

Ecology and Behavior

Plasticity in Oviposition Site Selection Behavior in *Drosophila suzukii* (Diptera: Drosophilidae) in Relation to Adult Density and Host Distribution and Quality

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Subject Editor: Nikolaos Papadopoulos

Received 6 January 2021; Editorial decision 7 May 2021

Abstract

Flexibility in oviposition site selection under temporally shifting environmental conditions is an important trait that allows many polyphagous insects to flourish. Population density has been shown to affect egg-laying and offspring fitness throughout the animal kingdom. The effects of population density in insects have been suggested to be mutualistic at low densities, whereas intraspecific competition is exhibited at high densities. Here, we explore the effects of adult crowding and spatial resource variation on oviposition rate in the invasive pest *Drosophila suzukii* (Matsumura). In a series of laboratory experiments, we varied the density of adult males and females while holding oviposition substrate availability constant and measured per female oviposition rate using high and low-quality substrates. We found that oviposition behavior was affected more by substrate than adult density, though both variables had significant effects. When we varied the spatial arrangement of whole raspberries, we observed differences in oviposition rate and egg distribution between the grouped and solitary female treatments. Our results suggest that social interactions encourage oviposition, especially when exposed to unfamiliar or unnatural substrates. These results highlight the compensating effect of increased oviposition rate per female as adult populations decline. They will help researchers and crop managers better understand in-field population dynamics throughout the season as population densities change.

Key words: sociality, reproductive plasticity, population ecology, invasive species

Behavioral plasticity is a key trait of invasive insect species (Jaenike and Papaj 1992, Little et al. 2020). This adaptive ability allows individuals to adjust to dynamic and unpredictable environmental conditions, interactions with unknown species, and potentially harmful founder effects. Polyphagous invasive species can exhibit considerable behavioral plasticity in host selection. A part of host selection in herbivorous species, oviposition site selection (OSS), involves a decision process by which females integrate a wide variety of informational inputs to decide where to place their eggs. The number of factors females can use in OSS is substantial, including but not limited to extrinsic information, such as abiotic elements and inter-specific dynamics, and intrinsic information like mating history and previous experience (as reviewed in Gibbs and Van Dyck 2009). In

this paper, we focus on the elements of intraspecific interaction, host quality, and host availability in an invasive frugivorous insect.

Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) is a global fruit pest with a wide trophic range of domesticated and wild-growing plants (Lee et al. 2015, Poyet et al. 2015, Elsensohn and Loeb 2018). Females cause economic damage by laying eggs in ripening and ripe fruit, and the larvae then consume the inner flesh of the fruit. Females are thought to randomly distribute single eggs in one or more oviposition sequences in individual fruits (Mitsui et al. 2006). In most temperate growing regions, *D. suzukii* populations generally are low in the spring and increase throughout the year, with the highest population densities, infestation rates, and damage to fruit in late season crops (Drummond et al. 2019, Papanastasiou et al. 2020).

As a result, at each successive generation of *D. suzukii*, adults are faced with different population density dynamics and environmental conditions.

Female insects often compete for limited high-quality substrates while intrasexual competition in males focuses on mating with females or guarding and monopolizing potential mating locations such as attractive oviposition sites. In *D. suzukii* females, a variety of social signals may impact host selection and promote skip oviposition, potentially leading to increased foraging and use of less preferred oviposition sites, as in the apple maggot *Rhagoletis pomonella* (Walsh) (Averill and Prokopy 1989). Laboratory experiments have revealed that marking pheromones deposited on oviposition substrates by both sexes of *D. suzukii* influence subsequent oviposition by naïve females (Tait et al. 2020, Elsensohn et al. 2021). However, females do not appear to use egg-related cues in OSS (Elsensohn et al. 2021). Previous observations also suggest that as adult population size increases, oviposition and thus infestation in less ripe, suboptimal fruit occurs only after infestation in ripe fruit (Swoboda-Bhattarai and Burrack 2015, Elsensohn 2020). Because both the number of adults and available host fruits vary through time and space, females in time-limited species like *D. suzukii* must choose between laying an egg in fruit or retaining the egg given the information and circumstances available to them.

