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Fresh banana as an alternative host for mass rearing *Drosophila suzukii*

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Abstract

During 2013-2015, four species of local parasitoids were collected in Mexico intending to develop biological programs for the spotted wing drosophila, *Drosophila suzukii* (Matsumura). However, to increase the productivity of this fly and to subsequently establish the mass rearing of its collected parasitoids, it was evaluated the developmental time of its life stages as well as some characteristics of its pupae and adults when reared in five fresh fruits (apple *Malus domestica* Borkh., banana *Musa paradisiaca* L., blackberry *Rubus fruticosus* L., grape *Vitis vinifera* L. and peach *Prunus persica* L.) and an artificial diet. Among the six tested rearing medium, the banana resulted the shorter time to *D. suzukii* adult emergence and produced the largest number of adults. These adults had higher size (except from specimens reared on blackberry) ($p \leq 0.05$). These results indicate that among the tested substrates, the banana is the best one to rear *D. suzukii*, and thus, allowing the mass rearing of the local parasitoids.

Key words: spotted wing drosophila, native parasitoids, augmentative biological control.

Introduction

The spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera Drosophilidae), was first detected in Mexico in 2011 (CABI, 2015). Since then, it has put at a high risk the berry industry (blackberries, blueberries, raspberries, strawberries, among others) because, due to its serrated ovipositor, this fly is able to lay eggs on ripening or ripe fruit (Cini *et al*., 2012). So far, there are no studies in Mexico of economic damage caused by this pest, but in the United States were reported losses up to 50% of crop value (Bolda *et al*., 2010). The berry industry in Mexico is estimated at 596,593 ton/year of harvested fruit and an economic value of 1,501 million USD (SIAP, 2016). Worldwide and in Mexico, the berry crops are protected mainly through the use of chemical products (Cini *et al*., 2012; Haye *et al*., 2016), a method that entails risks to human health and possible loss of its effectiveness through time (Haye *et al*., 2016).

To complement the control of *D. suzukii* in Mexico, the Centro Nacional de Referencia de Control Biológico (CNRCB) implemented a program of exploration and evaluation of native parasitoids associated with this pest. In a first stage, a colony of this fly was established in March 2013. Since then and up to May 2015, this colony was reared on store-bought grape [*Vitis vinifera* L., Red Globe variety (Vitales Vitaceae)] (Moreno-Carrillo *et al*., 2015) and acceptable results were obtained. That is to say, it provided enough larvae and pupae (used as sentinel hosts) to carried out explorations from March 2013 to February 2015 in Colima, Mexico, and from those explorations were collected four species: the larval parasitoid *Leptopilina boulardi* Barbotin, Carton et Kelner-Pillault (Hymenoptera Figitidae) and the pupal *Pachycrepoideus vindemmiae* Rondani (Hymenoptera Pteromalidae), *Spalangia simplex* Perkins (Hymenoptera Pteromalidae) and *Trichopria drosophilae* Perkins (Hymenoptera Diapriidae) (Garcia-Cancino *et al*., 2015; Moreno-Carrillo *et al*., 2015). However, despite the fact that during this period the grape as host of *D. suzukii* proved to be adequate, this substrate showed some disadvantages: throughout November to January there were days of scarcity and its cost increased up to three times higher than normal.

To increase the productivity of the colony of *D. suzukii* at the CNRCB and to subsequently establish the mass rearing of the local collected parasitoids, there were evaluated the duration of the life stages of this fly as well as some characteristics of its pupae and adults in five fresh fruits and an artificial diet.

Materials and methods

Place of study and source of biological materials

The study was conducted from May 2015 to March 2017 at the CNRCB (laboratory #2 of Entomophagous Insects: 22 ± 1 °C and 40 ± 5% relative humidity), a governmental institution belonging to the Direccion General de Sanidad Vegetal (DGSV) of SENASICA and located in Tecomán Colima, Mexico (18°55'37.73"N 103°53'0.41"W). The specimens of *D. suzukii* were obtained from the colony of the CNRCB. This colony was started in March 2013 with 3000 specimens collected in blackberry [*Rubus fruticosus* L. (Rosales Rosaceae)] at the state of Colima (Moreno-Carrillo *et al*., 2015), and its rearing methodology was described by García-Cancino *et al*. (2015) and Moreno-Carrillo *et al*. (2015).

