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Resident parasitoids associated with Drosophilidae in Michigan tart cherry orchards and woodland edges

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Abstract

Knowledge of the distribution and abundance of resident parasitoid species of *Drosophila* flies constitutes an important base for developing and implementing a biological control program for *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), an invasive pest that attacks ripening thin-skinned wild and cultivated berries and stone fruits. For this purpose, a field survey was conducted to identify the parasitoid community associated with *D. suzukii* infested sites in tart cherry orchards and woodlots in west, northwest, and central Michigan. Sentinel traps baited with *D. suzukii* larvae and pupae in banana slices were deployed in the center of tart cherry orchards, in woodlots adjacent to tart cherry orchards, and in woodlots isolated from any known commercial host of *D. suzukii*. Traps were placed from the beginning of July to the end of October 2021. Three parasitoid species that are known to use drosophilids as hosts were recovered from these traps. *Pachycrepoideus vindemiae* (Rondani) (Hymenoptera: Pteromalidae) and *Leptopilina boulardi* Barbotin, Carton & Keiner-Pillault (Hymenoptera: Figitidae) emerged from the infested bananas. *Leptopilina heterotoma* (Thomson) was collected as an adult in a sentinel trap. Among these wasps, only *P. vindemiae* successfully parasitized *D. suzukii* pupae in the laboratory. This pupal parasitoid was abundant and widely distributed in both cherry orchards and woodlots. The highest number of *P. vindemiae* was collected from orchards, followed by woodlots adjacent to orchards, with woodlots without nearby cultivated fruit having the lowest detections. These findings suggest that future release of augmentative or classical biological control agents for *D. suzukii* could be successful in orchards post-harvest to control late-season populations of this pest.

Keywords
biological control, sentinel trap, spotted wing drosophila, wasp

Introduction

Since its first detection in Michigan in 2010, spotted-wing *Drosophila*, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), has rapidly become the number one pest of small fruits and cherries in Michigan. Due to a zero tolerance for detectable larvae in these fruits, growers have relied almost entirely on calendar-based spray programs to protect their valuable crops from *D. suzukii* damage prior to harvest (Van Timmeren and Issacs 2013). These intensive spray programs target the adult flies but have also disrupted existing IPM programs developed for other insect pests, with potential cascading effects on the environment, especially non-target organisms (Tait et al. 2021). Moreover, these high-input control strategies are costly for growers, particularly those that are growing processing fruits with a lower profit margin such as tart cherry. All these factors have motivated researchers to search for effective and environmentally sustainable long-term solutions to prevent crop damage by *D. suzukii*.

Biological control is a vital component of an IPM strategy of invasive insect pests (Lee et al. 2019) and remained unutilized in the framework of *D. suzukii* management until recently (Gabarra et al. 2015; Woltz et al. 2015). Explorations for potential classical biological control agents in Japan, South Korea, and China, where *D. suzukii* is native or endemic, found three dominant larval parasitoids that predominately attack *D. suzukii*: *Asobara japonica* Belokobylskij (Hymenoptera: Braconidae), *Ganaspis brasiliensis* (Ihering) and *Leptopilina japonica* Novkovic & Kimura (Hymenoptera: Figitidae) (Wang et al. 2020a). In addition, over 50 frugivorous drosophila parasitoids have been reported worldwide, and several common larval drosophila parasitoids in the Americas and Europe have been evaluated against *D. suzukii* with most of them failing to develop from this host due to its strong host immune defense (Wang et al. 2020b). In the meantime, both *G. brasiliensis* and *L. japonica* have been unexpectedly detected in British Columbia, Canada and the state of Washington outside their presumed native range of Asia (Abram et al. 2020).

Of the known pupal parasitoids of drosophilids, two have been found to attack and develop successfully from *D. suzukii*: *Pachycrepoideus vindemiae* (Rondani) (Hymenoptera: Pteromalidae) and *Trichopria drosophilae* Perkins (Hymenoptera: Diapriidae) in Spain (Gabarra et al. 2015), Italy (Miller et al. 2015), Mexico (Garcia-Cancino et al. 2020), California (Wang et al. 2016), and Oregon (Miller et al. 2015). Both species are cosmopolitan and readily parasitize *D. suzukii* pupae inside fruit as well as in soil (Wang et al. 2016).

In comparison, information is currently lacking regarding the composition and distribution of resident parasitoid species of Drosophilidae in Michigan. This knowledge is important for the implementation of biological control against *D. suzukii*, especially prior to classical biological control agent releases. Moreover, this information

will also aid in determining any possible non-target effects of parasitoids new to this region, with potential spillover effects to other Drosophilidae as well as potential interactions among resident and future introduced parasitoids (Hougardy et al. 2020). Thus, the objective of our survey was to determine the presence and biological control status of resident parasitoid populations in Michigan cherry orchards and natural habitats using sentinel banana traps infested with larvae or pupae of *D. suzukii* as hosts, in support of the development of biological control programs that may include future augmentative releases of parasitoids to suppress populations of *D. suzukii*.

