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Biological control agents for spotted wing drosophila in Virginia

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Within the last decade, two species of exotic drosophilids have invaded North America. Since its arrival in 2008, the spotted wing drosophila (*Drosophila suzukii,* henceforth SWD) has become a widespread economic pest of small soft-skinned fruits due to its ability to oviposit in intact, ripening fruit (Hauser 2011, Walsh et al. 2011). Less known is the African fig fly (*Zaprionus indianus,* henceforth AFF), which was discovered in Florida in 2005 (van der Linde et al. 2006). This tropical-subtropical species was already established in South America as an important pest of fig production, but also has a wide host range and has been documented as a highly adaptable species (van der Linde et al. 2006, da Mata et al. 2010). In Virginia, AFF is now often observed concurrently with late-season infestations of SWD, especially in vineyards (Pfeiffer 2012). Though SWD is the more important pest in North America, due to interactions with AFF, it was deemed appropriate to include AFF in this study.

This study investigates the potential for using native parasitoids of drosophilids in Virginia as conservation biological control agents against SWD and AFF, and provides baseline information for future biological control endeavors regarding drosophilids. The project includes two objectives: 1) Use sentinel traps to determine which parasitoids of drosophilids are present in southwestern VA small fruit production, and if they can successfully attack SWD and/or AFF in the field, and 2) Perform parasitization bioassays in the laboratory to determine if native parasitoids can successfully attack SWD and/or AFF under controlled conditions.

Sentinel Trapping Methodology:

During the 2015 field season, sentinel traps were placed in a cherry orchard, a caneberry field, a blueberry farm, and two vineyards in southwestern Virginia. Half of the sentinel traps contained a Petri dish with ~50 g of banana, the other half contained ~50 g of the same type of fruit as the cropping system. Prior to placement in the field, fruit was seeded with larvae of SWD, AFF, or *Drosophila melanogaster*, or left uninfested for control traps. Twelve to sixteen traps were placed on the edge and interior of each cropping system. Bait dishes were collected after 3-4 d in the field and subsequently replaced. Larvae/pupae were allowed to complete development in the laboratory and observed for fly/parasitoid emergence. For each crop type, 21-24 trapping days were accumulated per trap, excluding AFF sentinel traps, which

had 6-8 trapping days per trap (Table 1). AFF sentinel traps were only placed out later in the season once AFF was detected in the system, so no AFF sentinel traps were placed in the cherry orchard because cherries are an early season crop.

Table 1. Date ranges and accumulated trapping days for sentinel trapping during 2015 field season.

Crop Type	Date Range (2015)	Trapping Days (per trap)
Cherry	5/18 – 6/26	24
Caneberry	7/6 – 10/9	23
+*Z. indianus*	9/17 – 21, 10/6 – 9	(7)
Blueberry	8/6 – 9/15	21
+*Z. indianus*	9/4 – 8, 9/11 – 15	(8)
Grape	8/4 – 9/18	22
+*Z. indianus*	9/8 – 11, 9/15 – 18	(6)
All	5/18 – 10/9	92

Sentinel Trapping Results:

The larval parasitoid *Leptopilina* spp. (Figitidae) was the most abundant parasitoid reared from the traps, and the generalist pupal parasitoid *Pachycrepoideus vindemiae* (Pteromalidae) was reared in lower numbers (Figure 1). Parasitoids emerged from baits that were in the cherry orchard and caneberry field, but not the blueberry farm or vineyards. In the cherry orchard, only one individual of *P. vindemiae* was reared from SWD, and this species has previously been documented to parasitize SWD in the field (Stacconi et al. 2013). All other parasitoids from both sites were reared from *D. melanogaster* or other ambient drosophilids that had infested the traps in the field. None were reared from AFF.

Interestingly, in the cherry orchard, more parasitoids were reared from the banana bait dishes than the cherry bait dishes, while in the caneberry field, parasitoids were only reared from the caneberry bait dishes and not from the banana bait dishes (Figure 1). This raises the question, are parasitoids of drosophilids attracted to certain fruit odors more than others when it is time to oviposit?

There was also an edge effect observed in that more parasitoids were reared from traps placed on the edge of the cropping system than those in the interior (Figure 2). There are several possible explanations for this observation: the parasitoids might be entering the system from the nearby wooded habitats; the edges of these sites were shadier, and therefore somewhat cooler than the interiors, and the parasitoids may prefer the shade/cooler temperature; there could simply be more wild hosts around the edges, so there would naturally be more parasitoids around the edges as well.

