SPECIAL ISSUE – INSECT-PLANT INTERACTIONS: HOST SELECTION, HERBIVORY, AND PLANT RESISTANCE

Fruit firmness, superficial damage, and location modulate infestation by *Drosophila suzukii* and *Zaprionus indianus*: the case of guava in Veracruz, Mexico

Rodrigo Lasa[1]*, Eduardo Tadeo[1], Luis A. Dinorín[2], Itzel Lima[1] & Trevor Williams[1]

[1]*Red de Manejo Biorracional de Plagas y Vectores, Instituto de Ecología AC, Xalapa, Veracruz 91070, Mexico, and* [2]*Instituto Tecnológico de Martínez de la Torre, Miguel Hidalgo 101, Col. Adolfo Ruíz Cortines, Martínez de la Torre, Veracruz 93600, Mexico*

Abstract

The exotic pestiferous flies *Drosophila suzukii* (Matsumura) and *Zaprionus indianus* Gupta (Diptera: Drosophilidae) were recently identified in traps used for monitoring tephritid pests of guava, *Psidium guajava* L. (Myrtaceae), in Veracruz, Mexico. To determine whether both drosophilids were infesting guava, a previously unreported host, samples were taken from fruits on trees and fallen fruits on the ground. Overall, 74 and 36% of visually intact fruits attached to the tree were infested by *D. suzukii* and *Z. indianus*, respectively. Under laboratory conditions, small artificial punctures on the surface of ripe guavas did not result in increased oviposition by *D. suzukii* compared to undamaged fruit, whereas *Z. indianus* almost completely avoided oviposition, or were not capable of developing in fruit. Females of *D. suzukii* were capable of ovipositing in early ripe guavas in laboratory tests (23% of fruits were used for oviposition), although a high penetration force is required to pierce fruit (mean ± SEM = 89.0 ± 3.0 cN). Fully ripe and overripe guavas were softer (52.2–53.5 cN penetration force) and were more frequently infested (ca. 60% infestation). Numbers of females that developed in guavas were not influenced by ripeness/firmness, whereas male development was reduced in early ripe fruit compared to ripe and overripe fruit. In laboratory choice experiments with crushed fruits, *D. suzukii* adults were equally attracted to guava and blueberry, independent of gender and age. However, raspberry was more attractive than guava. This study demonstrates that *D. suzukii* is attracted to guava, is capable of ovipositing in fruit and, under field conditions, is more abundant in fruits still attached to the tree compared to fallen fruit that remain intact. In contrast, *Z. indianus* was not capable of developing in intact guavas and, although present in fruits attached to the tree, was most abundant in fallen damaged fruits.

Introduction

Invasive pest species represent a major challenge to many countries as a result of trade globalization. Mexico is a megadiverse country that forms part of the Mesoamerican corridor connecting continental North and South America and is exposed to invading insect pests that threaten crops and biodiversity (Williams et al., 2013). Two invasive pests, *Drosophila suzukii* (Matsumura) and the African fig fly, *Zaprionus indianus* Gupta (both Diptera: Drosophilidae), were recently found in traps used for monitoring tephritid pests (*Anastrepha* spp.) of guava, *Psidium guajava* L. (Myrtaceae), in the state of Veracruz, Mexico (Lasa & Tadeo, 2015), although their ability to infest guava was not determined. The presence of *D. suzukii* in the crop canopy of guava trees was previously reported in a trap-based study in Baja California, Mexico, but fruit infestation was not registered (de los Santos Ramos et al., 2014).

*Correspondence: Rodrigo Lasa, Instituto de Ecología AC, Apartado Postal 63, Xalapa, Veracruz 91070, Mexico. E-mail: rodrilasa@gmail.com

Guava, together with more than 74 species from 31 plant families (Lachaise & Tsacas, 1983), has been reported as a host for *Z. indianus* in Brazil (Vilela & Goñi, 2015) and Florida, USA (van der Linde et al., 2006), although infestations were limited to damaged fruits. Similarly, a great variety of wild and cultivated hosts have been found to support the development of *D. suzukii* (Mitsui et al., 2010; Walsh et al., 2011; Cini et al., 2012; Lee et al., 2015). However, guava has not been reported as a host for this pest. Host selection by *D. suzukii* differs among host species and among varieties, as fruit firmness, or more specifically the force required for ovipositor penetration of a host, is believed to be of key importance in modulating fruit infestation (Burrack et al., 2013). Although *D. suzukii* has a serrated ovipositor that allows females to oviposit in ripening fruits (Atallah et al., 2014), in some crops such as cranberries and peach, superficial wounds on the surface of fruit can favor oviposition by *D. suzukii* (Steffan et al., 2013; Stewart et al., 2014).

Annual production of guava in Mexico is estimated at ca. 303 000 tons over an area of ca. 21 000 ha. However, the state of Veracruz is a marginal producer, with just over 200 tons per year of guava (SIAP, 2014), but with a high presence of trees growing in backyards in urban and rural locations. In this study, the presence of *D. suzukii*, *Z. indianus*, and other drosophilid species in guava fruits collected directly from the tree canopy was compared with fallen fruits to determine foraging and infestation preferences of these pests. Additional laboratory experiments were performed to determine whether factors such as fruit maturity, surface penetration force, and surface damage significantly influenced guava infestation patterns by *D. suzukii* and *Z. indianus*.

