Proceedings of the North American spotted-wing drosophila biological control working group

December 9 - December 10, 2020

1 Outline

1.1 Day 1 – December 9, 2020

9:00 – Introduction

9:05 Paul Abram, Michelle Franklin, Tracy Hueppelsheuser – Report on 2020 surveys for *Leptopilina japonica* and *Ganaspis brasiliensis* in crop and non-crop habitats in BC.

9:45 Tracy Hueppelsheuser, Michelle Franklin, Paul Abram – Lessons learned in 2020 on rearing Asian parasitoids of SWD from field-collected fruit samples.

10:00 Matt Buffington – Identifying *Leptopilina japonica* and *Ganaspis brasiliensis*, and distinguishing them from similar native parasitoid species.

10:40 BREAK

10:50 Amanda Stout, Xingeng Wang, Kim Hoelmer – Laboratory rearing of two Asian larval parasitoids for biological control of spotted-wing drosophila

11:00 Justin Renkema – Surveys for SWD parasitoids in Ontario, Canada in 2020

11:10 Juli Carrillo, Matt Tsuruda, Pierre Girod, Martina Clausen, Sadie Larter, Paul Abram – Surveys for SWD parasitoids in Coastal BC, Canada in 2020

11:20 Chandra Moffat, Tyler Nelson, Mairi Robertson – Surveys for SWD parasitoids in Interior BC, Canada in 2020/Identity and phenology of non-target *Drosophila*

11:40 Chia-Hua Lue, Matthew Buffington, Sonja Scheffer, Matthew Lewis, Amy Driskell, Anna Jandova, Kimura Masahito, Yves Carton, Robert Kula, Todd Schlenke, Mariana Mateos, Shubha Govind, Julien Varaldi, Bregje Wertheim, Emilio Guerrieri, Massimo Giorgini, Xingeng Wang, Kim Hoelmer, Kent Daane, Paul Abram, Marylene Poirie, Frank Jiggins, Daniel W. Tracey, Jeremy S. Davis, Owen T. Lewis, Jeff Leips, Phillip P. A. Staniczenko, Jan Hrcek – DROP: Molecular voucher database for identification of *Drosophila* parasitoids

11:55 Discussion, wrap-up

1.2 Day 2 – December 10, 2020

9:00 Introduction

9:05 Marianna Szucs – Surveys for SWD parasitoids in Michigan and experimental evolution on native parasitoids

9:15 Jana Lee – Releases of *Muscidifurax* and *Pachycrepoideus* in Oregon

9:25 Michael Culshaw-Maurer – The potential for enemy risk effects to play a role in biological control of SWD

9:40 Jade Sherwood, Paul Abram – Preliminary evidence for enemy risk effects on SWD from *Leptopilina japonica*

9:50 Kent Daane, Brian Hogg, Xingeng Wang, Kim Hoelmer – Update on petition for *Ganaspis brasiliensis*; releases of pupal parasitoids in California.

10:30 BREAK

10:40 Discussion

- What are our expectations for the contribution of Asian larval parasitoids to SWD suppression in an IPM context?
- What do we still need to know about non-target effects of *G. brasiliensis* and *L. japonica*?
- Plans for future releases or redistributions of *G. brasiliensis* or *L. japonica*?
- The future of SWD pupal parasitoid releases
- Coordinating multi-group survey efforts and developing a survey methodology paper
- Identifying knowledge and research gaps, coordinating future research efforts
- Proposed common names for *L. japonica* and *G. brasiliensis*
- Future working group meetings, incorporation as an IOBC-NRS working group
- Next meeting in Spring 2021: Proposed topic – SWD predators, pathogens, and symbionts.