Population density of conspecifics is known to affect individual oviposition rates. Adult density has downstream consequences on fecundity and offspring fitness when resources are limited (Barker 1973, Averill and Prokopy 1987). In *Musca domestica* L., total egg number positively correlated with density, whereas fecundity (eggs per female) had a negative correlation with fly density when oviposition substrate and nutritional resources were limited (Pastor et al. 2011). In *D. suzukii*, larval crowding at varying densities and nutritional resource quality show that density effects are correlated with host quality (Hardin et al. 2015, Kienzle et al. 2020). High-quality resources have fewer fitness tradeoffs than lower-quality resources at higher larval densities. Crowding at later larval stages leads larvae to seek pupation sites detached from their host, a behavior that carried fitness benefits under laboratory conditions (Da Silva et al. 2019) but may incur significant costs in the field.

Field-scale density studies of *D. suzukii* are constrained by variable environmental conditions, variable demography of the adult population, and our limited ability to accurately estimate population size. Thus, laboratory experiments serve as the first step toward understanding OSS under different scenarios of population or resource size, as might be seen throughout the year or as a function of pest management interventions. We sought to characterize the effects of adult density and spatial arrangement of resources on the propensity of females to oviposit. A better understanding of how females respond to different population conditions may lead to improved modeling of population dynamics, resource use, and competition in this invasive pest.

Materials and Methods

Oviposition Substrates

In choice experiments, members of the genus *Rubus*, including raspberries and blackberries, are among the most preferred hosts for attraction and oviposition, traits that positively correlate with larval performance in *D. suzukii* (Bellamy et al. 2013, Burrack et al. 2013, Kenis et al. 2016). We, therefore, employed raspberry-based substrates for our experiments. All fruit used in experiments were organic raspberries (Driscolls, Product of Mexico, or the USA)

purchased no more than 24 hrs before the start of each experiment. Two experimental substrates were used that varied in texture and nutritional quality. The high-quality substrate consisted of four whole raspberries (~20 g). Unlike other fruit that is picked at the stem, raspberries detach from the stem and receptacle when ripe, creating a bowl-shaped fruit. Cotton was stuffed into the empty interior of the fruit to prevent oviposition on this side and the fruit was then placed cotton-side down on a 60 mm petri dish. The lower quality substrate was raspberry juice agar, which was made by straining berries through the fine mesh, separating the pulp and seeds from the juice. A 1% agarose juice solution was prepared using equal parts water and raspberry juice, and 1% each of agar, and the anti-fungal agents Tegosept (methylparaben [CAS: 99-76-3]), and propionic acid (CAS:79-09-4). The agarose solution (~20 ml) was poured into 60 mm sterile Petri dishes (Falcon, Fisher Scientific, Waltham, MA) and cooled to room temperature.

Flies

The lab colony was established in 2011 from adults reared from naturally infested fruit in North Carolina, with genetic variation maintained through annual additions of wild-caught adults. The colony was maintained on a cornmeal agar diet (Hardin et al. 2015) at 20 °C (range 17 – 23°C) and 65% (range 55 – 75%) relative humidity and a 12:12 L:D photoperiod. Fly density in the colony averaged 20–30 flies per 25 × 95 mm polystyrene vial (Genesee Scientific, San Diego, CA). Experimental female and male flies were 7–10 d old sexually mature and presumed to be mated as they were collected from mixed-sex colony containers. Flies were not starved before experimentation.

Male and Female Density

A petri dish containing a single substrate was placed in the center of a 473 ml round plastic arena (PFS Sales, Raleigh, NC) that was modified with two 2.5 × 2.5 cm screen-covered air vents. Flies were added to the arena at specified densities and removed after 4 hrs. The experiment was randomly blocked by replicate and 12 total replicates were conducted for each treatment. For the male density experiments, the following treatments were used: 0, 2, 4, 10, 18, or 30 males, with two females added to each male treatment. For the female density experiments, females were tested at 1, 2, 5, 10, 20, or 50 females per container, with no males, added to remove the potential for harassment, especially at low female density.