Material and methods

Insect colony and fruits

A laboratory colony of *D. suzukii* was started in an insectary at the Instituto de Ecología AC, Xalapa, Veracruz, Mexico, using adults that emerged from naturally infested wild blackberry, *Rubus fruticosus* L., collected at Xico, Veracruz (19°25′59.92″N, 97°1′58.88″W, 1 385 m altitude) in June 2015. The laboratory colony of *Z. indianus* was started using adults that emerged from naturally infested chico zapote, *Manilkara zapota* L., collected at Apazapan, Veracruz (19°19′2.80″N, 96°43′23.87″W) in March 2015. Adults of both species were allowed to oviposit in a cornmeal-based artificial diet (Dalton et al., 2011), dispensed into 300-ml plastic cups and covered with a fine nylon gauze. The colonies were maintained at 24 ± 1 °C, $60 \pm 10\%$ r.h., and L12:D12 photoperiod, with a light intensity of 3 500–4 500 lux, measured using a YK-10LX light meter (LT Lutron, Taipei, Taiwan). Females and males were kept together in cages from emergence until required for experiments. Flies that were used 3 days after emergence were considered unmated, whereas flies used after 8 days of emergence were considered to have mated.

Pesticide-free guava fruits (var. Calvillo) were bought from a local supplier and were used immediately for oviposition studies or where stored at 4 °C for 1 day prior to use. All fruits were carefully inspected prior to experiments; guavas showing any degree of superficial damage were discarded. Penetration force of the fruit epidermis was determined at three points along the equatorial region for each of 30 fruits per maturity stage using a portable penetrometer (Wagner Instruments, Greenwich, CT, USA) modified to be used with a no. 3 entomological pin (Elephant, Austria) (Lee et al., 2016). Readings from the penetrometer are reported in centiNewtons (cN). Penetration force measures were averaged for each fruit and used to classify fruits according to their maturity stage which was classified into one of three classes: green-yellow (from here onwards described as early ripe), ripe yellow, and overripe yellow guavas.

Collection of fruits from the field

Guava fruits (var. Criolla) were collected from a single guava orchard at weekly intervals from 30 September to 15 October 2015 at Xico, Veracruz (19°25′8.21″N, 96°58′30.74″W, 1 183 m altitude), close to where this fly was detected in traps in 2014 (Lasa & Tadeo, 2015). On each collection date, samples of 30 fruits were randomly selected from a pooled batch of fruits collected in three locations in the guava orchard: (1) fallen fruits collected from the ground that clearly had broken or damaged skin (total n = 90) and that were selected from recently fallen fruit that had no signs of decomposition, (2) fruits in which the skin was unbroken and undamaged by visual inspection collected from the ground (n = 90), and (3) fruits collected directly from the tree canopy which had an unbroken and undamaged skin by visual inspection (n = 90). To collect fruits from the tree, branches were shaken using an attached rope and fruits were allowed to fall on to a blanket suspended above the ground to prevent damage. For selection, a visual inspection of fruits was performed carefully by the same observer and with reference to a previously defined standard. Fruits were taken to the laboratory and individually placed in 200-ml cups with a thin layer of vermiculite, covered with a 0.1-mm nylon mesh lid and maintained under laboratory conditions described above. Among all treatments, drosophilid emergence was registered in damaged guavas collected from the ground at 7–8 days after collection, assuming that no drosophilids had already left the fruit at the time the fruits

were collected in the field. In all cases, 10 days after guavas had been individualized, cups were inspected every other day and emerged drosophilids were placed in 1.5-ml microcentrifuge tubes with 70% ethanol. At day 22, all drosophilids had emerged and almost all tephritid (*Anastrepha* spp.) larvae had pupated in the vermiculite layer. At this time, all fruits were dissected and larvae or pupae of *Anastrepha* that were found in the fruit were transferred to vermiculite. Cups containing *Anastrepha* pupae were moistened with 0.3% (wt/vol) sodium benzoate solution every other day to allow adult emergence. The number of *Anastrepha* pupae, adult emergence, and sex ratio were recorded for each guava fruit.

The total numbers of male and female *D. suzukii*, *Z. indianus*, and other drosophilid species (both sexes pooled) were assessed for each individual guava and proportions of infested fruits within each type of sample were compared. In addition, mean numbers of drosophilids per infested fruit were calculated based on fruits from which at least one adult emerged of the species in question.

