Canadian Agri-Science Cluster for Horticulture 3

Update to Industry

2019-2020

Activity title: Integrated management of the pepper weevil, an invasive pest of greenhouse pepper crops in Canada

Name of Lead Researcher: Roselyne Labbe, PhD, Agriculture and Agri-Food Canada

Names of Collaborators and Institutions:

- Félix Longpré, Insect rearing biologist, London Research and Development Centre, AAFC
- Cara McCreary, Greenhouse Vegetable Integrated Pest Management Specialist, Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA)
- Miyuki Santiago, Business Development, Koppert Biological Systems
- Niki Bennett, Science Coordinator, Ontario Greenhouse Vegetable Growers
- Dr. Cynthia Scott-Dupree, Bayer Chair in Sustainable Pest Management School of Environmental Sciences University of Guelph, working with Bruce Power, Canadian nuclear generator, providing isotopes for potential Sterile Insect Technique research.

Activity Objectives (as per approved workplan):

1. Develop improved rearing methods for the pepper weevil.
2. Evaluate reduced-risk conventional and biopesticides for the management of the pepper weevil.
3. Evaluate non-target impacts of management products on beneficial insects used in greenhouse pepper biological control.
4. Establish the efficacy of parasitoid *Jaliscoa hunteri* for the management of the pepper weevil. Establish and compare the efficacy of rearing methods for *J. hunteri* production. Explore alternate crop delivery methods for *J. hunteri.*
5. Conduct laboratory and greenhouse trials investigating the potential of the Sterile Insect Technique (SIT) for managing the pepper weevil.

Research Progress to Date (use plain language):

Executive Summary

Challenge: The pepper weevil, *Anthonomus eugenii* is one of the most important pests of pepper crops in North America. In 2016, the Canadian greenhouse pepper industry suffered a massive crop loss amounting to an estimated $83 million due to infestation by the pepper weevil (Ontario Greenhouse Vegetable Growers). This loss was attributable to a number of factors including annual introductions through imported fruit, the density of greenhouse and field pepper production in certain localities, mild winters and hot summers, and the lack of effective management tactics which represent ongoing challenges for the future of greenhouse pepper production in Canada.

At present, the use of chemical insecticides, along with crop scouting and cultural management are the main strategies used to manage weevil populations. However, these strategies are very labor intensive, costly, and inefficient. Few products are currently registered in Canada and existing products are insufficient for yearend cleanup which may result in the yearly recurrence of the pepper weevil, despite massive efforts to eliminate the pest. Furthermore, insecticides offer only temporary suppression of adult populations and are unable to target immature life stages of the pepper weevil. There is also a need to address concerns regarding the negative impacts products have on non-target biological control agents used to manage other important greenhouse pepper pests. For all of these reasons, it is essential to identify new tools, including reduced risk conventional agents, biopesticides and biological control organisms that may contribute to achieving good season-round integrated management of the pepper weevil.

Prior surveys of the natural enemies of the pepper weevil in Mexico and Canada, has led to renewed interest in biological control of the pest. In Mexico, three wasp species, *Triaspis eugenii*, *Urosigalphus* sp., and *Jaliscoa hunteri* account for 96% of all parasitoids that attack the pepper weevil (Rodríguez-Leyva et al. 2007). In Canada, at least seven parasitoid species attack and kill the pepper weevil, including *J. hunteri* and *Pteromalus anthonomi*. Trials performed in Florida have demonstrated that *J. hunteri* can significantly reduce the number of weevil-infested bell pepper fruit (Schuster 2012). Jaliscoa hunteri in particular has the potential to be developed as a biological control agent of the pepper weevil and requires evaluation.

Furthermore, new technologies such as the Sterile Insect Technique also have the potential to control the pepper weevil, although this possibility has yet to be examined. With virtually no off-target effects, and minimal environmental risk, this technology may very well alter the way we approach pest management.

Objectives: Towards meeting the challenges of managing the pepper weevil on Canadian greenhouse pepper crops, this proposed study aims to: 1) develop improved rearing methods for the pepper weevil; 2) evaluate reduced risk conventional and biopesticides for the management of the pepper weevil; 3) assess the non-target impacts of management products on beneficial arthropods used in greenhouse pepper production; 4) develop rearing and delivery methods for *J. hunteri* and assess its efficacy in suppressing pepper weevil on greenhouse crops; 5) investigate the potential of the Sterile Insect Technique (SIT) for managing the pepper weevil.

