THE IMPACT OF LABORATORY SELECTION AND GEOGRAPHIC VARIATION OF RESISTANCE ON *DROSOPHILA SUZUKII* BIOLOGICAL CONTROL

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Entomology – Master of Science

2023

ABSTRACT

The focus of this research is to better understand the interactions between the invasive pest *Drosophila suzukii* and the parasitoid wasps that target it. The first chapter evaluates whether a colony of the generalist pupal parasitoid *Trichopria drosophilae* that had been selected in the laboratory over several generations for increased performance on *D. suzukii* would also show improved performance under more natural conditions than the artificial rearing environment. To test this, adapted and non-adapted populations of *T. drosophilae* were used to parasitize *D. suzukii* in three different species of fruit. More wasps and fewer flies emerged from the adapted colony in every fruit type, which indicates that several generations of laboratory rearing did not impose an apparent cost when making the shift to a natural environment. Subsequently, when the same adapted and non-adapted *T. drosophilae* were compared alongside a co-evolved larval parasitoid, *Ganaspis brasiliensis*, using raspberry plants, *G. brasiliensis* had more wasps emerge than either *T. drosophilae* colony, but the same number of *D. suzukii* emerged as the adapted population. These results show that experimentally reared generalist parasitoids still have potential as a biological control agent for reducing an invasive species' population, even with lower developmental success. The second chapter investigates how *D. suzukii* resistance to parasitism by *G. brasiliensis* varies with geography and time. Individuals from eight geographically distinct populations of *D. suzukii* across Michigan and Oregon showed different rates of parasitism and encapsulation during resistance assays. Flies collected in August from these locations also demonstrated differences in parasitism and encapsulation rates to the same populations sampled just a month later. These results demonstrate that it is possible for resistance-related traits to vary geographically and temporally which may impact the performance of *G. brasiliensis* as a biological agent.

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CHAPTER 1: Biological Control Potential of a Laboratory Selected Generalist Parasitoid Versus a co-Evolved Specialist Parasitoid Against the Invasive *Drosophila suzukii*

Introduction

Invasive species are most effectively controlled in the long-term by the introduction of co-adapted natural enemies from their native ranges. Since the testing and approval of natural enemies for these so-called classical biological control introductions can take a long time, often decades, and because there has been a steep decline in such programs (Heimpel & Cock, 2018; Van Driesche et al., 2020) alternative approaches are being explored. One such alternative is to use natural enemies that are already present in the introduced ranges (Jarrett et al., 2022; Kruitwagen et al., 2018; Linder et al., 2022). Even though native species often lack adaptations to effectively attack exotic species, an increasing number of studies demonstrate that a few generations of laboratory selection can significantly improve the developmental success of natural enemies on novel hosts (Henry et al., 2008; Hopper et al., 2021; Jarrett et al., 2022; Jones et al., 2015; Linder et al., 2022). These proof-of-concept studies represent the first steps towards developing laboratory selection as a tool for biological control of invasive pests. However, it is largely unknown whether the improved performance measured under artificial laboratory conditions translates to increased attack rate and developmental success of the invasive targets in more natural environments. In order to contextualize the importance of this laboratory-enhanced performance, we also need to understand how the biocontrol potential of laboratory-improved native natural enemies compares to classical biological control agents that are often co-evolved specialists on the target invasive species.

Insect parasitoids that lay their eggs inside or on the host and whose developing larvae eventually kills the host are the most commonly used natural enemies to control invasive insect pests (Van Driesche et al., 2020). Parasitoids use visual and chemical cues both from the insect host and from the plants their hosts feed on to locate targets on the landscape (Dicke & Baldwin, 2010; Gandolfi et al., 2003; Turlings et al., 1991; Vet & Dicke, 1992; Vet et al., 1995). However, during laboratory rearing these cues can be largely absent or significantly altered. For example, for the rearing of drosophilid flies that are the subject of our investigations, a cornmeal, yeast and sugar based artificial diet is used instead of real fruit. Parasitoids are then released in small plastic vials with artificial diet that contain the developing fly larvae or pupae to be parasitized. To build up populations for mass release or experiments, both the parasitoids and flies spend tens or hundreds of generations in laboratory rearing under these artificial conditions. Such long-term laboratory rearing was shown to alter courtship behavior, host finding and foraging behaviors in parasitoids, and the artificial rearing substrates can impact parasitoid vibrational communication and host acceptance (Bautista & Harris, 1997; Canale & Benelli, 2012; Gandolfi et al., 2003; Ghaemmaghami et al., 2022; Joyce et al., 2008; Naranjo-Guevara et al., 2020). The fitness of parasitoids may also be reduced with prolonged laboratory rearing as they adapt to the rearing environment and undergo demographic and genetic changes (Hopper et al., 1993; Nunney, 2003; Szűcs et al., 2019). In cases when the laboratory colony is initiated with few individuals and/or the populations pass through bottlenecks, genetic diversity can be reduced, genetic load can increase via drift, and inbreeding can ensue upon mating between related individuals (Fauvergue et al., 2012; Lenteren, 2003). Because of the negative effects of prolonged laboratory rearing periodical augmentation using wild caught individuals is recommended to restore fitness of mass reared parasitoids (Bartlett, 2018; Ghaemmaghami et al., 2022; Hoekstra, 2003). However, this may not be a feasible approach when laboratory selection is used to improve certain traits because gene flow between the added wild-type parasitoids and the selected individuals could swamp the targeted adaptations (Lenormand, 2002; Tallmon et al., 2004; Whiteley et al., 2015). Thus, it is important to test parasitoids that have undergone selection and long-term laboratory rearing without augmentation of wild-type individuals for their ability to find and attack target species that are developing in real host plants.

Classical biological control introductions most often use specialist endoparasitoids (Van Driesche et al., 2020). Development inside a host requires unique adaptations to overcome host defenses (Godfray, 1994). These specialized adaptations usually make the parasitoids quite host-specific which is a necessary component of classical biological control programs to avoid non-target effects on native species (Hajek & Eilenberg, 2018; Heimpel & Mills, 2017). Highly specialized parasitoids also tend to have fine-tuned adaptations to locate the narrow range of hosts they are able to exploit (Godfray, 1994). These traits can make specialist parasitoids very efficient biocontrol agents with relatively high attack rates of the target species (Godfray, 1994; Hajek & Eilenberg, 2018; Heimpel & Mills, 2017; Van Driesche et al., 2020). However, when an exotic host invades a novel habitat, generalist parasitoids are usually the first to attack them (Abram et al., 2019; Cornell & Hawkins, 1993; Grabenweger et al., 2010; Gröbler & Lewis, 2008; Lee et al., 2019) and it is usually generalist parasitoids that respond rapidly to selection on novel hosts (Golec et al., 2019; Henry et al., 2008; Jarrett et al., 2022; Jones et al., 2015; Linder et al., 2022). Even though generalists, by definition, are adapted to attack a wide range of species, their parasitism rates can vary widely both on native and exotic hosts (Cornell & Hawkins, 1993; Grabenweger et al., 2010; Gröbler & Lewis, 2008). Attack rates of exotic hosts by parasitoid communities dominated by generalist species in the introduced range tend to be lower than parasitism rates in their native ranges where they are attacked by a combination of specialist and generalist parasitoids (Cornell & Hawkins, 1993; Grabenweger et al., 2010; but see

Vindstad et al., 2013). It is unknown how the performance of an experimentally improved generalist parasitoid would compare to a co-evolved specialist parasitoid from the native range of the exotic species.

The focal pest of this study is spotted wing drosophila (*Drosophila suzukii*) (Matsumura) (Diptera: Drosophilidae), a polyphagous pest of soft fruits, native to Southeast Asia that invaded Europe and continental North America in 2008 (Asplen et al., 2015; Lee et al., 2019). In the introduced range of the United States, only two generalist pupal parasitoids, *Pachycrepoideus vindemmiae* (Rondani) (Hymenoptera: Pteromalidae) and *Trichopria drosophilae* (Perkins) (Hymenoptera: Diapriidae), are known to attack *D. suzukii* at low levels (< 10% parasitism rates) (Lee et al., 2019). Previous studies showed that laboratory selection could significantly increase the developmental success of these two parasitoid species on *D. suzukii* (Jarrett et al., 2022; Linder et al., 2022). Recently, a classical biological control agent, the specialist larval parasitoid, *Ganaspis brasiliensis* (Ihering) (Hymenoptera: Figitidae) was approved for field releases in North America against the invasive *D. suzukii* (Daane et al., 2021). Here, we explore whether a laboratory selected population of *T. drosophilae* also shows increased attack rates in more natural environments by comparing their performance with that of non-adapted *T. drosophilae* reared on *D. melanogaster* in three different fruit types that *D. suzukii* are known to infest (raspberries, cherries, and blueberries). We also assess how release density influences parasitism and how prolonged laboratory rearing may have affected foraging behavior by testing parasitism rates of fruit that is either placed on the ground (i.e., fallen fruit) or elevated (i.e., fruit on the plant). In addition, we compare the biocontrol potential of laboratory improved generalist parasitoids and of a specialist biocontrol agent by evaluating their parasitism rates on raspberry plants.

Materials and Methods

Fly colonies

The origin and rearing procedures of the *D. suzukii* and *D. melanogaster* colonies were described in Jarrett et al., (2022). Briefly, the flies were reared using a standard DSSC cornmeal diet at 25 ± 2 °C, 16L:8D photoperiod, and at 85 ± 5% humidity. Population sizes of >1000 individuals were maintained in standard drosophila vials of 2.5 x 9.5 cm (Genesee Scientific, San Diego, CA, USA) at densities of around 30 flies per vial.

Parasitoid colonies

Trichopria drosophilae is a solitary, cosmopolitan pupal parasitoid of flies in the Drosophilidae family (Carton et al., 1986). Our laboratory colony was founded by 30 individuals from a North American population as described in Jarrett et al. (2022). Three replicate populations of *T. drosophilae* were reared for 10 generations on *D. suzukii* as part of previous selection experiments and three populations were reared on *D. melanogaster* representing the control treatment for those experiments (Jarrett et al., 2022; Linder et al., 2022). The populations that responded positively to selection for increased developmental success on *D. suzukii* in these prior experiments will be referred to as 'adapted' or 'improved' and the controls as 'non-adapted' populations. As the selection experiments concluded, the adapted populations of *T. drosophilae* were moved back onto the natal host, *D. melanogaster* for rearing for five generations for ease of maintenance. Two generations prior to the start of the current experiments, the three replicate adapted *T. drosophilae* populations were moved back onto the invasive host, *D. suzukii,* while the three non-adapted populations remained on *D. melanogaster*. Parasitoids were reared by releasing ~25 adults in vials that contained 5-day old pupae of the respective fly species. Parasitoids were provided with a strip of paper towel infused with honey and left in the vials for 48 hours to parasitize fly pupae. Adult parasitoids emerged within five weeks and were either used for experiments or to maintain colonies.

