Effects of non-crop habitat and patterns of movement by *Drosophila suzukii* on fruit infestation in commercial blackberry fields

Final report

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OBJECTIVES

1. To determine if proximity to non-crop habitat affects fruit infestation levels in commercial blackberry fields.

2. To determine if there are patterns associated with *D. suzukii* movement into and out of crop fields.

JUSTIFICATION

Drosophila suzukii (Matsumura), the spotted wing drosophila, is a highly invasive vinegar fly that was first discovered in the eastern United States in 2009¹. Female *D. suzukii* use their saw-like ovipositor to lay eggs in ripe or ripening fruit². Larval feeding damages fruit and decreases shelf life, while egg laying can facilitate secondary infection by bacteria, fungi, and yeast³. *D. suzukii* severely threatens the viability of berry production, most significantly impacting raspberries, blackberries, blueberries, cherries, and strawberries. There is currently a zero tolerance threshold for *D. suzukii* larvae in fresh market fruit and a single infested fruit can result in the rejection of an entire shipment. As a result, many growers are suffering economic losses, while also facing increased production costs related to labor and insecticide use⁴. For example, many caneberry growers in North Carolina made one or no insecticide applications during harvest in 2010, but may now make 12 or more applications of the broad-spectrum insecticides 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 fruit⁵, suggesting that insecticides alone may not provide effective SWD control in the rainy and humid southeastern US.

Understanding the movement patterns and diurnal activity patterns of *D. suzukii* populations is essential for the development of effective, landscape-level management programs for *D. suzukii*. However, we know very little basic information about the population dynamics of *D. suzukii* in agroecosystems. *D. suzukii* has a wide host range and can alternate between hosts that ripen in sequence or at different times of the year, and likely use wild or ornamental hosts and non-crop habitat as refuges and overwintering habitat. Mated females in reproductive diapause are assumed to be the overwintering stage of *D. suzukii*^{6,7}, although we know very little about where they overwinter. In order to reduce infestation rates in fruit crops, we must identify

sources of early season populations and determine when *D. suzukii* move into and out of crop fields and between crop fields and adjacent natural habitats. We also need to identify elements of non-crop habitat, such as wooded edges and water sources, which might be used to help predict patterns of infestation. Managing *D. suzukii* in crops and adjacent natural habitats by understanding movement and activity patterns will help growers, land managers, and researchers design strategies to reduce *D. suzukii* infestations and help minimize economic losses and decrease pesticide use.

METHODOLOGIES

Objective 1: To determine if proximity to non-crop habitat affects fruit infestation levels in commercial blackberry fields.

We hypothesized that infestation levels would decrease as the distance from non-crop habitat increased. To test our hypothesis, we deployed traps and collected fruit samples along transects running from wooded edges and water sources, such as irrigation ponds and streams, into crop fields. From June-August 2013, we deployed three to four transects per experiment, spaced ≥ 20 m apart, and deployed standard *D. suzukii* traps (32 fl oz deli cups) with yeast/sugar/water bait at ~20 m intervals along each transect. Based on our preliminary results from 2013, we updated our experimental design slightly in 2014 and deployed traps at ~30 m intervals along transects, spaced ~30 m apart, running from wooded edges into crop fields. We deployed traps at ~15 m intervals along transects, spaced ~30 m apart, running from water sources into crop fields. In both years, we collected and replaced the trap bait weekly and identified, sexed, and counted all of the *D. suzukii* caught in each trap. We also collected ~40 ripe fruit around each trap weekly and reared any larvae that were present to adults using standard methods⁵. All of the *D. suzukii* present were identified, sexed, and counted.

In 2013, we conducted these experiments in Ouachita and Navaho blackberry varieties at two commercial blackberry farms in Cleveland and Lincoln Counties in western North Carolina. At each farm, we conducted one experiment testing the effects of a wooded edge on fruit infestation within the crop field and one experiment testing the effects of a water source. The farm in Lincoln County where we worked in 2013 sustained considerable cold damage in early 2014 and we were forced to find a replacement. Therefore, in 2014 we conducted our experiments at two commercial farms in Cleveland County, NC. At both farms, we conducted one experiment testing the effects of a water source on fruit infestation. We conducted one experiment testing the effects of a wooded edge on infestation at the farm used in 2013 (orange circles; Fig. 1). At the new farm, we were able to conduct two experiments testing the effects of a wooded edge in Ouachita and Navaho varieties and one experiment in Prime-Ark® 45. We set up transects on 27-28 May at both farms, and serviced the traps and collected ripe fruit weekly throughout the fruiting season. We removed a subset of transects from each experiment at approximately one month after final harvest. To date, we continue to collect postharvest trap capture data from the remaining transects every other week and plan to collect data throughout the winter and spring seasons.