Evaluated hosts

The study consisted of two stages. In the first, five store-bought fresh fruits (i.e., five hosts) were compared: apple [*Malus domestica* Borkh var. Golden Delicious (Rosales Rosaceae)], banana [*Musa paradisiaca* L. var. Colima (Zingiberales Musaceae)], blackberry [*R. fruticosus* var. Tupy], grape [*V. vinifera* var. Red Globe] and peach [*Prunus persica* L. Batsch var. Diamond (Rosales Rosaceae)]. These hosts were selected based on their range preference by *D. suzukii*. Apple and peach: less preferred hosts (Lee *et al*., 2015); blackberries: reported and observed as a preferred host; grape: the host that has being used in the stock colony, and banana: a non reported host but used as a lure in traps to collect both this dipteran and its parasitoids (Chabert *et al*., 2012; Rossi-Stacconi *et al*., 2015; Mazzetto *et al*., 2016).

The second stage was performed after the results of the first group of evaluations were obtained, and therein, the fruit with higher production potential was compared with an artificial diet (i.e., a sixth host), which was reported by Bautista-Martinez (2004) but with the following modifications: 560 ml of purified water, 8 gram of sodium benzoate (El Harinero, Colima, Mexico), 100% corn flour Maseca® (GRUMA, SAB de CV, DF, Mexico) instead of soybean flour, and 20 gram of ground sugarcane bagasse were replaced with 67 gram of natural wheat germ (Natural Mexico, Jalisco, Mexico). This diet was used in the CNRCB by Weiler (2017) to produce *D. suzukii* during 2016, determining five population parameters of *L. boulardi*, among them, the net reproductive rate (Ro) and the intrinsic growth rate (r_m). Although this diet is still in the development stage, it was included in these comparisons because it uses low-cost ingredients, and it has a lot of potential for the advancement of mass rearing insects, in other words, the availability of an efficient diet can help to increase production volume and quality (Cohen, 2004).

Life stages of *D. suzukii*

The number of days required by *D. suzukii* to complete its various life stages (i.e., egg, larva, pupa and adult) was evaluated separately for each host as follows: First, 1000 g of wet diet were distributed homogeneously in 10 Petri dishes of 9.4 cm in diameter by 1.2 cm in height, and simultaneously, the same amount of each fresh fruit were rinsed with tap water, air-dried for 1 h and cut into pieces (except blackberry and grape). Second, the 1000 g of each host were introduced during 4 hours into one cage (70 × 70 × 70 cm) for oviposition by *D. suzukii* (1000 randomly adults ≤ 4 days old). Third, all material from each host were extracted and evenly distributed into ten transparent plastic trays (i.e., 100 g of fruit or diet per container). These trays (17 cm in diameter by 8 cm in height, R10®, Reyma, Guanajuato, Mexico) had the upper lid perforated for ventilation (hole of 8 cm diameter covered with organza fabric) and newspaper strips (25 cm length and 2 cm width) to absorb the humidity (condensation of up to 25 ml at the end of the trial if these strips were not included), additionally, only the artificial diet trays had inside 25 ml of distilled water, otherwise, the larvae would die by dehydration. Four, every 24 hours and through a stereoscopic microscope, 20 randomly specimens (egg, larva, pupa or adult) (i.e., replicates per measurement for each treatment) were drawn from the pool of 10 trays R10®, recorded individually for its current life stages and afterwards were discarded. Finalizing these measurements until only adult emergence holes were observed in the pupae. This entire evaluation process was carried out three times.

Pupae and adults of *D. suzukii*

The characteristics of pupae (length and width) and adults (hind tibia length, quantity and sex) of *D. suzukii* were evaluated separately for each host as follows: 1000 gram of fruit or diet (same presentation as previous experiment) were placed homogeneously into 10 R10® trays (same conditions previously described) with 20 mated females of *D. suzukii* (≤ 4 days old); 48 hours later, the adult dipterans were removed. Subsequently, when the deposited eggs reached their pupal stage, 5 pupae (≤ 24 hours old) were drawn per tray (i.e., 50 replicates per treatment), and using both a stereomicroscope SteREO-Discovery® V8 (ZEISS Group, DF, Mexico) and a millimeter rule, their body length and width were measured. Finally, when all adults had emerged and died, the number of females and males per tray was determined (i.e., 10 replicates per treatment), and using both a compound microscope (DM300®, Leica Microsystems, Wetzlar, Germany) and a millimeter ruler, the length of the hind tibia of five females were measured (i.e., 50 replicates per treatment). This evaluation process and all of its replications per treatment was performed three times (i.e., 150 pupae, 30 adult offsprings and 150 hind tibiae).