Ganaspis brasiliensis is an Asian solitary larval parasitoid (Daane et al., 2021; Wang et al., 2020). An East Asian strain (G1) of *G. brasiliensis* has a narrow host range that is restricted to a few species within the genus *Drosophila* and had been approved for field releases in the continental USA as a classical biological control agent against *D. suzukii* (Daane et al., 2021; Hougardy et al., 2022; Wang et al., 2020). A colony of *G. brasiliensis* was initiated at Michigan State University (MSU) using 50 female and 50 male adults from a USDA-APHIS laboratory in Newark, NJ. The *G. brasiliensis* colony used in the experiments described below were initiated by 15 females and 15 males from the MSU core colony. To rear *G. brasiliensis* store-bought, conventionally grown blueberries were infested by *D. suzukii* at densities of 100 flies per 300 grams of fruit in clear plastic containers (25x19x25cm; PrepNaturals, Philadelphia, PA, USA). Berries were lightly dusted by instant dry yeast (Fleischmann's ActiveDry yeast) to increase attraction of flies and to prevent mold formation during the rearing process (Rossi-Stacconi et al., 2022). Two days following infestation when the fly larvae were in the first instar stages, 30 *G. brasiliensis* adults were released into the containers with the *D. suzukii* for five days for parasitization. Adults were reused to parasitize infested fruit up to three times. The next generation of parasitoids emerged in five weeks. The rearing took place in the same incubators used for fly rearing using the same temperature and light settings.

Experiment 1 – Testing the effectiveness of adapted versus non-adapted populations of Trichopria drosophilae *in real fruit*

Two generations after the three replicate adapted populations were moved back from *D. melanogaster* onto *D. suzukii* for rearing we retested their developmental success on the invasive host. For this test 20 female parasitoids were drawn randomly from each of the three replicated adapted and non-adapted populations. Each female parasitoid was provided with 10 *D. suzukii* pupae for 48 hours to parasitize. The pupae were removed from the artificial diet used for fly rearing and presented in a clear Petri dish (60x15mm; Falcon, Corning, NY, USA) for parasitization. The numbers of emerging flies and parasitoids were monitored for five weeks. This test confirmed that differences in development success between the selected and control lines on *D. suzukii* were maintained despite the five generations of rearing of both on *D. melanogaster* (for details see Supplementary Materials and Fig. S1-2). Following this test, the three replicate selection populations were merged into a single adapted population for further experimentation. The same was done for the three replicate non-adapted populations.

The performance of adapted and non-adapted populations (selection treatments) of *T. drosophilae* were compared in three types of fruit (raspberries, sweet cherries, and blueberries) placed at two different heights (on the ground or elevated) at two different parasitoid densities (50 or 100). There were eight replicates for each selection treatment for each fruit type and for each parasitoid densities tested (2 selection treatments x 8 replicates x 3 fruit types x 2 parasitoid densities = 96 replicates). In addition, there were eight replications that were used to test *D. suzukii* emergence success from each fruit type without any parasitism (controls). The eight replications for each treatment combination were split evenly between two temporal blocks in two consecutive weeks for logistical reasons. Each fruit type tested was store bought and organically grown. After a thorough wash, 50 grams of fruit were placed in plastic containers (25cm x 19cm x 25cm, PrepNaturals) and dusted lightly with yeast (see rearing methods for *G. brasiliensis*). These containers were then placed at the bottom of mesh cages (30cm x 30cm x 30cm, Restcloud) with two containers per cage (per replicate) on a tabletop in the laboratory at ambient temperature (22-25 °C). In each cage, 100 *D. suzukii* adults were released to infest the fruit for 48 hours. Flies were provided with honey water via a cotton wick placed in a 59 ml cup during infestation. After two days the flies were removed, and each plastic container was fitted with a lid that had a 12 x 8 cm square mesh screen for ventilation. The containers with the infested fruit were then placed into the incubators used for fly rearing for 72 hours to allow the fly larvae to mature into pupae. These containers were then placed back into the mesh cages used to infest them with flies. In each cage one of the containers was placed on the ground and the other container was suspended 20 cm from the bottom of the cage using plastic supports secured to the mesh cage walls. The cages then received either 50 or 100 *T. drosophilae* adult parasitoids from either the adapted or non-adapted populations or no parasitoids. After 48 hours of parasitism, the containers with the fruit were removed from the cages and labeled, noting the replication number, selection treatment, parasitoid density treatment and the location during parasitism. They were fitted with lids with fine mesh squares and placed into the rearing incubators. Twice a week for five weeks, the numbers of emerging flies and parasitoids in each container were recorded and the insects were removed from the containers.

Experiment 2 – Testing the effectiveness of adapted populations of Trichopria drosophila *versus the specialist* Ganaspis brasiliensis *on raspberry plants*

To compare the performance of laboratory selected populations of the generalist *Trichopria drosophilae* and the specialist *Ganaspis brasiliensis*, raspberry plants were introduced alongside the fruit to provide morecues related to foraging for hosts than the fruit tested in the previous experiment could provide on its own. Parasitism levels of three different parasitoid treatments (adapted and non-adapted *T. drosophilae*, and *G. brasiliensis*) were tested on one fruit type (raspberry) that was placed on both the ground and elevated to the fruiting level of the raspberry plant to offer a choice in foraging height. There were 12 replications for each parasitoid treatment and 12 additional replications were used to test emergence success of *D. suzukii* from fruit not exposed to parasitism. In April 2022, one-year-old bare root 'Heritage' raspberries (*Rubus idaeus,* Indiana Berry and Plant Co.) were planted into 8.517 L plastic pots using Suremix Perlite potting mix (Cleveland, OH, USA). The potted plants were kept in the greenhouse until mid-May when they were taken outside and dug into the soil at the MSU Entomology farm up to the rim of the pots. In late September the plants with the pots were lifted from the ground and used for experiments. At this point, plants were 40-60 cm tall with some fruit and flowers. Since the amount of fruit differed among plants, we removed the berries and instead standardized the amount of store-bought raspberries added to the cages for the experiment.

The potted plants were placed individually into tall mesh cages (61 cm x 40 cm x 40 cm, Restcloud) in a greenhouse at 22℃. Infestation of raspberries proceeded as described for experiment 1 by placing 50 grams of fruit into plastic containers and releasing 100 *D. suzukii* individuals in each cage to lay eggs for 48 hours. However, given that *T. drosophilae* is a pupal parasitoid and *G. brasiliensis* is a larval parasitoid, the infested fruit was not incubated prior to the release of *G. brasiliensis* but was incubated for five days prior to the release of *T. drosophilae* to reach the pupal stage. In each cage, one of the containers with the infested fruit was placed on the bottom of the cage and the other container was elevated to the canopy level of the raspberry plants. Fifty parasitoids of the given treatments were released in each cage and left to parasitize for 48 hours. The containers with the fruit were then removed from the cages and placed into the rearing incubators where *D. suzukii* and parasitoid emergence was checked twice a week for five weeks.

Statistical analyses

All analyses were conducted using R software 4.2.3 (R core team, 2022). Linear mixed effects models (lmer) were constructed in the *lme4* package and the ANOVA method was used to compute test statistics (Bates et al., 2015). Histograms and the Shapiro-Wilk test were used to check the distribution of the data. Data were square root transformed to reduce skewness and normalize distribution when necessary. Akaike Information Criterion (AIC) was used to select the model with the best fit by comparing the simplest model without any interactions to models with all possible combinations of two-way and three-way interactions. All post-hoc pairwise comparisons were performed using the *emmeans* package with Tukey-adjustment (Lenth, 2021).

For the first experiment, the number of emerging parasitoids was compared by including selection treatment (adapted vs. non-adapted *T. drosophilae*), fruit type (raspberry, cherry or blueberry), parasitoid density (50 or 100), the position of fruit during parasitism (ground vs. elevated) and the interactions between fruit type and selection treatment, and fruit type and fruit position as fixed effects. Temporal block was included as a random effect. To assess the effect of different parasitoid treatments on the number of flies emerging, we included the control treatment with no parasitoid release in the analysis and compared this to the treatments that used adapted and non-adapted parasitoid releases. Parasitoid treatment and fruit type were fixed effects and temporal block was a random effect in the model.

For the second experiment, to compare the number of parasitoids emerging, the three parasitoid treatments (adapted vs. non-adapted *T. drosophilae,* and *G. brasiliensis*) and the position of fruit during parasitism (ground vs. elevated) were the main factors in the model. To test how parasitism affected fly emergence, the three parasitoid treatments were included as main factors in the model.

Results

Experiment 1 – Testing the effectiveness of adapted versus non-adapted populations of Trichopria drosophilae *in real fruit*

The number of parasitoids emerging of the adapted and non-adapted *T. drosophilae* populations from *D. suzukii*-infested fruit was significantly different ($F_{1,181} = 37.24$ $p < 0.0001$). The number of parasitoids emerging from blueberries and cherries was higher when attacked by the adapted compared to the non-adapted population of *T. drosophilae* (pairwise comparison in blueberry: $p < 0.0001$, and in cherry: $p = 0.003$) (Fig. 1.1). Parasitoid emergence in raspberries showed the same trend of higher emergence of the adapted population but it was not statistically significant from the non-adapted population (pairwise comparison: $p = 0.32$) (Fig. 1.1). The latter result may be due to the overall lower parasitoid emergence from raspberries (25.6 ± 5.07, back-transformed mean ± SE) compared to blueberries (48.4 ± 6.97) or cherries (54.4 ± 7.39) ($F_{2,181} = 17.85$ $p < 0.0001$). Neither the placement of fruit on or off ground ($F_{1,181} = 2.04$ $p = 0.16$) nor the number of parasitoids released ($F_{1,181} = 0.25$ $p = 0.62$) affected parasitoid emergence. The number of *D. suzukii* emerging differed among parasitoid treatments ($F_{2,210} = 2.77$ $p < 0.0001$) but not among the different fruit types ($F_{2,210} = 2.77$, $p = 0.06$) (Fig. 1.2). The greatest number of flies emerged from the control treatment (71.2 ± 6.07) that received no parasitoids. Parasitism by the non-adapted *T. drosophilae* population reduced fly emergence by 71% (20.4 ± 1.67), and parasitism by the adapted population reduced fly emergence by 89% (8.0 ± 1.05) (all pairwise comparisons are significant at < 0.05) (Fig. 1.2).

