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Behavioral Interactions Between Spotted-Wing Drosophila (Drosophila Suzukii) and It's Parasitoid the Samba Wasp (Ganaspis Brasiliensis)

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BEHAVIORAL INTERACTIONS BETWEEN SPOTTED-WING DROSOPHILA
(*DROSOPHILA SUZUKII*) AND ITS PARASITOID THE SAMBA WASP (*GANASPIS*
BRASILIENSIS)

by

Dominic Crowley

A Thesis Submitted in Partial Fulfillment
of the Requirements for a Degree with Honors
(Biology)

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ABSTRACT

Past research has found that female *Drosophila* generally decrease their reproductive output in response to exposure to predators, including parasitoid wasps. However, no studies on behavioral changes induced by the endoparasitic wasp *Ganaspis brasiliensis* have been documented in the literature. *G. brasiliensis* has been identified as a biocontrol agent candidate against *D. suzukii*, with field trials currently underway across the contiguous United States. In this thesis, two experiments were performed: a behavioral observation assay measuring reproductive behaviors and an oviposition assay, a measure of reproductive activity. Female *D. suzukii* exposed to *G. brasiliensis* were observed to have depressed oviposition, producing fewer offspring than the unexposed. Decreased oviposition upon exposure to larval predators may be a strategy to increase survival of offspring, implicating an evolutionary tradeoff between offspring quality and quantity. These results indicate that the mere presence of *G. brasiliensis* alone may suppress *D. suzukii* populations, providing evidence for the suitable use of *G. brasiliensis* as a biocontrol agent.

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INTRODUCTION

Spotted-wing drosophila (SWD), *Drosophila suzukii* (Diptera: Drosophilidae) is an invasive vinegar fly that can cause significant damage to soft fruits. Unlike other drosophila, which primarily infest overripe and damaged fruit, female *D. suzukii* can infest healthy fruits using a serrated ovipositor. Additionally, damage associated with oviposition and the subsequent emergence of larvae from the fruit renders it vulnerable to infection by bacterial and fungal pathogens (Asplen et al., 2015). Since its first detection in California, *D. suzukii* has become a key pest in blueberries, blackberries, raspberries, strawberries, wine grapes, and cherries throughout the USA. These are of great economic significance in the United States and losses due to *D. suzukii* exceed \$718 million annually (Bolda et al., 2010; Farnsworth et al., 2017)). The species has fast development, a high reproductive capacity, and an expansive host range including the utilization of many wild hosts in the surrounding landscape from which they can readily migrate into crop fields (Asplen et al., 2015).

Insecticides have been the main method used to control *D. suzukii* (Van Timmeren & Isaacs, 2013). In blueberries, insecticide applications have doubled since the *D. suzukii* invasion, and growers of late-season sweet cherries and berries report requiring as many as 11-16 additional insecticide applications to harvest a marketable crop (Van Timmeren & Isaacs, 2013). These increases in sprays for *D. suzukii* have reversed a trend of reducing the usage of broad-spectrum insecticides, with a 45% increase in the percentage area treated between 2011 and 2019 (Johnson & Fanning, Unpublished Data). There are significant non-target effects of intensive management for *D. suzukii*, including secondary pest outbreaks due to insecticides disrupting the biological control of other

pests, such as the blueberry stem gall wasp in Michigan and scale insects in the Southeastern states of Georgia and Florida. For some pollinator-dependent crops such as fall raspberry, where plants are flowering while susceptible ripe fruit is present, there are also concerns regarding non-target impacts on pollination services. Due to the intensive use of insecticides, resistance, primarily to Spinosad has been documented in California (Gress & Zalom, 2019); Ganjisaffar et al., 2022). Resistance to spinosad and pyrethroid pesticides has also been induced in the laboratory setting (Deans & Hutchison, 2022). This research has serious implications and is particularly troubling for organic blueberry farmers, who rely on Spinosyn and Pyrethrum.

Cultural controls have shown some success in *D. suzukii* control, but are inadequate to provide sufficient control, particularly in areas with heavy infestation (Schöneberg et al., 2021). These practices include sanitation measures, the timing of harvest, pruning of canopies, modifying irrigation, mulching underneath the crop, and exclusion netting. Many of these practices alter the temperature and humidity of the cropping area, abiotic factors critical in the development and survivorship of *D. suzukii* (Fanning et al., 2019; Tochen et al., 2014). Fanning et al. (2019) demonstrated that *D. suzukii* sense and orientate to higher humidity in bioassays, indicating that the adult fly behavior can be modified based on their surrounding environment. This innate behavior by *D. suzukii* is exploited in the cultural control list above. A barrier to the adaptation of cultural controls is the time and labor associated with its implementation (Schöneberg et al., 2021). These factors often render the use of cultural controls more expensive than competing methods, making its adaptation challenging.

