2017

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Published in *Journal of Economic Entomology,* 110:4 (2017), pp 1648–1652.
doi 10.1093/jee/tox150

Submitted 16 February 2017; accepted 2 May 2017; published 24 May 2017.

Reproductive Status of *Drosophila suzukii* (Diptera: Drosophilidae) Females Influences Attraction to Fermentation-Based Baits and Ripe Fruits

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Abstract
Drosophila suzukii (Matsumura) is an invasive species that is a devastating pest of soft-skinned fruit crops. Although much effort has been directed toward developing traps and attractants to monitor for *D. suzukii,* current monitoring tools do not reliably predict fruit infestation. The objective of this study was to determine if *D. suzukii* females at different developmental stages are differentially attracted to monitoring traps with fermentation-based baits and ripe fruits. Females were collected on the surface of traps, within traps, and on ripe fruits during three experiments at field locations in North Carolina, USA, and were dissected to determine their reproductive status. In general, females collected on ripe fruits were more likely to have mature eggs present in their ovaries and had higher numbers of mature eggs than females collected on the surface of or within monitoring traps. The results of this study have implications for *D. suzukii* monitoring and the development of effective baits for use in integrated pest management programs.

Keywords: monitoring, raspberry, blackberry, invasive species

The invasive vinegar fly *Drosophila suzukii* (Matsumura) attacks and can cause substantial economic damage to soft-skinned fruit crops such as raspberries, blackberries, blueberries, cherries, and strawberries (Bolda et al. 2010, Lee et al. 2011). Much effort has been directed to developing traps (Lee et al. 2012, 2013; Iglesias et al. 2014) and attractants (Cha et al. 2012, 2013; Landolt et al. 2012; Basoalto et al. 2013) to monitor for *D. suzukii*, but captures in current traps are poorly correlated to fruit infestation. When six fermentation-based attractants were compared within four host crops across 10 states (Burrack et al. 2015), five of the six attractants detected the presence of *D. suzukii* before the development of fruit infestation. However, once ripe fruits were available, captured flies had fewer mature eggs within their ovaries. One explanation for this result is that females with mature eggs may be more attracted to ripe fruits than to traps with fermentation-based baits. If so, such baits may underestimate the presence of egg-laying *D. suzukii* females when ripe fruits are available and may help explain why captures in currently used monitoring traps do not effectively predict fruit infestation.

Female *Drosophila* must feed to mature eggs (Markow and O'Grady 2008). Yeasts, a common component of fermentation-based baits, are an important source of nutrients for *Drosophila* species that can dramatically impact their ability to produce eggs (Chippindale et al. 1993, Chippindale et al. 1997, Simmons and Bradley 1997, Ganter 2006). During previous experiments, we observed male courting behavior, male–male aggression, and mating pairs on the surface of traps containing a yeast, sugar, and water mixture, which suggested to us that flies may be using traps to locate mates.

Based on these observations, we developed a series of hypotheses. First, young, reproductively immature females will be more attracted to fermentation-based baits than older, reproductively mature, egg-laying females. Next, older flies will be more attracted to ripe fruits than to baits. Finally, flies orienting to traps will spatially partition this resource. Specifically, flies within traps will more likely be young females seeking food to mature eggs, whereas females on the surface of traps will be seeking mates and be more likely to already have mature eggs.

Our objective was to determine if reproductive status affects female *D. suzukii* attraction to and capture in monitoring traps containing fermentation-based baits, and whether flies collected directly from ripe fruits differed in reproductive status from those collected on and within traps. Determining if there are patterns associated with female attraction to fermentation-based baits and ripe fruits will lead to improved monitoring tools and a better understanding of how to interpret fermentation-based trap captures and their use in IPM programs.

Materials and Methods

Sample Collection

We conducted three experiments. The first compared flies collected within traps with those collected on ripe fruits. Three traps (fermenting cup bait; Burrack et al. 2015) were deployed at least 3 m apart within a mixed planting of raspberry and blackberry cultivars at the Upper Mountain Research Station (UMRS) in Ashe County, North Carolina, from 11–12 October 2013. Three traps were also deployed along a brush-lined creek that ran parallel to the planting; each trap was located 10 m away from a trap within the planting. Flies were collected from within traps 24 h after they were deployed, whereas flies were periodically aspirated off ripe raspberries and blackberries located near traps.