2019-2020 Report Executive Summary

In this reporting period year, we have completed work associated with assessing numerous insecticidal products for the control of the pepper weevil. This insect is now fully assessed for its survival to 16 compounds both in laboratory bioassay spray trials, as well as in the greenhouse environment. This is considered a success as we were able to identify new and reduced-risk products such as kaolin clay and mineral oil, that perform better even than the neonicotinoid positive control, thiamethoxam – an industry standard for pepper weevil control. This will further provide the data required to support new product registrations and label expansions needed for achieving pepper weevil control in both greenhouse as well as field settings in Canada. We have now begun work to assess the non-target effects of such key products on biocontrol organisms commonly applied for suppression of other greenhouse pests. Together, this will help guide the development of best practices for control of pepper weevil and serve to enhance the environmental performance of greenhouse crop production in Canada.

Other developments in this project year were the identification of a model plant system in which to better study the impact of parasitoid, Jaliscoa hunteri, for pepper weevil suppression. This has been instrumental in our ability to quantify the effects of this parasitoid on pepper weevil populations, which were up to now, very unclear. Early trial results with such a system have shown that parasitoids can reduce pepper weevil emergence in pepper buds by up to 68% relative to an untreated control, which suggests that biocontrol may be an effective strategy for contributing to pepper weevil control.

Also, our development of molecular diagnostic tools, such as pepper weevil specific PCR primers, represent important tools for our future quantification of the incidence of host feeding by J. hunteri. Such an innovation will allow us better understand how J. hunteri can effect important levels of pest suppression, including those we have previously observed in laboratory and greenhouse environments.

During this reporting period, we have recruited two new HQP, including MSc student Serena Leo, who will continue to elucidate the function of parasitoid J. hunteri for pepper weevil suppression. We have also, as of summer 2020, welcomed MSc student, Jacob Basso who will begin work on evaluating how the Sterile Insect Technique (SIT) may function to reduce pepper weevil populations on greenhouse pepper crops.

Together, the innovations we have achieved through this project to date are likely to guide the development of best practices for pepper weevil control and improve the agricultural productivity of Canadian greenhouse crops, particularly when faced with the costly threat of pepper weevil crop invasion.

Objectives

01 - Develop rearing methods for the mass rearing of the pepper weevil using artificial media.

Description: Develop rearing methods for the mass rearing of the pepper weevil using artificial media.
Outcome: Generate an improved pepper weevil rearing system with which to mass rear parasitoid hosts for biological control and for product testing.

Performance Summary: (Partially Met) ----------
In this reporting period, trials have been conducted which examined the potential for ornamental pepper plants to serve as hosts for pepper weevil and as a way to minimize the cost and input required for maintaining colonies of this insect. Pepper weevil introductions were conducted onto four different varieties of ornamental pepper plants grown to the bud formation stage. These were subsequently followed by counting of the total number of weevil offspring produced per plant up to four weeks after the initial weevil introduction.

02 - Evaluation of reduced risk conventional and biopesticides for the management of the pepper weevil.

Description: Evaluation of reduced risk conventional and biopesticides for the management of the pepper weevil.
Outcome: Identify products most appropriate for pest management on active crops and at end of crop cleanup.
Performance Summary: (Met) ----------
In this project year, we have completed work associated with assessing a total of 16 different products for the control of the pepper weevil. This insect is now fully assessed for its survival both in laboratory bioassay spray trials, as well as in small cage greenhouse trials. This work enabled us to identify new, reduced-risk products that are effective for pepper weevil suppression and control including kaolin clay and mineral oil, both products which performed better than our thiamethoxam positive controls. It is anticipated that results from these trials will directly contribute to label expansions or new registrations in Canada for products that are effective for pepper weevil control.

03 - Evaluation of non-target impacts of management products on beneficial insects used in greenhouse pepper biological control.

Description: Evaluation of non-target impacts of management products on beneficial insects used in greenhouse pepper biological control.
Outcome: Identify the impact of pepper weevil control products on commonly used pepper biological control agents.
Performance Summary:
Preparations are now underway to initiate laboratory trials assessing the non-target effects of insecticidal agents useful for suppressing pepper weevil in a greenhouse environment, on biological control agents typically also applied in this setting. Test species will include, *Amblyseius swirskii*, *Neoseiulus cucumeris*, *Phytoseiulus persimilis* as well as *Orius insidiosus*.