Biological control has been studied intensively since *D. suzukii* was first discovered in the United States (Lee et al., 2019). Naturally occurring predators consume and remove a substantial portion of *D. suzukii* (Woltz et al., 2015; Woltz & Lee, 2017). Carabid beetles, crickets, green lacewing larvae, and rove beetles are likely predators (Lee et al., 2019). Despite the activity of these native predators, *D. suzukii* continues to cause major economic damage indicating that they are insufficient to keep populations below economic thresholds.

In addition to predators, resident parasitoids have been surveyed across North America by collecting and rearing them from infested fruits or from artificial diet baited with *D. suzukii*. However, these initial surveys have shown that few naturally occurring parasitoids attack *D. suzukii* in the USA (Daane et al., 2016; Kaçar et al., 2017). Two pupal parasitoids, *Pachycrepoideus vindemmiae* (Rondani) (Hymenoptera: Pteromalidae) and *Trichopria drosophilae* (Perkins) (Hymenoptera: Diapriidae) have been commonly collected (Wang et al., 2018). However, naturally occurring parasitism of *D. suzukii* populations by these two pupal parasitoids is generally below 10% (Lee et al., 2019). Native larval parasitoids cannot overcome the defense response of *D. suzukii* in which the parasitoid egg/larva is encased (encapsulated) with melanized cells. However, if the parasitoids lay eggs on *D. suzukii* larvae, they can still reduce the survival of *D. suzukii* to adulthood (Kacsoh & Schlenke, 2012).

The most promising biological control option is the introduction of a specific parasitoid from the native range of *D. suzukii*. Explorations in South Korea, China, and Japan have discovered at least 19 larval parasitoids (Lee et al., 2019). Multiple species of parasitoids successfully parasitize *D. suzukii* immature life stages in the region of origin

(Mitsui et al., 2007). Over 75% of immature stages are parasitized by multiple parasitoid species in China and Japan (Girod et al., 2018). Of the Asian parasitoids surveyed, three larval parasitoids, *Ganaspis brasiliensis* (Ihering), *Leptopilina j. japonica*, and *Asobara japonica* Belokobylskij, were chosen based on frequent occurrence and imported into quarantine labs for evaluation as classical biological control agents (Daane et al., 2016; Wang et al., 2019; Wang et al., 2018). These parasitoids were systematically evaluated in quarantine and *G. brasiliensis* was found to be one of the most efficient and host-specific parasitoids of *D. suzukii* (Giorgini et al., 2019; Lee et al., 2019). The species was approved for release as a classical biocontrol agent (United States: National Archives and Records Administration: Office of the Federal Register et al., 2023), and field trials are underway throughout the contiguous US.

In biological control parasitoids and predators primarily impact their prey or host through direct computation; however, additionally there are also non-consumptive effects, also referred to as non-lethal effects, risk effects, or trait-mediated interaction that result in changes in prey biology driven by predation threat. These non-consumptive effects as reviewed by *Hermann and Landis* includes behavioral changes, life history adjustments, and physiological changes (Hermann & Landis, 2017). The primary impact of *G. brasiliensis* on *D. suzukii* is through the parasitism of its larvae, which has been well-investigated. Research on *D. melanogaster* and other figitid wasps suggests that there may be non-lethal effects that have been yet to be elucidated (Fleury et al., 2004; Lefèvre et al., 2011). In *D. melanogaster*, exposure to the figitid wasp, *Leptopilina boulandi*, produces unusual reproductive behavior in female flies (Lefèvre et al., 2011). Exposure to *L. boulandi* has been shown to result in a dramatic acceleration of copulatory

behavior (Ebrahim et al., 2021). Interestingly, this effect was not reproduced when *D. melanogaster* was exposed to insects that were non-predators, such as other species of drosophila or parasitoid wasps that do not parasitize drosophila (Ebrahim et al., 2021). This response could indicate an evolutionary tradeoff between offspring quantity and survival, with the wasp-exposed females choosing to mate quickly to produce a greater quantity of surviving offspring. Given the lethality of *G. brasiliensis* on *D. suzukii*, this trait could be under strong selective pressures.