Next, we compared flies collected on the surface of traps with those collected within traps. Four traps (yeast and sugar bait; Burrack et al. 2015) were deployed within a commercial blackberry field in Cleveland County (CC), NC, on 25–26 July and 30–31 August 2014. Four traps were also deployed between the field and an adjacent wooded edge, 20 m away from traps within the field. Many *D. suzukii* and other drosophilids were observed on the surface of traps but did not necessarily enter them, and these flies were aspirated from the surface of traps for 1 min before flies were collected within traps. Flies were collected hourly for 24 h, except during darkness.

Finally, we compared flies collected in all three locations: on the surface of traps, within traps, and on ripe fruits. Eight traps (yeast and sugar bait; Burrack et al. 2015) were deployed within a mixed planting of blackberry cultivars at the Piedmont Research Station (PRS) in Rowan County, NC, on 20–21 July 2016. Traps were placed 12 m apart within three rows, and were checked for fly activity every 30 m from 5:30pm until dark and again from 6:45–9 am. Flies were aspirated from the surface of each trap for 1 min before flies were collected from within. Flies were then collected from ripe berries surrounding the trap by blowing them into small 10- by 18-cm mesh bags with drawstring closures.

Flies collected in all three experiments were preserved in 70% ethanol and dissected under a stereomicroscope (Olympus SZX10, Center Valley, PA). The total number of mature eggs in both ovaries were counted; eggs were considered mature when they possessed fully formed respiratory filaments (**Fig. 1**).

Fig. 1. *Drosophila suzukii* reproductive tract containing developing and mature eggs.

Data Analysis

Females were compared according to 1) the likelihood that they had one or more mature egg(s), and 2) the total number of mature eggs present in their ovaries. Trap placement (CC, UMRS) and time of year (CC) were also tested to determine if they affected mature egg counts. Analyses were conducted using generalized linear mixed models via PROC GLIMMIX in SAS v. 9.4 (**Table 1**), and post hoc means comparisons made using the Tukey–Kramer test.

Results

The likelihood of having one or more mature eggs(s) present differed among females. Those collected on ripe berries at UMRS were more likely to have one or more mature eggs (range = 0–14 per female) than females within traps (range = 0–7 per female; $F_{1,50}$ = 16.19, P = 0.0002). Similarly,

Table 1. Response and explanatory variables, including fixed and random effects, for all generalized linear mixed models and the distribution of response variables.

Site(s)	*Fixed effects*	*Random effects*	*Distribution/link*
Response variable: Likelihood of having one or more mature egg(s) present in ovaries			
All	Collection location (on trap surface/within trap/on ripe fruit)		Binomial/logit
Response variable: No. of mature eggs present in ovaries			
UMRS	1. Collection location (within trap/on ripe fruit)		Lognormal/identity
	2. Trap placement (within planting/along creek)	Trap	Lognormal/identity
CC	1. Collection location (on trap surface/within trap) | trap placement (within crop field/between field & edge)	Trap	Lognormal/identity
	2. Collection location (on trap surface/within trap) | collection period (harvest/postharvest)	Trap placement + trap	Lognormal/identity
PRS	1. Collection location (on trap surface/within trap/on ripe fruit)	Time of day (30-m interval) + trap	Lognormal/identity

females collected on ripe berries (range = 0–19 per female) and on the surface of traps (range = 0–10 per female) at PRS were more likely to have one or more mature eggs than females collected within traps (range = 0–7 per female; $F_{2,227}$ = 8.64, P = 0.0002). However, females collected on the surface of (range = 0–10 per female) and within traps (range = 0–2 per female) at CC were equally likely to have one or more mature eggs ($F_{1,67}$ = 0.05, P = 0.83).

Females collected on ripe berries generally had more mature eggs than females on the surface of and within traps, whereas females on the surface of traps had more mature eggs than females within traps; however, the magnitude and significance of these relationships differed between sites (**Fig. 2**). Females collected on ripe berries at UMRS had over five times more mature eggs on average than females within traps ($F_{1,50}$ = 27.09, P<0.0001). Although females collected on ripe berries and on the surface of traps at PRS had similar numbers of mature eggs, females collected in both locations had over twice as many mature eggs as females within traps ($F_{2,198}$ = 10.98, P<0.0001). However, females collected on the surface of traps at CC did not have more mature eggs than females within traps ($F_{1,59}$ = 1.05, P = 0.31).