Objective 2: To determine if there are patterns associated with D. suzukii movement into and out of crop fields.

In concert with the 2014 experiments outlined above, we used flight interception traps to determine if there are patterns associated with *D. suzukii* movement into and out of crop fields and between crop fields and adjacent non-crop habitats. We used ez-Migration traps (BugDorm,

Taiwan), which are 2-headed Malaise traps that are equipped to simultaneously capture insects moving in two opposite directions. In this style of trap, insects moving in one direction are funneled into a single collection canister containing 70% ethanol, while insects moving in the opposite direction are funneled into a separate collection canister. On each sampling date, we set up four Malaise traps adjacent to one of the fields where we conducted transect experiments testing the effects of a wooded edge on fruit infestation. Traps were centered on rows that were halfway between transect rows, and were set up perpendicular to and at ~3 m away from the start of the row (Fig. 1). We aimed to sample each site once every two weeks and to alternate weeks between the two farms over the course of the summer. During each sampling period, we checked each trap hourly, on the hour, for 24 hours, except during darkness. In the evening, we checked the traps until it was completely dark following sunset (usually 10 PM); in the morning, we started to check traps while it was still completely dark before sunrise (usually 5 AM). We collected samples whenever a Drosophila-like insect was captured or after every four hours. Samples were collected using soft forceps after the contents of the canister were poured through a standard, handheld kitchen strainer and were stored in 70% ethanol. For each sample, all of the D. suzukii caught were identified, sexed, and counted, and were preserved in 70% for future use. All of the other Drosophila species caught were sexed and counted, and were preserved in 70% ethanol for later identification to species.

In 2014, we also sought to determine the diurnal activity patterns of D. suzukii by measuring their attraction, over the course of 24 hours, to standard traps baited with yeast/sugar/water like those used in the transect experiments. We conducted this experiment in concert with the Malaise trap experiment. On each sample date, four yeast/sugar traps were set up between the crop field and the wooded edge, in line with the Malaise traps. These traps were centered on rows that were halfway between the Malaise trap and transect rows, and were set up ~3 m away from the start of the row (blue circles; Fig. 1). In addition, four yeast/sugar traps were set up within the crop rows, located ~30 meters away from the wooded edge to match the position of the traps along the established transects (pink circles; Fig. 1). 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; the contents of each trap were then poured through a standard, handheld kitchen strainer to look for flies that may have drowned in the bait. In addition, starting on 25-26 July at Site 1, flies were aspirated off the surface of traps using a standard handheld aspirator (BioQuip, Rancho Dominguez, CA) for one minute before traps were opened and flies were collected from within the trap. For each sample, all of the D. suzukii caught were identified, sexed, counted, and preserved in 70%. All other Drosophila species caught were sexed, counted, and preserved in 70% ethanol for later identification to species.

Data analyses

For the third and fourth sampling dates at Site 1, we tested to see if there were differences between the numbers of *D. suzukii* collected in traps placed between the crop field and wooded edge and the numbers of *D. suzukii* collected in traps placed ~30 m into the crop rows. We analyzed data collected during the three hours preceding darkness (7-10 PM on 25 July and 6-9 PM on 30 August) and the two hours following sunrise (6-8 AM on 26 July and 7-9 AM on 31 August). We analyzed the data using ANOVA via PROC MIXED in SAS 9.4 with trap placement and date as fixed effects, and also used Levene's test to assess the equality of variances. We used the same approach to test for differences between the numbers of *D. suzukii*

that were aspirated off the surface of traps and the numbers of *D. suzukii* that were collected from within traps during the same dates and times described above, with collection method and date as fixed effects.

RESULTS

Objective 1: To determine if proximity to non-crop habitat affects fruit infestation levels in commercial blackberry fields.