2 Abstracts

2.1 Day 1 – December 9, 2020

Report on 2020 surveys for *Leptopilina japonica* and *Ganaspis brasiliensis* in crop and non-crop habitats in BC

Paul Abram, Michelle Franklin, Tracy Hueppelsheuser

Two Asian larval parasitoids of the invasive fruit pest spotted-wing drosophila, *Drosophila suzukii* (Diptera: Drosophilidae) (SWD) were recently found to have established in British Columbia, Canada. In 2020, we conducted the first study of the ecology of these two parasitoids, *Ganaspis brasiliensis* and *Leptopilina japonica* (Hymenoptera: Figitidae) in their new North American range. We measured parasitism levels of SWD by these parasitoids across several crop and non-crop host plants at multiple crop and non-crop field sites throughout the growing season. We found that, in unmanaged habitats, the two parasitoids are well established and abundant in British Columbia and have re-formed a close association with SWD across the diverse array of host plants it attacks over the course of the season in their new geographic range. Interestingly, parasitism was often absent or very low in the time period immediately following the colonization of a host plant whose fruit had recently ripened. Parasitism was very low in crop fields, even when it was present in adjacent non-crop host plants at the same site. In 2020, we also conducted a pilot study to harvest SWD pupae from dropped and fresh fruit in a blackberry field. Here, we found that: (i) there is proportionally more parasitism of SWD by *G. brasiliensis* (vs. *L. japonica*) in fresh fruit; (ii) both

parasitoid species often emerged from single fruit; and (iii) parasitism within individual fruit rarely exceeded 80%, implying that some proportion of SWD larvae within each fruit may be able to escape parasitism. Based on host pupal morphology, we also confirmed that an as-yet unidentified Asobara sp. is in fact attacking SWD larvae in BC.

Lessons learned in 2020 on rearing Asian parasitoids of SWD from field-collected fruit samples

Tracy Hueppelsheuser, Michelle Franklin, Paul Abram

Below is a brief overview of the presentation:

- Highlighted goals of the project previously discussed at spring 2020 meeting
- Provided an update on the sampling protocol outlined in spring 2020 meeting and highlighted successful methods and those that required fine tuning
- Briefly described results from Red Elderberry (*Sambucus*) and Himalayan Blackberry (*Rubus*): emergence pattern from fruit showed SWD emergence peak first, followed by male ¬*L. japonica*, then female *L. japonica* and male *G. brasiliensis*, and finally female *G. brasiliensis*
- Remarks after first season of sampling:
  - Keep samples for >6 weeks
  - Plan for high emergence in August and September
  - Limit sampling of overripe fruit

Identifying *Leptopilina japonica* and *Ganaspis brasiliensis*, and distinguishing them from similar native parasitoid species

Matt Buffington

Identifying natural enemies of spotted-wing drosophila is critical to slow the spread of the species across North America. Further, as foreign exploration and host range testing for natural enemies increases, the identification of native vs foreign natural enemies becomes even more critical. Here I review the basic morphology and biology of the eucoilines *Leptopilina japonica* (the ronin wasp) and *Ganaspis brasiliensis* (the samba wasp). Key features of these wasps are morphological variation on the scutellum and metapleuron; basic diagnostics of males and females are provided, and distinguishing these two species from *Leptopilina heterotoma* and *Ganaspis xanthpoda* were discussed. The presentation was concluded with general discussion of these genera, as well as the braconid *Asobara*. The basic collection data for specimens was summarized, and an organized plan to collect and submit specimens to the National Insect Collection was agreed upon.

Laboratory rearing of two Asian larval parasitoids for biological control of spotted-wing drosophila

Amanda Stout, Xingeng Wang, Kim Hoelmer

spotted-wing drosophila, *Drosophila suzukii* or SWD, is a major crop pest species in North America and Europe. SWD is unique in that it can attack ripening, rather than decaying fruit, unlike other species of fruit flies. Several parasitic species of wasps have been investigated and selected as an effective biological control agent for SWD. Two larval parasitoids, *Ganaspis brasiliensis* and *Leptopilina japonica*, have been selected as the most effective.