Material and methods

Insect colony

A *D. suzukii* colony was established, originating from adult flies collected from infested cherry fruit from a commercial orchard near South Haven, Michigan during the summer of 2020. The colony was maintained on a standard solid cornmeal diet (Dalton et al. 2011) in 50 ml polystyrene vials (Genesee Scientific, CA) in a growth chamber set to 22 °C, 45% relative humidity (RH), and a 16:8 (L:D) photoperiod.

Preparation of sentinel traps

Larval and pupal parasitoids of *D. suzukii* were sampled using sentinel traps described by Miller et al. 2015 with some modifications. About 20–24 fresh banana slices (2–3 cm thick, 20–25 g total) were exposed to 300 to 400 adult *D. suzukii* in a white mesh cage (34 × 34 × 61 cm with a vinyl window, BioQuip Products, Compton, CA) in a rearing room kept at 22 °C and 55% RH, with a 16:8 (L:D) photoperiod. These flies were allowed to oviposit directly onto the banana slices for 2–3 days. Afterwards, each infested banana slice was transferred into a plastic cup (118 ml) with a small piece of sponge (2 × 4 × 5 cm) at the bottom to absorb excess liquid and a piece of paper towel lining the sides of the cup to provide pupation sites. Each cup was then covered by a lid with a hole (2.5 cm diam.) in the center plugged with a foam plug (Genesee Scientific, San Diego, CA) for air exchange. Half of the cups were kept in the rearing room for 4–5 days, allowing larvae to develop into the pupal stage; the other half were held in a growth chamber at 13 °C and 55% RH, with a 16:8 (L:D) photoperiod to slow larval development and ensure there would be first or second instar stages present when deployed in the field. Prior to field deployment, lids with plugs were replaced with lids modified with a 4 cm diameter opening covered with a piece of nylon mesh (mesh opening: 830 micron, 55% open area; Component Supply, Sparta, TN) through which parasitoids could pass while adult *D. suzukii* were kept inside based on the laboratory observation. All sentinel traps consisted of a pair of these sentinel trap cups, each with a banana slice infested with *D. suzukii* larvae or pupae, placed in an orange delta trap (Great Lakes IPM, Vestaburg, MI) which served as a shelter in the field.

Sampling locations and duration

The survey was carried out at 3 sites in west Michigan, 7 sites in central Michigan, and 6 sites in northwest Michigan, with a total of 26 sentinel traps deployed (see Suppl. material 1). Nine traps were deployed at the edge of woodlots, not adjacent to any tart cherry orchards, instead surrounded by corn and/or soybean fields. The remaining traps were deployed either all within or adjacent to tart cherry orchards. Each site was at least 280 m apart from any other site being monitored in this study. At 3 cherry orchards in west Michigan, however, two traps were placed in or adjacent to cherry orchards to compare wasp capture in these habitats. Here, one trap was placed in the center of the orchard, and the other was placed at the edge of an adjacent woodlot at least 60 m apart. Traps in central and west Michigan were deployed on 9 July and taken down on 15 October 2021. In northwest Michigan, nine sentinel traps were deployed in 9 different natural habitats containing wild mulberry, raspberry, blackberry, pin cherry, and/or honeysuckle, all directly adjacent to tart cherry orchards, from 22 July to 15 September 2021. All sentinel cups in each trap were collected and replaced weekly.

Rearing and identifying parasitoids

After one week in the field, sentinel cups from west and central Michigan were transported back to the rearing room at Michigan State University campus and carefully examined for possible trapped adult parasitoids before their lids were switched to those with foam plugs described as above to prevent exit or entry of any insects. Four weeks later, cups were examined every couple days, for up to 2 weeks, and all parasitoids emerged were collected and counted. Representative morphospecies of emerging parasitoids were collected using an aspirator and identified to their family or genus level, then presented with numerous 1–2 instar larvae or at least 20 pupae of *D. suzukii* reared in a diet for oviposition for 4 days to test their ability in parasitizing these *D. suzukii* life stages. In the meantime, voucher specimens emerged from sentinel cups were placed in 70% ethanol before being sent to Dr. Matthew Buffington (USDA-ARS, Beltsville, MD) for species identification.

Sentinel cups collected from northwest Michigan were transported to a laboratory at the Northwest Michigan Horticulture Research Center in Traverse City and assembled in the same way described as above. Once wasps started to emerge, these cups were delivered to campus of Michigan State University for identification and ovipositional tests, however the number of emerging wasps over time was not counted; only the presence or absence of wasp species was recorded.

