Canadian Agri-Science Cluster for Horticulture 3

Update to Industry

2019-2020

Activity title: Evaluating biological control strategies for the tomato leaf mining moth (*Tuta absoluta*), a potential invasive greenhouse pest in Canada (Horticulture Cluster CHC IPM)

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

Names of Collaborators and Institutions:
Cara McCreary, Greenhouse integrated pest management specialist, Ontario Ministry of Agriculture Food and Rural Affairs (OMAFRA); Dr. Sherah VanLaerhoven, University of Windsor, co-director of MSc student working on this project; Nature Conservancy of Canada; Thames Talbot Land Trust; Parks Canada

Activity Objectives (as per approved workplan):
1. Field survey for native natural enemies of the tomato leaf miner, *Tuta absoluta* (predators and parasitoids)
2. Establish rearing methods for predators and parasitoids
3. Define the life history, predatory capacity and biological control potential of new agents on greenhouse crops.
4. Identify and apply novel molecular tools for identification and monitoring of the tomato leaf miner.

Research Progress to Date (use plain language):

Executive Summary

Tuta absoluta is an invasive and serious pest of tomato crops worldwide. Tomato yield loss by the leaf miner can reach up to 100% for both fresh market and processing tomatoes grown in fields and greenhouses. Yield loss is directly linked to larval injury where the larvae feed by burrowing into tomato plant leaves, stems, flowers, fruits, and growing apex. *Tuta absoluta* was introduced in 2006 into Europe and is only now controlled by integrated pest management, including an emphasis on intensive crop scouting using pheromone traps, selective insecticide application, and the use of pathogens, predators and parasitoids. Some of these agents have proven to be particularly effective including inoculation of crops with generalist mirid predators such as *Nesidiocoris tenuis*, *Macrolophus pygmaeus* or *Dicyphus errans* which can feed on eggs and larvae of the tomato leaf miner. These predators are among the most abundant natural enemies adapted to living on tomato and other solanaceous plants. While the tomato leaf miner has not yet made its way into North America, it has an enormous potential for population growth in any newly invading area as seen with its extensive establishment in Europe and North Africa. Towards preparing for the possibility of an inadvertent introduction of *T. absoluta* into Canada, we propose to; 1) Survey plants that are closely related or closely resemble tomatoes or mirid hosts for potential new biological control organisms against *T. absoluta*; 2) Develop pest management tools that can be integrated with current commercial vegetable production standards; 3) Examine the potential for the application of native predators and parasitoids to control the tomato leaf miner through assays using other native Gelechiidae leaf miner species as an indicator for success; 4) Develop rapid molecular diagnostic identification tools so that

management strategies for *T. absoluta* can be quickly deployed. This project could lead to the identification, study and development of novel agents for the biological control of this pest in Canada.

2019-2020 Report Executive Summary

In this second 2019-2020 project year, we continued our survey of multiple natural and agricultural areas in Ontario Canada, for the presence of novel hemipteran predator species. This survey year was characterized by the collection of 486 number of specimens, with at least 15 individual species representing potentially good predatory biological control agents.

We have also made significant headway in developing effective mass rearing approaches for two hemipteran predators, *Nabis americoferus* and *Hoplistocelis pallescens*. These are based on creating isolated rearing cells which greatly minimize predator mortality, insuring that most predators complete nymphal development and reach adulthood. In this project year, we also completed characterizing the developmental time and survival rate to adulthood for both *N*. *americoferus* as well as *H*. *pallescens*. We are now working on characterizing the functional response of *N*. *americoferus* to various greenhouse pest and prey types including the greenhouse whitefly *Trialeurodes vaporariorum*, the lepidopteran pest *Udea rubigalis*, as well as eggs of the lepidopteran species *Ephestia kuehniella*. Together these trials will greatly assist us in quantifying the predatory capacity of *N*. *americoferus* and *H*. *pallescens*, as it is likely to relate to control of the invasive tomato leafminer, *Tuta absoluta*.