Fruit puncture damage tests

Non-choice oviposition tests were applied to determine whether small puncture wounds on the surface of guava fruits could facilitate oviposition of either invasive species. Non-choice tests were performed because no other known hosts were fruiting during the period of the study in this region. The puncture wound was designed to simulate the damage that might result from the oviposition of *Anastrepha fraterculus* (Wiedemann), which commonly attacks guava in Mexico, or other minor superficial wounds derived from the feeding of insects that interact with this crop. Moreover, puncture wounds could also favor maturation and increase drosophilid infestation. Two treatments were compared: (1) yellow ripe guavas that were selected among intact fruits with unbroken skin by a careful visual inspection, and (2) similar yellow ripe guavas that had been carefully punctured to a depth of 5–8 mm at 12 points on the surface using a 00 entomological pin of 0.3 mm diameter (Original Elephant, Emil Arlt, Parrish, FL, USA). Immediately after treatment, guavas were placed individually in 550-ml plastic cups with a thin layer of vermiculite and covered with a fine nylon mesh lid. Four male + female pairs, 1 week old, were released inside each cup. Adults were given continuous access to a cotton pad moistened with 10% (wt/vol) honey solution placed on the gauze lid of the cup, and were allowed to oviposit for a 72 h period. Cotton pads were re-moistening at 24-h intervals. After exposure, flies were discarded and guavas were individualized in 200-ml cups with a thin layer of vermiculite, covered with a 0.1-mm mesh lid and incubated under laboratory conditions. Adult emergence was

checked every other day, from day 10 to day 22 following exposure to adult flies. A similar number of guavas was not exposed to *D. suzukii* as a control in case of an existing infestation. The percentage of fruits that were infested and the number of adults of each sex that emerged were recorded. A total of 50 replicates per treatment were performed. Fruit firmness, measured as surface penetration force, was evaluated using a randomly selected sample of 30 additional guavas at the same maturity stages. An identical experiment was performed using *Z. indianus* under similar conditions but with a total of 30 replicates per treatment including a control treatment with unexposed fruit. The width of the ovipositor of five females of *A. fraterculus* was measured with a Nikon microscope and Nis-Advanced Research v.3.2 Image software (Nikon, Tokyo, Japan).

Influence of guava firmness (ripeness) on *Drosophila suzukii* infestation

A no-choice test was performed to evaluate how changes in fruit firmness during ripening influenced the susceptibility of guavas to infestation by *D. suzukii*. For this, three stages of physiological maturity of guavas were compared: early ripe, yellow ripe, and overripe guavas. Overripe guavas were obtained by allowing yellow ripe guavas to mature under laboratory conditions (24 °C) for 1 week. Guava firmness was evaluated by surface penetration force with a no. 3 entomological pin in a random sample of 30 additional guavas of each maturity stage. Fruits were exposed to oviposition by *D. suzukii* as described in the previous test. Four male + female pairs, 1 week old, were released inside a 550-ml cup containing one guava and allowed to oviposit during 72 h. After this period fruits were individually incubated in 200-ml plastic cups with vermiculite for up to 22 days to allow emergence of adult flies. In total 30 replicates were performed of each guava maturity stage. The percentage of infested fruits was recorded as well as the number of male and female adults that emerged.

Guava attraction under cage conditions

Multiple-choice tests were developed to compare attraction to *D. suzukii* of guava and other berry crops commonly infested by the pest. Commercial fruits of raspberry and blueberry (both Driscoll's, Jalisco, Mexico) were bought from a local supplier and directly processed together with yellow ripe guava described above. A total of 140 g of each fruit was crushed using a ceramic mortar, samples of 3 g crushed fruit were placed into small plastic cups (2 cm diameter, 1 cm deep) and frozen until use. Mean maturity stage for each fruit was estimated in degrees Brix (°Bx) using a refractometer (model 300051;

Sper Scientific, Scottsdale, AZ, USA) and a randomly selected sample of 20 individual fruits. A fruit juice extractor (Liquafruit, Taurus, Mexico) was used to extract guava juice for analysis.

Small traps were constructed from 120-ml plastic cups (35 mm diameter, 87 mm high) that were drilled with three equidistant lateral holes through which translucent conical tubes (9 mm external diameter, 6 mm internal diameter, 20 mm deep) were inserted to decrease the frequency of fly escape once inside the trap. Holes were placed at 45 mm from the base. The plastic cup was covered with cream-colored masking tape to facilitate landing on the surface of the trap and to avoid any effect of different fruit colors. Traps were baited with one of four treatments: 3 g raspberry, 3 g guava, 3 g blueberry, or 3 ml water dispensed on a small piece of cotton as a control. Traps were placed at a height of 11.5 cm at the corners of Plexiglas cages (25 × 25 × 25 cm) with 0.1-mm nylon mesh sides. Traps were initially positioned at random and subsequently rotated clockwise in position for each new replicate. Forty non-starved flies (20 females and 20 males) were released inside the cage at 17:00 hours. At 23 h after the flies were released, traps were removed from cages and flies were knocked down by freezing at −20 °C for 15 min. The flies captured in each trap were counted and sorted by sex. The remaining flies inside the cage were discarded. Two independent tests were performed with flies selected 3 days after emergence and considered unmated and those selected 8 days after emergence which were considered that have mated. A total of 16 replicates were performed for each age group under laboratory conditions described above.