In this presentation, rearing methods for both parasitoids on artificial diet will be discussed. Both parasitoids have been effectively mass reared on artificial diet mixed with blueberry using simple laboratory equipment and temperature chambers. Adults parasitoids are then stored in small tubes for mating, long term storage, and shipment.

Surveys for SWD parasitoids in Ontario, Canada in 2020

Justin Renkema

Below is a brief overview of the presentation:

- Sampled fruit from six sites in the Niagara Region and Norfolk County
- Recorded no parasitoids emerging from sampled fruits following protocols from P.K. Abram & T. Hueppelsheuser
- High SWD numbers in blackberry collections (100s) and few in other fruits collected (<10)

Surveys for SWD parasitoids in Coastal BC, Canada in 2020

Juli Carrillo, Matt Tsuruda, Pierre Girod, Martina Clausen, Sadie Larter, Paul Abram

Our research is conducted at two locations: the organic University of British Columbia (UBC) farm in Vancouver that is geographically isolated from the rest of the berry production and in Delta at growers' fields following conventional methods.

At UBC, we sampled weekly from July to December on crop (blueberry) and non-crop plants (Himalayan blackberry, wild raspberry, thimbleberry or snowberry). In total, 9,382 spotted-wing drosophila (SWD) and 1,912 parasitoids emerged. Overall, more *Leptopilina* sp. were caught than *G. brasiliensis*. We also retrieved few *Asobara* sp. and *Pachycrepoideus vindemmiae*. An interesting result is the presence of the latter on snowberry in November. In strawberry production at UBC, experimental alleys with intercrop plants were evaluated (sweet alyssum vs. control: rye grass/clover) in order to mitigate SWD infestation. Ripe strawberries were sampled weekly in June/July. In total, 3,350 SWD and 192 parasitoids emerged (140 *Leptopilina* sp., 38 *G. brasiliensis*, 4 *Asobara* sp. and 6 *P. vindemmiae*). Our analysis showed no statistical difference between the control and sweet alyssum treatments in the average number of SWD or parasitoids per fruit.

In Delta, non-crop hosts plants were sampled within different habitat amendments adjacent to agricultural sites. In total, 2,878 SWD and 64 parasitoids were identified (25 *Leptopilina* sp., 28 *G. brasiliensis* and 12 *P. vindemmiae*).

In conclusion, we are confirming first records of *Ganaspis brasiliensis* on coastal B.C. Overall, Himalayan blackberry seems to be the favourite non-crop host plant. For 2021, we will keep sampling berries and monitor parasitoids spread. For intercropping we will evaluate the impact of other plants. Finally, we will test the efficacy of different mulches (plastic and alginate based) in strawberry and/or blueberry on SWD and parasitoid populations.

Surveys for SWD parasitoids in Interior BC, Canada in 2020/Identity and phenology of non-target *Drosophila*

Chandra Moffat, Tyler Nelson, Mairi Robertson

Ensuring non-target safety via host specificity testing is paramount to the approval and introduction of classical biological control agents. As part of a larger project on ecological pest management and biological control of SWD, we have been leading the Canadian efforts to quantify the physiological and ecological host ranges of *Leptopilina japonica* and *Ganaspis brasiliensis*, both proposed for importation to various eco-regions across Canada. To accomplish this goal, we initiated a project to record the species diversity of drosophilids endemic or naturalized in southern British Columbia. Using a series of baited (lure or vinegar) McPhail traps in both cultivated (cherry) and wild host environments, we have monitored the seasonal phenology of these non-target drosophilids from March through November of 2018-2020. Flies caught in traps were identified using *Drosophila* of the Midwest and Northeast Version 2 (Werner, Steenwinkle and Jaenike 2018). Eighteen species of vinegar flies (16 *Drosophila* and 2 *Chymomyza*) were recovered from traps. All species detected were obtained from traps placed in cherry orchards, whereas reduced species composition sets were obtained from traps in each of the non-crop host environments. Physiological host range testing done to date in the USA and Europe is highly relevant to Canadian drosophilids. Further physiological and ecological host range testing of both *L. japonica* and *G. brasiliensis* is planned for representatives of drosophilids recovered in our surveys, particularly those that have not yet been previously tested by other groups. As of December 2020, neither species of parasitoid has been recovered in Interior British Columbia, despite surveying multiple crop and non-crop *Drosophila* host plant species.