Together, these findings directly contribute to advancing sector strategies for the Canadian horticultural industry including enhancing its environmental performance. Specifically, this research contributes directly to the development of new, effective and sustainable tools for the biological control of both existing and invasive arthropod pests, to the benefit of Canadian greenhouse growers and horticultural field crop producers.

Objectives

01 - Field survey for native natural enemies of *Tuta absoluta* (predators and parasitoids)

Description: Field survey for native natural enemies of *Tuta absoluta* (predators and parasitoids)
Outcome: Identification of new arthropod species associated with the predation or parasitism of Gelechiidae moths.

Performance Summary:

Over the course of surveys conducted in 2019, we have collected over 486 hemipteran bug specimens, which include at least 50 unique hemipteran species, and a minimum of 15 predatory species which may represent potentially good biocontrol agents. Over the next project year, we will employ both morphological and DNA barcode analysis to confirm identifications of such specimens. This will also offer some measure of the taxonomic diversity of the hemipterans present in this region of Canada and may facilitate the study of other predatory species in future.

In this project year, as with the previous, specific areas surveyed were selected which were closely associated with agricultural and naturalized areas. As such, we once again surveyed Nature Conservancy Canada (NCC) and Thames Talbot Land Trust (TTLT) properties, but this year also added new survey sites including Point Pelee National park as well as Ojibway Nature Centre and Park. Also new to this survey year was the incorporation of tap sheets as a method for hemipteran collections on plants and one in addition to sweep netting. Once again, plants selected for sampling included known hosts for predatory mirids, including hairy plants such as common mullein, stem hedge nettle, and hairy nightshade. In addition to these, in this project year, we also added other plant types for sampling including tree hosts of the *Hyaliodes* bugs, which was a particularly fruitful strategy.

Starting in June 2019, we surveyed three Thames Talbot Land Trust properties including Hawk cliff Woods, Meadow Lilly Woods as well as Newport Forest. From these early summer surveys, we identified predators including Blepharidopterus sp., *Hyaliodes* sp., *Miris dolabratus* and *Stenotus binotatus*.

In 2019, we were also fortunate to have the opportunity to work alongside Dr. Steven Paiero, curator of the University of Guelph insect collection, who was conducting a survey of the Hemiptera at Ojibway Nature Centre & Park. In total, we conducted three outings with Dr. Paiero at Ojibway in July and August, which covered both grassland and forest habitats. These brought to light a sizable abundance of *Hyaliodes* predators which were collected from oak and Sassafras trees. This association of *Hyaliodes* with tree hosts was remarkably consistent and may have been one of the reasons for why this species failed to adapt to tomato plants as hosts after we had brought specimens back to our Harrow RDC laboratory this year. During these surveys, we also collected multiple *Ilnacora*, *Geocoris* and Nabidae predators whom also hold considerable predatory potential with relevance to agricultural systems.

In July 2019, we also surveyed NCC lands on Pelee Island including the Florian Diamante Nature Reserve, Middle Point Woods, as well as the Richard & Beryl Ivey NCC properties. In these locations, we predominantly collected bug species belonging to genera *Hoplistocelis*, *Neurocolpus* and *Zelus*. Through this collection, we were able to establish a colony of *Hoplistocelis pallescens* which we are currently successfully maintaining on tomato. In the same month, collections of this species were also made at Point Pelee National park, as well as the presence of *Blepharidopterus* sp. and *Neurocolpus* sp. predators which were also collected for taxonomic analysis.

Most importantly, in 2019, we continued to improve on rearing strategies for the predator Nabis americoferus. We were also able to adapt the protocol established for *N. americoferus* species to rear the tall-plant dwelling nabid *H*. *pallescens*. This predator has adapted remarkably well to tomato as an oviposition host and the colony for this new species is thriving. This new predator also appears to be an avid predator of crop pests including aphids, and feeds readily on eggs of the flour moth, *Ephestia kuehniella*. For these reasons, we believe this predator has a considerable potential for its development as a biological control agent for the tomato leaf miner Tuta absoluta. Together, *N. americoferus* and *H*. *pallescens* will serve as our two principal subject species for ongoing life history and functional response studies.