Statistical analysis

The percentages of infested fruits within each type of sample were compared by χ^2 test of independence. Mean number of flies per fruit within each type of sample were normalized by rank transformation (Conover & Iman, 1981) and compared by one-way ANOVA. A t-test was used to compare mean numbers of females and males that emerged from intact or punctured fruits. Mean numbers of males and females that emerged from fruit maturity treatments, force, and the brix value of the three fruit maturity stages were compared by one-way ANOVA. Maturity, in degrees Brix (°Bx), and the surface penetration force was $\sqrt{x}$ transformed to obtain homogeneity of variance prior to analysis by one-way ANOVA. In all cases, means separation was performed by Tukey test. Attraction to crushed fruit was also rank transformed (Conover & Iman, 1981) and compared by two-way ANOVA. All analyses were performed using SPSS v.17 (SPSS, Chicago, IL, USA).

Results

Flies recovered from guava fruits from the field

In total 6 790 drosophilids were reared from guavas collected in the field. Of these, 1 071 flies emerged from intact fruits collected from the tree (83% of fruits infested by at least one drosophilid), 1 144 flies from intact fruits collected from the ground (80% infested by at least one drosophilid), and 4 575 flies from damaged fruits collected from the ground (100% infested by at least one drosophilid). The percentage of intact guavas collected from the tree that were infested with *D. suzukii* (74%) was markedly higher than the percentages of guavas infested with *Z. indianus* or other drosophilid species (χ^2 = 37.5, d.f. = 2, P<0.01; Table 1). In contrast, guavas collected from the ground had similar percentages of infestation by *D. suzukii*, *Z. indianus*, and other drosophilids, regardless whether they had broken skin (χ^2 = 3.905, d.f. = 2, P = 0.14) or unbroken skin (χ^2 = 0.745, d.f. = 2, P = 0.69; Table 1). On average the numbers of *D. suzukii* and *Z. indianus* reared from each fruit taken from the tree were ca. 3- to 4-fold higher than the number of other drosophilids ($F_{2,126}$ = 9.59, P<0.01), whereas mean numbers of each species did not differ for insects reared from fallen unbroken skin fruits ($F_{2,142}$ = 2.22, P = 0.11). In contrast, other drosophilid species were more abundant than *D. suzukii* or *Z. indianus* in fallen damaged fruit ($F_{2,239}$ = 31.84, P<0.01; Table 1). Adult sex ratio was consistently female-biased (58.2–68.2% females) in *D. suzukii* reared from fruits collected from different locations (Table 1), whereas this ratio tended to be closer to equality in *Z. indianus* (48.7–56.1%).

Between 87 and 95% of guavas that were infested with drosophilids (all species) were also infested by *Anastrepha* spp. fruit flies. The percentage of infestation by *Anastrepha* spp. was similar for guavas collected from the tree (89%), and broken (94%) or unbroken skin fruits (94%) collected from the ground (χ^2 = 2.700, d.f. = 2, P = 0.26). On average, 4.3 ± 0.2 (mean ± SE) *Anastrepha* spp. pupae were collected from each fruit. In the laboratory, 77% (n = 827) of tephritid pupae produced adults, of which 99% (n = 820) were identified as *A. fraterculus* and 1% (n = 7) were *A. striata*.

Fruit puncture tests

A similar percentage of guavas was infested by *D. suzukii* when fruits were visually intact (58%) or when previously punctured with an entomological pin (64%) (χ^2 = 0.378, d.f. = 1, P = 0.54; Table 2). No differences were observed in the mean number of females (t = 0.411, d.f. = 59, P = 0.68) or males (t = 0.217, d.f. = 59, P = 0.83) recovered from each infested fruit, in both intact and punctured

Table 1 Mean percentage of guava fruits that were infested by *Drosophila suzukii*, *Zaprionus indianus*, and other drosophilid species, and mean (± SEM) number of drosophilid flies and adult sex ratio per infested fruit, collected from the tree canopy (unbroken skin) and the ground (broken and unbroken skin). Per type of location 90 fruits were sampled

Origen of sample and species		% fruits with drosophilids present (n)	No. flies per infested fruit	Adult sex ratio (% females)
Unbroken skin fruits from the tree	*D. suzukii*	74a (67)	8.5 ± 1.1a	58.2 ± 3.3
	Z. indianus	36b (33)	11.1 ± 2.0a	48.7 ± 4.7
	Other drosophilids	33b (30)	3.4 ± 0.8b	
Unbroken skin fruits from the ground	*D. suzukii*	56a (50)	6.3 ± 0.8a	68.2 ± 4.2
	Z. indianus	50a (45)	10.8 ± 1.7a	52.0 ± 4.6
	Other drosophilids	56a (50)	6.9 ± 2.3a	
Broken skin fruits from the ground	*D. suzukii*	86a (77)	7.4 ± 0.8a	66.7 ± 3.5
	Z. indianus	89a (80)	15.3 ± 2.0b	56.1 ± 1.2
	Other drosophilids	94a (85)	32.7 ± 4.0c	

Means within a sample type followed by the same letter did not differ significantly (fruit percentages: χ^2 test; fly numbers: Tukey test, both P>0.05).