04 - Establish the efficacy of parasitoid *Jaliscoa hunteri* for the management of the pepper weevil. Establish and compare the efficacy of rearing methods for *J. hunteri* production. Explore alternate crop delivery methods for *J. hunteri*. Perform

Description: Establish the efficacy of parasitoid *Jaliscoa hunteri* for the management of the pepper weevil. Establish and compare the efficacy of rearing methods for *J. hunteri* production. Explore alternate crop delivery methods for *J. hunteri*. Perform commercial trial with *J. hunteri* for the suppression of the pepper weevil.

Outcome: Identifying the potential for *J. hunteri* to serve as an effective greenhouse biological control agent.
Performance Summary: (Partially Met) ----------
While wasp *Jaliscoa hunteri* is recognized for its potential to parasitize and kill the pepper weevil, little is known about how this is accomplished on pepper plants. For instance, the infrequent emergence of wasps from large bell peppers suggests that the effect this species has is probably occurring at earlier stages of fruit development. In this reporting period, we conducted cage trials with four varieties of ornamental pepper plants in which pepper weevil adults were first introduced, followed by exposure of their offspring within pepper buds to *Jaliscoa hunteri* adult male and female wasps. Through this assays, we could show that pepper weevil was being attacked at the bud and small fruit stage. The timing for wasp introductions in these assays were also critical, as it is known *J. hunteri* are most able to parasitize and develop upon 3rd larval instars of the pepper weevil. Following these wasp treatments, which were compared to pepper weevil infested plants unexposed to the parasitoid, pepper weevil offspring emergence, bud abortion, and adult wasp emergence were recorded for a period of over four weeks. These trial were critical to our understanding the impact of *J. hunteri* wasps on weevil density, with important implications for the development of this species as a commercial agent for weevil biological control in Canada and elsewhere.

05 - Conduct laboratory and greenhouse trials investigating the potential of the Sterile Insect Technique (SIT) for managing the pepper weevil.
Description: Conduct laboratory trials investigating the impact of different radiation doses on male weevil survival, activity and competitive behaviour. Greenhouse trials will also be conducted that investigate the function of Sterile Insect Technique for weevil management.
Outcome: Generate data on the efficacy of the Sterile Insect Technique for pepper weevil suppression in the greenhouse environment.
Performance Summary:
The work associated with objective 5 will be initiated in the next project year in 2020-2021.

Milestones
01 - Improved pepper weevil rearing methods
Activity: Generate improved rearing methods for pepper weevil to increase overall density of laboratory colony.
Milestone / Deliverable: Evaluation of various rearing environments and substrates for optimizing pepper weevil production in the laboratory.
Progress Summary: (In Progress) ----------
In this reporting period, work has been initiated to evaluate the feasibility of using ornamental peppers for the rearing and studying pepper weevil. Trials with four ornament varieties of *Capsicum anuum* showed that pepper weevil adults have a strong affinity to oviposit within pepper buds, the youngest of fruiting pepper stages. Furthermore, surprisingly small buds were able to produce weevil adults, with up to 97 per plant being observed in our studies. Considering the small amount of space and maintenance time that ornamental pepper plants require, this work demonstrates that maintaining pepper weevil colonies on such plants can be both practical and effective. In future project years, this method will be compared to others to determine their cost and time effectiveness.

02 - Product efficacy trials
Activity: Perform laboratory assays to identify effective insecticidal control agents for the control of the pepper weevil including quantification of direct and indirect effects. Perform greenhouse trials to confirm efficacy of these products on pepper plants.
Milestone / Deliverable: Generate a list of effective biological and conventional agents able to control the pepper weevil, with detail on the mechanism of activity. Validate the efficacy of agents in greenhouse cage trials on pepper.
Progress Summary: (Completed) ----------
In this study, a series of insecticidal products covering a broad spectrum of modes of action were assessed for their potential in managing the pepper weevil under laboratory and greenhouse conditions. To accomplish this, laboratory mini-spray tower and greenhouse cage trials were conducted that assessed the efficacy of 16

conventional, reduced-risk and microbial insecticides. In laboratory trials, adult weevils were sprayed with insecticides, placed on treated leaves within a cup cage, and were monitored for their survival over 10 days.