In the meantime, we continue to work with taxonomist Dr. Michael Schwartz at the Canadian National Collection and Dr. Steven Paiero from University of Guelph to identify some of the hundreds of hemipteran specimens we have collected to date within the context of this study. Through this work, we hope to document the localization and diversity of important predatory hemipteran predator species within Canada which may serve in the development of novel biological control programs in future. In addition to this, our collection of leaf mining microlepidopteran larvae continued in 2019. From Pelee Island and on Harrow RDC site we collected *Chrysoesthia sexguttella*, a leaf miner from the same family as *Tuta absoluta* that feeds on *Chenopodium* sp (goosefoot) plants, which are a secondary host for *Tuta absoluta*. In future project years, we will continue rearing and identifying the native Canadian parasitoids of leaf miners for potential development as biological control agents of *T. absoluta*.

02 - Establish rearing methods for predators and parasitoids

Description: Establish rearing methods for predators and parasitoids

Outcome: Development of effective ways to rear new predatory or parasitoid biological contorl species.

Performance Summary:

In April 2019, a decision was made to focus our rearing efforts on one of the two Nabis predator species *Nabis americoferus* instead of *N*. *roseipennis* which we had previously established in 2018. This was in part due to the long developmental time of N. roseipennis and the challenges we experienced with keeping colonies of this species thriving. However, in August 2019, a colony of another nabid predator *H. pallescens*, was also established and has adapted very well to the rearing protocol we have established for *N. americoferus*. Thus, both *N. americoferus* and *H. pallescens* are now being reared on tomato as a host plant. For both of these species, work is currently underway to improve colony rearing efficiency through adopting a number of time saving mass rearing strategies.

In addition, methods for rearing hemipteran predators *Nabis americoferus* and *Hoplistocelis pallescens* were refined in this reporting period. Also, due to the long developmental time and poor establishment of a third predator colony for *Nabis roseipennis*, which was established in the first project year, we made the decision to terminate this species for future study and focus our attention on *N. americoferus* and *H. pallescens*. We have also started a mass rearing protocol in larger cages to see if both predators can survive in larger cages.

03 - Define the life history, predatory capacity and biological control potential of new agents on greenhouse crops.

Description: Define the life history, predatory capacity and biological control potential of new agents on greenhouse crops.

Outcome: Identify life history attributes that may lend a particular predator or parasitoid well to greenhouse biological control of *Tuta absoluta*.

Performance Summary:

Work is currently underway to define the life history and predatory capacity of *Nabis americoferus* and *N. roseipennis*, two predators found to be naturally associated with tomato crops.

Laboratory life history studies: In this reporting period, we continued to define the life history parameters of predators *Nabis americoferus* as well as *Hoplistocelis pallescens* when reared on tomato host plants and aphids. To date, the developmental time from egg to adult for both *Nabis americoferus* and *Hoplistocelis pallescens* was assessed for 50 and 37 individuals of each species respectively. For these studies, predators were individually maintained in Petri dishes were fed *Myzus persicae* aphids and were monitored through their entire developmental period. While developmental time for *N*. *americoferus* is on average 18 days long, the time to development for *Hoplistocelis pallescens* was considerably longer, at 26 days.

Laboratory predatory capacity trials: In laboratory trials, second instar and adult female *Nabis americoferus* were tested for their predatory capacities, following a 24-hour starvation period. Individuals were kept singly in a Petri-dish and consumption rate of either prey whitefly (*Trialeurodes vaporariorum*) or aphids (*Myzus persicae*) at treatment rates of 1, 2, 4, 8 or 16 of one prey type was measured after a 24-hour period. To date, we have shown that both 2nd instar nymphs and adults of *N. americoferus* are able to consume over 16 whitefly or aphid individuals within a 24 hour period. Work is ongoing to identify the upper limit of prey consumption for each of these life stages of this predator species so that their functional response can be better quantified. Over the course of the next project year, we also anticipate starting such functional response trials with our newest Hoplistocelis pallescens predator colony and are also beginning trials for the predatory capacity of both predators of eggs of the flour moth, *Ephestia kuehniella*, another small lepidopteran species which his currently serving as our proxy for the non-native invasive pest, *Tuta absoluta*. Together, these studies will help lay the groundwork for understanding the bionomics of such predators in the context of crop protection as well as the resilience of agricultural systems in light of their invasion by crop pests.