When ripe fruits were readily available, monitoring traps attracted females with low mature egg counts regardless of whether they were located within or outside of the crop. Females collected within traps placed inside the crop planting and within traps placed along the creek at UMRS had similarly low numbers of mature eggs ($F_{1,55}$ = 0.57, P = 0.45), as did females collected at traps placed within the crop field and between the crop field and wooded edge during the harvest period at CC ($F_{1,6}$ = 0.04, P = 0.84; Table 2). During both the harvest and postharvest periods at CC, females collected on the

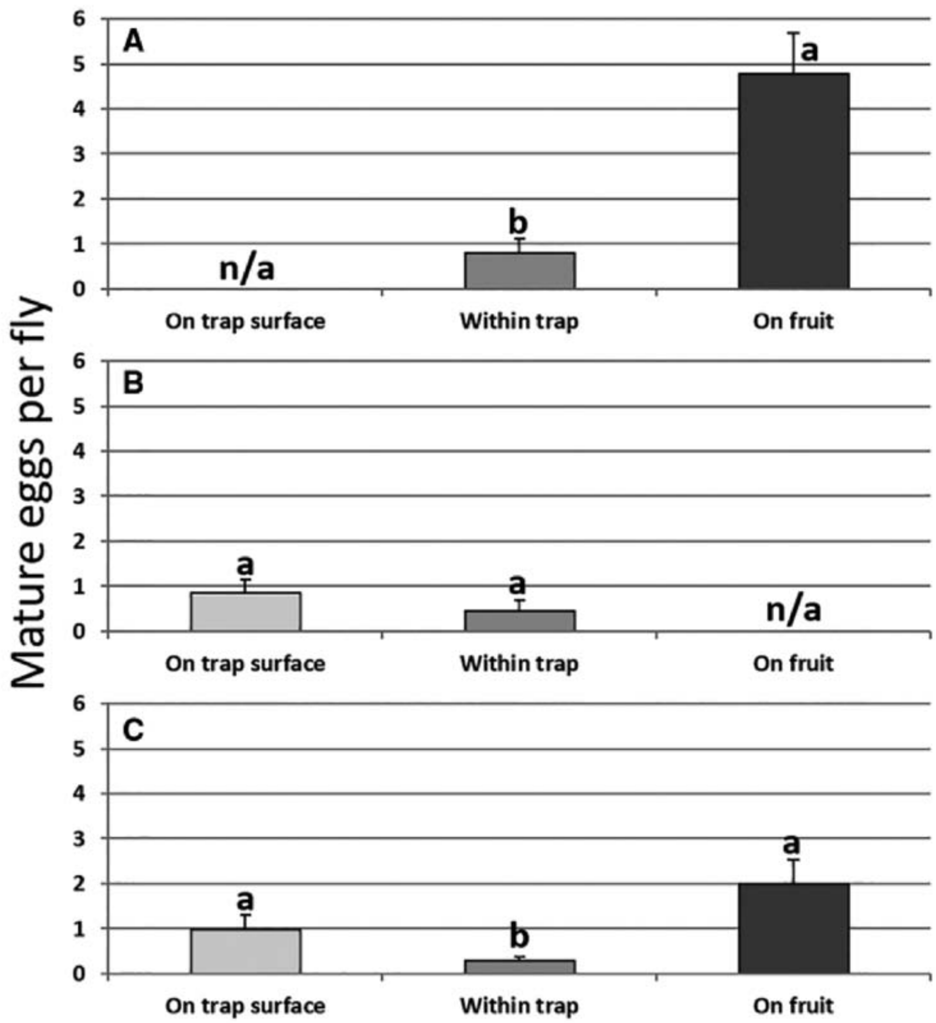

Fig. 2. Mean number of mature eggs present in *D. suzukii* females collected on the surface or within monitoring traps and on ripe fruit at (**A**) Upper Mountain Research Station (UMRS) in 2013, (**B**) a commercial blackberry farm in Cleveland County (CC) in 2014, and (**C**) Piedmont Research Station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

surface and within traps had similar numbers of mature eggs ($F_{1,69}$ = 1.46, P = 0.23), although females collected at traps during the postharvest period had nearly three times as many mature eggs as females collected during harvest ($F_{1,10}$ = 5.51, P = 0.041; **Table 2**).

Table 2. Effects of trap placement and harvest period on the mean number of mature eggs per fly collected on the surface of and within monitoring traps with a fermentation-based bait at Upper Mountain Research Station (UMRS) in Ashe County, NC, in 2013 and a commercial blackberry farm in Cleveland County (CC), NC, in 2014.