Of the 16 insecticides tested, eight provided greater than 60% weevil mortality, a threshold considered necessary for achieving pest suppression. These agents were subsequently evaluated for weevil suppression on greenhouse pepper crops.

In greenhouse trials, adult weevil mortality, bud and foliar damage, bud and fruit abortion and subsequent weevil offspring emergence were measured following each of three weekly insecticide applications. The most efficacious insecticides included kaolin clay and mineral oil, which performed as well as the thiamethoxam positive control, and incurred 70 and 55% of adult weevil mortality, respectively. Additionally, kaolin clay and mineral oil reduced offspring weevil emergence by 59 and 54%, respectively, compared to untreated controls. Despite the clear challenge that controlling this pest represents, this work has identified useful new tools for the integrated management of the pepper weevil, which will greatly accelerate their future registration for use in greenhouse and field pepper production in Canada.

03 - Non-target testing of agents

Activity: Laboratory and small cage greenhouse trials performed to evaluate the non-target effects of microbial and conventional agents on biocontrol organisms.

Milestone / Deliverable: Generate compatibility data for use of microbial and conventional agents in the context of a biocontrol programme on greenhouse pepper crops.

Progress Summary: (In Progress) ----------

Work is currently underway to test the impact of pepper weevil control products on non-target biological control agents also frequently applied to greenhouse pepper crops. For these assays, agents including kaolin clay, mineral oil, *Metarhizium robustii* (Met52), *Beauveria bassiana* strain ANT-03, *Bacillus thuringiensis* var. *galleriae* and a thiamethoxam positive control will be evaluated for their effects on biocontrol agents. The mortality of predators and parasitoids including *Amblyseius swirskii*, *Phytoseiulus persimilis*, *Orius insidiosus* and *Aphidius colemani* will be assessed both by direct cuticular spray, as well as by exposure to product dipped leaves. This work will be instrumental to generating the best practices for the concurrent management of pepper weevil along with other crop pests of greenhouse pepper.

04 - Parasitism of the pepper weevil

Activity: Novel methods for rearing and delivering the pepper weevil parasitoid, *Jaliscoa hunteri* to greenhouse pepper crops.

Milestone / Deliverable: Complete lab trials to identify the most suitable host for rearing *J. hunteri*. Complete caged and commercial greenhouse trials evaluating the efficacy of parastioids and release methods for pepper weevil suppression.

Progress Summary: (In Progress) ----------

In 2019-2020, tests were conducted to quantify the effect of *Jaliscoa hunteri* on pepper weevil development within pepper buds. For this purpose, controlled environment trials were conducted in summer 2019 whereby pepper weevil adults (3 males, 3 females) were introduced into a microperforated bag around the foliar and bud components of four ornamental pepper varieties of *Capsicum anuum* including cultivars Blaze, Hot Pops, Medusa and Wicked. Pepper weevil were allowed to oviposit for 7 days, after which, resulting larvae were allowed to develop to the 3rd instar as predicted by known life history. Thereafter, 10 adults of the parasitoid wasp, *Jaliscoa hunteri* (five males and five females) were introduced and allowed to effect pepper weevil mortality. Through this method, and comparing to unparasizided control plants (all treatments in triplicate) we were able to show that *Jaliscoa hunteri* could incur up to a 68% reduction in pepper weevil emergence from buds, a result which was consistent among the plant cultivars tested. Furthermore, we were able to show that *Jaliscoa hunteri* could successfully develop to adulthood on pepper weevils from this system. This trial serves as a proof-of-principle for the potential effect *Jaliscoa hunteri* can have on weevil populations. Future work will elucidate the mechanisms employed by wasps in effecting weevil mortality, either through direct parasitism, or likely more importantly, though host feeding.

In this reporting period, we have also developed important molecular tools to detect the occurrence of host feeding by *Jalsicoa hunteri* on immature pepper weevils. To date, early tests have shown that the pepper weevil specific PCR primers were able to identify wasps which have actively fed upon immature pepper weevils in pepper buds and fruits. This behaviour is occurring especially often among female wasps and is suspected to serve as a form of nutrient acquisition that allows them to develop the eggs necessary for successful parasitism. Such molecular tools will be instrumental to furthering our understanding of how such biological control agents work to suppress economically important pests including the pepper weevil.