DROP: Molecular voucher database for identification of *Drosophila* parasitoids

Chia-Hua Lue, Matthew Buffington, Sonja Scheffer, Matthew Lewis, Amy Driskell, Anna Jandova, Kimura Masahito, Yves Carton, Robert Kula, Todd Schlenke, Mariana Mateos, Shubha Govind, Julien Varaldi, Bregje Wertheim, Emilio Guerrieri, Massimo Giorgini, Xingeng Wang, Kim Hoelmer, Kent Daane, Paul Abram,

Marylene Poirie, Frank Jiggins, Daniel W. Tracey, Jeremy S. Davis, Owen T. Lewis, Jeff Leips, Phillip P. A. Staniczenko, Jan Hrcek

Molecular identification is widely used as it can hugely speed-up biodiversity surveys and laboratory experiments. However, some groups of organisms cannot be reliably identified using general databases like GenBank or BOLD. Here we address this problem for parasitoids of *Drosophila*, a group of parasitic wasps which are well studied but whose diversity is severely underestimated. We introduce a new curated molecular reference database for ***Dro****sophila* **p**arasitoids (DROP), where sequences are linked to voucher specimens, and the voucher specimens are identified by taxonomists (`https://github.com/janhrcek/DROP/`). To initiate DROP, we curated 876 vouchers, 540 DNA sequences, 16 genomes, and 4 transcriptomes and from 113 described *Drosophila* parasitoid species, 69 provisional species, and 147 laboratory strains. Additionally, we provide an updated catalogue of *Drosophila* parasitoid species as a taxonomic reference for the research community. We expect that our effort will catalyze research using this practical model system by allowing reliable molecular identification and improving cross-referencing between individual studies. Our study can also serve as an example for researchers experiencing similar problems with molecular identification of organisms they study.

2.2 Day 2 – December 10, 2020

Surveys for SWD parasitoids in Michigan and experimental evolution on native parasitoids

Marianna Szucs, Shelley Linder, Rufus Isaacs, Benjamin J.M. Jarrett

We are in an era where the number of invasive species is steadily increasing while simultaneously classical biological control introductions are decreasing due to increasing regulations and costs. These trends are unlikely to change, and thus alternative methods need to be developed to control invasive pests that are safe, sustainable and publicly accepted. Native natural enemies represent a potential alternative as they often try to adopt invasive species but typically have low success rates on them. Laboratory selection can be used to speed up evolution and improve the performance of native parasitoids on exotic species within a few generations. We have started assessing the potential of experimental evolution to increase the virulence of native parasitoids on spotted-wing drosophila (*Drosophila suzukii*). The larval parasitoid, *Leptopilina heterotoma* was not able to develop, while two pupal parasitoids *Pachycrepoideus vindemmiae* and *Trichopria drosophilae* were successful at parasitizing spotted-wing drosophila and they showed increases in virulence just after three generations of selection. If this approach proves successful for multiple parasitoid species, it could provide a safe and effective alternative or complimentary method to classical biological control.

Releases of *Muscidifurax* and *Pachycrepoideus* in Oregon

Jana Lee

In 2019-20, augmentative releases were tested in caneberry hoop houses, and wild Himalayan borders with four control and four release plots. *Pachycrepoides vindemiae* were released weekly and reared on site in a banker system with 'augmentorium boxes'. In 2020, *P. vindemiae* was released initially with an augmentorium box, followed by two large releases of *Muscidifurax raptorellus*, a commercially available wasps for filth fly control. Parasitism was compared by percent parasitism of sentinel pupae, number of parasitized traps, and emergence of new wasps. In general, there was some indication of increased parasitism activity in release over control plots. However, there was no evidence of decreased infestation among sampled fruit nor presence of adult SWD in baited traps.