A. Effect of trap placement on mature egg counts

Period	Trap placement at UMRS	Mature eggs ± SE	Trap placement at CC	Mature eggs ± SE
Harvest	Within crop (*n* = 29)	0.793 ± 0.334a	Within crop (*n* = 16)	1.687 ± 0.746a
	Outside crop (*n* = 30)	1.500 ± 0.596a	Outside crop (*n* = 53)	0.528 ± 0.194a

B. Effect of harvest season on mature egg counts

Period[a]	Collection location at UMRS	Mature eggs ± SE	Collection location at CC	Mature eggs ± SE
Harvest	Trap surface (*n* = 58)	0.862 ± 0.272a	Combined[b] (*n* = 69)	0.797 ± 0.232b
	Within trap (*n* = 11)	0.454 ± 0.247a		
Postharvest	Trap surface (*n* = 6)	1.333 ± 0.715a	Combined (*n* = 15)	2.267 ± 0.720a
	Within trap (*n* = 9)	2.889 ± 1.086a		

Within sections A and B, values followed by the same letter within a column are not significantly different (α = 0.05, Tukey–Kramer adjustment).

a. Harvest and postharvest periods at CC were 25–26 July 2014 and 30–31 August 2014, respectively.

b. Includes flies collected on the surface of and within traps.

Discussion

Our results support the idea that reproductively mature females with higher numbers of mature eggs are more attracted to ripe fruits than to fermentation-based baits, although the olfactory responses of individual females were likely affected by their physiological state. Female *D. suzukii* raised on diet in the laboratory were sensitive to fruit volatiles in two recent studies (Keesey et al. 2015, Revadi et al. 2015a). However, headspace volatiles from vinegar were attractive to *Drosophila melanogaster* Meigen regardless of age, sex, and mating status, provided the flies had been starved (Becher et al. 2010). Reproductively mature *D. suzukii* females may therefore be more attracted to ripe fruits for oviposition if they are well-fed, but more attracted to fermentation odors from rotting fruits or monitoring traps when hungry or unable to find sufficient nutrients to maintain egg maturation. In fact, *D. suzukii* females became more attracted to both fruit and yeast odors following mating (Mori et al. 2017), suggesting the importance of being able to locate both resources.

Wild *D. suzukii* females had a low degree of egg maturation compared with females reared under controlled laboratory conditions (Plantamp et al. 2016), which suggests that wild flies may not necessarily achieve full reproductive potential. In our study, *D. suzukii* females collected on ripe fruits at UMRS had twice as many mature eggs as females collected on ripe fruits at PRS. Ripe fruits at UMRS were not harvested and fermented and rotted in the field, which may have provided food for flies, as we observed several *D.*

suzukii females collected on ripe fruits had red and purple midguts when dissected. Conversely, ripe fruits at PRS were removed for use in other experiments and all females collected on ripe fruits had clear midguts. Our results support the hypothesis that females with higher numbers of mature eggs were attracted to monitoring traps once oviposition and food resources were depleted during the postharvest period at CC (Table 2). It is therefore likely that both resource quality and abundance can affect the responses of reproductively mature *D. suzukii* females to olfactory cues in the field.

Our results also support the idea that reproductively immature females with low numbers of mature eggs are more attracted to fermentation-based baits than to ripe fruits. Regardless of whether such females are looking for nutrients to mature eggs or potential mates, the fermentation-based attractants used in monitoring traps may be useful in removing reproductively immature females from the field before they mate and commence egg-laying. Laboratory studies suggest that *D. suzukii* females can become inseminated within 24 h of emergence, but do not produce offspring until 2.5 d after emergence (Revadi et al. 2015b), which agrees with an earlier estimate of 1–4 d following emergence for the onset of offspring production (Kanzawa 1935). If wild *D. suzukii* behave similarly, under optimal conditions, females have ~2–4 d to mate, find a protein-rich food source to start egg maturation, and find a suitable oviposition substrate. Therefore, there is potentially a 4-d window in which mass trapping with fermentation-based baits could remove reproductively immature females before they start to lay eggs in crop fields, perhaps using a yeast species or strain attractive to *D. suzukii* (Hamby et al. 2012, Scheidler et al. 2015).

Our results point to differing levels of attraction to ripe fruits and monitoring traps with fermentation-based baits by *D. suzukii* females at different developmental stages, and provide needed context to interpret results of previous studies in which traps with fermentation-based baits were used to monitor for *D. suzukii*. Future research should address whether the mating status of females on the surface of monitoring traps differs from those within traps. Controlled laboratory studies that compare newly emerged, virgin females and mated, reproductively mature females for differences in sensitivity to fermentation volatiles and volatiles associated with ripe fruits would also be helpful in further defining differential attraction.

Acknowledgments — We would like to thank the grower who graciously allowed us to conduct experiments on his farm, the staff at Upper Mountain and Piedmont Research Stations, and Tiffany Moore for assisting with fly dissections. Research was funded by the North American Raspberry and Blackberry Association and the North Carolina Agricultural Foundation.

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