Statistical analysis

Wasp emergence over a 14-week sampling period from sentinel traps in Michigan was combined and square root transformed to meet assumptions of normality and homoscedasticity (SAS Institute 2021). An ANOVA compared the numbers of *P. vindemiae* emerged: 1) between 5 traps in orchards vs. 12 traps at the edge of woodlots, and 2)

between 3 traps in woodlots adjacent to orchards vs. 9 traps from woodlots without adjacent orchards (SAS Institute 2021). Analysis was not possible on any other species captured due to insufficient sample size.

Results

In total, 1 adult parasitoid was captured, at least 1876 parasitoids were reared out from the sentinel cups. Three species of parasitoids were either found in traps or emerged from cups baited with sliced banana infested with drosophila larvae or pupae: *Pachycrepoideus vindemiae* Rondani (Hymenoptera: Pteromalidae), *Leptopilina boulardi* Barbotin, Carton & Keiner-Pillault, and *Leptopilina heterotoma* (Thomoson) (Hymenoptera: Figitidae). Large numbers of *P. vindemiae* and *L. boulardi* emerged from sentinel cups within 3–4 weeks after initial field collection. *Pachycrepoideus vindemiae* was present in all trapping sites, but *L. boulardi* was only found in northwest Michigan. A single specimen of *L. heterotoma* was found in a sentinel cup immediately following field collection in a commercial cherry orchard in west Michigan.

The first emergence of *P. vindemiae* was from sentinel traps deployed at central Michigan sites during the week of 23 July. This species was abundant both in west and central Michigan throughout the sampling period (Fig. 1) and present in all 26 sentinel traps including those deployed in the northwest region at least once during the survey period. In total, 1319 *P. vindemiae* emerged from sentinel traps at sites in west Michigan compared to 557 *P. vindemiae* in central Michigan. Sentinel traps placed in orchards had significantly higher *P. vindemiae* emergence than those in the woodlots ($F_{1,15} = 5.14$, $P = 0.04$) (Fig. 2A). Woodlots adjacent to orchards had significantly more *P. vindemiae* emerged than woodlots without cherry orchards nearby ($F_{1,10} = 7.83$, $P = 0.02$) (Fig. 2B).

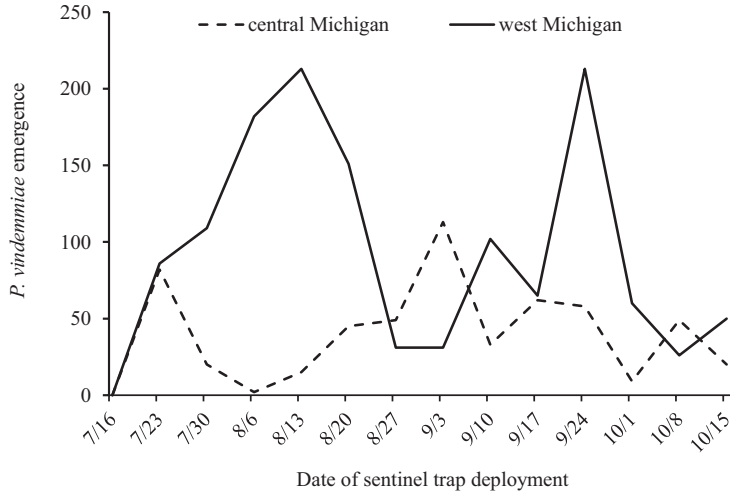

Figure 1. Total *P. vindemiae* emergence from weekly *D. suzukii* pupal sentinel traps in west Michigan (n = 6) and central Michigan (n = 11).

Pachycrepoideus vindemiae successfully reproduced using *D. suzukii* pupae as its host under laboratory conditions. Hundreds of *L. boulardi* emerged from sentinel cups deployed in the northwest region, but none of them were able to reproduce on *D. suzukii* larvae.

Discussion

This survey presents the first baseline data on the current field presence and seasonal occurrence of resident parasitoids of Drosophilid flies in Michigan. As expected, *P. vindemiae* was abundant and prevalent in Michigan since it attacks other pupae of many Dipteran fly families including Drosophilidae (Wang et al. 2004; Rossi Stacconi et al. 2013; Miller 2015). Under controlled and optimal laboratory conditions, females of this species are capable of parasitizing nearly 600 *D. suzukii* pupae during their lifetime (Bezerra Da Silva et al. 2019). However, a field survey revealed low levels of

