**North American Bramble Growers Research Foundation**

**Proposal Category:** Production Research; Pest Management Strategies

**Final Report**

**Title**: Effects of the mating and reproductive status of spotted wing drosophila females on their attraction to fermentation-based baits and ripe fruits

**Name, Mailing and Email Address of Principal Investigator(s):**
Hannah J. Burrack
Department of Entomology
North Carolina State University
Raleigh, NC 27695-7634
Email: hjburrac@ncsu.edu

Katharine A. Swoboda Bhattarai
Department of Entomology
North Carolina State University
Raleigh, NC 27695-7634
Email: kaswobod@ncsu.edu

Office phone: 919-513-4344
Fax: 919-515-3748

**Rationale**

*Drosophila suzukii* (Matsumura), the spotted wing drosophila (SWD), is a highly invasive vinegar fly first detected in the eastern United States in 2009[1]. Females use their saw-like ovipositor to lay eggs in ripe and ripening fruit and severely threaten the viability of raspberry, blackberry, blueberry, cherry, and strawberry production[2]. Currently, there is zero tolerance for larvae in fresh market fruit and a single infested fruit can result in the rejection of an entire shipment. Current management programs for SWD rely heavily on insecticide applications and are not economically or environmentally sustainable[3]. For example, many caneberry growers in North Carolina made one or no insecticide applications during harvest in 2010, but may now make eight or more applications of broad-spectrum insecticides such as malathion, zeta-cypermethrin, and spinetoram in rotation throughout the long fruiting season. Despite this dramatic increase in insecticide use, some fields that have been sprayed multiple times still contain larvae in 100% of fruit[4], suggesting that insecticides alone may not provide effective SWD control in the rainy and humid southeastern US.

Monitoring programs that reliably predict infestation risk for growers are not currently available, although several studies have tested the effectiveness of different trap designs[5,6] and bait formulations[7,8,9] at catching SWD. In the most comprehensive study to date, Burrack et al. (2015) compared how attractive six fermentation-based baits were to SWD when deployed in a variety of cropping systems in ten US states. Five of the tested baits caught SWD between 1 and 2 weeks earlier than apple cider vinegar, a commonly used standard, and detected the presence of SWD prior to the development of fruit infestation. However, fewer females with mature eggs in their ovaries (Fig. 1) were captured in traps after ripe fruits were available in the field, suggesting that females with mature eggs may be more attracted to ripe fruit when it is present than to traps with fermentation-based baits[10]. Therefore, such baits may underestimate the presence of egg-laying female flies in the presence of ripe fruit.

**The goals of this research were to determine if reproductive and mating status affect the attraction of SWD females to fermentation-based baits and ripe fruit, and to obtain important information about the mating system of SWD.** This research has broad implications for SWD monitoring and the development of effective baits. This work will also provide needed context to results of previous studies in which traps with fermentation-based baits were used to catch SWD. More broadly, determining if there are patterns associated with SWD attraction to fermentation-based baits and oviposition behavior is essential for the development of effective, landscape-level management programs for SWD. Such information would help improve SWD monitoring tools and help growers, land managers, and researchers target tools to reduce SWD infestations, decrease pesticide use, and minimize economic losses.

**Objectives**

1. Determine if mating and reproductive status differs between SWD females collected on the surface of monitoring traps, within traps, and on ripe fruit.
2. Estimate SWD sperm loads by determining the number of progeny produced by 1) females after a single copulation in the laboratory and 2) wild-caught females.

