Final report

Title: Tracking the movement of spotted wing drosophila (*Drosophila suzukii*) over space and through time to improve management programs

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Introduction

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 one and two 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 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, baits may underestimate the presence of egg-laying female flies in the presence of ripe fruit.

Tracking the movement and activity patterns of SWD over space and through time will lead to improved monitoring programs and, ultimately, make it easier for growers to predict and prevent infestation in crop fields. Understanding the timing and direction of SWD movement between areas of non-crop habitat and crop fields, and between co-occurring hosts, will allow growers to apply insecticides when SWD are present and attracted to a host crop. This is in contrast to the current management strategy, which advises growers to begin treatment when fruit starts to ripen and to continue through the end of harvest. Similarly, determining when and where SWD females infest fruit within crop fields will allow growers to more effectively time and apply insecticides. Determining when and where SWD are attracted to monitoring traps will help growers know when and where to most effectively monitor for SWD, and subsequently help them make more informed management decisions. Finally, based on the observation that baits may underestimate the presence of egg-laying females when ripe fruit are available, determining if the SWD females that are captured in monitoring traps are the same ones that are infesting fruit in crop fields is of paramount importance. Therefore, the objectives of this study were to determine if there are daily and/or seasonal patterns associated with 1) SWD movement into and out of crop fields, 2) SWD attraction to a fermentation-based bait commonly used in monitoring traps, and 3) SWD oviposition behavior. Addressing these three interrelated objectives is essential for the development of effective, landscape-level management programs for SWD.

Objectives and Methods

1. Determine if there are patterns associated with SWD movement into and out of crop fields.

We used bi-directional Malaise traps (BugDorm, Taiwan) to determine when SWD move into and out of crop fields. These traps simultaneously capture insects moving in two opposite directions by funneling them into separate collection canisters filled with 70% ethanol.

Figure 1. Plot maps showing the placement and orientation of Malaise traps (white rectangles) and yeast/sugar traps (pink and blue circles) used to determine the movement and diurnal activity patterns of SWD at Farm 1 in 2014 and 2015.

In June-August 2014, we collected samples at two commercial blackberry farms in Cleveland County, NC, where a wooded edge ran adjacent to a crop field. On each sampling date, we set up four Malaise traps in the area between the crop field and the wooded edge, located ~3 m away from the start of the crop rows (Fig. 1). We aimed to sample at each farm once every two weeks over the course of the season. During each sampling period, we checked each trap hourly, on the hour, for 24 hours, except during darkness. We checked the traps until it was completely dark following sunset (usually 10pm), and started to check traps again in the morning before sunrise (usually 5am). All SWD and other *Drosophila* captured in the Malaise traps were identified, sexed, and counted, and were preserved in 70% for future use. In 2015, we repeated the experiment at the same farms used in 2014.

We caught far more SWD and other *Drosophila* moving into crop fields than out of crop fields in 2014. In 2015, we experimented with deploying another type of trap higher in the air column to catch any flies may be flying above the Malaise traps on their way out of the field. We constructed four traps, each with four transparent, sticky panels set in a frame made from PVC pipe that was positioned on a T-post and anchored to the ground using paracord. Each panel was sticky on both sides and measured approximately 1.5' wide and 1' tall; altogether, the four panels in each trap covered an area of 6 ft^2 and were deployed between 6 and 8.5' above the ground (Figure 2). Panels were composed of sections of Clear Rollertrap (Great Lakes IPM, Inc., Vestaburg, MI) or transparencies coated with Spray-on Tangle-Trap® (The Tanglefoot Company, Grand Rapids, MI). When deployed, each trap was set up next to a Malaise trap and was positioned to catch insects flying into and out of the crop field. Traps were deployed during periods of high SWD activity, from 6-10pm and/or from 6-10am, on some sample dates depending on the weather.

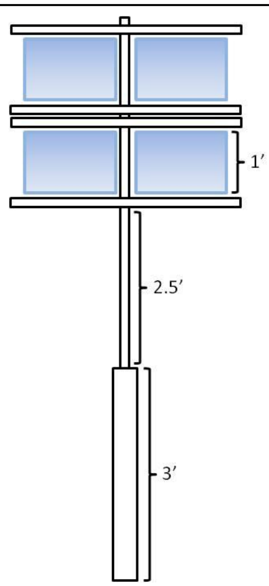

Figure 2. Sticky panel trap design.