In order to test the effects of a water source on fruit infestation within crop fields, we collected data along four transects at Site 1 and three transects at Site 2. Between the two sites, we serviced 34 traps weekly and collected ripe fruit around 23 traps when it was available (the other traps were located outside of the fields). To test the effects of a wooded edge on fruit infestation within crop fields, we collected data along four transects at Site 1 and nine transects total at Site 2, five located within one field and four within another. Between the two sites, we serviced 65 traps weekly and collected ripe fruit around 52 traps when it was available. Over the course of the season, we were able to add a significant amount of data to that which we collected in 2013. To date, data collection and analysis are ongoing.

Objective 2: To determine if there are patterns associated with D. suzukii movement into and out of crop fields.

We collected samples on four dates at Site 1 and three dates at Site 2 (Table 1). We started to collect Malaise trap and yeast/sugar trap data at Site 2 because we collected 2 male *D. suzukii* in transect traps on 3 June, but did not collect any *D. suzukii* in transect traps at Site 1 until 10 June.

No *D. suzukii* were caught in the Malaise traps at Site 2 on any of the three sampling dates. A handful of *D. suzukii* were caught in Malaise traps at Site 1 as follows: 1 female was caught moving into the field at 10 AM on 11 July; 1 female was caught moving out of the field at 9 PM on July 25th; and 1 male was caught moving out of the field at 5 AM on 26 July. In contrast, males and females of other *Drosophila* species were captured in the Malaise traps on all of the sampling dates at both sites (Table 2). The highest numbers of other *Drosophila* individuals were caught on the first sampling date at Site 1, while equally high numbers were caught on the first and third sampling dates at Site 2. Overall, far more individuals were caught moving into the crop field than moving out of the crop field. The proportions of males and females caught while moving into the crop field ranged from 0.50-1 across the three sampling dates at Site 1 and 0.60-0.92 across the four sampling dates at Site 2 (Table 2).

Despite the fact that we caught *D. suzukii* males in the transect traps at Site 2 on 3 June, we did not catch any *D. suzukii* females in the yeast/sugar/water-baited traps at Site 2 on any of the three sampling dates (Fig. 2). In contrast, we caught seven females of other *Drosophila* species in traps located between the wooded edge and the crop field at 7 and 8 AM on 7 June, and continued to catch them on the following two sampling dates. Overall, all *Drosophila* females were caught between 6 PM and sunset or between sunrise and 9 AM, except for a single female who was caught in a trap placed within a row at 12 PM on 6 July. On the second and third sampling dates, the highest numbers of female *Drosophila* were collected at 9 PM.

We did not catch any male *D. suzukii* until the third sampling date at Site 2, when we caught a single male *D. suzukii* at 9 PM on 5 July in a trap placed within the row (Fig. 3). In contrast, we caught two males of other *Drosophila* species during the early morning hours of the first sampling date and continued to collect *Drosophila* males throughout the experiment. Overall, all males were collected between 7 PM and sunset or between sunrise and 8 AM. As

was also true for *Drosophila* females at Site 2, the highest numbers of males were caught at 9PM on the third sampling date at Site 2.

The first *D. suzukii* females caught at Site 1 were two females caught at 9 PM on 11 July (second sampling date) in traps placed between the crop field and the wooded edge (Fig. 4). The highest numbers of *D. suzukii* females were caught on the third sampling date, during which almost 25 females were caught at 9 PM on 25 July in traps placed between the crop field and the wooded edge. We continued to collect considerable numbers of *D. suzukii* females during the fourth sampling date on 30-31 August, which occurred during the postharvest period. Overall, all *D. suzukii* females were caught between 8 PM and sunset or between sunrise and10 AM, except for a single female collected before sunrise at 5 AM on 26 July. As for females of other *Drosophila* species, a single individual was caught during the first sampling date at 8 PM in a trap located within the crop row. In general, very few *Drosophila* females were caught until the final sampling date at Site 1, during which several females were caught in traps placed between the crop field and the wooded edge.