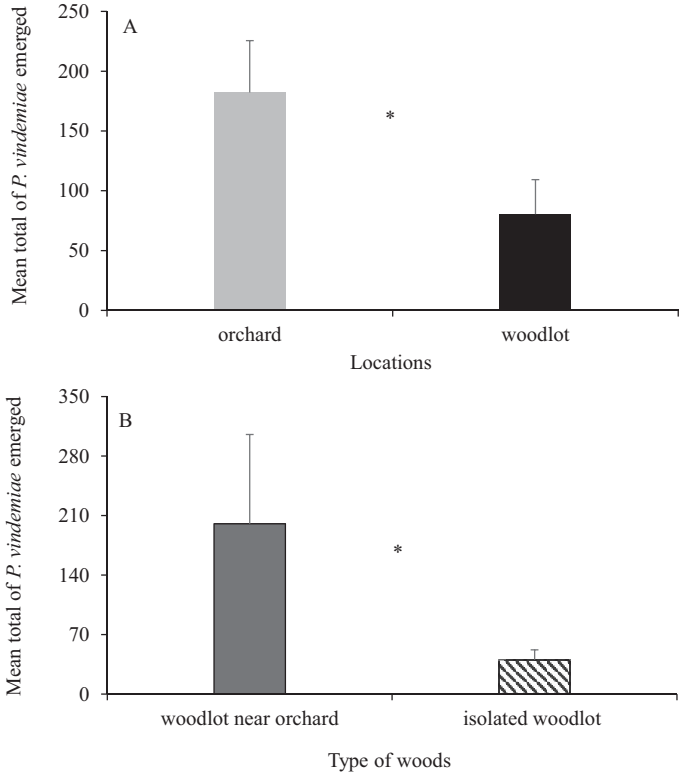

Figure 2. Mean total numbers of *P. vindemiae* (+SEM) emerged during a 14-week sampling period between sentinel traps placed in orchards vs. in woodlots regardless of orchards nearby (**A**), and between sentinel traps placed in woodlots adjacent to orchards and in those without orchards nearby (**B**). Asterisk indicates a significant difference between trap locations ($P < 0.05$).

parasitism of *D. suzukii* pupae in both North America and Europe (Lee et al. 2019; Wang et al. 2021), likely due in part to its relatively wide host range (Wang et al. 2021).

Larval parasitoids such as *L. boulardi*, *L. heterotoma*, and *Asobara tabida* (Nees) are important natural enemies of many endemic Drosophilidae species, but rarely successfully parasitize *D. suzukii* larvae (Girod et al. 2018). Although, hundreds of *L. boulardi* emerged from sentinel traps originally baited with *D. suzukii* larvae in northwest Michigan, none of them successfully reproduced on *D. suzukii* in the laboratory due to a strong immune response by the host. This indicates that other drosophilid species re-colonized *D. suzukii* infested banana slices, attracting larval parasitoids such as *L. boulardi* and *L. heterotoma* for oviposition. Indeed, other Drosophilidae including *D. melanogaster* were observed emerging from some of the sentinel traps after being incubated in the laboratory.

One potential reason why *D. suzukii* is so destructive to cultivated fruit crops is the absence of a significant natural enemy in invaded regions. Recently, *G. brasiliensis*, a species-specific larval parasitoid imported from Asia (Daane et al. 2016; Giorgini et al. 2019) that attacks early instar *D. suzukii* larvae (Wang et al. 2018), has been approved for release in Michigan as a classical biological control agent. While it is expected to take time for new natural enemies such as *G. brasiliensis* to suppress *D. suzukii* populations, resident pupal parasitoids such as *P. vindemiae* and *T. drosophilae* could play an important role through augmentative releases in combination with releases of new natural enemies. Interestingly, *P. vindemiae* has recently been found to parasitize and successfully develop on all preimaginal stages of *G. brasiliensis* (Hougardy et al. 2022), which could also potentially affect the establishment and success of *G. brasiliensis*.

Importantly, *P. vindemiae* was found most abundantly within cherry orchards after harvest, suggesting that they can navigate this agroecosystem despite presumable remnants of chemical applications commonly present in these systems. However, this may also imply that they are present during the growing season when regular insecticide applications are applied, suggesting possible disruption of this biological control from chemical exposure. More information is needed to elucidate the effects of pesticide, both in-season and post-harvest, on the success of current and future biological controls for *D. suzukii*. Additionally, *P. vindemiae* was more commonly associated with woodlots adjacent to cultivated cherry, likely because *D. suzukii* and other *Drosophila* populations are higher in areas with greater access to host fruit. This study serves as baseline information for the resident parasitoid community of Drosophilidae in the tart cherry agroecosystem and provides guidance on landscape factors that influence establishment of future augmentative and classical biological control releases.

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Supplementary material 1

Endemic parasitoids associated with Drosophilidae in Michigan tart cherry orchards and woodland edges

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Data type: table (docx. file)

Explanation note: The location and description of each sentinel trap.

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