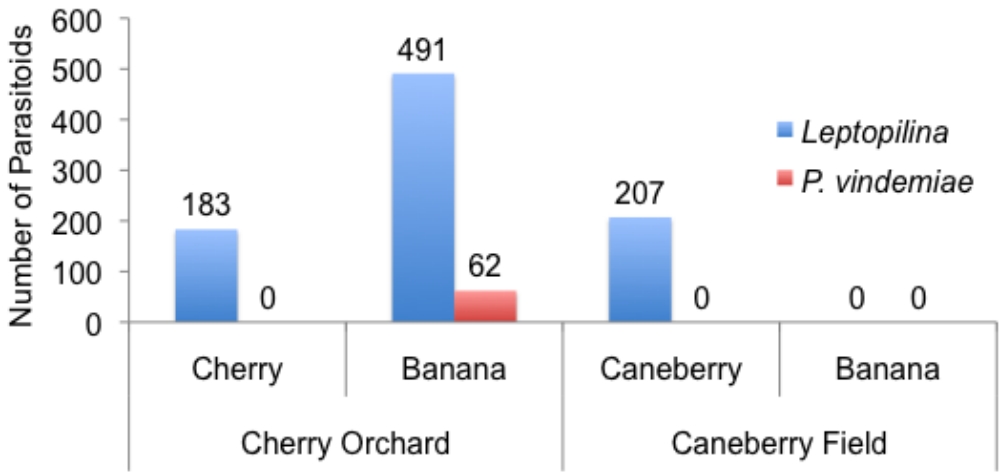

Figure 1. Parasitoid emergence from sentinel traps with respect to bait type.

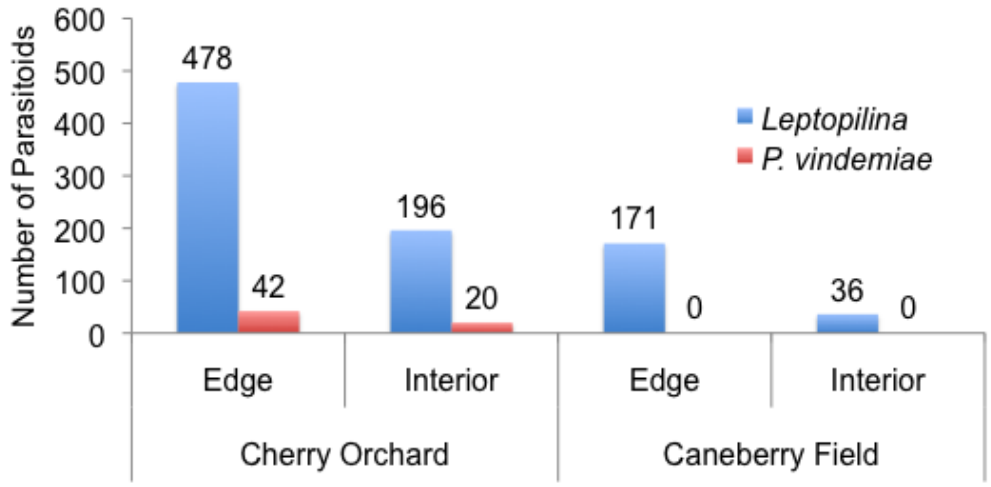

Figure 2. Parasitoid emergence from sentinel traps with respect to trap placement.

Parasitization Bioassay Methodology:

Fifty 2nd-instar larvae of *D. melanogaster*, SWD, or AFF were placed in a 35-mm Petri dish filled with ~1 mm depth of rearing media. The larvae were then exposed to three mated females of *Leptopilina* for 72 h in a rearing bottle at 26ºC, with 12-h daylength. After 72 h, the parasitoids were removed from the bottle, and 10 “wandering” maggots were collected and observed under the microscope for parasitoid eggs and encapsulation (Figure 3). The remaining larvae were allowed to complete development in the incubator. This experiment was modeled after Kacsoh and Schlenke (2012), and had six replications for each fly species.

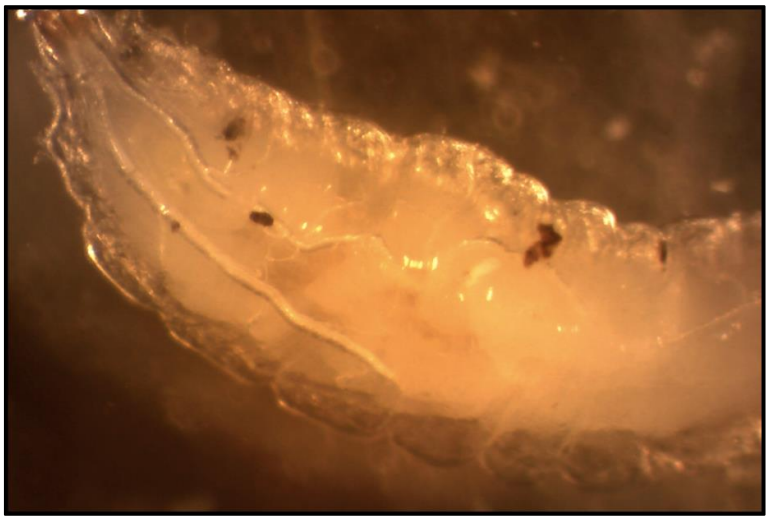

Figure 3. Posterior end of a parasitized *Drosophila* larva. Parasitoid eggs have been encapsulated, indicated by the black spots.