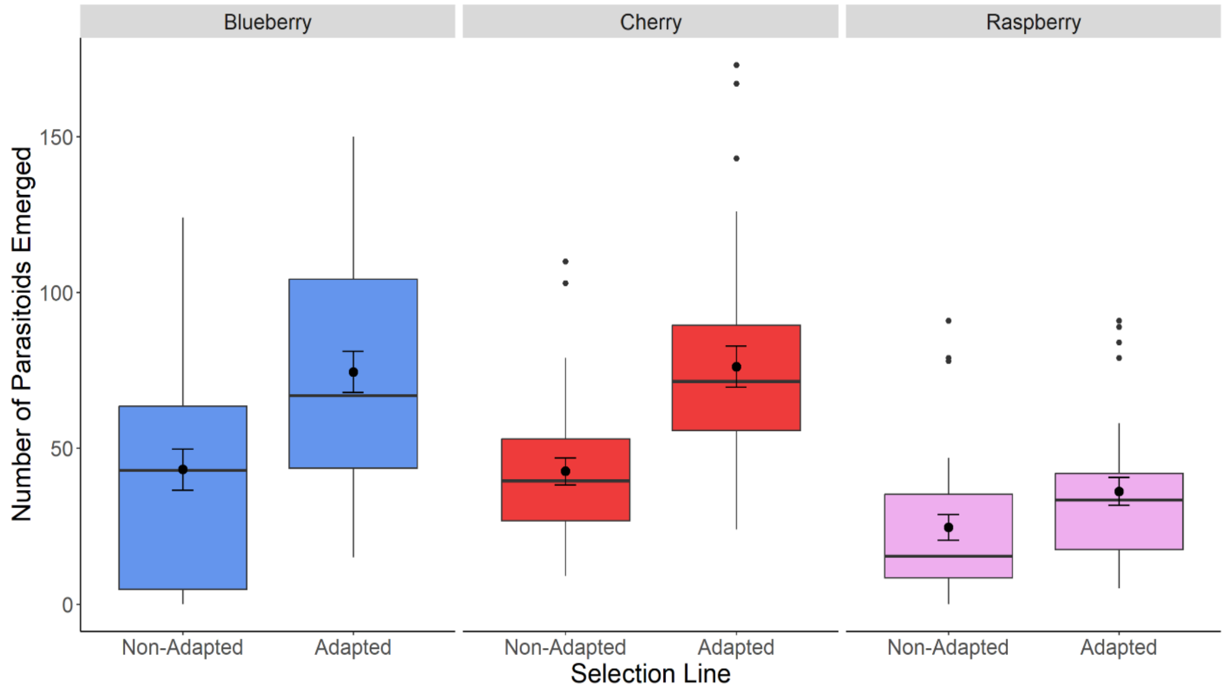

Figure 1.1. The number of *Trichopria drosophilae* adult parasitoids emerging from three types of fruit (blueberries – blue boxes, sweet cherries – red boxes, and raspberries – pink boxes) infested by *Drosophila suzukii*. *Trichopria drosophilae* populations had previously been either selected (Adapted) or had not been selected (Non-Adapted) for improved developmental success on *D. suzukii*. Dots indicate outlier observations, the horizontal line indicates the median with the box representing the interquartile range, and vertical lines are 1.5 times the interquartile range. Means and standard errors are shown within each box plot.

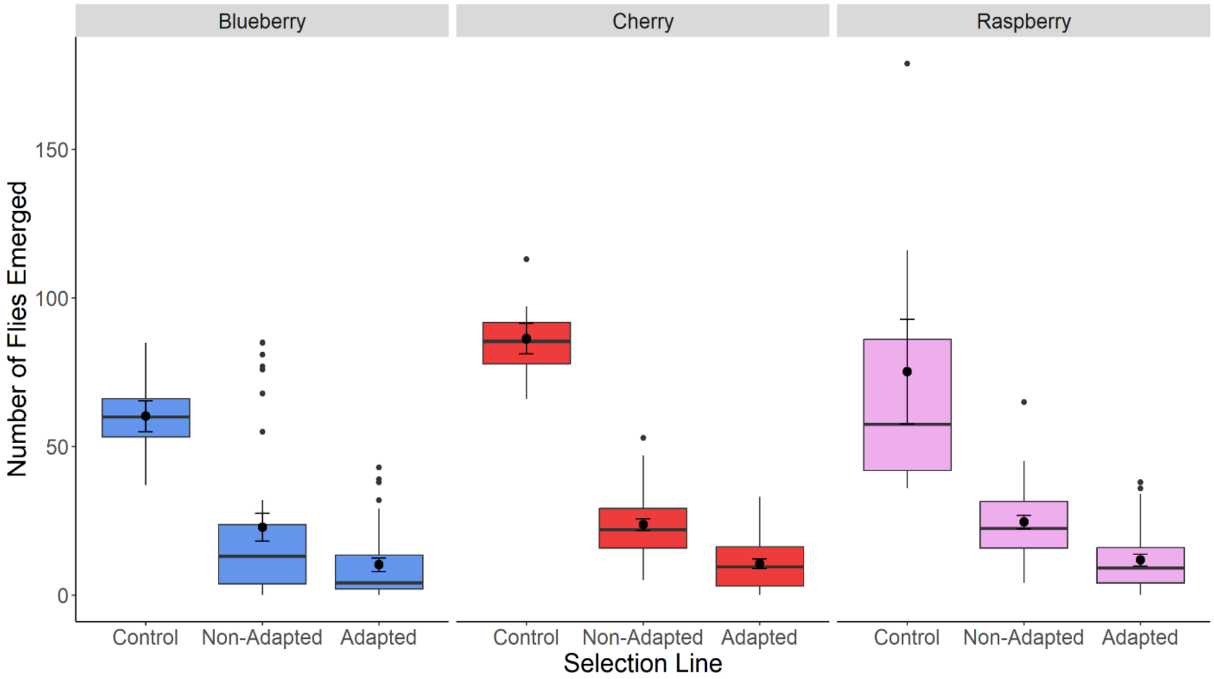

Figure 1.2. The number of *Drosophila suzukii* adult flies emerging from three types of fruit (blueberries – blue boxes, sweet cherries – red boxes, and raspberries – pink boxes). Infested fruit was either not attacked by parasitoids (Control) or were attacked by *Trichopria drosophilae* that had previously been selected (Adapted) or had not been selected (Non-Adapted) for improved developmental success on *D. suzukii*. Dots indicate outlier observations, the horizontal line indicates the median with the box representing the interquartile range, and vertical lines are 1.5 times the interquartile range. Means and standard errors are shown within each box plot.

Experiment 2 – Testing the effectiveness of adapted populations of Trichopria drosophila *versus the specialist* Ganaspis brasiliensis *with live plants*

The number of emerging parasitoids from *D. suzukii* infested raspberries differed among the three parasitoid treatments ($F_2 = 69.41$ $p < 0.0001$). The specialist *G. brasiliensis* emerged at the highest numbers (62.1 ± 2.01; mean ± SE), followed by the adapted population of *T. drosophilae* (40.0 ± 2.01), with the non-adapted *T. drosophilae* population yielding the fewest parasitoids (29.2 ± 2.01) (all pairwise comparisons are significant at < 0.05) (Fig. 1.3). The placement of the fruit on or off ground had no effect on parasitoid emergence ($F_2 = 0.15$ $p = 0.70$). Most flies emerged from the control treatment (82.1 ± 2.34) that received no parasitoids (Fig. 1.4). Parasitism by the non-adapted population of *T. drosophilae* reduced fly emergence by 67%

(27.2 ± 1.66) (pairwise comparison: $p < 0.0001$). Interestingly, the number of emerging *D. suzukii* were similar for *G. brasiliensis* (12.7 ± 1.66) and the adapted population of *T. drosophilae* (13.4 ± 1.66) (pairwise comparison: $p = 0.9919$). Attack by either of these two parasitoid populations reduced fly emergence by 84-85% compared to the controls (both pairwise comparisons are < 0.05) (Fig. 1.4).

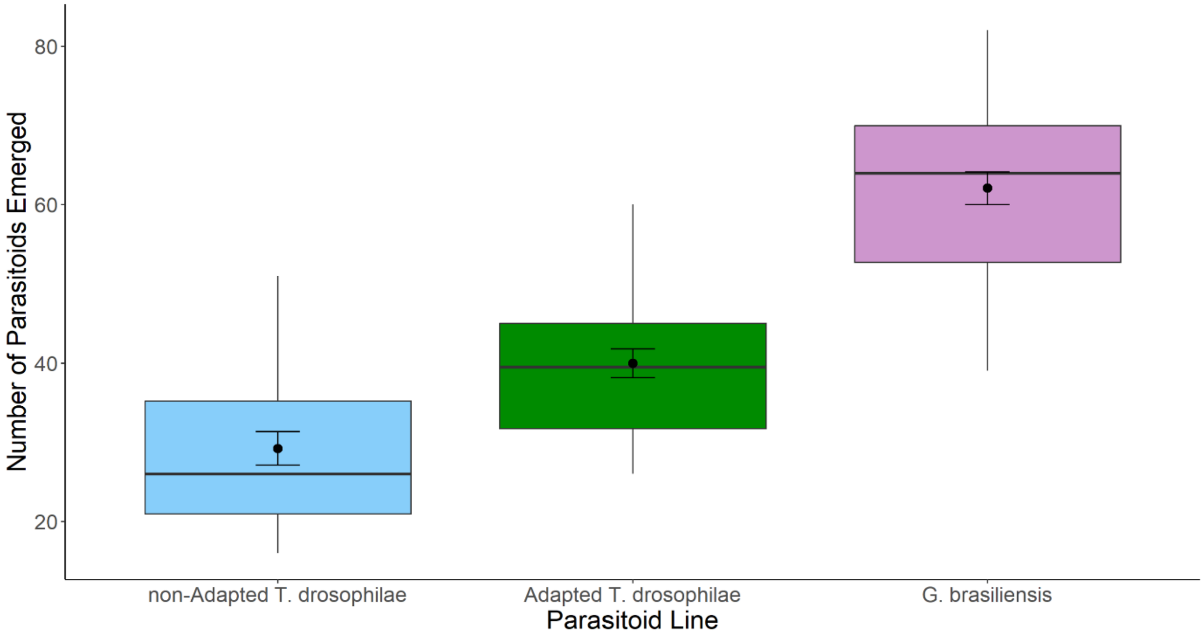

Figure 1.3. The number of *Trichopria drosophilae* or *Ganaspis brasiliensis* adult parasitoids emerging from raspberries infested by *Drosophila suzukii*. *Trichopria drosophilae* populations had previously been either selected (Adapted) or had not been selected (Non-Adapted) for improved developmental success on *D. suzukii*. Dots indicate outlier observations, the horizontal line indicates the median with the box representing the interquartile range, and vertical lines are 1.5 times the interquartile range. Means and standard errors are shown within each box plot.

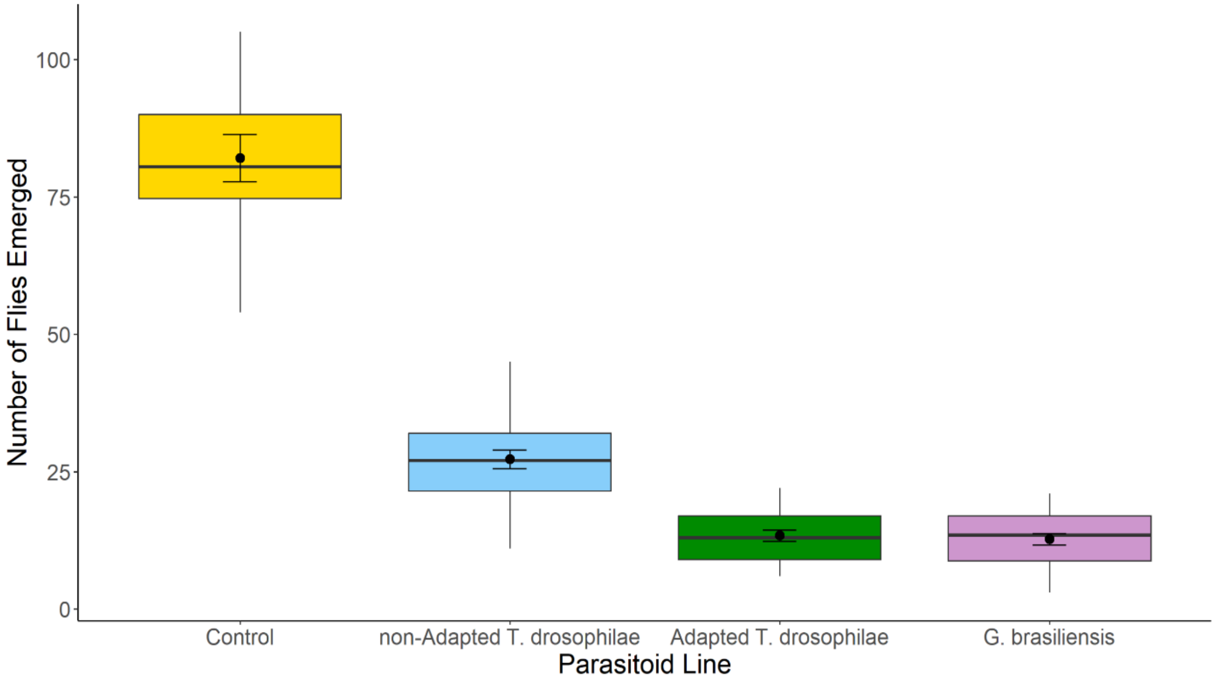

Figure 1.4. The number of *Drosophila suzukii* adult flies emerging from raspberries. Infested raspberries were either not attacked by parasitoids (Control) or were attacked by three different parasitoid populations: 1) *Trichopria drosophilae* that had previously been selected (Adapted) 2) or had not been selected (Non-Adapted) for improved developmental success on *D. suzukii*, and 3) *Ganaspis brasiliensis* that is a specialist larval parasitoid. Dots indicate outlier observations, the horizontal line indicates the median with the box representing the interquartile range, and vertical lines are 1.5 times the interquartile range. Means and standard errors are shown within each box plot.