Egg Load

Due to the relatively low oviposition rate observed in the population density experiments on raspberry agar, we dissected females to quantify their egg load. Experimental females from these trials were saved in 70% ethanol and a subsample from three density types was dissected. To ensure we were examining females that had the opportunity to lay eggs during the experimental window, we only examined treatments with five or fewer females from both density experiments. Using fine-tip forceps, the reproductive organs were isolated from the abdomen under a stereomicroscope. All mature eggs, as defined by the presence of respiratory filaments, were counted. A total of 25 females were dissected.

Spatial Variation

A 0.3 × 0.3 × 0.3 m cage was used to test oviposition rate under changing host availability under two spatially explicit scenarios. Five cotton-stuffed raspberry fruits were arranged in two ways:

1) clustered together in the middle of the arena, forming a tight X-shape, or, 2) fruits were distributed in the cage, with four fruits placed approximately 50 mm from each of the cage corners and a single berry in the middle (see Fig. 3 for visual representation). Experimental groups of one or five gravid female adults ($n = 12$) were exposed to each spatial grouping for 2 hrs, after which fruit was removed and the eggs per fruit were counted. Fruit placement within the cage was recorded to assess oviposition bias and the distribution of eggs throughout the cage.

Data Analysis

SAS 9.4 statistical software was used to analyze all experimental data (SAS Institute 2013). For both male density experiments, the eggs per female values were log-transformed to adjust for assumptions of normality and analyzed with a generalized linear mixed model (GLIMMIX) fit a Poisson distribution. The female density experiment with raspberry juice agar data was log-transformed and analyzed by GLIMMIX fit to a log-normal distribution. The female density experiment with whole fruit was log-transformed and analyzed with a mixed model (MIXED) using a Satterthwaite correction. All analyses used treatment as the fixed effect, with replicate and experiment date as random effects.

A mixed model using log-transformed data evaluated the effects of female number and spatial arrangement of the oviposition resource on the eggs per female (oviposition rate). Resource (fruit) position within the arena was assessed through a mixed model, subsetting the data into two groups of clustered or spread out. Female number and fruit position were fixed effects, with replicate and date as random effects. The proportion of fruit attacked was analyzed with GLIMMIX using a binomial distribution. Female number and fruit position were fixed effects, with replicate, time, and date as random effects. Adjusted means were compared using the Tukey–Kramer adjustment.

Egg load was analyzed with linear regression, with the number of mature oocytes as the dependent variable and the number of eggs laid during the experimental period divided by the number of females in that replicate, if necessary.

Results

Male Density

Male density significantly affected the oviposition rate of two females in the raspberry agar substrate ($F_{5,55} = 3.81, P = 0.005$), but not in whole fruits ($F_{5,55} = 1.86, P = 0.117$), as females laid a similar number of eggs on the higher quality, whole fruit substrate regardless of male density (Fig. 1). An average of 12.27 ± 0.22 eggs per female (\pm SEM) were laid in whole raspberries. Females oviposited fewer eggs per female on average in the low-quality raspberry agar, with the lowest oviposition rate (1.46 ± 0.51 eggs per female) occurring when no males were present in the agar treatment, and the highest oviposition rate at intermediate male densities of 10 and 18 males (4.25 ± 1.10 and 3.92 ± 0.82 eggs per female, respectively). Thus, the presence of males appeared to increase the likelihood of oviposition on the suboptimal agar resource to a point, with the largest male density (30 males) suppressing oviposition by 35–40% compared to the highest oviposition rates.

Female Density

On the high-quality whole raspberries, the oviposition rate decreased as the number of females per container increased (Fig. 2). Single females laid significantly more eggs than females housed at 20 and 50