Table 2 Mean percentage of guava fruits infested and mean (± SEM) numbers of female and male flies recovered per infested fruit, for intact fruits or fruits previously punctured with an entomological pin and exposed to *Drosophila suzukii* and *Zaprionus indianus* under laboratory conditions

Treatment		% fruits infested (n)	No. females per infested fruit	No. males per infested fruit
D. suzukii	Intact yellow ripe guavas	58a (29)	3.9 ± 0.9a	3.5 ± 0.8a
	Punctured yellow ripe guavas	64a (32)	4.4 ± 0.9a	3.8 ± 0.9a
Z. indianus	Intact yellow ripe guavas	3.3 (1)	(1 female)	0
	Punctured yellow ripe guavas	0	0	0

Means within a column followed by the same letter did not differ significantly (fruit percentages: χ^2 test; fly numbers: Student's t-test, all P>0.05) (only applies to *D. suzukii*). Means are based on 50 replicate samples in the case of *D. suzukii* and 30 replicates in the case of *Z. indianus*.

fruits (Table 2). No infestation was observed in any of the control guavas that were not exposed to *D. suzukii*. Of *Z. indianus*, only a single adult female emerged from a single guava from the intact fruit treatment. No infestation was observed in any of the control guavas that had not been exposed to *Z. indianus*. The mean (± SE) penetration force of the fruit epidermis of yellow ripe guavas was measured at 53.5 ± 2.1 cN. The ovipositor of *A. fraterculus* was narrower than the entomological pin (mean ± SE = 0.126 ± 0.002 vs. 0.3 mm).

Influence of guava firmness on *Drosophila suzukii* infestation
The mean penetration force of the epidermis differed among fruits of different physiological maturity stages ($F_{2,87}$ = 78.79, P<0.01). No differences were observed between ripe and overripe guava (Tukey test: P = 0.75), whereas yellow-green stage fruit were significantly firmer than the other ripeness stages (Table 3). Ripening, measured as fruit firmness (which did not consider other internal and external fruit changes such as color or volatiles emitted), influenced infestation by *D. suzukii*, with a lower percentage of green-yellow fruits infested compared to yellow ripe or yellow overripe fruits (χ^2 = 9.91, d.f. = 2, P<0.01). Yellow ripe and yellow overripe fruits, with similar firmness values, were also similar in their susceptibility to infestation (χ^2 = 0.07, d.f. = 1, P = 0.79). The mean number of female flies reared from each fruit was similar for all treatments ($F_{2,39}$ = 0.583, P = 0.56), but the mean number of males per fruit differed ($F_{2,39}$ = 3.27, P = 0.049; Table 3).

Guava attraction under cage conditions
Mean (± SE) sugar content differed among fruit types: raspberry 9.3 ± 0.2 °Bx, blueberry 14.1 ± 0.3 °Bx, and yellow ripe guava 12.0 ± 0.2 °Bx ($F_{2,57}$ = 100.04, P<0.01). In both tests, the two-way ANOVA indicated a significant

Table 3 Mean percentage of guava fruits infested and mean (± SEM) skin penetration force and numbers of *Drosophila suzukii* females and males that emerged from infested fruits of three ripeness stages under laboratory conditions. In total 30 fruits (replicates) were tested per ripeness category

Guava fruits	Penetration force (cN)	% fruits infested (n)	No. females per infested fruit	No. males per infested fruit
Early ripe	89.0 ± 3.0a	23a (7)	3.7 ± 1.0a	1.0 ± 0.3a
Yellow ripe	53.5 ± 2.1b	60b (18)	6.1 ± 1.3a	5.6 ± 1.0b
Yellow overripe	52.2 ± 1.6b	57b (17)	4.8 ± 1.3a	3.9 ± 1.2b

Means within a column followed by the same letter were not significantly different (fruit percentages: χ^2 test; penetration force and fly numbers: Tukey test, all P>0.05).
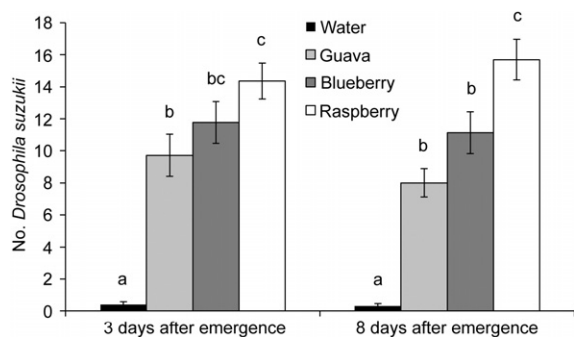

Figure 1 Mean (± SEM) total number of *Drosophila suzukii* flies captured in traps baited with crushed guava, blueberry, or raspberry fruit in choice experiments (water as a control). A total of 20 males and 20 females were released in each cage. Flies were tested 3 and 8 days after emergence (they were considered unmated and mated, respectively). Means within a group of flies capped with the same letter were not significantly different (Tukey test: P>0.05).

effect of fruit odor but not of sex or their interaction. The water control treatment was less attractive than any of the fruit odors ($F_{3,96}$ = 74.03, P<0.01) for flies at 8 days after emergence, irrespective of sex ($F_{1,96}$ = 0.450, P = 0.83) or fruit*sex ($F_{3,96}$ = 2.63, P = 0.054). Similarly, for flies of 3 days post-emergence, the water control was less attractive than any of the fruit odors ($F_{3,96}$ = 55.44, P<0.01) with no effect of sex ($F_{1,96}$ = 0.498, P = 0.88) or fruit*sex ($F_{3,96}$ = 0.765, P = 0.52). The attraction of flies was similar for crushed fruits of guava and blueberry for flies of 8 (Tukey test: P = 0.068) and 3 days (Tukey test: P = 0.83) post-emergence (Figure 1). However, in both maturity stages, crushed guava fruits were less attractive than raspberry (Tukey test: P<0.01 in both cases) (Figure 1).