Discussion

In this study, I found that the improved parasitism success achieved by laboratory selection of the generalist parasitoid *T. drosophilae* on the invasive *D. suzukii* is maintained on the long term even if the rearing host is changed to *D. melanogaster* for several generations. The adapted *T. drosophilae* showed increased biocontrol efficacy under more realistic conditions, including in multiple different types of fruit and when fruit is offered alongside a live plant. We also found that the co-evolved specialist parasitoid *G. brasiliensis* had higher adult emergence success from *D. suzukii* than the laboratory improved population of *T. drosophilae.* Nevertheless, these two parasitoid populations were able to reduce emerging *D. suzukii* numbers to the same extent, which indicates that they may have similar biocontrol potential despite their different life histories.

One of the main concerns during laboratory rearing is behavioral changes in parasitoids including the deterioration of host searching and oviposition behaviors especially when artificial rearing substrates are used (Gandolfi et al., 2003; Lewis & Tumlinson, 1988; Lenteren, 2003; Vet & Dicke, 1992; Vet et al., 1995). For example, the fruit fly parasitoid *Diachasmimorpha longicaudata* (Ashmead) that was reared for over 160 generations on oriental fruit flies (*Bactrocera dorsalis* Hendel) raised on a semi-synthetic wheat diet preferred to oviposit in flies that developed in the wheat diet compared to flies that developed in papaya (Bautista & Harris, 1997). Rearing of codling moth (*Cydia pomonella* L.) on artificial diet for 30 generations also changed the host searching behavior of the larval parasitoid *Hyssopus pallidus* compared to when the moth host was raised on a natural apple diet (Gandolfi et al., 2003). The frass produced by moth larvae while feeding on apples serves as a cue to locate hosts in nature but the parasitoids that were reared under artificial conditions had a 53% lower search response to this cue than those reared in the natural system with apples (Gandolfi et al., 2003). Yet, we found that *T. drosophilae* has maintained its ability to locate *D. suzukii* in multiple types of real fruit despite the long-term laboratory rearing during which hosts were offered in an artificial diet. The foraging behavior of parasitoids did not change either as they were able to locate hosts on the ground and at elevated positions similarly as they forage in nature (Rossi Stacconi et al., 2019). On average across different fruits the improved *T. drosophila* population reduced *D. suzukii* emergence by 89% (Fig. 1.2). When a raspberry plant was present to provide additional foraging cues, the adapted *T. drosophilae* population and the specialist *G. brasiliensis* both reduced fly emergence by 84-85% (Fig. 1.4). These results are encouraging regarding the potential effectiveness of laboratory improved parasitoids for release in the wild.

Previous experimental results have shown that changes in parasitoid behavior in artificial rearing environments may be transient plastic responses that can be reversed or improved rapidly with learning upon exposure to natural plant-related cues (Gandolfi et al., 2003; Lewis & Tumlinson, 1988; Vet & Dicke, 1992). In the codling moth system, for example, *H. pallidus* parasitoids quickly learned to associate cues from apples with the presence of hosts and exposure to apple cues during development or as early adults for a single generation was enough to revert the deterioration in their host searching behaviors (Gandolfi et al., 2003). While plant-related cues may be plastic traits, host-related cues are more reliable from a parasitoid's perspective, and they are likely to be genetically based (Lewis & Tumlinson, 1988; Vet & Dicke, 1992). Therefore, prolonged exposure to altered host cues could be subject to rapid evolution, for example, in cases when factitious hosts are used during laboratory rearing (Bourchier et al., 1993; Gowda et al., 2021; Lenteren, 2003). However, if the target host is used for rearing the foraging potential of laboratory reared parasitoids should not be compromised significantly.

The adapted population of *T. drosophilae* had consistently higher emergence rates in each tested fruit compared to the non-adapted population (Figs. 1.1, 1.3). Thus, adaptation to *D. suzukii* has been maintained despite multiple generations of rearing on their natal host *D. melanogaster* in between the end of the original selection experiment (Jarrett et al., 2022; Linder et al., 2022) and the beginning of the real fruit assays. Attack rates of *D. suzukii* were high across all types of fruit (Figs. 1.2, 1.4) and the number of parasitoids emerging were relatively high in blueberries and cherries considering the number of flies available for parasitism (Figs. 1.1, 1.3). The low parasitoid emergence from raspberries was likely due to the quick deterioration of this thin-skinned soft fruit during the rearing process resulting in liquification and excessive mold that could have killed many parasitoids. These results indicate that the fitness of parasitoid populations has not declined during long-term laboratory rearing. Alternatively, the relatively high fitness of both the adapted and non-adapted *T. drosophilae* population could be because we combined the three replicate selection populations into a single large adapted population and did the same for the control populations before testing them on the different fruits. The merging of populations may have resulted in both demographic and genetic rescue as it increased population sizes, has likely alleviated genetic load, and mating between individuals that may have had disparate adaptations to the new host could have created new, successful variants (Hufbauer et al., 2015; Tallmon et al., 2004; Whiteley et al., 2015). Similar approaches have been recommended to maintain parasitoid fitness during mass rearing that include the maintenance of isofemale inbred lines that are merged prior to release. Another approach uses compartmentalization where the population is separated in multiple vials that are combined at set intervals to facilitate interbreeding among lineages (Bartlett, 2018; Nunney, 2003; van de Zande et al., 2014). Recent experimental research in Trinidadian guppies (*Poecilia reticulata*) shows that gene flow does not necessarily overwhelm local adaptive traits (Fitzpatrick et al., 2020). Therefore, introduction of a few field-collected individuals into a laboratory colony may not erase host adaptation. However, it seems that fitness may be preserved without adding wild-type individuals via the maintenance of isolated replicate populations that could be used to create outbreeding events.

In terms of adult emergence, the newly approved biological control agent *G. brasiliensis* was more successful than the laboratory improved population of *T. drosophilae*. These results are not surprising given that the strain of *G. brasiliensis* that is approved for field releases in the

USA and was used in our experiments is a co-evolved specialist on *D. suzukii* (Daane et al., 2021; Hougardy et al., 2022). We also know from previous studies that despite substantial improvement of developmental success in response to laboratory selection, *T. drosophilae* was not able to reach similar emergence rates on *D. suzukii* than another generalist pupal parasitoid, *P. vindemmiae* (Jarrett et al., 2022; Linder et al., 2022). This was likely because *T. drosophilae* is an endoparasitoid and *P. vindemmiae* is an ectoparasitoid (Wang et al., 2020). Endoparasitoids need adaptations such as specialized venoms to overcome host defenses since they develop inside the host (Godfray, 1994; Wang et al., 2016; Wang et al., 2020). Even though it is difficult to directly compare larval and pupal parasitoids, both *G. brasiliensis* and *T. drosophilae* are endoparasitoids and thus need adaptations to contend with host defenses. It is likely that *G. brasiliensis,* with its long-term co-evolutionary history, is more effective at overcoming *D. suzukii* host defenses than *T. drosophilae* that was subject to relatively short-term selection to improve its development success on this novel host.

However, developmental success is not the only measure to assess biocontrol potential of parasitoids. One of the potential outcomes of parasitism is the death of hosts without the successful development of parasitoids (Abram et al., 2016; Abram et al., 2019). This so-called nonreproductive host killing happens often when native parasitoids try to attack novel invasive hosts that they are not necessarily able to develop on (Abram et al., 2016; Abram et al., 2019; Kaser et al., 2018; Kruitwagen et al., 2021). Non-reproductive host killing is a heritable trait that can respond to selection (Kruitwagen et al., 2021). When the native larval parasitoid, *Leptopilina heterotoma* (Hymenoptera: Figitidae) was subjected to laboratory selection, its killing rate of *D. suzukii* improved somewhat but not its developmental success (Kruitwagen et al., 2021). We found that *G. brasiliensis* and the improved population of *T. drosophilae* reduced *D. suzukii* emergence to the same extent (Fig. 1.4). This indicates that, although fewer *T. drosophilae* successfully developed and emerged on *D. suzukii*, they killed the same proportion of flies as the co-evolved *G. brasiliensis*. . The increased killing rate that is detected between the adapted and non-adapted population of *T. drosophilae* on the different types of fruit (Figs. 1.3, 1.4) could be a correlated response to selection for improved developmental success on *D. suzukii*.

This study demonstrates that improved parasitoid performance on a novel host accomplished in the laboratory under artificial conditions can translate to increased attack rates in more natural environments. We also show that a generalist pupal parasitoid can achieve similar biocontrol potential as a specialist larval parasitoid with the combined result of improvements in developmental success and of nonreproductive host killing rate. Thus, the use of laboratory selection to improve the performance of native natural enemies may be a viable alternative approach for biological control of invasive species. In the case of *D. suzukii*, augmentative releases of *T. drosophilae* have already been underway (Gonzalez-Cabrera et al., 2021; Rossi-Stacconi et al., 2019) but with the approval of *G. brasiliensis* for release in multiple US states, the focus will likely shift to this specialist parasitoid. Nevertheless, *T. drosophilae* and *G. brasiliensis* will encounter each other in nature and interspecific competition can reduce effectiveness of both (Hougardy et al., 2022). On the other hand, developing a biological control program with multiple available natural enemies can be beneficial as there can be differences in species abundance and performance locally, regionally and over time.