05 - Evaluation of the Sterile Insect Technique for pepper weevil control

Activity: Evaluate the potential of the Sterile Insect Technique (SIT) for control the pepper weevil through laboratory and greenhouse trials.

Milestone / Deliverable: Evaluate the potential of the Sterile Insect Technique for pepper weevil control in lab and greenhouse trials.

Progress Summary: (Not Started) ----------

In early 2020, we have recruited an MSc student, Jacob Basso, to work with us at the Harrow Research and Development Centre, and at the University of Guelph with Dr. Cynthia Scott-Dupree, to evaluate the effectiveness of the sterile insect technique for management of the pepper weevil. This work will start in earnest in May 2020.

Extension Activities (presentations to growers, articles, poster presentations, etc.):

1. Fernández*, C., Vanlaerhoven, S. and Labbe. R. 2019. Choosy weevil? Alternate hosts and oviposition preference of *Anthonomus eugenii* in southern Ontario. Canadian Society of Zoologists AGM. Windsor, ON. (Oral Presentation – May 13, 2019)
2. Fernández*, C. Van Laerhoven, S. Labbé, R. 2019. Genetic diversity of Anthonomus eugenii in North America through COI gene barcode analysis. Entomological Society of Canada Joint Annual Meeting, Fredericton, NB. (Oral Presentation – September 4, 2019)
3. Rizzato*, R., Fernandez, D.C., Gagnier, D. Zhang, Y., Labbe, R. 2019. Using parasitoid wasp Jaliscoa hunteri to target a major pepper crop pest in Canada. Entomological Society of Ontario Annual Meeting, Bark Lake, ON. (Oral Presentation – November 1, 2019)
4. Labbe*, R., Gagnier, D., Rizzato, R., Tracey, A. and McCreary, C. 2019. Efficacy of reduced risk, microbial and conventional agents for suppression and control of the pepper weevil, Anthonomus eugenii. Entomology 2019. St. Louis, Missouri. (Poster - November 11 -14, 2019)
5. Labbé*, R.M., and Marchand, G. 2019. Greenhouse Crop Protection Research Summary. Regional Research Users Meeting at the Harrow Research and Development Centre. December 12, 2019.
6. Labbé*, R.M., Developing tools for the sustainable management of greenhouse pests. Department of Integrative Biology, University of Windsor (Seminar presentation - January 24, 2020)
7. Labbe, R., Gagnier, D., Rizzato, R., Tracey, A. and McCreary*, C. 2019. Efficacy of reduced risk, microbial and conventional agents for suppression and control of the pepper weevil, *Anthonomus eugenii.* Ontario Fruit and Vegetable Growers Association AGM, Niagara Falls, ON. (Poster- February 19, 2020)

* Denotes presenter

Early Outcomes (if any) or Challenges:

Issues:

1. In 2019, our team member, Felix Longpré took a one year leave, which affected his ability to contribute to this project.
2. In addition, faced with the lack of pepper weevil infestation in outdoor areas, the collection of know native parasitoids of the pepper weevil limited our ability to investigate their biological control potential (Former objective 5).

Impact:

1. In response to Felix's time away, we initiated work associated with evaluating whether ornamental peppers would serve as a suitable oviposition and rearing substrate.
2. Furthermore, in discussion with Dr. Cynthia Scott-Dupree at the University of Guelph, the CHC as well as the OIPC and RDT at AAFC, we were able to change our fifth project objective to the evaluation of the Sterile

Insect Technique for pepper weevil control.

Action Plan:

1. This work was successful and will assist us to achieve other project milestones. As of February 2020, the return of Felix to AAFC means that he will be able to continue work associated with several objectives in this research project.
2. We have now recruited a MSc student, Jacob Basso from the University of Guelph, who will begin work on evaluating the SIT as part of the revised objective 5. No change in the budget is anticipated as a consequence of these changes to the work plan.

Key Message(s):

For this reporting period, our recent research on the impact of *Jaliscoa hunteri* on pepper weevil infested ornamental plants has demonstrated to us that biological control of this pest species can indeed represent an effective strategy for achieving weevil control. In combination with the use of other management tools developed through this project, the use of such a biocontrol agent has the potential to considerably increase the effectiveness integrated management practices aimed at this pest, and can result in a net pest suppression relative to the use of conventional agents alone.

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