The potential for enemy risk effects to play a role in biological control of SWD

Michael Culshaw-Maurer

Enemy-risk effects, often referred to as non-consumptive effects (NCEs), are an important concept in natural enemy ecology, but their significance has had little impact on the conceptual underpinning or practice of biological control. I begin by presenting a brief overview of enemy-risk effects, including their potential interactions with biological control systems, then move to a conceptual overview of varying levels of enemy-risk effects, including a discussion of terminology frequently used in different areas of the literature. I then outline a key biological control example using *Pseudacteon* phorid flies to control fire ant populations, and extend this case study to the topic of evaluating biological control agents and methods that capture or don't capture enemy-risk effects. Finally, I present a number of possible enemy-risk effects in the SWD system, including both within-fruit, within-crop, and between-crop dynamics. The SWD system includes a wide array of spatial and temporal scales on which enemy-risk effects may occur, presenting an exciting opportunity to incorporate risk effects into the framework of an emerging biocontrol program.

Preliminary evidence for enemy risk effects on SWD from *Leptopilina japonica*

Jade Sherwood, Paul Abram

In biological control, success is often linked to direct consumption of hosts. However, there are other impacts of natural enemies on hosts, such as enemy risk effects, that are not as widely studied. Past studies of *Drosophila*-larval parasitoid associations have shown that artificial chemical washes from *Leptopilina* sp. parasitoids induced adult and larval avoidance behavior in *Drosophila* species, but have not conducted more realistic tests of exposure to cues from live male and female parasitoids. In a home laboratory, we found that *D. suzukii* avoided settling on food substrate previously exposed to live male and female *L. japonica*, but only at times of the day corresponding with peak *D. suzukii* activity levels. Additionally, there was subsequently lower *D. suzukii* adult emergence from parasitoid-exposed substrate, suggesting that flies avoided laying eggs in substrate that had previously been exposed to parasitoids. Interestingly, both male and female *L. japonica* parasitoids induced avoidance responses in *D. suzukii* and reduced the number of eggs they laid, indicating

that male parasitoids may have potential to contribute to biological control of *D. suzukii*. However, further research is suggested to evaluate whether these candidate enemy risk effects contribute to biological control of *D. suzukii* under field conditions.

Update on petition for *Ganaspis brasiliensis*; releases of pupal parasitoids in California

Kent Daane, Brian Hogg, Xingeng Wang, Kim Hoelmer

Biological controls for the spotted-wing drosophila (SWD) were discussed for two different programs. First, an update was provided on the importation and release of *Ganaspis brasiliensis*. The petition was positively reviewed by NAPPO and is now back to the USDA APHIS for internal review for "Environmental Compliance" via National Environmental Policy Act (NEPA) as well as Tribal Consultation. The Petition will then be published in Federal Register for Public Comment this winter, after which we hope to clear these final hurdles and have permission to move *Ganaspis* out of quarantine by August 2021. The second update described the augmentative release of the pupal parasitoid *Pachycrepoideus vindemiae* in organic cane berry fields in Watsonville, CA. Releases were made at four farms, each paired with control plots at least 200 m away. To mimic the natural movement of parasitoids into berry fields from non-crop source habitats, parasitoids are being released at the edge of berry fields in semi-natural habitats. Pre-release surveys found low (<20%) parasitism and few (<6%) *P. vindemiae*. In 2020, we released ~200,000 *P. vindemiae* from August to November and by October had a dramatic increase in parasitism to 20-60% and a shift to from *Trichopria drosophilae* to *P. vindemiae*. Work in 2021 will look at the crop impact of this level or parasitism, movement of the released parasitoids and possible 'carry-over' of parasitism from 2020 to 2021.