Parasitization Bioassay Results:

Based on microscope observations, *Leptopilina* attacked SWD and *D. melanogaster* at a greater rate than they attacked AFF (Table 2). Observations indicated that *D. melanogaster* and AFF had a greater encapsulation rate than SWD, which is inconsistent with Kacsoh and Schlenke (2012), which showed SWD to be much more successful at encapsulation than *D. melanogaster*. It is unclear why these results were so different. However, once the remaining larvae completed development, results were more as expected (Figure 4). *Leptopilina* was able to successfully parasitize *D. melanogaster*, but not SWD or AFF. Why then were the microscope observations so inconsistent? Perhaps the environmental conditions were not optimal for SWD, and it took a longer time for larvae to complete the encapsulation process. Furthermore, in the case of *D. melanogaster*, where >80% of observed parasitoid eggs were encapsulated and yet 65% of flies were successfully parasitized, perhaps encapsulation occurs regardless and some parasitoid eggs/larvae are able to overcome it in *D. melanogaster,* but not in SWD or AFF.

Table 2. Attack rates of *Leptopilina* on three drosophilid species and larval encapsulation rates of *Leptopilina* eggs by those species, based on microscope observations.

Fly Species	Mean Parasitized Larvae	Attack Rate	Mean No. Eggs Laid	Mean encapsulated eggs	Encapsulation Rate
D. melanogaster	4.8	48%	10.2	8.5	83%

D. suzukii	5.7	57%	13.8	8.3	60%
Z. indianus	1.2	12%	1.5	1.3	87%

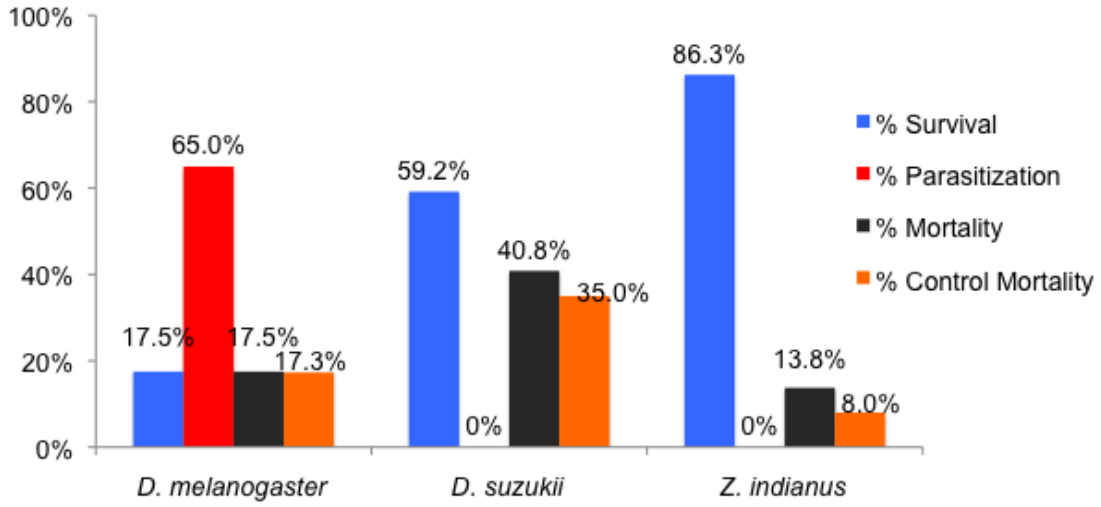

Figure 4. Survival, parasitization, and mortality rates of drosophilids exposed to 3 mated females of *Leptopilina* spp. for 72 h.

Conclusions:

Based on results from this study, we can conclude that native parasitoids in Virginia are not likely to be effective biological control agents against SWD or AFF. Sentinel traps did not produce a parasitoid that could attack either fly species with much success, and laboratory trials showed SWD and AFF are both unsuitable hosts for the native *Leptopilina* spp. that was tested. However, these results do support the case for classical biological control. Furthermore, the edge effect observed in our field study should be something to take note of when utilizing biological control for these pests.

Next Steps:

We will complete statistical analyses for the data presented here, and perform another parasitization bioassay using *P. vindemiae*. We will also investigate the relative attractiveness of different fruit odors to parasitoids of drosophilids with olfactometer bioassays.

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