Discussion

Unlike most of the species in the genus *Drosophila*, which have preference for overripe, rotten, or fermenting fruits, *D. suzukii* has the ability to attack ripening fruits that may

still be attached to the host plant (Mitsui et al., 2006). This study reveals the first evidence that *D. suzukii* flies are attracted to guava, that guava is a viable host in which *D. suzukii* can oviposit and can complete its development, and that this crop is naturally infested at high levels by this pest species in this part of the Gulf region of Mexico. In this study, 74% of visually intact fruits collected from the tree canopy were found to be infested by *D. suzukii*. Our field results also indicate that *D. suzukii* tend to forage in the tree canopy, with a similar prevalence of infestation in fruits from the tree canopy as on fallen fruits. It is important to note that guava fruits collected from trees were at least 3.5–5.5 m above the ground, much higher than the fruits of most cultivated berry crops. However, fruits in the crop canopy may have been slightly overestimated, as infested fruits tend to fall off branches more readily than non-infested fruits.

Drosophila suzukii had previously been reared from rotting strawberry guava fruits, *Psidium cattleianum* Sabine, collected from trees and from the ground in Hawaii, USA (Kido et al., 1996). Moreover, *D. suzukii* was one of the most frequently captured insects in methyl eugenol traps in Hawaii and its abundance was always positively correlated with captures of the tephritid *Bactrocera dorsalis* (Hendel), and coincident with the fruiting cycles of wild guava (Newell & Haramoto, 1968; Vargas et al., 1989). Although *D. suzukii* was associated with *A. fraterculus* in guava, a positive relationship between these two species seems unlikely as small superficial punctures did not result in increased oviposition by *D. suzukii*. Entomological pin punctures of 0.3 mm performed by us were wider than the mean (± SE) diameter of the *A. fraterculus* ovipositor (0.126 ± 0.002 mm), or the mean width of the *D. suzukii* egg (0.212 ± 0.004 mm; Stewart et al., 2014). Revision of fruits under a dissecting microscope following laboratory exposure to *D. suzukii* indicated that eggs were located at points unrelated to puncture wounds or damaged areas of the guava exocarp. Stewart et al. (2014) showed that the size of damaged sections of peach played a role in

D. suzukii oviposition, although they only observed oviposition in punctures of 1 mm; a wound far larger than the width of the egg or the female's ovipositor. Nevertheless, tephritid oviposition accelerates fruit ripening which could reduce fruit firmness although our results indicate that this did not increase its susceptibility to attack by *D. suzukii*. The high prevalence of *A. fraterculus* in fruits collected from guava trees may reflect high levels of this tephritid in the area and/or the tendency for *Anastrepha*-infested fruits to fall off branches more readily than non-infested fruits (Christenson & Foote, 1960).

Studies on cherry and American black cherry have reported that *D. suzukii* tends to oviposit more frequently in fruits that are still attached to the host plant than on fruits that have fallen to the ground (Mitsui et al., 2006; Poyet et al., 2014). Keesey et al. (2015) suggested that the presence of this fly in the tree canopy could be explained by attraction to green leaf volatiles, particularly β-cyclocitral – a behavior that could favor the attack of fruits attached to the tree. As such, fruit volatiles, leaf volatiles, and volatile compounds produced by microorganisms associated with guava should be evaluated to better understand the role of semiochemicals in host location and selection by this pest.

Although some fruit features, such as pH or sugar content, can influence *D. suzukii* infestation (Ioriatti et al., 2015; Lee et al., 2016), surface penetration force has been identified as a very important variable driving oviposition in *D. suzukii*. Oviposition tends to increase as fruit penetration force decreases (Burrack et al., 2013; Ioriatti et al., 2015; Lee et al., 2016). Studies with soft fruits and artificial diet reported oviposition in surfaces with a penetration force of up to 52 cN, although higher values were possible if softer fruits were not available (Burrack et al., 2013). For wine grapes, an upper threshold of 41 g (40 cN) has been suggested based on observations of field-infested grapes (Ioriatti et al., 2015). However, a recent study was unable to clearly define an upper threshold for when oviposition would not occur (Lee et al., 2016). Our results demonstrate that *D. suzukii* populations attacking guava in Mexico are capable of ovipositing in this species, even in early ripe guava. This occurs despite the high force required to penetrate the guava epidermis, in the range of 52.2–89.0 cN, which is higher than previously described. However, in ripening guava, softness could vary considerably over the surface of each fruit and adult females may have the capacity to assess firmness at various points on the surface. No significant differences were observed in the number of females that emerged per fruit in any of the three maturity stages, but significantly fewer males emerged from early ripe guavas. It is unclear why early

ripe fruits could affect male emergence and additional studies are required to clarify this issue. Indeed, the unusual shape and serrated morphology of the *D. suzukii* ovipositor appear to be key features that allow it to attack ripening fruit, resulting in its major pest status in many parts of the world (Atallah et al., 2014).