Reduced oviposition in the face of threat by parasitoid wasps has been documented in *D. melanogaster*, but not *D. simulans*. Reduced oviposition occurring in *D. melanogaster* but not *D. simulans* under exposure to *L. bouhardi* was hypothesized to represent an evolutionary trade-off between physiologic (melanocytic encapsulation) and behavioral defense (Lefèvre et al., 2011). *D. suzukii* is known to have a robust immune response, demonstrating high rates of encapsulation against a large range of parasitoids (Chabert et al., 2012; Poyet et al., 2013). Although *D. suzukii*'s ability to encapsulate *G. brasiliensis* is poor, it has been noted to have a high hemocyte load that confers it a high level of resistance against other endoparasites, containing five times the level of hemocytes in *D. melanogaster* (Kacsoh & Schlenke, 2012). Under the behavioral-immunological tradeoff hypothesis, one would expect the absence of a behavioral response in *D. suzukii*.

In this study, we aim to determine if there are differences in the behavior of adult *D. suzukii* in the presence of *G. brasiliensis* and if there are impacts on egg-laying leading to differences in the presence of larva in the fruit. I hypothesize that the presence

of *G. brasiliensis* will impact the behavior of *D. suzukii* and reduce females' egg-laying thus reducing the quantity of larvae in the fruit.

METHODS

Drosophila Rearing and Husbandry

The *D. suzukii* strains used were originally collected from several agricultural sites in the Downeast region of Maine, United States. For all experiments, flies were reared on 5–7 ml standard cornmeal diet (Drosophila Stock Center, San Diego, CA, USA), consisting of cornmeal (62.5 g), white sugar (100 g), nutritional yeast flakes (35 g), agar (22.5 g), distilled water (1700 ml), propionic acid (8.85 ml), methyl paraben (1.65 g) and ethanol (19.65 ml). Flies were maintained in drosophila vials (2.5 × 9.5 cm; Lab-Express, Ann Arbor, MI, USA) with foam stoppers (Genesee Scientific, San Diego, CA, USA). These vials were maintained in growth chambers (Percival, Percival Scientific Inc., Boone, IA, USA) at 25°C, 16:8 light:dark photoperiod and 75% relative humidity.

Prior to experiments, flies were anesthetized with carbon dioxide (CO₂) gas in accordance with standard practice. Once anesthesia was achieved, as indicated by the cessation of movement, flies were transferred onto a staging pad supplied with CO₂ to allow for the continuation of anesthesia. Flies were sexed visually with the aid of a dissecting microscope based on the presence of *D. suzukii*'s characteristic spots and/or the presence of an ovipositor, indicating the presence of a male or female (Asplen et al., 2015). Once flies were sorted by sex, they were transferred to new vials and maintained under previous conditions until the initiation of the experiment.

Ganaspis Rearing and Husbandry

A laboratory line of *G. brasiliensis* of the G1 lineage, originating from a USDA Agricultural Research Service colony, was used. Wasps are reared by infesting store-

bought blueberries with *D. suzukii* for 2-3 days, after which infested fruit was exposed to *G. brasiliensis* for 2-3 days. Fruit infested with parasitized larva was incubated in a growth chamber (Percival, Percival Scientific Inc., Boone, IA, USA) at 25°C, 16:8 light:dark photoperiod and 75% relative humidity. Wasp emergence was checked daily and emerging wasps were collected using an aspirator. Newly emerged wasps were collected and maintained in vials with honey strips and a moistened toilet-paper substrate in an incubator as described above. All wasps utilized were directly obtained from mixed-sex vials and were sexually mature.

Behavioral Assay

Virgin adult *D. suzukii* reared in accordance with previously specified methods were placed in 20 mm diameter petri dishes (Corning Inc., Corning, NY, USA) alone or in the presence of *G. brasiliensis*. Insects were transferred without anesthesia, utilizing a pipette tip assemblage tool to move each insect individually (Yang, 2019). A five-minute acclimatization period followed the transfers to prevent impacts of the handling on behavior. High-definition video was recorded using a document camera positioned overhead. Individual arenas (petri dishes) were placed on a labelled cardboard grid to allow for future identification of specific replicates.