2. Determine the diurnal activity patterns of SWD associated with their attraction to fermentation-based baits.

We used standard SWD monitoring traps (32 fl oz. clear plastic cups) to determine when SWD are attracted to fermentation-based baits. In concert with the Malaise trap experiment described above, we set up four traps with yeast/sugar bait between the crop field and wooded edge (blue circles; Fig. 1) and four traps in the crop field located ~30 m away from the wooded edge (pink circles; Fig. 1). In 2014, we deployed yeast/sugar traps alongside the Malaise traps because another experiment was being conducted in the same field. However, because the yeast/sugar traps proved to be very attractive to SWD in 2014, we placed the Malaise and yeast/sugar traps farther apart in 2015 to reduce the likelihood of interference occurring between the two types of traps. We also added an extra pair of yeast/sugar traps so that each Malaise trap was flanked by two yeast/sugar traps.

Traps were checked hourly, on the hour, for 24 hours, except during darkness as described above. Each hour, all flies were collected from the surface of the bait using soft forceps before the contents of each trap were poured through a small kitchen strainer to look for drowned flies. As we collected samples, we noticed that many flies were present on the outside of the traps but did not necessarily enter them. Therefore, halfway through the 2014 season, we started to aspirate flies off the surface of traps for one minute before we collected flies from within the traps, and continued to do so throughout the 2015 season. All of the SWD caught were identified, sexed, counted, and preserved in 70% ethanol. All other *Drosophila* species caught were sexed, counted, and preserved for later identification to species.

3. Determine if there is a relationship between the timing of fruit infestation and the diurnal activity patterns of SWD associated with their attraction to fermentation-based baits.

To determine when SWD females lay eggs in fruit throughout the day, we compared infestation rates in blackberries that were exposed to ovipositing females from 6-10am, 10am-2pm, 2-6pm, 6-10pm, or overnight from 10pm-6am. On 20 July at Farm 1 and 25 July at Farm 2, we placed small 5-7" mesh bags over clusters of unripe blackberries to prevent oviposition. Six clusters were bagged in each of the 10 rows between the two northernmost Malaise traps at Farm 1 (Fig. 1) and the equivalent rows at Farm 2. Clusters were bagged in the first trellis section of each row, closest to the wooded edge, and were left to ripen in the bags. During the next sampling period, one haphazardly-selected bag was removed in each row during each time period. At the end of each time period, all of the ripe berries on each cluster were collected, while any purple or red berries were marked with flagging, counted, and re-bagged. Red and purple berries were allowed to ripen in the bags and were collected once they were ripe. Ripe berries were brought back to the lab and weighed, and were individually suspended in an organza sling inside a 2 oz. plastic portion cup with holes poked in the bottom to promote juice drainage. Berries were held in a growth chamber at 20°C for 10 days, at which time they were dissected and all pupae moved to a small Petri dish with a moistened paper towel square until adults emerged and were confirmed to be SWD.

To determine if fermentation-based baits are equally attractive to young, reproductively immature females and older, egg-laying females, we dissected all of the SWD females caught on the surface of or within the yeast/sugar traps in 2014. We determined the reproductive status and mature egg load of each female using methods developed previously by our laboratory[10].

Results

1. Determine if there are patterns associated with SWD movement into and out of crop fields.

We collected Malaise trap samples on four dates at Farm 1 and three dates at Farm 2 in 2014, and caught a total of three SWD in the traps: 1 female moving into the field at 10 AM on 11 July; 1 female moving out of the field at 9 PM on July 25th; and 1 male SWD moving out of the field at 5 AM on 26 July.

In 2015, we collected samples on seven dates at Farm 1 and four dates at Farm 2 (Table 1). We sampled at each farm every two weeks starting before ripe berries were present and continuing through the postharvest period, with the exception of two weeks at Farm 2. We were able to sample more often in 2015 because we had more flexibility to accommodate weather patterns and on-farm activities. We are still processing samples collected in 2015, but did catch SWD moving into and out of crop fields during the early morning and evening hours.

We deployed windowpane traps on four dates at Farm 1 and one date at Farm 2 (Table 1), but did not catch SWD during any of the sampling periods.

Table 1. Dates when Malaise traps and yeast/sugar-baited traps were deployed at two blackberry farms in 2015, along with the time the 24-hr sampling period began, the approximate fruiting period, and the sunset and sunrise times for Cleveland County, NC.

Farm	Week	Date	Start time	Fruiting period	Sunset	Sunrise
1	1	6-7 June*	2 PM	Pre-fruiting	8:37 PM	6:11 AM
	3	20-21 June*	2 PM	Some ripe fruit present	8:43 PM	6:11 AM
	5	6-7 July*	11 AM	Full fruiting, harvest period	8:44 PM	6:17 AM
	7	19-20 July	10 AM	Full fruiting, harvest period	8:39 PM	6:25 AM
	9	3-4 August*	10 AM	Full fruiting, harvest period	8:28 PM	6:36 AM
	11	17-18 August	11AM	Some ripe fruit remaining	8:13 PM	6:47 AM
	13	29-30 August	11 AM	Postharvest	7:58 PM	6:56 AM
2	2	12-13 June*	1 PM	Some ripe fruit present	8:41 PM	6:11 AM
	4	28-29 June	3 PM	Full fruiting, harvest period	8:45 PM	6:14 AM
	6	--	--			
	8	24-25 July	2 PM	Full fruiting, harvest period	8:37 PM	6:29 AM
	10	--	--			
	12	24-25 August	11AM	Postharvest	8:06 PM	6:53 AM

*Dates when windowpane traps were deployed.