**Procedure**

*Objective 1: Determine if mating and reproductive status differs between SWD females collected on the surface of traps, in traps, and on ripe fruit.*

Prior activities

In October 2013, we collected SWD females within traps containing a fermentation-based bait[10] and aspirated SWD females off ripe raspberries and blackberries at Upper Mountain Research Station (UMRS) in Ashe County, NC. In 2014 and 2015, we deployed SWD monitoring traps within crop fields and in areas between crop fields and an adjacent wooded edge biweekly from June-August at two commercial blackberry farms in Cleveland County (CC), NC. We checked the traps, which contained a homemade fermentation-based bait made from yeast and sugar[10], hourly for 24 hours except when it was dark. During the experiment, we noticed that many SWD and other drosophilid flies were present on the outside of traps but did not necessarily enter them, and observed male courting behavior, male-male aggression, and mating pairs on the surface of some traps. Therefore, starting halfway through the 2014 season, we aspirated flies off the surface of traps for one minute before we collected flies from within the traps. During both studies, we identified, sexed, and counted all the SWD caught at monitoring traps and on ripe fruits and preserved them in 70% ethanol.

While encouraging, these experiments were "snapshots" over a limited geographic area and provided us with an incomplete understanding of how the reproductive status of SWD females affects their attraction to monitoring traps and/or ripe fruits. Therefore, we proposed to develop a truly representative dataset by 1) catching additional females for dissection during a focused field experiment during the 2016 growing season, and 2) completing additional dissections of previously captured SWD females to determine mating status in addition to reproductive status (i.e., the number of mature eggs present in the ovaries).

Project activities

On 20-21 July 2016, we conducted an experiment in a mixed research planting of 'Ouachita' and 'Von' blackberries at Piedmont Research Station (PRS) in Rowan County, NC, in which we concurrently collected SWD females on the surface of monitoring traps, within traps, and on ripe berries. We set up eight monitoring traps within the planting; traps consisted of 32 fl. oz. clear plastic cups and lids (DeliPRO brand, Tri-pack Industrial USA, White Plains, NY) with 10 equidistant holes drilled near the top and were baited with 1.69 g of dry active yeast, 8.45 g of sugar, and 150 ml of water. Traps were suspended within the canopy on 48" plastic step-in fence posts and positioned along on the northern, shady side of three ~36m long rows of 'Von' blackberries. Traps were placed ~12m apart from each other, with three traps placed in two of the rows and two traps placed in the third row.

Flies were collected by walking between traps in a loop until all eight traps had been checked for activity. At each trap, we aspirated flies off the surface of the trap for one minute using a handheld aspirator fitted into a 50ml conical centrifuge tube for one minute. Flies were then collected from within the traps; all flies were collected from the surface of the bait using soft forceps, after which the contents of each trap were poured through a handheld kitchen strainer to obtain any flies that may have drowned. We also collected flies from ripe berries located in the general vicinity of each trap by placing a small ~4x7" mesh bag behind a berry with flies on it, blowing the flies into the bag, and quickly placing a small piece of foam within the drawstring closure to trap flies within the bag. Flies collected at all three locations were transferred to vials with 70% ethanol for preservation.

Traps were set up by 4:45pm on 20 July and were checked for fly activity every half hour starting at 5:30pm. Flies started to become active around 7pm and we continued to collect flies while daylight persisted (sunset was at 8:34pm, while last light occurred at 9:03pm). We returned in the morning on 21 July and collected flies from 6:45 – 9am (first light occurred at 5:52am, while sunrise was at 6:21am). The clouds burned off by ~8:20am and there was very little activity observed at the monitoring traps by ~8:40am.

All SWD females that were caught on the surface of monitoring traps, within traps, or on ripe fruits during the three experiments were dissected to determine their reproductive status and mature egg load using methods previously developed by our laboratory[10]. Females were dissected under a stereomicroscope (Olympus SZX10, Center Valley, PA) and the total number of mature eggs in both ovaries were counted; eggs are mature once they possessed fully formed respiratory filaments (Fig. 1). Ovarian development was also ranked as follows: 1) No eggs present and ovaries are small; 2) Only developing, immature eggs present; 3) Mature and immature eggs present; 4) Only mature eggs present. For each female, we preserved the paired spermathecae, which is the storage organ in which flies hold sperm from previous mating events, in 70% ethanol. We will determine the mating status of each female by crushing the spermathecae in a 2% aceto-orecin solution and examining them for the presence of sperm, which will determine whether females were mated or unmated when collected. We will then combine information about mating status and reproductive status of individual females to determine if females that were captured on the surface of traps, within traps, or on ripe fruit differ per this combined metric.