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APPENDIX

Supplementary Materials

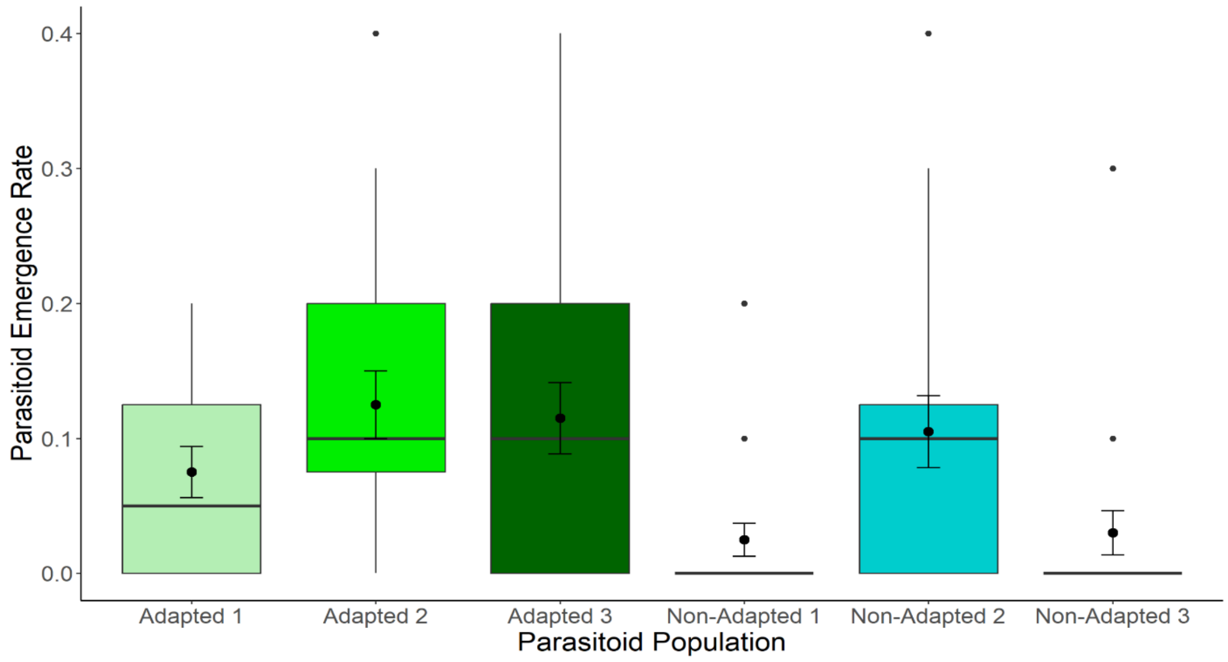

Figure S1. The proportion of *Drosophila suzukii* pupae that had successful emergence of *Trichopria drosophilae.* Three replicate populations of *T. drosophilae* had previously been either selected (Adapted 1-3) or had not been selected (Non-Adapted 1-3) for improved developmental success on *D. suzukii.* Dots indicate outlier observations, the horizontal line indicates the median with the box representing the interquartile range, and vertical lines are 1.5 times the interquartile range. Means and standard errors are shown within each box plot.

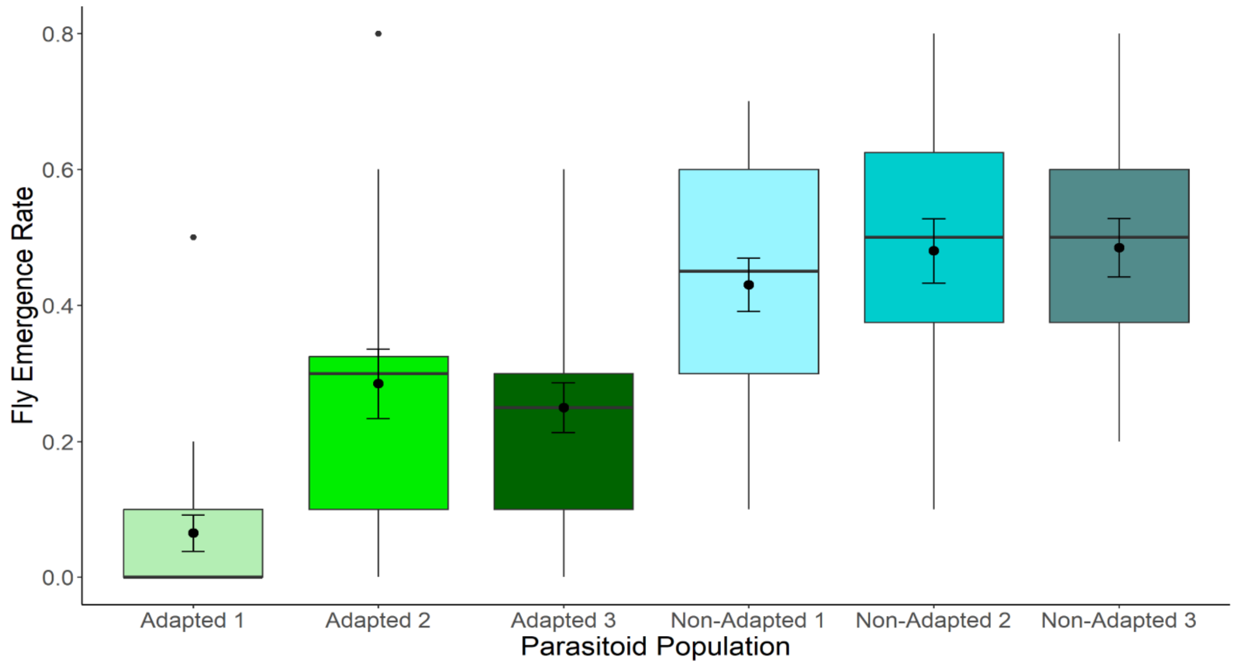

Figure S2. The proportion of *Drosophila suzukii* pupae that yielded adult flies when attacked by three replicate populations of *T. drosophilae* that had previously been either selected (Adapted 1-3) or had not been selected (Non-Adapted 1-3) for improved developmental success on *D. suzukii*. Dots indicate outlier observations, the horizontal line indicates the median with the box representing the interquartile range, and vertical lines are 1.5 times the interquartile range. Means and standard errors are shown within each box plot.

CHAPTER 2: Geographic Variation in Resistance of the Invasive *Drosophila suzukii* to Parasitism by the Biological Control Agent, *Ganaspis brasiliensis*

Introduction

Insect host-parasitoid systems have provided classic models for research on coevolutionary dynamics (Kraaijeveld et al., 1995; Kraaijeveld et al., 1998; Thompson, 2005; Thompson, 2005). Insect parasitoids lay their eggs inside or on the host and feeding by the developing larvae eventually kills the host. Since only one party survives this interaction, there is strong selection pressure for prey to escape parasitism by mounting physiological and behavioral defenses (resistance) and for parasitoids to overcome host defenses and increase developmental success (virulence) (Godfray 1994). Research focusing on European *Drosophila* species and their native parasitoids shows that there can be regional differences in virulence and resistance (Kraaijeveld et al., 1995; Kraaijeveld & Godfray, 1999). This indicates that it is not solely interactions between parasitoids and hosts that can lead to changes in these important traits, but that the environmental effects on a host-parasitoid community may play a role as well. Both host resistance and parasitoid virulence can also evolve rapidly (Jalvingh et al., 2014; Cavigliasso et al., 2019; Moiroux et al., 2018), and the maintenance of both can be costly (Fellowes et al., 1998; Kraaijeveld & Godfray, 2002; Kraaijeveld & Godray, 1997; Kraaijeveld et al., 2001).

Invasion theories posit that escape from specialist natural enemies (Enemy Release Hypothesis - ERH) (Keane & Crawley, 2002) may allow for the re-allocation of costly defenses towards growth and development (Evolution of Increased Competitive Ability - EICA) (Honor & Colautti, 2020; Blossey & Notzold, 1995). Thus, the combined prediction of ERH and EICA is that invasive species will evolve lower defenses in the introduced range in the absence of specialist parasitoids compared to the native range. Invasive insect pests are often the target of classical biological control programs that focus on the introduction of specialized natural enemies from an invasive species' native range. As invasive hosts are reunited with their co-evolved natural enemies, reciprocal selection pressures are restored and a new coevolutionary arms race can ensue in the introduced range. The outcome of this new arms race can determine the success or failure of the biocontrol program and thus it is important to understand the mechanisms that influence host-parasitoid interactions in the introduced ranges.

The focus of this study is *Drosophila suzukii* (Diptera: Drosophilidae) that invaded the Americas and Europe in 2008 (Asplen et al., 2015). *Drosophila suzukii* has a wide host range, attacking economically important crops such as raspberries, blueberries, strawberries, and cherries, as well as numerous wild hosts, such as dogwood, pokeweed, choke cherry, and elderberry (Asplen et al., 2013; Poyet et al., 2015; Lee et al., 2019). The extreme polyphagy coupled with high fecundity (> 600 eggs per female) and short generation times (~14 days at 22 °C) make *D. suzukii* difficult to control (Wang et al., 2018). Furthermore, the large population sizes and multiple generations per season also provide ideal conditions for rapid evolution.

Drosophila suzukii differs from native North American and European drosophilids in two important traits. Firstly, it has a serrated ovipositor that allows for the piercing of harder, still ripening fruit (Asplen et al. 2005). Therefore, it can occupy a novel niche that is unavailable to other drosophilids that can only lay eggs in overripe, fallen or rotting fruit (Asplen et al., 2015; Lee et al., 2019). Secondly, *D. suzukii* has a much stronger immune response to parasitism than native drosophilids (Kacsoh & Schlenke, 2012; Poyet et al., 2013). Only two cosmopolitan, generalist pupal parasitoids have been able to develop on *D. suzukii* in the introduced ranges, but their attack rates have remained below 10% leaving *D. suzukii* largely free from parasitism (Chabert et al. 2012; Lee et al. 2019). The eggs and/or larvae of native larval parasitoids are frequently encapsulated and killed by *D. suzukii* as it mounts a cellular and humoral immune response (Poyet et al., 2013; Kacsoh & Schlenke, 2012). *Drosophila suzukii* has a high haemocyte load that is shown to correlate with increased levels of resistance to parasitism (McGonigle et al. 2018, Poyet et al. 2013). However, maintaining high levels of such constitutive resistance is costly and can lead to trade-offs in the form of increased larval mortality and slower development in competitive environments and when food is limited (Fellowes et al., 1998; Kraaijeveld & Godfray, 1997; Kraaijeveld et al., 2001; McGonigle et al., 2017).

Given the scarcity of co-evolved specialist parasitoids and the cost of maintaining high levels of defenses, the expectation is that *D. suzukii* may have evolved lower resistance against parasitism in its introduced ranges compared to the native range. There is little data to confirm such predictions, however, and the one study that has directly compared resistance of introduced and native *D. suzukii* populations showed the opposite pattern. Poyet et al. (2013) found that haemocyte loads in three invasive *D. suzukii* populations from France were twice as high as two native populations from Japan. The higher haemocyte levels correlated with higher encapsulation rates of a European larval parasitoid, *Leptopilina heterotoma* (Poyet et al. 2013).

In this study, we investigated spatial, temporal and host-plant related patterns of resistance of *D. suzukii* populations by sampling eight geographically distinct populations in Michigan and Oregon over a two-month period in three different types of host plants. We assayed resistance-related traits using a recently-approved biological control agent, the larval parasitoid *Ganaspis brasiliensis* (Hymenopters: Figitidae), that was reared in the laboratory and allowed to interact with *D. suzukii* from each collected fruit sample. The aim of this investigation was to better understand mechanisms mediating host resistance in a novel environment in the absence of specialist natural enemies and to establish baseline levels of resistance prior to the release of a recently approved biological control agent, the larval parasitoid *Ganaspis brasiliensis* (Hymenoptera: Figitidae).