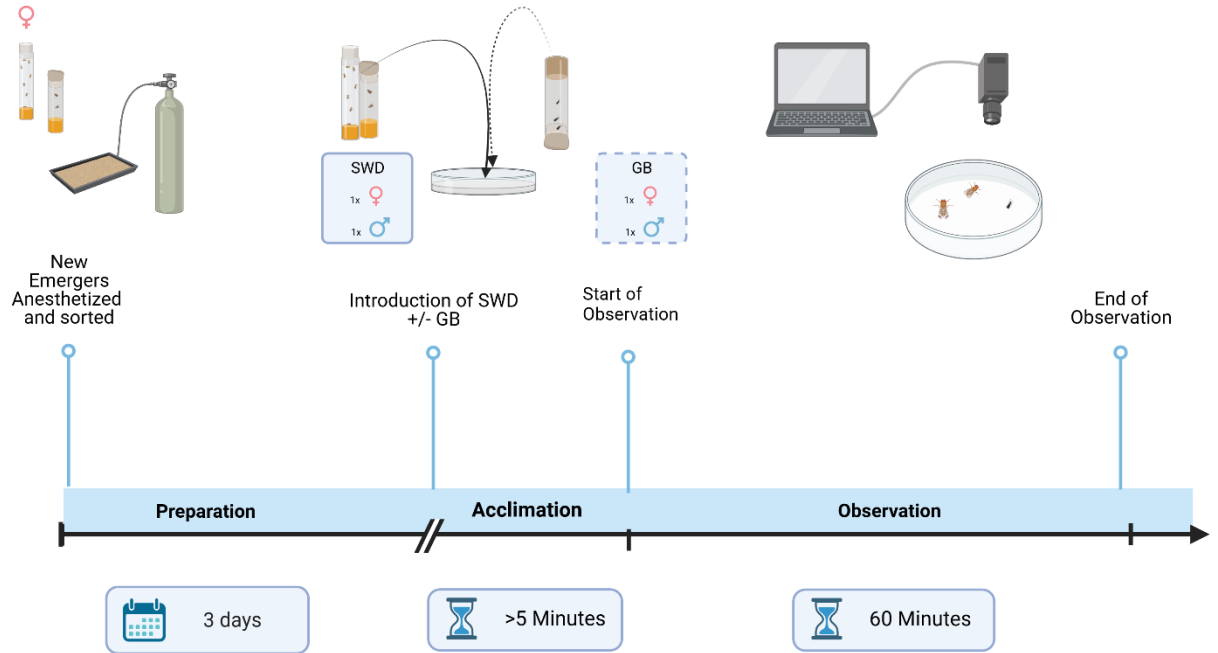


Figure 1. Timeline of Preparation & Experiment for Behavioral Assay.

Recorded videos were viewed and event coded using Behavioral Observation Research and Interaction Software (BORIS) (Friard & Gamba, 2016). Videos recordings were reviewed at least twice for each recording, with the initial coding reviewed for accuracy during the second viewing. Coding took place by the selection of keyboards mapped to the applicable events within BORIS. The duration of observation was time limited to the defined period (60 minutes). Observations took place in one of two forms: point events, describing an instance at a particular timepoint, and state events, which record the duration of an event (Friard & Gamba, 2016). Events were recorded as being assigned to a defined focal subject (the replicate the behavior was occurring in).

The following point events were recorded: physical interaction between *G. brasiliensis* and *D. suzukii* (i.e. antennation), courtship behavior (between *D. suzukii*) and mating. Courtship behavior was defined as any of the behaviors described in the

literature: orienting, tapping, singing, and mounting (Bontonou & Wicker-Thomas, 2014). A single state event, significant locomotion, was recorded. This was defined as a movement of three or more steps in close succession. The event was recorded as having begun at the initiation of movement and stopped when movement ceased.

	Time	Frame index	Subject	Code	Type	Modifier	Comment
23	00:08:25.233	15157	B1	GR	STOP		
24	00:08:25.967	15179	B1	CS			Would be good...
25	00:09:12.567	16577	C2	GR	START		
26	00:09:24.733	16942	C2	GR	STOP		
27	00:10:42.567	19277	B1	GR	START		
28	00:11:05.000	19950	C2	GR	START		
29	00:11:21.867	20456	B1	XI			
30	00:12:16.900	22107	C1	GR	START		
31	00:12:24.667	22340	C2	GR	STOP		
32	00:12:48.733	23062	B1	GR	STOP		
33	00:13:23.600	24108	B1	GR	START		
34	00:14:40.767	26423	C1	GR	STOP		
35	00:15:11.100	27333	C2	GR	START		
36	00:15:13.567	27407	B1	CS			
37	00:15:28.800	27864	A1	GR	START		
38	00:15:40.200	28206	B1	GR	STOP		
39	00:15:40.767	28223	B1	CS			
40	00:16:03.767	28913	A1	GR	STOP		
41	00:16:06.600	28998	C2	GR	STOP		
42	00:17:47.600	33038	B1	GR	START		

Figure 2. Example of Event Recordings in BORIS.