Greenhouse crop establishment and predator vertical distribution trials: In fall of 2019, we conducted a series of small cage greenhouse trials at the Harrow RDC which allowed us for the first time, to observe the successful establishment and reproduction of predator *N. americoferus* on both tomato or pepper crops. This study also allowed us to investigate whether predators were able to contribute to the suppression of pests on these crops including the greenhouse whitefly (*Trialeurodes vaporariorum*) on tomato, as well as the green peach aphid (*Myzus persicae*) on pepper plants. This study also offered us an opportunity to examine how supplemental foods such as eggs of the flour moth, *Ephestia kuehniella*, might function to improve predator crop establishment.

While these trials were successful in showing the establishment, and even the replication of predators on both experimental crop types, as second generation nymphs were observed by trial end, they have also shown us that the number of predators released as a ratio of the number of pests present, matters enormously as to whether pest populations can remain in check. To date, we have shown that aphid populations can be suppressed by *N.*

americoferus even when only four predators are initially introduced onto a cage full of aphids. However, in future, the number of predators will need to be increased as aphid populations grew at a very rapid rate during this trial, which may have been attributed to the one-week predator-free condition we employed. In future, predators will be released synchronously with aphid pests. In contrast, on tomato plants, the number of whitefly per plant was clearly too low to observe any sizeable impact of the predator on populations of this pest. In future, more whitefly will be inoculated earlier onto crop plants, which should improve the chances of observing significant predation of this pest on tomato crops. Together, with release strategy optimized, these types of trials will be instrumental in developing best management practices for the establishment of predators on crops.

04 - Identify and apply novel molecular tools for identification and monitoring *Tuta absoluta*.
Description: Identify and apply novel molecular tools for identification and monitoring *Tuta absoluta*.
Outcome: Development of an assay that would allow for the rapid identification detection of the quarantinable *Tuta absoluta* pest.
Performance Summary: Work associated with this research objective will begin in the new fiscal year starting in 2020.

Milestones
01 - Field survey for native natural enemies
Activity: Perform field survey to identify and assess the suitability of mirid and parasitoids to suppress Gelechiidae moth populations.
Milestone / Deliverable: Identification of novel mirid and other potential natural enemies.
Progress Summary: (In Progress)
Our hemipteran predator survey and collection continued in 2019 with the addition of new Parks Canada and Ojibway Park sites. Collections over summer and fall were also conducted at several of the Nature Conservancy and Thames Talbot Land Trust sites for a total of 14 different sites surveyed from June to September. Through these surveys, we identified a number of interesting and newly reported predatory hemipteran species, including *Hoplistocelis pallescens* for which we were able to establish a colony.

02 - Rearing natural enemies
Activity: Evaluate rearing methods for the maintenance of colonies of predators and potential parasitoids.
Milestone / Deliverable: Identify the factors that optimize natural enemy colonies.
Progress Summary: (In Progress)
Methods for rearing two hemipteran predators, *Nabis americoferus* and *Hoplistocelis pallescens* were refined in this second project year, with the development of segregated cell units to maximize predator survival to adulthood. We have also explored the use of alternate food sources for maintaining predators including the green peach aphid, the cherry oat aphid as well as on a lepidopteran pest insect, *Udea rubigalis*. We have found that predators maintained on green peach aphid as well as on tomato as an oviposition substrate works well and has led to the flourishing of the *Nabis americoferus* colony. Work is currently underway to optimize conditions for rearing *Hoplistocelis pallescens*. For both predatory species we have started employing a mass rearing protocol whereby predators are reared in large greenhouse cages.