Materials and Methods

Parasitoid rearing

A laboratory colony of *G. brasiliensis* was established at Michigan State University (MSU) using 50 adult males and 50 adult females from a USDA-APHIS laboratory in Newark, NJ in February 2022. The *G. brasiliensis* population used in our experiments was established using 15 adult males and 15 adult females from this MSU-based colony in May 2022. To rear *G. brasiliensis*, 150 grams of store bought conventionally grown blueberries were placed into 25cm x 19cm x 25cm plastic containers (PrepNaturals) and sprinkled with 1 teaspoon of instant dry yeast (Fleischmann's Active Dry Yeast) each to reduce mold buildup and ensure infestation from flies (Rossi-Stacconi et al., 2022). Two containers were placed without lids in a 30cm x 30cm x 30cm mesh cage (RestCloud), and 100 *D. suzukii* flies (50 males and 50 females) were released to infest the blueberries for 48 hours. The *D. suzukii* used to infest the fruit originated from our laboratory colony that was funded in 2018 and augmented with wild-caught individuals each year. The fly rearing proceeded as described in Jarrett et al., (2022) using the standard DSSC cornmeal diet in incubators set at 25±2°C, 70±5% relative humidity, and a 16L:8D photoperiod.

The containers with the fly infested blueberries were then removed from the cages, and 30 *G. brasiliensis* individuals (15 male and 15 female) were added to each to parasitize *D. suzukii* larvae for five days. During parasitism containers were covered with lids that had fine mesh windows for ventilation and a strip of honey underneath for the adult parasitoids to feed on. The parasitoids were removed after 5 days and reused for a next round of parasitization of a new batch of *D. suzukii* infested blueberries. This process was repeated 3-4 times until most adult parasitoids died to maximize population growth. Following parasitization, the plastic containers were held in the same incubators used for fly rearing and were checked twice a week to remove any emerging *D. suzukii*. After approximately 28-35 days *G. brasiliensis* adults emerged, which were then used to start the next parasitoid generation.

Field Sampling of Drosophila suzukii *populations*

We sampled seven *D. suzukii* populations in Michigan in 2022 following a north to south and east to west grid (Fig. 2.1) and received samples from one location in Corvallis, Oregon. The distance between the northernmost and southernmost field sites in Michigan was 400 km, and the westernmost and easternmost sites were about 320 km apart. Most sites were conventionally managed mixed orchards, while the site in Oregon and one site in Michigan were organically managed mixed fruit and vegetable farms (Table 2.1). *Drosophila suzukii* was collected from each location once in August and in September. At each date three types of fruit samples were collected: 1) managed fruit (cherries, raspberries, or blueberries), 2) unmanaged, wild fruit, and 3) for a standardized sample we used banana traps. For the managed fruit collection 0.5 kg of conventionally grown (i.e., pesticide sprayed) fruit was picked from cultivated plants and fallen fruit from the ground. For the unmanaged fruit samples, 0.5 kg of fruit was collected from brambles and other wild soft fruit bearing plants (i.e., pokeweed) that were located along field edges. Each fruit sample was collected by hand and from a minimum of three different plants. For the banana traps, one halved banana was placed in a 454 ml lidded red solo cup with several holes drilled into the bottom and lid to allow flies to enter and rainwater to leave. Wires were threaded through holes at the top of the cup and used to hang the traps from trees located along the edge of orchards. At each site three banana traps were placed and left for 48 hours to be infested by fruit flies. An eighth field site located in Corvallis, Oregon was sampled similarly in August and September 2022 and the collected fruit was mailed to MSU to rear out fruit flies and for subsequent resistance assays.

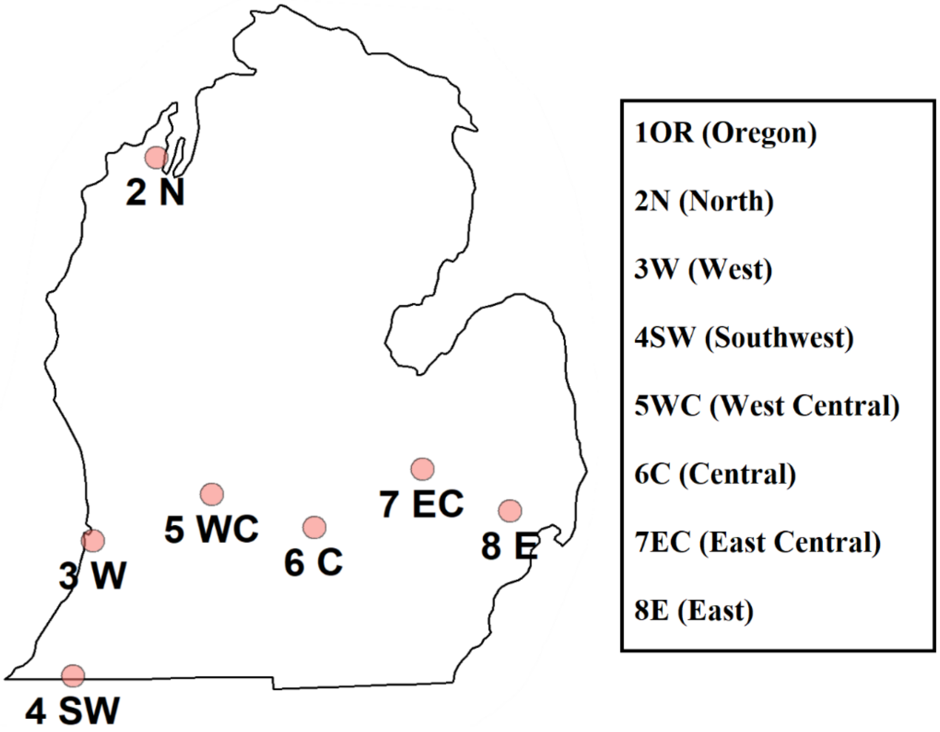

Figure 2.1. Sites sampled and assayed for *Drosophila suzukii* resistance in 2022. An additional location was sampled in Corvallis, OR. For coordinates, site and sampling information see Table 2.1.

Table 2.1. Field site sampling information

Site	Closest City	Latitude	Longitude	Crop Type	Management	Sample 1 Date	Sample 2 Date
1OR	Corvallis, OR	44.61024	-123.22518	Mix. Fruits and Veg.	Organic	8/16/2022	9/14/2022
2N	Bingham, MI	44.88324	-85.67508	Mixed Fruits	Conventional	8/11/2022	9/19/2022
3W	Fennville, MI	42.59455	-86.15549	Mixed Fruits	Conventional	8/8/2022	9/14/2022
4SW	Niles, MI	41.78807	-86.30464	Mixed Fruits	Conventional	8/8/2022	9/14/2022
5WC	Clarksville, MI	42.87345	-85.25865	Mix. Fruits and Veg.	Conventional	8/8/2022	9/24/2022
6C	East Lansing, MI	42.67491	-84.48975	Mix. Fruits and Veg.	Organic	8/12/2022	9/19/2022
7EC	Flint, MI	43.02399	-83.67534	Mixed Fruits	Conventional	8/9/2022	9/16/2022
8E	Washington, MI	42.77274	-83.01852	Mixed Fruits	Conventional	8/9/2022	9/16/2022

Resistance Assays

Fruit from each site and sample type was placed in separate plastic containers (25cm x 19cm x 25cm, PrepNaturals) in an incubator (25±2°C, 70%RH, 16L:8D) for 10 days. Emerging adult *D. suzukii* were identified based on morphology, separated from other fruit fly species, and transferred to drosophila vials (2.5 x 9.5 cm) (Genesee Scientific, San Diego, CA, USA) containing 6 mL standard DSSC cornmeal diet. Adult flies were allowed to mate and oviposit in the artificial diet for 48 hours. Three days after the adult flies were removed, 50 early second instar larvae were extracted from the artificial diet from each sample. The 50 larvae were divided into five replicates of ten larvae each for each location and sample type combination. An additional set of five replicates was created using second instar larvae from our lab-reared colony of *D. suzukii* to test how reusing the same females may have affected parasitism.

The larvae for each replicate were placed atop a halved blueberry to prevent dehydration inside a 60x15mm plastic Petri dish (Falcon, Corning, NY, USA). Each Petri dish then received one female *G. brasiliensis* wasp that had been isolated with a single male in a drosophila vial for 24 hours to mate. The female wasp was left in the Petri dish for four hours to parasitize D. *suzukii* larvae. Because of the limited availability of *G. brasiliensis* females, we had to reuse the same individuals for multiple rounds of parasitism. Individual females were first used to parasitize *D. suzukii* larvae from each of the eight field sites of the five replicates of the banana traps (n = 40 females). The same females were then moved onto the *D. suzukii* samples collected from managed fruit to parasitize for four hours and after that onto the samples from unmanaged fruit for four hours. An additional five parasitoid females were used in three consecutive times to parasitize *D. suzukii* larvae originating from our laboratory colony to test the effect of reusing females on parasitism rates.

The *D. suzukii* larvae were left in the halved blueberries for 72 hours to allow the effects of parasitism and encapsulation to become more visible. The larvae were then removed and examined under a microscope (Nikon SMZ1000, 10x-80x magnification) for evidence of parasitization (the presence of parasitoid eggs) and encapsulation of parasitoid eggs that is indicated by the darkening of the egg. This process allowed us to more quickly see the results of our resistance assays than if we had waited to examine *D. suzukii* adults after they emerged. For most larvae, these signs were visible under a microscope with sufficient light, but if no evidence of parasitism or encapsulation was immediately clear, larvae were dissected with ophthalmological spring scissors (Fine Science Tools). In each replicate, the number of larvae that were parasitized and the number of encapsulated parasitoid eggs were recorded.

Statistical Analyses

All statistical analyses were performed using R software 4.2.3 (R Core Team, 2013). Generalized linear models (GLM) from the lme4 package were constructed to determine the impact of fixed effects on our dependent variables, while comparison between effects was performed using the emmeans package (Bates et al., 2015; Lenth et al., 2021). Akaike Information Criterion (AIC) was used with the *aictab* function to select the model with the best fit by comparing the simplest model without any interactions to models with all possible two-way and three-way interactions. All post-hoc pairwise comparisons were performed using the *emmeans* package with Tukey-adjustment (Lenth, 2021). The dependent variables (parasitism rate and encapsulation rate) were treated as binomial outcomes since the proportional values were bounded between 0 and 1. Parasitism was calculated for each female by dividing the number of parasitized hosts (n_p) by the number of hosts offered ($n_0 = 10$) to create a parasitism rate (PR): PR = n_p/n_0. Encapsulation rate (ER) was calculated by dividing the number of encapsulated eggs in parasitized hosts (n_e) by the number of parasitized hosts (n_p): ER = n_e/n_p.

First, to test the effect of reusing the same females on parasitism rate and encapsulation rate GLMs were constructed using month (August or September) and the sequence of parasitism (first, second, or third) in the resistance assays as fixed effects. For these resistance assays, *D. suzukii* larvae from our laboratory colony were offered consecutively three times, with the first parasitism event corresponding to tests conducted on field collected samples from banana traps, the second on managed fruit, and the third on unmanaged fruit samples. Parasitism rate and encapsulation rate of *D. suzukii* larvae collected at different locations, in different fruit samples and at different times were compared using GLMs. In these models, location (the eight field sites), sample type (banana trap, managed fruit, and unmanaged fruit), month (August or September) and the interaction between location and month were included as fixed effects. In the model for parasitism rate, the unmanaged fruit samples were removed from analyses because those had been affected by the reuse of parasitoids in the resistance assays.

