, the Colorado potato beetle. By evaluating nearly 140 populations of Colorado potato beetles collected over five years from five regions of Canada, we documented that reduced susceptibility and development of insecticide resistance are highest for the spinosyn organic insecticides across regions, even amongst populations where the neonicotinoid or diamide products are more commonly used. High levels of reduced susceptibility are also seen to the neonicotinoids and increasing levels of reduced susceptibility to diamides. Molecular diagnostic studies as part of our project yielded a number of new candidate genes for consideration as targets for RNAi based pest management tools. Cross resistance was found in some populations and resistance management should continue to prioritize rotating among insecticide classes and between years to extend the longevity of all classes of insecticides for control of Colorado potato beetle. These data will be viewable in an online interactive mapping database in final stage development for growers, extension specialists and other stakeholders to access regionally relevant results to each product evaluated.

This project is generously funded through the Canadian Agri-Science Cluster for Horticulture 3, in cooperation with Agriculture and Agri-Food Canada's AgriScience Program, a Canadian Agricultural Partnership initiative, The Fruit & Vegetable Growers of Canada and industry contributors.

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