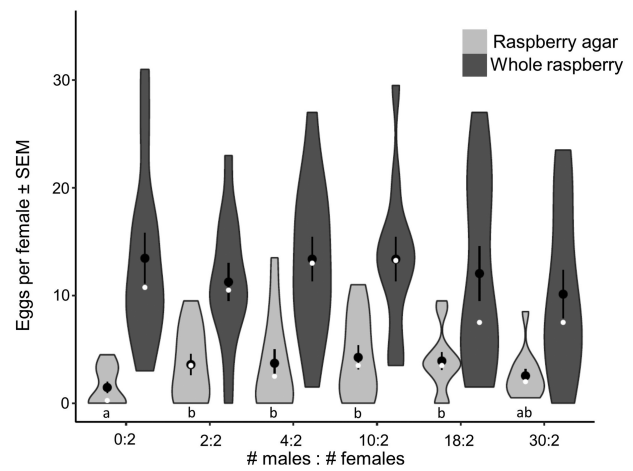


Fig. 1. Mean a number of eggs per female (\pm SEM) laid into a whole fruit and raspberry juice agar under different male densities. The black dot represents the mean number, the vertical line is the standard error, and a white dot is the median value. Male density varied (0, 2, 4, 10, 18, or 30 males), and two females were present in each assay. Violin plots show the probability density of the data at different values, smoothed by a kernel density estimator. Plots sharing the same letter are not significantly different from each other (ANOVA with Tukey–Kramer test, $n = 12$). No significant differences were observed among densities in the whole raspberry treatment.

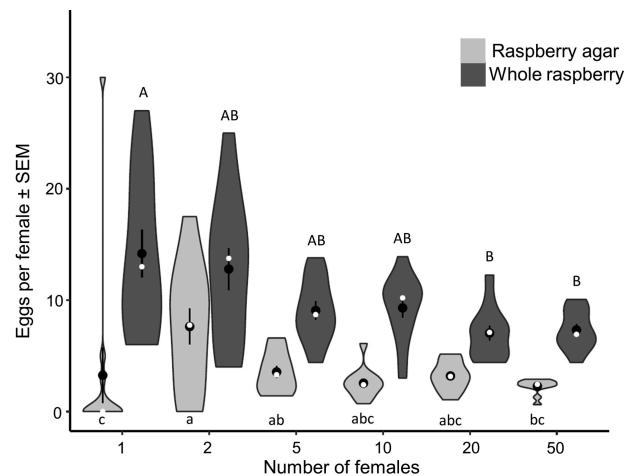


Fig. 2. the mean a number of eggs per female (\pm SEM) laid into a whole fruit and raspberry juice agar when the density of females varied. The black dot represents the mean number, the vertical line is the standard error, and the white dot is the median value. No males were present in these assays. Violin plots show the probability density of the data at different values, smoothed by a kernel density estimator. Plots sharing the same letter and capitalization are not significantly different from each other (ANOVA with Tukey–Kramer test, $n = 12$).

females per container ($F_{5,55} = 5.03, P = 0.0007$). A similar pattern was evident on the lower quality raspberry juice agar substrate, but more eggs were laid per female at a density of two females per container ($F_{5,55} = 5.23, P = 0.0005$). As in the male density experiment, the number of eggs laid into raspberry juice agar was substantially lower than the number laid into whole raspberry fruit. Given the higher variation among treatments in the female density experiment than in the male one, oviposition may be more influenced by the presence of other females, and less by males in a given area.

Female egg load had no significant effect on whether a female chose to oviposit during the experiment ($R^2 = 0.0001, P = 0.956$,

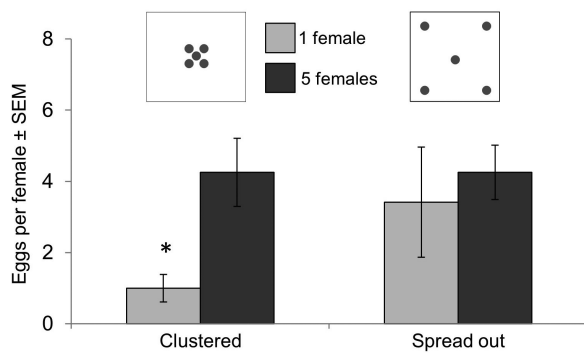


Fig. 3. Effects of the spatial distribution of fruit and female density on mean (\pm SEM) eggs oviposited per female. In the 'spread out' treatment fruit were distributed throughout the arena, whereas in the 'clustered' treatment fruit was grouped in the center of the arena, but fruit were not touching. Light gray bars represent a single female in each assay, whereas dark gray bars represent five females per assay (GLM, with Tukey–Kramer test, $n = 12$).

$n = 25$). Mature oocytes were found in treatments both with and without oviposition. Sample females from experiments in which no eggs were laid contained on average 6.4 ± 1.86 mature eggs, suggesting the reticence to oviposit was due to factors other than the availability of mature eggs.