The first *D. suzukii* males caught at Site 1 were not caught until the third sampling date, during which several males were caught in traps placed between the crop field and the wooded edge (Fig. 5). We continued to collect *D. suzukii* males during the postharvest period on 30-31 August, all of which were caught in traps placed between the crop field and wooded edge. All *D. suzukii* males were caught between 7 PM and sunset or between sunrise and 8 AM. As for males of other *Drosophila* species, the first individuals were caught during the first sampling date on 14 June. However, numbers remained low until the last sampling date when comparatively high numbers of *Drosophila* males were caught in traps placed between the crop field and the wooded edge, especially during the evening hours.

Overall, there were significant differences between the numbers of *D. suzukii* collected in traps placed between the crop field and wooded edge and those collected in traps placed ~30 m into the crop rows at Site 1 on 25-26 July and 30-31 August. Results from Levene's test supported the null hypothesis that the population variances were equal ($F_{1,100} = 3.87$, P = 0.0517), and there was not a significant interaction between trap placement and date ($F_{1,108} = 3.10$, P = 0.0810). More *D. suzukii* were collected in traps placed between the crop field and wooded edge than in traps placed ~30 m into the crop rows ($F_{1,108} = 12.89$, P = 0.0005). In addition, more *D. suzukii* were caught on 25-26 July than on 30-31 August ($F_{1,108} = 7.34$, P = 0.0078).

Starting on the third sampling date at Site 1, flies were aspirated off the surface of traps before flies were collected from within the trap. During this sampling period, more *D. suzukii* and other *Drosophila* species were aspirated off the surface of traps than were collected within the traps (Fig. 6). This pattern was the same for traps placed between the crop field and wooded edge and for traps placed within the crop rows, although the differences were more dramatic for traps placed between the crop field and wooded edge. The crop field was treated with an insecticide just prior to the fourth sampling date; therefore, traps were not placed within the crop rows until the reentry interval ended at 9PM on 30 August. In general during this sampling period, more *D. suzukii* and other *Drosophila* species were collected at 8 and 9 PM on 30 August in traps placed between the crop field and wooded edge (Fig. 7). Overall, there were significant differences between the numbers of *D. suzukii* that were collected from within traps. Results from Levene's test supported the null hypothesis that the population variances were equal ($F_{1,222} = 3.64$, P =

0.0575). There was a significant interaction between collection method and date ($F_{1,222} = 8.66$, P = 0.0036). More *D. suzukii* were aspirated off the surface of traps on 25-26 July than were collected within traps on 25-26 July or were collected using either method on 30-31 August.

CONCLUSIONS

In 2013, we began a set of experiments to determine if proximity to non-crop habitat affects infestation in commercial blackberry fields in western North Carolina. We hypothesized that fruit infestation rates would decrease as the distance from non-crop habitat increased and found that this hypothesis was only supported when infestation rates were high in fruit located near a wooded edge. During these experiments, more females were caught in yeast/sugar/water-baited traps placed outside of crop fields than within crop fields. In addition, no relationship was observed between female trap captures and infestation rates within crop fields. Based on these preliminary data, we concluded that it would difficult to advise growers where sampling efforts should be focused to detect infestation, but that attention should be given to fruit located closer to wooded edges. Over the course of this past season, we were able to collect a significant amount of data to add to our 2013 data set and hope to gain a clearer understanding of how proximity to non-crop habitat affects infestation in commercial blackberry fields.

In an effort to determine whether our 2013 results were due to management tactics applied within crop fields or patterns associated with *D. suzukii* movement between crop fields and non-crop habitat, we conducted the Malaise trap and yeast/sugar/water-baited trap experiment described in this report. Overall, we only caught three *D. suzukii* in four Malaise traps deployed for 24 hours on seven sampling dates at two sites, all three of which were captured at Site 1. We caught one female moving into the field at 10 AM on 11 July. We caught one female moving out of the field at 9PM on 25 July and one male moving out of the field at 5 AM on 26 July. Because we caught very few *Drosophila* during the hours of darkness, it is possible that the male that was caught at 5 AM had been moving out of the field during the evening hours, but did not make its way into the alcohol canister until after the final collection at 10 PM. Based on these limited results, it is difficult to make many conclusions about the movement patterns of *D. suzukii* into and out of crop fields, except that they do appear to move between crop fields and adjacent wooded edges during the evening and early morning hours.