Following behavioral coding in BORIS, behavioral events were imported in Microsoft Excel (Microsoft Corporation, Redmonda, WA, USA., where tabulation of results was completed using pivot tables. This data was then exported as a comma separate values file (.csv) for import into R and JMP.

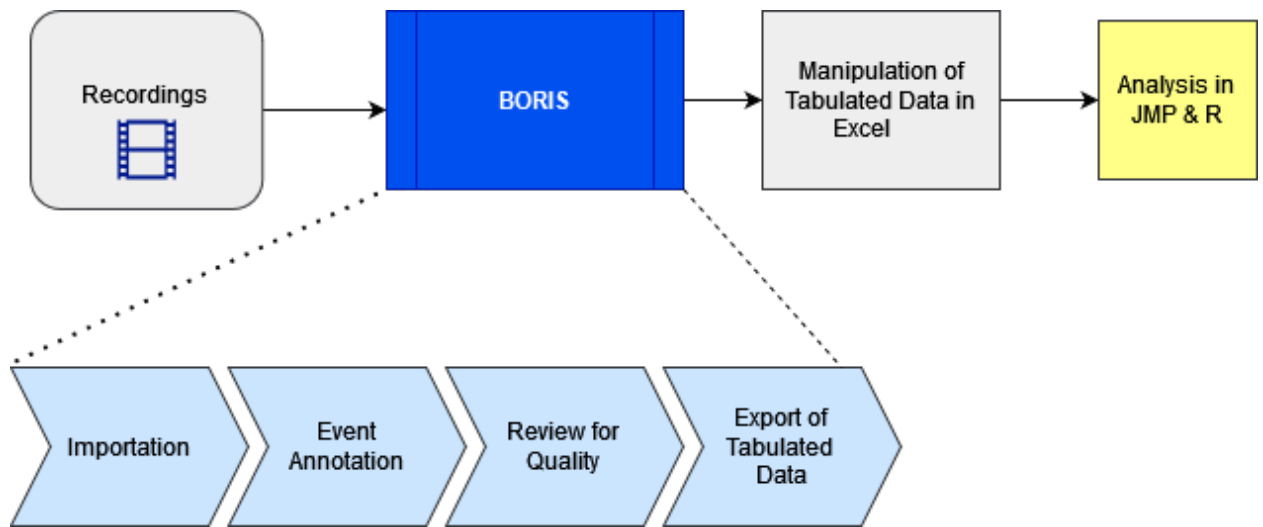


Figure 3. Steps of Behavioral Assay Data Processing & Analysis Process.

Oviposition Assay

To assess the impact of exposure to *G. brasiliensis* on oviposition, ten adult *D. suzukii* (5 male and 5 female) flies were used in an oviposition assay. All flies were 5-7 days old and females were gravid. Flies were anesthetized with CO₂ and placed inside an 8-ounce deli cup containing fifteen (15) conventionally grown blueberries obtained from a grocery store. Five female *G. brasiliensis* were added to the experimental replicates (n = 20). A similar number of *D. suzukii*-only cups were set up as controls (n = 18). Additionally, several fruit-only controls, containing only blueberries, were set up (n = 3). Cups were maintained in a growth chamber at 25°C on a 16:8 photoperiod for 48 hours to allow infestation to occur. After 48 hours elapsed, all adult *D. suzukii* and *G. brasiliensis* were removed via entomological aspirator. The cups were then returned to the incubator for 5 days to allow for larval growth.

The salt flotation method as described by *VanTimmeren et al*, 2017 was used to assess the presence of larvae in fruit as a marker of oviposition. In brief, a saline solution

was made by combining 1 cup of household table salt (Iodized salt, Morton) in 1 gallon of warm tap water. Berries in each of the deli-cups were submerged in the saline solution for one hour at room temperature to allow larvae to emerge. After 1 hour, the solution was passed through a coffee filter to isolate the larva, which were counted using a dissecting microscope (Nikon, Nikon Corporation, Tokyo, JP).

Statistical Analysis

JMP®, Version 16 (SAS Institute Inc., Cary, NC, USA) was used for all statistical analysis. All data were tested for normality and deviations from homoscedasticity using the Shapiro-Wilk test and Levene's test, respectively. All data were determined to be non-normal, and transformations were not successfully able to correct the normality. For the behavioral bioassays, Wilcoxon tests were used to look at the difference in the record behaviors. Data for the Oviposition assays was assessed using a Kruskal Wallis test, with post-hoc being performed by Wilcoxon each pair test.