Further work on mating status, addressing the questions associated with Objective 2, will be conducted in the laboratory during winter 2017

**Results**

At UMRS in 2013, the 23 SWD females aspirated from ripe berries had an average of 4.78 mature eggs in their ovaries (range = 0-14), whereas the 29 SWD females caught within traps had an average of 0.79 mature eggs (range = 0-7). SWD females aspirated off the surface of ripe berries were more likely to have one or more mature eggs present in their ovaries ($F_{1,50}$ = 16.19, $P$ = 0.0002) and had higher numbers of mature eggs in their ovaries ($F_{1,50}$ = 29.35, $P$ < 0.0001) than females collected in traps (Fig. 2).

At one commercial blackberry farm sampled in 2014, on the sampling date when we caught the most SWD overall (25-26 July), we aspirated 58 SWD females off the surface of traps and collected 11 SWD females from within traps during the 24-hour collecting period. Most females had developing eggs present in their ovaries but contained no mature eggs. Female SWD collected on the outside vs. inside of traps were equally likely to have one or more mature eggs in their ovaries ($F_{1,67}$ = 0.05, $P$ = 0.83). Females aspirated off the surface of traps had 0.86 mature eggs present on average (range = 0-10), while females caught in traps had 0.45 mature eggs present on average (range = 0-2), but the number of mature eggs was not significantly different for SWD females collected on the outside vs. inside of traps ($F_{1,67}$ = 0.00, $P$ = 0.98) (Fig. 2).

At Piedmont Research Station in 2016, we collected 43 SWD females on the surface of monitoring traps and 189 SWD females – 132 of which were dissected – within traps, and aspirated 55 SWD females off the surface of ripe fruit. Female SWD collected in the three locations were not equally likely to have one or more mature eggs in their ovaries ($F_{2,227}$ = 8.64, $P$ = 0.0002). SWD females collected within traps had fewer mature eggs in their ovaries (average = 0.29 eggs, range = 0-7) than SWD females aspirated off the surface of traps (average = 0.98 eggs, range = 0-10) and SWD females aspirated off ripe fruits (average = 2.00 eggs, range = 0-19) ($F_{2,113}$ = 10.98, $P$ < 0.0001) (Fig. 2).

**Interpretation**

<u>It appears that SWD females with fewer mature eggs in their ovaries are predominately attracted to monitoring traps</u>. Conversely, SWD females collected on ripe fruits had at least twice as many mature eggs in their ovaries as flies captured at monitoring traps. This has practical implications for how we interpret monitoring trap data. We are not capturing the same flies that are infesting fruit. Instead, we are likely capturing flies before they are capable of infested fruit because they are seeking food sources necessary to develop eggs.

We had predicted that SWD females may be differentially attracted to traps with fermentation-based baits and ripe fruits as follows:
1) Recently emerged, unmated – more likely to be found on **outside of traps**; seeking mates
2) Mated, but not yet laying eggs – more likely to be found **within traps**; seeking nutrients
3) Mated, egg-laying – more likely to be found **on fruit**; seeking oviposition sites

However, data from the three experiments suggest a different pattern. Instead, it now seems as though the following revised hypothesis is likely:
1) Recently emerged, unmated – more likely to be found **within traps**; seeking nutrients to start the egg maturation process
2) Fed, but still unmated – more likely to be found on **outside of traps**; seeking mates
3) Mated, egg-laying – more likely to be found **on fruit**; seeking oviposition sites

We plan to dissect the spermathecae from collections during Spring 2016, which will provide important information that either supports or refutes our revised hypothesis and that helps to more completely explain our results.