Spatial Variation

Oviposition was significantly affected by interactions between female density and the distribution of the five raspberry fruits in the arena (Fig. 3). The number of females had the largest effect on the per female oviposition rate (female number: $F_{1,41} = 13.15$, $P = 0.0008$). When fruit was clustered in the middle of the arena, solitary flies laid significantly fewer eggs per female than in the 5-female cohort (female number: $F_{1,99} = 53.79$, $P < 0.0001$). We did not detect any oviposition bias with regard to where fruit were placed in the arena for either spatial arrangement (spread out: $F_{4,99} = 1.47$, $P = 0.217$; clustered: $F_{4,99} = 1.40$, $P = 0.238$). However, the proportion of fruit that contained eggs subsequent to exposure again was affected by female number ($F_{1,44} = 13.65$, $P = 0.0006$), but not by spatial arrangement ($F_{1,44} = 0.03$, $P = 0.965$). Solitary flies laid eggs in approximately only 15% of the fruit, while grouped females exploited approximately 72% of available fruit. These results suggest that solitary females are not likely to leave a high-quality resource once found, even if other high-quality hosts are nearby, as in the clustered setting. But because we did not track individual flies in the grouped treatment, we cannot infer based on these data whether grouped females exploited more fruit due to the additive effect of the number of flies, or because individual females were more likely to spread their eggs among the fruit under social conditions.

Discussion

Our results indicate that female *D. suzukii* vary their OSS under different environmental and social conditions. In no-choice assays, females oviposited fewer eggs in lower quality, raspberry juice agar substrate than in fresh fruit, independent of adult female or male density. These oviposition patterns suggest that optimal oviposition decisions vary based on the availability and quality of resources. Using whole fruit, single females displayed the highest oviposition rate, with eggs per female declining with increasing female density. With the lower quality raspberry juice agar substrate, the intermediate densities produced the highest oviposition rate.

These findings may suggest that intraspecific social facilitation on a lesser-known substrate may allow *D. suzukii* to exploit suboptimal plant species for population growth. This mechanism may come into play either when resource choice is limited (spatiotemporal heterogeneity) or due to spillover effects of intraspecific competition for oviposition sites at high population densities that result in oviposition in lesser quality substrates. Of course, these results should be investigated at a larger spatial scale, but until field population density can be estimated reliably, scaled-up laboratory experiments remain the best way to understand basic *D. suzukii* OSS.

Surprisingly, changes in male density, with a constant two females per assay, did not affect female egg-laying rates in fresh raspberry fruit. Therefore, male harassment of gravid females did not appear to be prominent under these experimental conditions. It is thought that mating takes place on and around oviposition substrates, as males are often found near or on ripening fruit that they presumably cannot exploit as a food resource without female assistance (Swoboda-Bhattarai et al. 2017). As such, females may be used to high male densities at oviposition sites, or they become highly motivated to oviposit on the relatively abundant high-quality fruit at low female density. It is also possible that the substrate led to a change in male behavior that facilitated female oviposition. The underlying factor for the lack of male influence with the high-quality substrate remains unclear and should be investigated by varying female numbers to male density. Regardless, our results suggest that the propensity of females at low density to oviposit at a high rate in high-quality substrates could be a factor in this species' exponential population growth in cultivated crops.

The lower quality raspberry juice agar substrate revealed patterns that were not apparent on fresh fruit, despite the much lower overall oviposition rate on agar. Male density appeared to influence oviposition by the two females in a 'concave-downward' pattern – the highest oviposition rate was observed with 10 or 18 males in the arena and the lowest oviposition rates were at both tails of the distribution when no males or 30 males were present. Thus, the factors that affect OSS differ on high and low-quality oviposition substrates, as well as when the densities of both adults *D. suzukii* sexes vary.