2. Determine the diurnal activity patterns of SWD associated with their attraction to fermentation-based baits.

In 2014, SWD exhibited very strong diurnal activity patterns associated with their attraction to fermentation-based baits. Overall, we caught SWD at yeast/sugar traps during two distinct time periods, in the morning between sunrise and mid-morning and in the evening between ~6pm and sunset. Very few flies were collected outside of these windows. The placement of the traps affected how attractive they were, and far more SWD were caught at traps located between the crop field and wooded edge (blue circles; Fig. 1) than at traps located within the crop field (pink circles; Fig. 1). Differences in trap captures between weeks suggested that patterns of

SWD attraction to fermentation-based baits may change throughout the season and during the postharvest period.

We are still processing samples collected in 2015. However, based on observations made in the field at both farms, we caught SWD at yeast/sugar traps during the early morning and evening hours and also caught more SWD at traps located between the crop field and wooded edge than at traps located within the crop fields.

3. Determine if there is a relationship between the timing of fruit infestation and the diurnal activity patterns of SWD associated with their attraction to fermentation-based baits.

We exposed berries to ovipositing SWD females at Farm 1 during five time periods on 3-4 August. We were not able to complete a second replicate at Farm 2 due to inclement weather and on-farm activities. At Farm 1, no SWD were reared from berries exposed to SWD from 10am-2pm and overnight from 10pm-6am (Table 2); these two time periods correspond with times of the day when little to no SWD activity was observed at yeast/sugar traps and when no SWD were caught in Malaise traps in 2014. Conversely, at least one SWD was reared from berries exposed to SWD during the remaining three time periods. Infestation rates were higher, although not significantly so, in berries exposed from 6-10pm and 6-10am (Table 2). Interestingly, these two time periods correspond with times of the day when SWD were caught at yeast/sugar traps and in Malaise traps in 2014.

Table 2. Proportions of infested berries and mean infestation rates in blackberries exposed to ovipositing SWD females during five time periods on 3-4 August 2015 at Farm 1.

Date	Time period	Proportion of berries infested (# exposed)	Mean SWD per infested berry
3 August	10am-2pm	0 (20)	0
3 August	2-6pm	0.05 (22)	1
3 August	6-10pm	0.33 (18)	1.5
3-4 August	10pm-6am	0 (17)	0
4 August	6-10am	0.18 (22)	1.25

All of the female SWD that were caught on the surface of or within traps in 2014 were dissected to determine their reproductive status and mature egg load. At Farm 1, where we were able to sample most consistently in 2014, on the sampling date when we caught the most SWD overall (25-26 July), we collected 58 SWD females off of the surface of traps and 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). However, the number of mature eggs was not significantly different for SWD females collected on the outside vs. inside of traps ($F_{1,67} = 0.00$, $P = 0.98$). Females collected at yeast/sugar traps in 2015 have not yet been dissected to determine their reproductive status and mature egg load.

Conclusions

Over the course of two years, we conducted experiments designed to track the movement of SWD over space and through time at two commercial blackberry farms in western North Carolina. Although we are not finished collecting data from 2015, our 2014 results clearly show that SWD move into and out of crop fields and are attracted to monitoring traps with a fermentation-based bait (yeast/sugar) during the evening and early morning hours. Our preliminary results from 2015 suggest that the timing of fruit infestation and the diurnal activity patterns of SWD associated with their attraction to fermentation-based baits may be similar. We observed the highest peaks of SWD activity at yeast/sugar traps during the evening and morning hours in 2014, and observed that more berries were infested, and were infested at higher rates, during the same time periods in 2015. Additional experiments will need to be conducted to determine if the timing of fruit infestation by SWD and their attraction to monitoring traps are related, and to determine how weather and other abiotic factors might affect oviposition behavior and attraction to fermentation-based baits. In 2014, the yeast/sugar traps attracted SWD females with very few mature eggs in their ovaries; most of the 69 SWD females that were collected on the surface of or within yeast/sugar traps had developing eggs present in their ovaries but no mature eggs. We will dissect SWD females caught in 2015 to confirm these results. However, additional experiments will need to be conducted to compare the reproductive status of SWD females caught at yeast/sugar traps with those of SWD females caught on ripe fruits to determine if fermentation-based baits underestimate the presence of egg-laying females when ripe fruit are available.

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