Zaprionus indianus is a polyphagous species that breeds on fallen fruits and fruits on the tree of many plants (van der Linde et al., 2006). It has only acquired pest status for one variety of fig, *Ficus carica* L. (Vilela & Goñi, 2015). Almost 90% of fallen and broken guavas were infested by *Z. indianus* compared to 50% fallen unbroken fruit and 37% of fruit attached to the tree. These findings indicate that *Z. indianus* is fully capable of oviposition in preharvest damaged fruits, but appears to favor foraging on fallen, preferably damaged fruit. All guavas infested with this pest were also infested with *D. suzukii*, *Anastrepha* spp., or both. Laboratory results indicated that this species was unable to oviposit and develop in guava fruits, even when punctured with an entomological pin. The infestation of fruits attached to the tree therefore was presumably related to fruit injuries that could not be detected by visual inspection. Whether or not other potential interactions could have ecological repercussions for these three sympatric species in guava requires further study. The presence of *Z. indianus* on fallen damaged or rotting fruit is now common in the state of Veracruz. We have detected this species in mango, soursop, and citrus orchards at many sites in Veracruz. We have also reared it from additional hosts not previously reported, such as *Spondias mombin* L. (Jalcomulco, 19°19′42.39″N, 96°45′26.90″W), *Spondias purpurea* L. (Tuzamapan, 19°25′4.51″N, 96°52′17.48″W), *Manilkara zapota* L. (Apazapan, 19°19′3.30″N, 96°43′24.33″W), and *Artocarpus heterophyllus* Lam. (Paso de Ovejas, 19°17′7.57″N, 96°27′22.05″W) (R Lasa & E Tadeo, unpubl.).

Despite the fact that raspberry and blueberry are not found together with guava in Veracruz, our choice tests revealed that volatiles from homogenized guava fruits were as attractive to adult males and females of *D. suzukii* as blueberry, a berry crop commonly attacked by this pest (Kinjo et al., 2013). Moreover, our results agree with previous findings that raspberries are highly attractive to this pest (Abraham et al., 2015), more so than guava in our case. This underlines the likely importance of fruit volatiles in the localization of adult feeding and oviposition resources.

Our results suggest clear spatial differences in the foraging habits of these two invasive drosophilid species. These findings also agree with our previous study in the same area in which traps baited with Ceratrap (Bioibérica, Barcelona, Spain), for monitoring *Anastrepha* spp.

populations, captured 2.1- and 2.9-fold more *D. suzukii* individuals than *Z. indianus* or other drosophilids, respectively (Lasa & Tadeo, 2015). Many fruits attached to the tree were attacked by *D. suzukii*. As guava fruits are available during September to November, this may be an important reservoir host for *D. suzukii* populations during the late fall and winter months which allow this insect to move onto blackberry fruits that subsequently appear in the spring. Integrated pest management tools in regions invaded by *D. suzukii* should take into account the presence of other commercial or wild hosts, even if the fruit characteristics of those species are not typical of fruits attacked by this pest. This is especially important as temporal asynchrony between primary and secondary hosts for *D. suzukii* indicate that the latter may serve as reservoir hosts between fruiting cycles.

Acknowledgements

References

Abraham J, Zhang A, Angeli S, Abubeker S, Caryn M et al. (2015) Behavioral and antennal responses of *Drosophila suzukii* (Diptera: Drosophilidae) to volatiles from fruit extracts. Environmental Entomology 44: 365–367.

Atallah J, Teixeira L, Salazar R, Zaragoza G & Kopp A (2014) The making of a pest: the evolution of a fruit-penetrating ovipositor in *Drosophila suzukii* and related species. Proceedings of the Royal Society B 281: 20132840.

Burrack HJ, Fernandez GE, Spivey T & Kraus DA (2013) Variation in selection and utilization of host crops in the field and laboratory by *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), an invasive frugivore. Pest Management Science 69: 1173–1180.

Christenson LD & Foote RH (1960) Biology of fruit flies. Annual Review of Entomology 5: 171–192.

Cini A, Ioraitti C & Anfora GA (2012) A review of the invasion of *Drosophila suzukii* in Europe and a draft research agenda for integrated pest management. Bulletin of Insectology 65: 149–160.

Conover WJ & Iman L (1981) Rank transformations as a bridge between parametric and nonparametric statistics. American Statistician 35: 124–132.

Dalton DT, Walton VM, Shearer PW, Walsh DB, Caprile J et al. (2011) Laboratory survival of *Drosophila suzukii* under simulated winter conditions of the Pacific Northwest and seasonal field trapping in five primary regions of small and stone fruit production in the United States. Pest Management Science 67: 1368–1374.