Results

Parasitism rate

Reusing the same females for parasitization had a significant effect on parasitism rate ($F_{2,27} = 5.04$, $p = 0.014$) which did not significantly change between months ($F_{1,26} = 0.36$, $p = 0.55$). Parasitism rate declined between the first (mean parasitism ± SE; 89% ± 3.1) and third (73% ± 4.4) use of the females (pairwise comparison: $p = 0.014$) but not between the first and second (80% ± 4.0) use (pairwise comparison: $p = 0.19$). As an extension of these results from our laboratory ‘control’ population, it is likely that parasitism rate of *D. suzukii* collected from banana traps (tested first) and from unmanaged fruit (tested third) significantly differed due to our experimental design. Therefore, data for unmanaged fruit samples were excluded from subsequent parasitism rate analyses.

Parasitism rate of *D. suzukii* by *G. brasiliensis* differed significantly among samples from different locations ($F_{7,230} = 6.89$, $p < 0.001$), between months ($F_{1,229} = 11.54$, $p < 0.001$), and between different sample types ($F_{2,237} = 4.38$, $P = 0.014$). The northernmost site (2N in Figure 1) (81% ± 2.29) had the highest parasitism rate that was significantly higher than the westernmost (3W) (62.5% ± 2.81), southwestern (4SW) (65.7% ± 2.75), west central (5WC) (62.4% ± 2.80) and east central (7EC) (66.3% ± 2.76) locations (all pairwise comparisons: $p < 0.05$). Samples from Oregon (1OR) (75.4%±2.49) had higher rates of parasitism than the westernmost and west central Michigan samples (pairwise comparisons: $p < 0.05$) (Figure 2.2). Overall, parasitism rates of *D. suzukii* tended to be higher for collections made in August (73.3% ± 1.33) than those collected in September (66.3% ± 1.38) (pairwise comparison: $p < 0.001$). However, these results were not consistent within locations (month*location interaction: $F_{7,222} = 273.96$, $p = 0.0026$). Pairwise comparisons of parasitism rates of August and September samples within sites were only significant at the easternmost site (8E) ($p = 0.002$) (Figure. 2.2). The type of sample used to collect *D. suzukii* also led to differences in parasitism rate as larvae collected using banana traps (72.8% ± 1.59) had higher rates of parasitism than those collected from managed fruits (66.1% ± 1.70) (pairwise comparison: $p = 0.0105$).

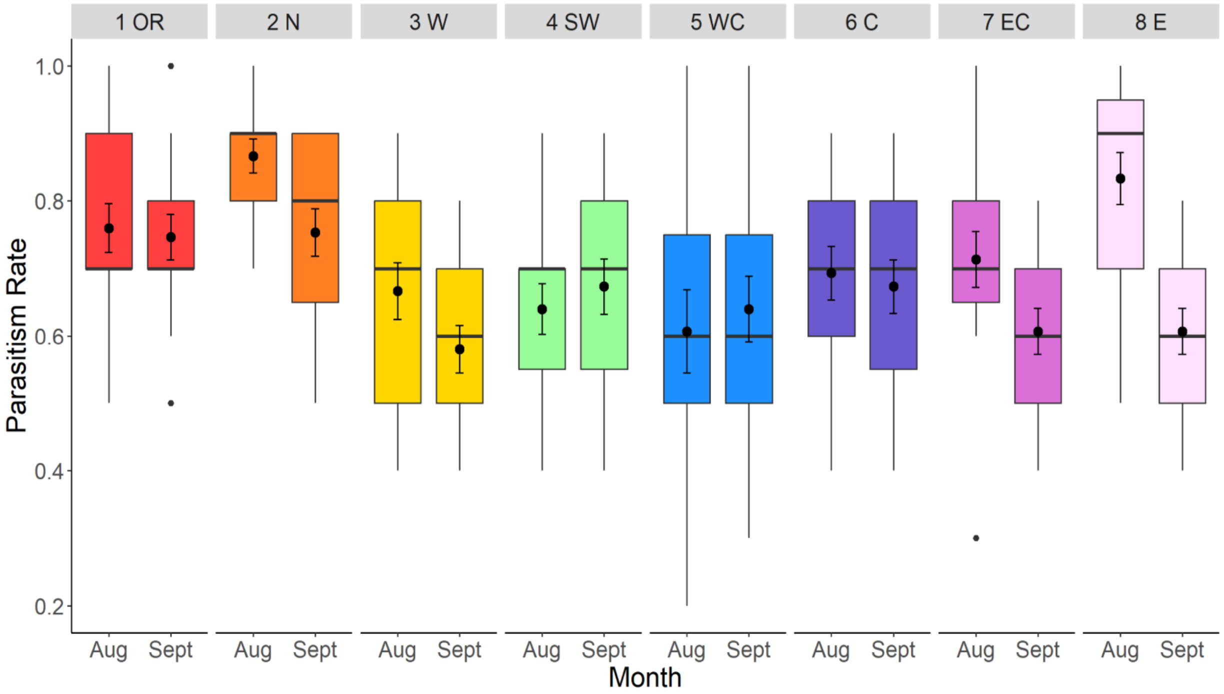

Figure 2.2. Parasitism rates of *Ganaspis brasiliensis* by eight populations of *D. suzukii* (1 population in Oregon – 1OR and seven populations in Michigan - 2N – 8E) sampled in August and September. The numbers on top indicate the sampling locations as shown in Figure 1. Dots indicate outlier observations, the horizontal line indicates the median with the box representing the interquartile range, and vertical lines are 1.5 times the interquartile range. Means and standard errors are shown within each box plot.

Encapsulation rate

Reusing females for parasitism did not affect encapsulation rate of parasitoid eggs by *D. suzukii* ($F_{2,27}$=3.24, p = 0.055). These results from our laboratory ‘control’ population indicate that reusing females likely did not affect encapsulation rates of *G. brasiliensis* in the field collected samples of *D. suzukii*. Therefore, all three sample types (banana trap, managed and unmanaged fruit) were included in encapsulation analyses of field samples. Encapsulation rates differed between months ($F_{1,26}$= 9.25, p < 0.001), being more than double in assays in September (25% ± 4.1) compared to August (9.3% ± 4.1%) (pairwise comparison: p = 0.0015). The temporal variability in encapsulation rate is likely the result of changes in the genetic makeup of our laboratory colony between August and September. Following the September assays, our long-term laboratory colony suffered a decline, indicating issues with the health of the flies. We augmented the population by adding 50 field collected *D. suzukii* from three different locations (150 flies total). Thus, the ‘control’ laboratory population used in the September assays may have already been experiencing negative genetic changes. Since the laboratory colony was used only to test the effects of reusing parasitoid females, these results have no effect on resistance assays conducted on the field sampled *D. suzukii* populations.

Encapsulation rates (resistance) differed among locations where *D. suzukii* was collected from ($F_{7,230} = 5.11$, $p < 0.0001$) and between months ($F_{1,229} = 10.938$, $p = 0.0011$) but not between fruit sample types ($F_{2,237} = 0.19$, $p = 0.83$). The northernmost site that had the highest parasitism rate showed the lowest encapsulation rate (16.1% ± 2.41). Encapsulation rates of the northernmost site were significantly lower than of the Oregon (33.4% ± 3.16), the westernmost (31.1% ± 3.39), and the central Michigan sites (6C) (32.4% ± 3.45) (all pairwise comparisons: $p < 0.05$) (Figure 2.3). The month of sampling affected resistance levels with *D. suzukii* collected in September (29.9% ± 1.66) demonstrating higher encapsulation rates than the August samples (22.3% ± 1.46) (pairwise comparison: $p = 0.0006$). However, the higher encapsulation rates later in the season were not consistent across sites (location*month interaction: $F_{7,222} = 3.868$, $p = 0.0005$). Of the eight sites only the central Michigan location showed significantly higher encapsulation rates in the September samples compared to the August samples (pairwise comparison: $p < 0.0001$) (Figure 2.3).

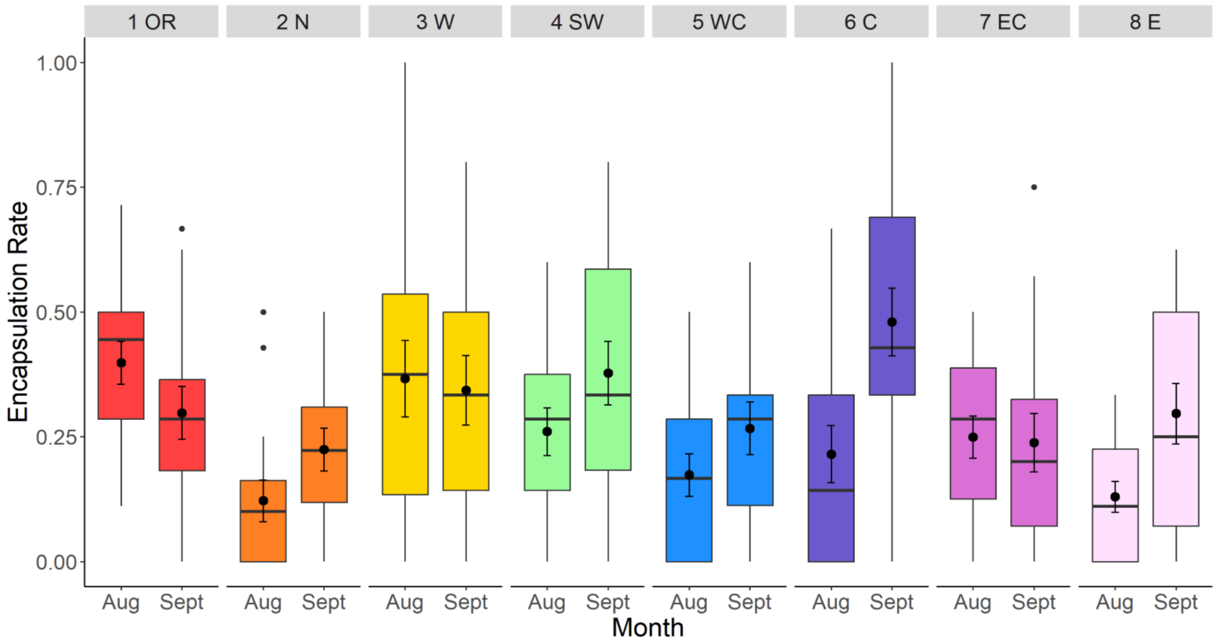

Figure 2.3. Encapsulation rates of *Ganaspis brasiliensis* by eight populations of *D. suzukii* (1 population in Oregon – 1OR and seven populations in Michigan - 2N – 8E) sampled in August and September. The numbers on top indicate the sampling locations as shown in Figure 1. Dots indicate outlier observations, the horizontal line indicates the median with the box representing the interquartile range, and vertical lines are 1.5 times the interquartile range. Means and standard errors are shown within each box plot.

Discussion

We found geographic and temporal differences in the levels of resistance of invasive *D. suzukii* populations in North America against the specialist larval parasitoid *G. brasiliensis*. Given that *G. brasiliensis* is being released *en masse* to control *D. suzukii* across the USA, these results can help to understand possibly differing outcomes of releases at different locations and at different times during the growing season.

Encapsulation rates of *G. brasiliensis* eggs by *D. suzukii* ranged between 12-39% in August and 22-48% in September regionally. Direct comparison of these results with other studies is difficult because of the different methods used to assess encapsulation. We dissected parasitized larvae to assess parasitism and encapsulation rates (Kacsoh & Schlenke, 2012). The two other studies that assessed the outcome of parasitization by *G. brasiliensis* examined the presence of black capsules in adult flies and found around 5% (Daane et al., 2021) and 15% of encapsulation rates by *D. suzukii* (Girod et al., 2018). These studies also used multiple *G. brasiliensis* populations that had differing host specificity, which can influence parasitism success (Daane et al., 2021; Girod et al., 2018). Even though the encapsulation rate assessments in this study do not account for mortality that could occur during development, the measured encapsulation rates are still relatively high considering that we used the most host specific strain of *G. brasiliensis* (G1) that co-evolved with *D. suzukii* in its native range and will be used for field releases across the USA (Daane et al., 2021; Hougardy et al., 2022).