There are several reasons why we may have failed to catch more D. suzukii in the Malaise traps. At Site 2, we were only able to sample three times due to rain on later sampling dates. Because we sampled for the last time at Site 2 on 5-6 July, we missed the main part of the D. suzukii season. It likely that we may have caught some D. suzukii in the Malaise traps if we had been able to sample later in the season, as there was still some ripe 'Navaho' fruit available in the field as late as 13 August. Secondly, it is possible that the placement of the Malaise traps may not have been ideal. Each trap was centered on the end of an individual crop row and was placed \sim 3 meters away from the end of the row. It is possible that the traps may need to be placed in the space between two rows or be placed closer to the end of the rows to catch insects moving out of the field. In addition, each mouth of the trap is fairly low to the ground (1.1 m tall by 1.8 m wide). It is possible that insects that are moving out of the field are flying higher than insects that are moving into the field. For example, if insects are using the top of the wooded edge for orientation, they may fly up and out of the field and miss the mouths of the Malaise traps that are facing the crop field. It may be necessary to augment our Malaise trap samples with some transparent sticky cards that can be deployed higher in the air to catch insects that may be flying above the Malaise traps. Finally, the yeast/sugar/water-baited traps were very attractive to both

D. suzukii and other *Drosophila* species in this study. Because we did not want to interfere with the transect experiment, we chose to place the yeast/sugar traps between the Malaise traps and the transect rows. In this orientation, the Malaise traps and the yeast/sugar traps that were placed between the crop field and the wooded edge were only ~7-8 m apart; it is possible that these yeast/sugar traps attracted *D. suzukii* individuals that may have been intercepted by the Malaise traps otherwise.

D. suzukii and other Drosophila species exhibited some very interesting diurnal activity patterns associated with their attraction to yeast/sugar traps over the course of 24 hours. In general, both D. suzukii and other Drosophila species were active during two distinct periods, between 6 PM and sunset and between sunrise and 10 AM on most sampling dates. Similar patterns have been observed for other species of Drosophila. In an early study of Drosophila diurnal activity conducted in 1950, it was observed that California species of Drosophila showed two peaks of diurnal activity, one in the morning and another before sunset in their natural habitats⁸. In another study conducted around the same time, it was suggested that temperature, humidity, and light might be factors that limit the periods during which Drosophila visit food sources⁹. In a more recent study, it was suggested that the high levels of activity exhibited by D. subobscura and D. pseudoobscura near sunrise and sunset could be explained by decreasing sun angles¹⁰. Regardless of the reasons for such behavior, we observed high levels of activity by both D. suzukii and other Drosophila species during the few hours before sunset and after sunrise that were consistent with the diurnal activity patterns observed for other Drosophila species. In addition, we observed male courting behavior, male-male aggression, and mating pairs on the surface of some of the traps, suggesting that *D. suzukii* and other *Drosophila* may come to traps not only to eat but to interact with conspecifics. All together, these findings may have significant implications for the management of D. suzukii in agroecosystems.

IMPACT STATEMENT

Understanding the timing and direction of movement between hosts will allow insecticides to be applied only when *D. suzukii* are present and attracted to a host crop, unlike the current management strategy, which advises growers to begin treatment when fruit starts to ripen and to continue through the end of harvest. Based on our preliminary results, it might be most effective for growers to apply insecticides during periods of high *D. suzukii* activity, i.e., late in the day or early in the morning, to increase the probability of *D. suzukii* adults coming into contact with a lethal dose of insecticide. Understanding the directionality of *D. suzukii* movement will also be useful for potential future management strategies, including attract and kill, mass trapping, and potential genetic pest management tactics. Based on our preliminary results, it might be effective to deploy attract and kill traps with a fermentation-based bait in the area between a wooded edge or other type of non-crop habitat and the crop field to be protected. Ultimately, such tactics would 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.

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Figures and Tables



Fig.1. A plot map showing the placement of traps along transects running from a wooded edge into a commercial blackberry field at Site 1 in 2014 (orange circles), the placement and orientation of Malaise traps (white squares) and yeast/sugar/water-baited traps (blue and pink circles) used to determine the movement and diurnal activity patterns of *D. suzukii* in 2014, and an image of a Malaise trap deployed at Site 2 in 2014.