RESULTS

Behavioral Assay

For the behavioral bioassays, the number of observed courtship (CS), significant movement (GR), cross species interaction (XI) and mating (MT) were determined and the percentage of each event type of the total number of recorded events was calculated. Overall cross species interaction in the SWD + GB treatment was low, and there was no significant difference in the percentage of courtship (CS) ($X^2 = 1.20$, d.f.= 1, $P < 0.2733$) and the significant movement (GR) ($X^2 = 0.36$, d.f.= 1, $P < 0.5475$). Despite observed courtship, no mating was observed in any of the bioassays. Thus, the duration of copulation events could not be obtained.

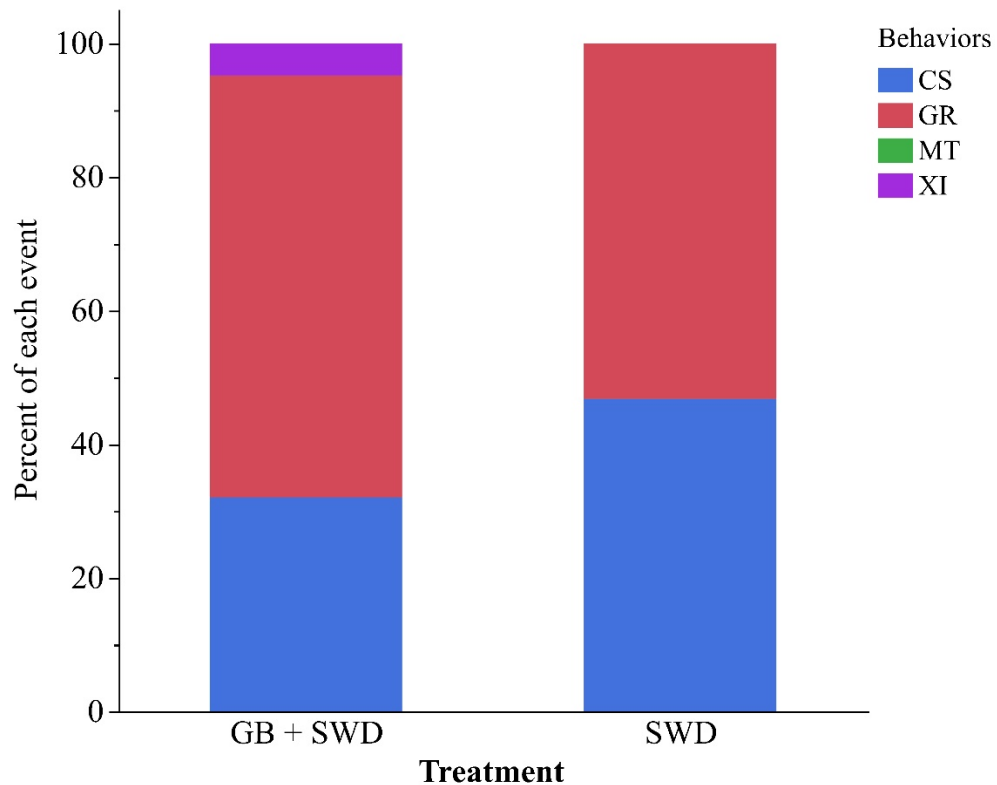


Figure 4. Average percentage of different behavioral events of observed courtship (CS), significant movement (GR), cross species interaction (XI) and mating (MT) in bioassay containers.

Oviposition Assay

In the oviposition assay there was significant difference by the different treatments ($X^2 = 28.48$, d.f.= 2, $P < 0.0001$) (Fig. 5). The highest infestation was seen in the *D. suzukii* only (n = 20) group that had an average (\pm S.E.) number of larva of 15.6 (\pm 1.75). This was significantly higher ($P < 0.05$, Wilcoxon test) than both the *D. suzukii* + *G. brasiliensis* (n = 18) and the fruit only groups (n = 3) that had an average (\pm S.E.) of 3.2 (\pm 0.73) and 0.6 (\pm 0.66) larvae, respectively. Some background infestation was seen in the fruit-only treatment, but just two larvae in one of the three replicates. This larva appeared to be of a drosophilid species.