Co-evolved, host specific parasitoids should have adaptations to effectively overcome host defenses as was shown for the larval parasitoid *Asobara japonica* (Hymenoptera: Braconidae) that only had 6-26% encapsulation rates by multiple strains of *D. suzukii* (Poyet et al., 2013). In contrast, the European parasitoid *L. heterotoma* that did not have a co-evolutionary history with *D. suzukii* was encapsulated at rates of 59-87% (Poyet et al., 2013). The strength of resistance correlated with hemocyte levels in the flies that were two times higher in the introduced compared to the native population of *D. suzukii* (Poyet et al., 2013). Thus, both the current study and the one by Poyet et al. (2013) indicate that introduced populations of *D. suzukii* have maintained relatively high levels of defenses against parasitism in contrast to the combined predictions of the ERH and EICA hypotheses (Keane & Crawley, 2002; Honor & Colautti, 2020; Blossey & Notzold, 1995). There is evidence that the maintenance of high haemocyte load is only costly in competitive environments. For example, when food is plentiful, there was no cost to resistance in three *Drosophila* species (McGonigle 2017). It is possible that *D. suzukii* can maintain high levels of defensive compounds without trade-offs because it currently experiences relatively low competition and high resource availability due to its ability to attack still ripening fruit with its serrated ovipositor (Poyet et al. 2013). In contrast, native drosophilids in Europe and North America are restricted to attacking overripe and fallen fruit and thus occupy different niches than *D. suzukii* (Asplen et al. 2015, Lee et al. 2019).

Encapsulation rates varied regionally across Michigan and Oregon. Similar variation in parasitoid resistance has been seen in other *Drosophila* species which have demonstrated population-level differences in encapsulation rates based on geographic location (Kraaijeveld & Van Alphen, 1995; Kraaijeveld & Godfray, 1999; Dubuffet et al., 2007). For example, *D. melanogaster* resistance to one of its larval parasitoids, *A. tabida,* is highest in central Europe (40-60% encapsulation rates) and lower in northern and southern Europe (Kraaijeveld and Godfray 1999). Seven geographically distinct populations of *D. yakuba*, a fly species native to Africa, also show varying encapsulation rates of the larval parasitoid *L. boulardi* that range between 6% and nearly 98% regionally (Dubuffet et al., 2007). Geographic structure in resistance can arise because of spatial and temporal differences in the strength of reciprocal selection between hosts and parasitoids, differences in the genetic variation available in local populations to respond to selection, differences in the wider host-parasitoid community, in abiotic conditions, and how the cost and benefits of resistance are balanced locally in terms of fitness (Kraaijeveld & Godfray, 1999; Kraaijeveld & Van Alphen 1995; Dubuffet et al., 2007). In native host-parasitoid communities where multiple parasitoid and host species interact, the regional difference in community structure can be the most important driver of geographic differences in host defenses (Kraaijeveld & Godfray, 1999). However, in the introduced ranges, host-parasitoid communities can be diminished, as is the case with *D. suzukii* that has experienced very low parasitism rates by native parasitoids over the last decade in North America (Lee et al., 2019). The recent discovery of an adventive larval parasitoid, *L. japonica,* in

Michigan (R. Isaacs pers. comm.) is unlikely to have had a substantial effect on *D. suzukii* resistance given that it is a newcomer and should have low population densities and limited distribution.

In the absence of widespread, high-density populations of specialist co-adapted parasitoids in the landscape abiotic factors may be the primary factors influencing resistance levels of *D. suzukii* populations. My results provide some evidence for that since the northernmost site in Michigan had the lowest encapsulation rate. This site is the coldest as it is located over 300 kilometers to the north of other sites in Michigan and lower temperatures have been associated with lower encapsulation ability in insect host-parasitoid interactions (Blumberg, 1991; Fellowes et al., 1999). There were also temporal differences in resistance of *D. suzukii* populations that show the opposite pattern, that is, somewhat increasing encapsulation rates later in the season. However, this difference was only significant between August and September for the central Michigan site (6C in Fig. 2.1) and *D. suzukii* collected in Oregon showed the reverse pattern of lower encapsulation in September than in August. Thus, it is likely that temperature effects on host resistance are not linear and are mediated by complex interactions between the host, their parasitoids, and the environment (Thomas & Blanford, 2003).

Parasitism rates of *D. suzukii* populations also differed regionally and over time, however, parasitism tended to show opposite patterns than encapsulation. For example, the northern site that demonstrated the lowest encapsulation rates showed the highest rates of parasitism. Similarly, the easternmost site that had low encapsulation rates had relatively high parasitism rates. While encapsulation rate increased from August to September, parasitism rate of the same populations decreased between those months. These patterns suggest that *G. brasiliensis* may be able to assess the resistance ability of larvae and lays fewer eggs in better defended individuals. Such correlations have been shown with the parasitoid *A. tabida* that tended to reject *D. melanogaster* larvae that had high resistance to parasitism (Kraaijeveld et al., 1995). Similarly, in 16 different Lepidopteran species caterpillars with the highest levels of resistance had the lowest levels of parasitism (Smilanich et al., 2009).

The above results indicate that there can be differences in the regional outcome of host-parasitoid interactions that may influence the efficacy of *G. brasiliensis* as a biological control agent of *D. suzukii*. At locations where *D. suzukii* have relatively low resistance the impact of biocontrol may be larger initially, while increased or geographically variable levels of resistance could render biocontrol less successful or more variable regionally. Our results could help to assess the mechanisms that may underlie biocontrol success or failure, and different management approaches may be recommended based on spatial differences in resistance of *D. suzukii* populations. For example, higher parasitoid releases may be necessary at locations that show relatively high levels of *D. suzukii* resistance to increase parasitoid pressure and to ensure that *G. brasiliensis* populations can maintain high densities despite their lower success of development. The higher population sizes and densities could help *G. brasiliensis* to maintain genetic diversity and adaptive potential and to evolve higher virulence over time. An alternative approach may be to release *D. suzukii* flies with low levels of resistance at sites that showed high encapsulation rates to reduce overall resistance by mixing populations. In any case, the baseline data collected here will be invaluable to further explore the eco-evolutionary dynamics of host-parasitoid interactions following the release of *G. brasiliensis* across Michigan.

LITERATURE CITED

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CHAPTER 3: Past Reflections and Future Directions

At the beginning of this research project, I relied heavily on trial-and-error. When I was first introduced to the topic and the actual work, I was a PhD student with the task of not only understanding the system at hand, but also thinking of in-depth questions to expand on it. While I welcomed this freedom and independence, I was unprepared for how many times my procedures would fail and require me to start over. As I began the rearing of two different fly colonies and two parasitoid wasp colonies, for example, I started to look for ways to make improvements to the system and increase efficiency. I would make a change to the container size from vials to glass jars, notice a negative effect on the insect colonies, and revert my changes while returning to the literature to see where I went wrong. For the next several months, I was consistently being challenged in the same way. This helped to show me many of the scientific flaws I came into this program bearing. I would assume my knowledge of the organisms and procedures I was attempting to carry out was sound, test a specific setup of flies and fruit within a mesh cage, and rework my steps when all the flies turned up dead a few days later without successfully infesting the fruit.

This process was discouraging at first and negatively interacted with my observations of the other graduate students around me who seemed much more capable and experienced than I was. As my attempts progressed, however, I managed to successfully complete my first real experiment involving different fruit types and their effect on *Trichopria drosophilae* parasitism. Scanning the data collected from this experiment and being able to use basic statistics to see any trends was genuinely exciting, and the many trials and errors that led to this conclusion made it feel more rewarding. This was one of the highlights of my graduate career and helped me to feel more like an actual scientist than I did in the somewhat isolated months leading up to it.

Although this was a positive development and provided motivation for me to continue working on this project, I was already starting to feel defeated by many of the other aspects of being a graduate student. Jumping into this PhD program immediately after finishing an undergraduate degree originally felt exciting, but I slowly started to feel mental fatigue overwhelming me. In an effort to preserve my mental health, I made the decision to switch my program from a PhD to an M.S. Continuing with a PhD would have surely had many of its own rewards but exiting with an M.S. and spending time outside of academia to figure out my actual ambitions and needs felt like a wiser use of the next several years. Making this change helped me to continue focusing on the projects that still needed completing without feeling the pressure of several more years' worth of decisions to make.

As I continued to rear insects and progressed onto performing experiments with live plants, I felt my reliance on trial-and-error waning. I was still making mistakes like my many failed attempts at designing a way to suspend fruit in a large mesh cage, but I was able to bounce back from them quickly and make necessary changes to progress. Even now looking back on elements of this project, I would have liked to spend more time deciding on the plant I would use and how it was going to be grown. As planting needed to happen in a specific timeframe to ensure I had old enough plants for my experiments, I felt rushed and did not spend as much time reading literature and considering possibilities before I had to decide and place orders. While I am pleased with the results, this part of the process would have been easier if I had been wiser with my time.

Overlapping with these raspberry plant experiments were the field site visits and preliminary assays associated with my *Drosophila suzukii* resistance experiments. Visiting field sites was one of the most positive elements of my graduate career and greatly contributed to my passion for this project. Up until this point, the entirety of my experiments was done in a laboratory or greenhouse, which made sense but offered little variety for the experimenter. Being able to collect the flies I would be experimenting with and determine how they would be sampled offered me a greater feeling of pride in this part of the research. Developing a working resistance assay procedure was similarly rewarding as I was able to combine some of the knowledge and tools I had familiarized myself with during my undergraduate career with the organisms I was now working with.

This process still involved several obstacles, however, like having to rear the sensitive *Ganaspis brasiliensis* parasitoid with limited space and determining the best combination of steps to make it work. I also learned from past mistakes and started testing procedures far in advance of the actual experiments. I tested many ways to offer *D. suzukii* larvae to *G. brasiliensis* females, which often led to dried out larvae, dead parasitoids, or both. I also tampered with many different lengths of time that the parasitoid could spend parasitizing before I removed her. Through a combination of reading similar procedures and testing different combinations, I finally put together a system that suited our needs.

The final data collected from these resistance assays provided another challenge that I required large amounts of guidance to address. While I was able to run statistical analyses and determine that there was significance between some of the effects I was measuring, being able to turn these numbers into a clear narrative proved much more difficult. Not only were these trends different from what I expected, but it was challenging to describe any reasonable pattern at all. Overcoming this obstacle required even more review of literature including papers that involved vastly different experimental procedures and study systems just to provide context to what I was looking at. I also relied heavily on regular conversations with my research mentor and other graduate students to ensure I was not overlooking anything. I plan to continue examining this data as I perform the same field collections and resistance assays in 2023. This will hopefully provide a more complete story for the data and allow larger inferences to be made.

Overall, I was able to learn more about myself and more about the scientific method through this process. Although there are elements of these experiments that could have gone more smoothly, I was able to use mistakes I made as a chance to learn and continue moving forward. Without these numerous examples of trial-and-error, I would not have been able to complete this project and acquire meaningful data that now contributes to a larger body of knowledge.