03 - Characterizing natural enemies
Activity: Conduct laboratory trials to establish the developmental and reproductive characteristics as well as the predatory potential for new natural enemies from established colonies.
Milestone / Deliverable: Generate data on the life history, predatory capacity and fecundity of natural enemies identified through this study.
Progress Summary: Work is currently underway aimed at characterizing the longevity, fecundity and predatory capacity of two hemipteran predators, *Hoplistocelis pallescens* and Nabis americoferus. To date, we now know that the complete development time for *N. americoferus* is just under 19 days, while it is longer at 33 days for *H. pallescens*.

The functional response of *N. americoferus* to eggs of the lepidopteran *Ephestia kuehniella* are nearly complete and we now know these predators are able to consume over 60 eggs within a single day as adults, and 48 as second instar nymphs. Further research on the response of this predator, as well as *H. pallescens* to this prey type will serve as an indicator for their biological control potential of the invasive lepidopteran moth, *Tuta absoluta*. Finally, as these predators have good potential as biocontrol agents for other crop pests including aphids, whiteflies and other lepidoptera, we are also currently conducting laboratory assays to evaluate these possibilities. To date, it is clear that *Nabis americoferus* adults can consume at least 16 greenhouse whitefly pupae within a 24 hour period.

Our results on predator life history and predatory capacity have to date been presented by poster at three scientific and grower directed meetings including the Entomological Society of Canada and the Entomological Society of Ontario Annual General Meetings in 2019, as well as the Ontario Fruit and Vegetable Growers Association meeting held in early 2020.

Small cage greenhouse trials performed in summer 2019 demonstrated to us that *N. americoferus* could actively reproduce on tomato and pepper crops. As such, they have the potential to provide long term inoculative biological control of crop pests when used as agents on greenhouse tomato or pepper. Future greenhouse trials will identify the conditions required to achieve good crop protection by *N. americoferus* and *H. pallescens*.

04 - Molecular identificaiton of *T. absoluta*

Activity: Develop new tools for the rapid identification and differentiation of *Tuta absoluta* from other related Gelechiidae and unrelated microlepidoptera.

Milestone / Deliverable: Develop a novel molecular diagnostic tool that will simplify and speed up the process of identification of field and greenhouse collected Gelechiidae specimens.

Progress Summary:

The research associated with this project milestone will begin in 2020-2021.

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

1. 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 17, 2019)

2. Mlynarek, J., LaFlair, A., Zhang, K., Vilcu, P., Sim, K., Rizzato, R., Gagnier, D. and Labbe, R. 2020. Predatory capacity and life histories of two native North American nabids, Ontario Fruit and Vegetable Growers Association AGM, Niagara Falls, ON. (Poster- February 19, 2020)

3. Mlynarek, J., LaFlair, A., Zhang, K., Vilcu, P., Sim, K., Rizzato, R., Gagnier*, D. and Labbe, R. 2019. Predatory capacity and life histories of two native North American nabids, Entomological Society of Ontario Annual Meeting, Bark Lake, ON. (Poster – November 1, 2019)

4. Mlynarek*, J., LaFlair, A., Zhang, K., Vilcu, P., Sim, K., Rizzato, R., Gagnier, D. and Labbe, R. 2019. Predatory capacity and life histories of two native North American nabids, Entomological Society of Canada Joint Annual Meeting, Fredericton, NB. (Poster – September 4, 2019)

* Denotes presenter

Early Outcomes (if any) or Challenges:

None to date.

Key Message(s):

In this second project year, we were able to successfully establish and continue to rear colonies of two hemipteran predators, *Nabis americoferus* and *Hoplistocelis pallescens*, both with an impressive potential for

greenhouse crop pest consumption. We have also identified a number of other predatory hemipteran species that may also be effective in the control of field and greenhouse crop pests, including the invasive tomato leaf. We are now in the midst of establishing important life-history parameters and predatory capacities for our two colony hemipteran predator species which may facilitate their future development as commercial biological control organisms.

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