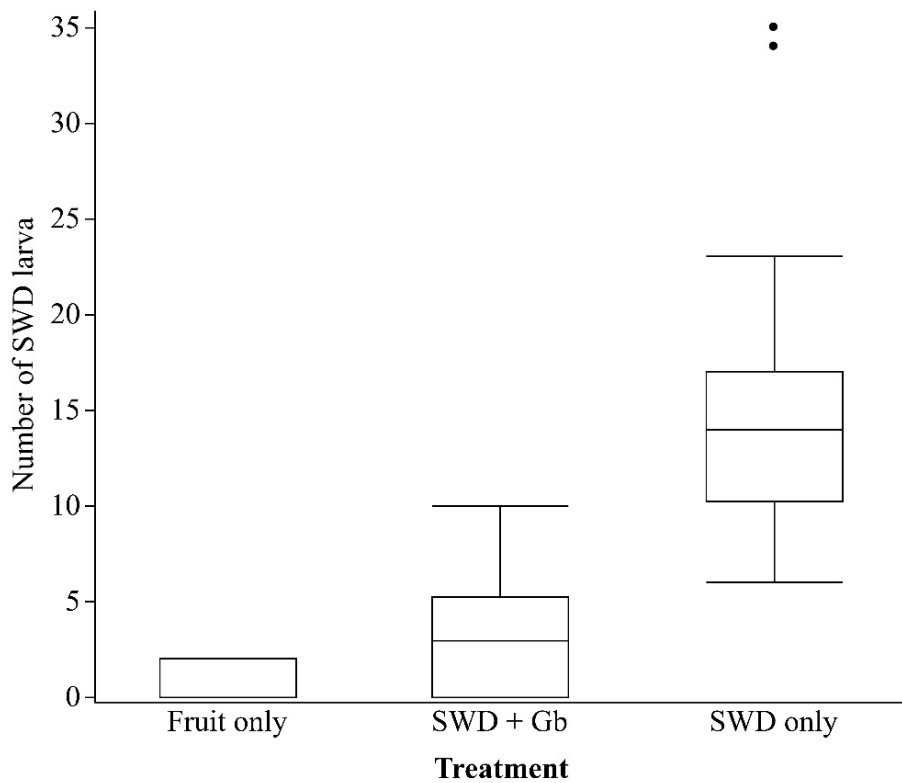


Figure 5. Average number of *Drosophila suzukii* (SWD) larva in blueberries with unexposed (Fruit only), exposed to *D. suzukii* only (SWD only), and exposed *D. suzukii* the presence of *Ganaspis brasiliensis* (SWD + Gb).

DISCUSSION

The interpretations that can be drawn from the behavioral assay in this experiment are limited but suggest that the presence of *G. brasiliensis* does not impact courtship behavior. This experiment had several notable limitations. Additionally, the behaviors observed demonstrated significant variance in the composition and frequency of the behaviors across replicates, making interpretation challenging. The behavioral observation assay used in this experiment was underpowered, limiting the conclusions that can be drawn from it. It is possible that using a larger quantity of replicates would be of greater utility.

The “bumping” of *G. brasiliensis* antennae with *D. suzukii*, a form of interspecific antennation, was observed, but did not appear to be associated with courtship or geotactic behavior in this experiment. This may indicate that adult *D. Suzukii* do not attempt to avoid *G. brasiliensis*, but the mating arena assay used was not designed to detect this before. The significance of direct physical interactions between parasitoids and adult *Drosophila* has not been well-characterized in the literature and may warrant further investigation. Interpretation of direct interspecific contact in this experiment was of limited utility due to the use of only one parasitoid and one host species.

The immune-behavioral trade-off hypothesis in *Drosophila* would predict poor behavioral avoidance in *Drosophila* species with greater immunological defense. An inverse correlation between changes in reproductive rate (as measured by oviposition) and immune response (as measured by eclosion) have been reported in *D. simulans* and *D. melanogaster* exposed to *L. bouvardi* (Lefèvre et al., 2011). However, a study of numerous *Drosophila* species (including *D. suzukii*) exposed to *L. bouvardi* and *L.*

heterotoma failed to replicate this association, questioning the validity of this hypothesis (Lynch et al., 2016). *D. suzukii* is known for having a robust immunological response to many endoparasites, as measured by eclosion rates and hemocyte load (Kacsoh & Schlenke, 2012). This experiment was inconsistent with that hypothesis, with the females exposed to *G. brasiliensis* displaying a significantly reduced rate of oviposition. The results of the oviposition assay in this experiment provide further evidence against the existence of tradeoff between immunological and behavioral defense against parasitism in *Drosophila*.