Site	Date	Start time	Fruiting period	Sunset	Sunrise
1	14-15 June	12 PM	Pre-fruiting	8:42 PM	6:11 AM
-	11-12 July	5 PM	Full fruiting, harvest period	8:43 PM	6:21 AM
	25-26 July	12 PM	Full fruiting, harvest period	8:36 PM	6:30 AM
	30-31 August	2 PM	Postharvest	7:57 PM	6:58 AM
2	6-7 June	7 PM	Pre-fruiting	8:38 PM	6:11 AM
	21-22 June	2 PM	Some ripe fruit present	8:44 PM	6:12 AM
	5-6 July	2 PM	Full fruiting, harvest period	8:45 PM	6:17 AM

Table 1. Dates when Malaise trap and yeast/sugar/water-baited trap data were collected at two sites in 2014, along with the time the 24-hr sampling period began, the approximate fruiting period, and the sunset and sunrise times for Cleveland County, NC.

Table 2. Numbers of male and female Drosophila species caught while moving into or out of the
crop field in four Malaise traps set up on four sampling dates at Site 1 and three sampling dates
at Site 2, and the proportions of males and females caught while moving into the crop field.

	14-15 June		11-1	11-12 July		25-26 July		30-31 August		
Site 1	Male	Female	Male	Female		Male	Female	 Male	Female	
Moving in	18	20	1	2		1	5	4	4	
Moving out	1	0	0	0		0	1	3	4	
Prop. moving in	0.95	1.00	1.00	1.00		1.00	0.83	0.57	0.50	
	6-7 June		21-22	21-22 June		5-6 July				
Site 2	Male	Female	Male	Female		Male	Female			
Moving in	6	16	3	5		11	12			
Moving out	2	4	2	4		1	4			
Prop. moving in	0.75	0.80	0.60	0.56		0.92	0.75			



Fig. 2. Number of females of *D. suzukii* (SWD) and other *Drosophila* species (Non) captured in four yeast/sugar/water-baited traps placed between the crop field and wooded edge (Between) and four traps placed ~30 into the crop row (In the row) over a 24 hour period on three sampling dates in 2014 at a commercial blackberry field in northern Cleveland County, NC. All females caught are shown on the graph, except for a single female who was caught in a trap placed within a row at 12 PM on 6 July. Traps were not set up within the crop rows on 6-7 June.



Fig. 3. Number of males of *D. suzukii* (SWD) and other *Drosophila* species (Non) captured in four yeast/sugar/water-baited traps placed between the crop field and wooded edge (Between) and four traps placed ~30 into the crop row (In the row) over a 24 hour period on three sampling dates in 2014 at a commercial blackberry field in northern Cleveland County, NC. All males caught are shown on the graph. Traps were not set up within the crop rows on 6-7 June.



Fig. 4. Number of females of *D. suzukii* (SWD) and other *Drosophila* species (Non) captured in four yeast/sugar/water-baited traps placed between the crop field and wooded edge (Between) and four traps placed ~30 into the crop row (In the row) over a 24 hour period on four sampling dates in 2014 at a commercial blackberry field in southeastern Cleveland County, NC. All females caught are shown on the graph.



Fig. 5. Number of males of *D. suzukii* (SWD) and other *Drosophila* species (Non) captured in four yeast/sugar/water-baited traps placed between the crop field and wooded edge (Between) and four traps placed ~30 into the crop row (In the row) over a 24 hour period on four sampling dates in 2014 at a commercial blackberry field in southeastern Cleveland County, NC. All males caught are shown on the graph.



Fig. 6. Total number of *D. suzukii* (SWD) and other *Drosophila* species (Non) that were aspirated off the surface of or caught within traps that were either placed between the crop field and wooded edge or were placed ~30 m into the crop rows over a 24 hour period on 25-26 July at Site 2. All individuals caught are shown on the graph.



Fig. 7. Total number of *D. suzukii* (SWD) and other *Drosophila* species (Non) that were aspirated off the surface of or caught within traps that were either placed between the crop field and wooded edge or were placed ~30 m into the crop rows over a 24 hour period on 30-31 August at Site 2. All individuals caught are shown on the graph. Traps were not placed within the crop rows until 9PM on 30 August.