Ioriatti C, Walton V, Dalton D, Anfora G, Grassi A et al. (2015) *Drosophila suzukii* (Diptera: Drosophilidae) and its potential impact to wine grapes during harvest in two cool climate wine grape production regions. Journal of Economic Entomology 108: 1148–1155.

Keesey IW, Knaden M & Hansson BS (2015) Olfactory specialization in *Drosophila suzukii* supports an ecological shift in host preference from rotten to fresh fruit. Journal of Chemical Ecology 41: 121–128.

Kido MH, Asquith A & Vargas RI (1996) Nontarget insect attraction to methyl eugenol traps used in male annihilation of the oriental fruit fly (Diptera: Tephritidae) in riparian Hawaiian stream habitat. Environmental Entomology 25: 1279–1289.

Kinjo H, Kunimi Y, Ban T & Nakai M (2013) Oviposition efficacy of *Drosophila suzukii* (Diptera: Drosophilidae) on different cultivars of blueberry. Journal of Economic Entomology 106: 1767–1771.

Lachaise D & Tsacas L (1983) Breeding sites in tropical African drosophilids. The Genetics and Biology of Drosophila (ed. by M Ashburner, HL Carson & JN Thompson), pp. 221–332. Academic Press, London, UK.

Lasa R & Tadeo E (2015) Invasive drosophilid pests *Drosophila suzukii* and *Zaprionus indianus* (Diptera: Drosophilidae) in Veracruz, Mexico. Florida Entomologist 98: 987–988.

Lee JC, Dreves AJ, Cave AM, Kawai S, Isaacs R et al. (2015) Infestation of wild and ornamental non-crop fruits by *Drosophila suzukii* (Diptera: Drosophilidae). Annals of the Entomological Society of America 108: 117–129.

Lee JC, Dalton DT, Swoboda-Bhattarai KA, Bruck DJ, Burrack HJ et al. (2016) Characterization and manipulation of fruit susceptibility to *Drosophila suzukii*. Journal of Pest Science 87: 771.

van der Linde K, Steck GJ, Hibbard K, Birdsley JS, Alonso LM et al. (2006) First records of *Zaprionus indianus* (Diptera, Drosophilidae), a pest species on commercial fruits, from Panama and the United States of America. Florida Entomologist 89: 402–404.

Mitsui H, Takahashi KH & Kimura MT (2006) Spatial distribution and clutch sizes of *Drosophila* species ovipositing on cherry fruits of different stages. Population Ecology 48: 233–237.

Mitsui H, Beppu K & Kimura MT (2010) Seasonal life cycles and resources uses of flower and fruit feeding drosophilid flies (Diptera: Drosophilidae) in central Japan. Entomological Science 13: 60–67.

Newell IM & Haramoto FH (1968) Biotic factors influencing populations of *Dacus dorsalis* in Hawaii. Proceedings of the Hawaiian Entomological Society 20: 81–139.

Poyet M, Eslin P, Heraude M, Le Roux V, Prevost G et al. (2014) Invasive host for invasive pest: when the Asiatic cherry fly (*Drosophila suzukii*) meets the American black cherry (*Prunus serotina*) in Europe. Agricultural and Forest Entomology 16: 251–259.

de los Santos Ramos M, Bello A, Gómez RJ, Hernández R & Iribe TJ (2014) Monitoring of spotted wing drosophila (*Drosophila suzukii* Mats.) and assessment of the new attractant

SuzukiiTrap® in Tijuana, Baja California, Mexico. Journal of Food, Agriculture and Environment 12: 349–355.

SIAP (2014) Servicio de Información Agropecuaria y Pesquera, Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación. México. Available at: http://www.siap.gob.mx/ (Accessed 23 January 2016).

Steffan SA, Lee JC, Singleton ME, Vilaire A, Walsh DB et al. (2013) Susceptibility of cranberries to *Drosophila suzukii* (Diptera: Drosophilidae). Journal of Economic Entomology 106: 2424–2427.

Stewart TJ, Wang SG, Molinar A & Daane KM (2014) Factors limiting peach as a potential host for *Drosophila suzukii* (Diptera: Drosophilidae). Journal of Economic Entomology 7: 1771–1779.

Vargas RI, Stark JD & Nishida T (1989) Abundance, distribution, and dispersion indices of the oriental fruit fly and melon fly (Diptera: Tephritidae) on Kuai, Hawaiian islands. Journal of Economic Entomology 82: 1609–1615.

Vilela CR & Goñi B (2015) Mosca-africana-do-figo, *Zaprionus indianus* (Diptera: Drosophilidae). Histórico e Impacto das Pragas Introduzidas no Brasil, 2nd edn (ed. by E Vilela, RA Zucchi & F Cantor), pp. 48–52. Holos Editora, Ribeirão Preto, Brazil.

Walsh DB, Bolda MP, Goodhue RE, Dreves AJ, Lee JC et al. (2011) *Drosophila suzukii* (Diptera: Drosophilidae): invasive pest of ripening soft fruit expanding its geographic range and damage potential. Journal of Integrated Pest Management 2: G1–G7.

Williams T, Arredondo-Bernal HC & Rodriguez del Bosque LA (2013) Biological pest control in Mexico. Annual Review of Entomology 58: 119–140.