Unfortunately, the behavioral observation study had to be altered significantly as the primary endpoint (copulation) originally designated failed to occur in the laboratory setting. Copulation frequency was the original endpoint intended to measure reproductive behavior. This variable is commonly used in the study of reproductive behavior in *drosophila* (Ejima & Griffith, 2007). Unfortunately, only a single instance of mating was observed in a pilot experiment. No instances of mating were observed in the behavioral observation experiment over the one-hour duration of observation across all replicates (n = 38).

Efforts were made in pilot experiments to adjust environmental parameters to encourage mating, such as the use of a smaller enclosure, but these efforts were unsuccessful. Additionally, virgin flies were used in an effort to increase the odds of successful mating, with literature suggesting that virgin males have greater reproductive activity than non-virgins in *D. melanogaster* (Koppik et al., 2018; Ruhmann et al., 2018). This too was unsuccessful. Across all groups, courtship behavior was readily observed (See Figure 4) but failed to progress to mating. The reason behind this is unknown and

may be multifactorial. The failure of *D. suzukii* to mate in the laboratory setting was unexpected and necessitated the use of alternative methods.

The failure of mating behavior to occur under laboratory conditions may be reflective of species-specific differences. At the time of writing, literature on *D. suzukii* reproduction is extremely limited, with no publications describing mating in laboratory conditions. Existing publications are focused on mortality and reproduction in field trials (Krüger et al., 2021; Revadi et al., 2015). *D. suzukii* will certainly mate in the laboratory setting, as there are several laboratories successfully maintaining colonies, but this normally occurs in media vials containing many drosophilae. It is possible that the seclusion of a single couple in close proximity may be detrimental to the mating process.

Environmental perturbations resulting from manipulation and rearing are another possible cause of the failure to mate. The inadvertent exposure of control group (unexposed) flies to pheromones is a possibility. Species were stored on different vertical levels, but the presence of some form of contamination is plausible. The role of olfaction in behavior response to predators in *Drosophila* has been well documented (Ebrahim et al., 2015). This issue would be specific to the behavioral observation assay, as the flies used in the oviposition assay were from a chamber only used to store drosophilae.

The anesthesia used in the sexing of newly emerged flies is another potential confounding factor. Anesthesia with carbon dioxide (CO₂) gas is well-known known to reduce copulation latency in *D. melanogaster* (Barron, 2000; Joachim & Curtsinger, 1990). There is no data available on when this effect dissipates completely, with the longest-duration recorded indicating that copulation depression was still present 20 hours later. (Barron, 2000). In totality, there are many variables that could have prompted the

lack of mating observed in the behavioral observation assay. Behavioral research specific to *D. sukukii*, particularly within the laboratory setting, is limited. This property made behavioral observation and it's the interpretation of this assay challenging.

CONCLUSION

This experiment had mixed results, most notably a failure of *D. sukuii* to mate in the laboratory environment. This resulted in a challenging behavioral assay producing results that demonstrated no impact among all measured behaviors (courtship and movement). However, the results of the oviposition assay were encouraging, demonstrating that female *D. sukuii* display decreased oviposition in this presence of *G. brasiliensis*. This reduction in reproductive output is a notable finding. Although oviposition depression in predators has been described in response to other parasitoids, this finding has not been reported in *G. brasiliensis*. This is an encouraging finding for integrated pest management (IPM) researchers who are seeking to employ *G. brasiliensis* as a biocontrol agent, suggesting that its mere presence may assist in the suppression of *D. sukuii*.

The results from the behavioral assay were disappointing, with no associations found. An inability to observe mating in the laboratory environment despite significant effort was unexpected. However, given the relative novelty of research on *D. sukuii*, such a finding may be reflective of its behavior differing from *D. melanogaster* because of its relative evolutionary distance.

In conclusion, the presence of *G. brasiliensis* was found to alter the oviposition behavior of the invasive pest *D. sukuii*, dampening its reproductive output. However, no changes in courtship behavior were observed in a behavioral assay. Oviposition depression in response to co-location with *G. brasiliensis* has not been described in the literature. *G. brasiliensis* may act on both adults (via behavioral response) and larvae (endoparasites) to decrease reproductive output of *D. sukuii*. This experiment

demonstrated that co-location with *G. brasiliensis* alters reproductive behavior in adult *D. suzukii*, lowering reproductive success and providing an encouraging sign for the suitability of *G. brasiliensis* as a biocontrol agent.

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