When a small cohort of female *D. suzukii* was exposed to a fixed number of oviposition hosts under two different spatial arrangements, the average oviposition rate for the 5-female group did not differ between spatially distributed and clumped fruit. Single females laid approximately the same number of eggs as the per-female rate with grouped females, but only when fruits were spread out. In contrast, oviposition by the single female was significantly reduced by >66% on clustered fruit. Even though solitary females laid more eggs when fruit was spread throughout the arena, the proportion of fruit containing eggs was not affected by their spatial arrangement. Other research suggests a potential oviposition preference for larger-appearing masses of fruit (Rice et al. 2016, Elsensohn 2020). However, the current observations suggest that OSS on clustered fruit by single females differs dramatically from OSS on social females. This difference may be related to host marking behavior and possibly even recognition of self-marking as distinct from other females' marking. More research is needed to disentangle the host type, size, volatile profile, and conspecific influence on oviposition choice.

This study provides evidence that multiple factors influence oviposition site selection in *D. suzukii*. It is important to acknowledge several limitations in our study design that could be addressed in future studies. First, we used naïve females, whereas in the field learning and memory may factor into subsequent oviposition events, as has been established in other dipteran species (Prokopy et al. 1986, Papaj and Prokopy 1989, Mery and Kawecki 2004,

Takahara and Takahashi 2017). Second, females were offered a single choice in these tests; multiple concurrent choices (fruits) of varying quality, as is common in the field, likely would affect OSS. Third, our design did not consider the per capita oviposition by individual females in each replicate. It can be inferred from the higher variability in the lower density treatments (which included some zeros) that not all the gravid females oviposited, even when given high-quality substrates for oviposition. We confirmed this inference by dissecting the females to count the number of mature eggs retained in the ovaries; there was no correlation between the number of retained eggs and the number of eggs laid in the trial. Finally, our studies quantified endpoints of assays conducted for 2 or 4 hrs. Early patterns might have been obscured by the accumulation of more eggs, especially on high-quality substrates. For example, in the male density experiment, it is possible that at high male density, harassment interfered with oviposition early in the experiment, but the two females in each 4 hrs assay overcame the harassment, resulting in similar high oviposition at all-male densities. Therefore, time-course assays or continuous video recordings might shed light on the dynamic nature of OSS.

The data presented here can be combined with results from larval density and resource use studies to gain a better understanding of how *D. suzukii* population dynamics may vary as different resources are utilized. Further work will be needed to assess oviposition under varying resource availability to better understand the interaction between individual or group behavior and the spatial distribution of hosts. Our experimental flies were obtained from a laboratory colony, which is maintained at high densities, typically 20 to 30 flies per vial. It would be interesting to conduct similar experiments with flies raised under lower density conditions or collected from field-infested fruit to see if results differ. While our findings confirm that host quality correlates with oviposition rate (Bellamy et al. 2013, Burrack et al. 2013, Kenis et al. 2016), they also highlight that resource quality affects how females are influenced by conspecifics. With a high-quality resource, solitary females had the highest oviposition rate of all the density treatments while the presence of males at any density did not affect oviposition. When a low-quality resource was offered though, the oviposition rate was much lower overall, and peak oviposition for both the male and female density trials was under grouped conditions. The presence of conspecific adults encourages individual egg-laying decisions in several insect species (Prokopy and Duan 1998, Díaz-Fleischer and Aluja 2003, Hoshizaki et al. 2020), although the underlying mechanism has not been explored for *D. suzukii* at this time.

Ultimately, even small numbers of *D. suzukii* can pose an economic threat, as fresh fruit markets have a zero-tolerance for insect infestation. Many studies have shown increased fruit infestation in cultivated hosts as a function of time and space (Drummond et al. 2019, Papanastasiou et al. 2020). Our results suggest, however, that reducing fly population densities may not necessarily reduce the number of eggs laid if females increase their per capita oviposition rate at low densities, especially in preferred hosts like raspberry fruits. Our results may also have implications for control strategies that alter the 50:50 sex ratio, such as the sterile insect technique (SIT). Further laboratory and field research will need to establish whether the patterns we observed hold under different and less controlled conditions. However, since several traditional and genetically engineered SIT techniques are in development for use against *D. suzukii* (Li and Scott 2016, Buchman et al. 2018, Nikolouli et al. 2018), it would be prudent to understand any unintentional consequences from altering densities and sex ratios.

Acknowledgments

We thank Aurora Toenison for help with the experimental set-up. Fellowship support for JEE was provided through National Science Foundation Integrative Graduate Education and Research Traineeship (grant number DGE-1068676).

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