Recent laboratory studies[20] suggest that females can get inseminated within 24-hours of emergence, but did not produce offspring until 2.5 days after emergence under the test conditions in both cases. Therefore, it appears that female SWD – under optimal conditions –

have around 2-3 days to find a protein-rich food source to jump start ovary maturation, find a mate (or more than one mate), and find suitable substrates within which to lay their maturing eggs. This may provide a window of opportunity for targeting young females before they are ready to start laying eggs in fruit crops.

Physiological state has been shown to affect attraction to volatile odor cues in related flies. In a study of long-range attraction, headspace volatile from vinegar attracted 62% of assayed *D. melanogaster* flies irrespective of age, sex, and mating status - provided the flies had been starved[21]. In the case of SWD, reproductively mature, egg-laying females might be attracted to odors produced by fermenting fruits – or monitoring traps – when feeding is the goal, but are likely more attracted to ripening and/or undamaged ripe fruits when oviposition is the goal[25,26]. Therefore, once females start laying eggs, they may be less attracted to monitoring traps, provided they can find sufficient food elsewhere in the system.

Our results support this idea. The plot at UMRS in 2013 was untreated and unmanaged, so ripe fruits were not harvested and remained in the field to ferment and rot, providing flies with food resources. Several SWD females collected on berries had purple bellies indicating that they had been eating juice from unharvested, rotting berries. In contrast, the plot at Piedmont in 2016 was untreated, but all ripe fruits were either bagged to prevent oviposition or collected for other experiments, so there was less food available in the plot compared to UMRS. All the SWD females dissected from Piedmont had clear bellies. This could explain why females collected on ripe fruit at UMRS in 2013 had twice as many mature eggs in their ovaries as SWD females collected at PRS in 2016. This could also explain why some females with quite a few mature eggs in their ovaries were collected on the surface of traps at PRS in 2016 (range was up to 10). In addition, environmental characteristics and condition likely affected our results, which may have been due in part to differences in temperature and relative humidity between the UMRS and PRS sites. Females caught on ripe berries at UMRS had twice as many mature eggs in their ovaries as female caught on ripe berries at PRS. Females were collected in early October at UMRS in 2013, whereas females were collected during late July at PRS in 2016. Temperature and relative humidity have individual and combined effects on SWD longevity and reproduction; for example, higher levels of relative humidity have been shown to increase the intrinsic rate of population increase in SWD, resulting in more progeny produced per female SWD[22].

In conclusion, our results provide evidence that points to differing levels of attraction to monitoring traps with a fermentation-based bait and to ripe fruit by females at different developmental stages. Future studies should be conducted in controlled laboratory environment to test newly emerged, unmated females and mated, reproductively mature females for differences in sensitivity to fermentation volatiles and volatiles associated with ripe and ripening fruit, and then test how starving affects these results.

**Implications**

Much time and effort has gone into developing fermentation-based baits for SWD, but they may not prove to be the best option if the goal is to predict infestation risk in crop fields.

Results of this study, coupled with our previous studies looking at SWD movement into and out of crop fields, will allow us to make better recommendations to growers about when and where to deploy traps and what kinds of bait to use. Understanding the attractiveness of fermentation-based baits to females at different life stages will also be useful for potential future management strategies, including attract and kill, mass trapping, and potential genetic engineering tactics. These tactics would also reduce the non-target effects of pesticides and reduce pesticide residues on fruit and in the environment, and would help to sustain the small fruits industry in the United States.

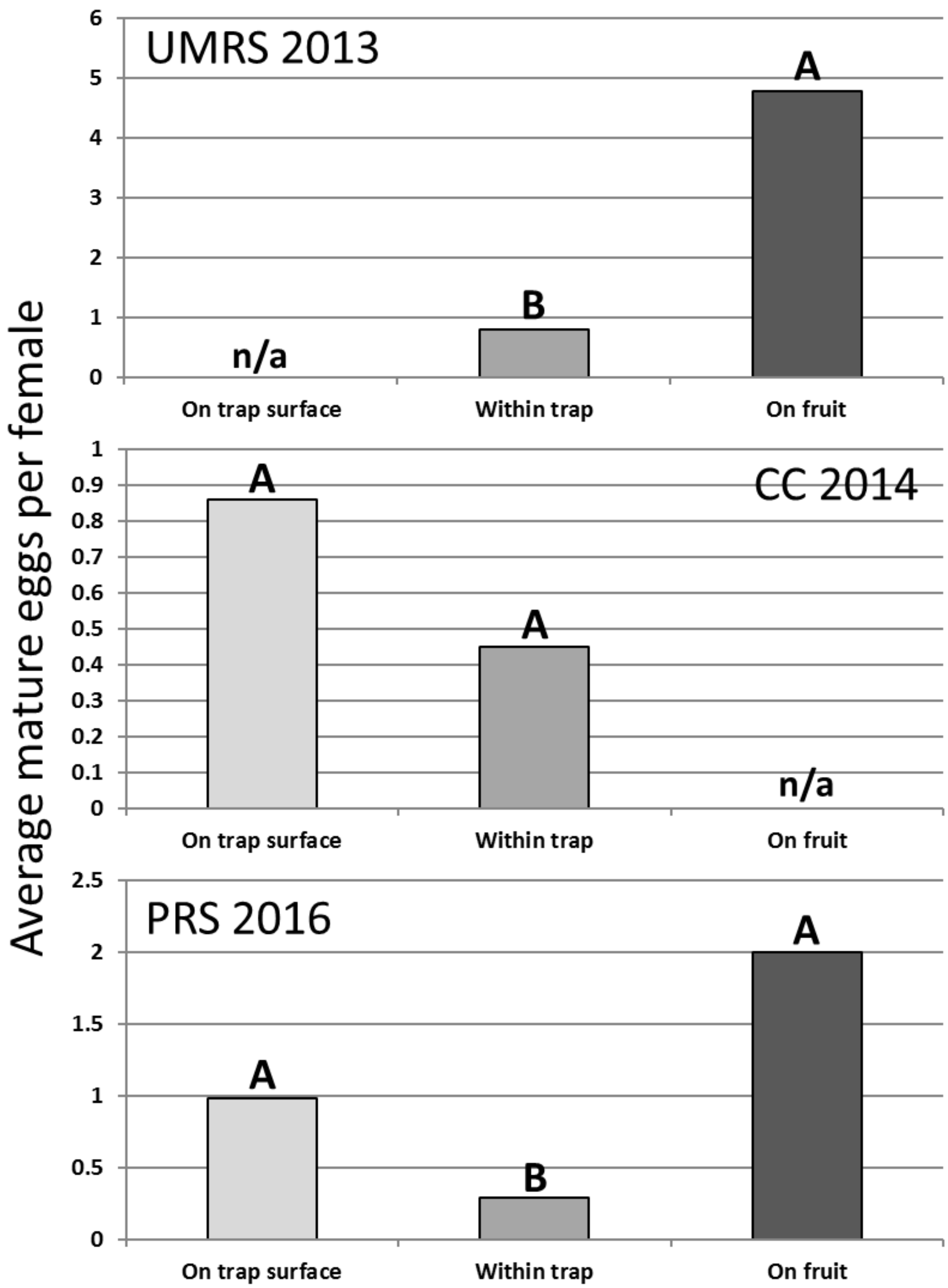


Figure 2. Average number of mature eggs present in SWD females collected on the surface of or within monitoring traps and on ripe fruit at Upper Mountain Research Station (UMRS) in 2013, at a commercial blackberry farm in Cleveland County (CC) in 2014, and at Piedmont Research Station (PRS) in 2016. Bars that share a letter within each graph are not significantly different at α=5%.

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