Statistical analysis

The first and second batch of evaluated hosts (treatments) were analyzed separately as follows: the statistical differences among treatments in the duration of life stages of *D. suzukii* were based on the Log rank and Wilcoxon tests from the PROC LIFETEST with Bonferroni adjustment, and the differences in the characteristics of pupae (length and width) and adults [hind tibia length, quantity and sex ratio (females/progeny)] were calculated by an analysis of variance test (ANOVA) and Tukey's multiple comparison tests. All data were analyzed using the statistical package SAS® V.9.2 (SAS Institute Inc. Cary North Caroline, USA) and $p \leq 0.05$.

Results and discussion

The eggs of *D. suzukii* deposited on the different hosts, except those deposited on the apple, required an equal number of days for their hatching, but their larval development was shorter in banana, grape and peach than in apple, artificial diet and blackberry (χ^2: Log rank ≤ 12.46, df ≤ 4, $p \leq 0.015$; Wilcoxon ≤ 10.26, df ≤ 4, $p \leq 0.036$) (table 1). On the other hand, both the pupal development and emergence of the adult had fewer days in the banana than in the other hosts (χ^2: Log rank ≤ 17.58, df ≤ 4, $p \leq 0.016$; Wilcoxon ≤ 22.41, df ≤ 4, $p \leq 0.025$), that is, the pupae were obtained 3, 1.66, 0.5, 0.33 and 0.33 days sooner than in apple, blackberry, artificial diet, grape and peach, respectively, and similarly in the banana, the adults emerged 10, 4, 3.2, 1.67 and 1.67 days faster than in the other hosts. To the best of our knowledge, there have not been reported studies on the development time of egg to adult of *D. suzukii* in banana, however, with the caveat that our experimental conditions were different from theirs, the duration recorded in this study falls within the range reported at

Table 1. Duration of life stages of *D. suzukii* developed in five fresh fruits and an artificial diet.

Hosts	Duration (days)			
	Egg	Larva	Pupa	Egg to adult
Apple	5.33 ± 0.33^{b}	8.00 ± 0.33^{c}	8.00 ± 1.00^{b}	21.33 ± 1.20^{d}
Banana	2.00 ± 0.33^{a}	4.33 ± 0.33^{a}	5.00 ± 0.33^{a}	11.33 ± 0.33^{a}
Blackberry	2.66 ± 0.33^{a}	6.00 ± 0.33^{b}	6.66 ± 0.33^{b}	15.33 ± 0.66^{c}
Grape	2.66 ± 0.33^{a}	5.00 ± 0.33^{a}	5.33 ± 0.33^{ab}	13.00 ± 0.33^{b}
Peach	2.66 ± 0.33^{a}	5.00 ± 0.33^{a}	5.33 ± 0.33^{ab}	13.00 ± 0.33^{b}
Banana	2.40 ± 0.15^{a}	4.26 ± 0.10^{a}	4.38 ± 0.09^{a}	11.05 ± 0.09^{a}
Artificial diet	1.96 ± 0.08^{a}	7.40 ± 0.28^{b}	4.88 ± 0.31^{b}	14.25 ± 0.31^{b}

Means ± SE (same column) followed by the same letter are not statistically different, within each subgroup of comparisons (separated by a thin dark line), by χ^2: Log rank and Wilcoxon tests ($p \leq 0.05$).

Table 2. Morphological characteristics of pupae and females of *D. suzukii* developed in five fresh fruits and an artificial diet.

Hosts	Pupa (mm)			Hind tibia of female (mm)
	Length	Wide	Length × wide	Length
Apple	3.13 ± 0.02^{c}	1.13 ± 0.06^{a}	3.54 ± 0.19^{a}	70.08 ± 0.64^{c}
Banana	3.45 ± 0.02^{b}	1.19 ± 0.06^{a}	4.11 ± 0.19^{a}	82.20 ± 0.64^{a}
Blackberry	3.53 ± 0.02^{a}	1.20 ± 0.06^{a}	4.27 ± 0.19^{a}	84.53 ± 0.64^{a}
Grape	3.54 ± 0.02^{a}	1.19 ± 0.06^{a}	4.26 ± 0.19^{a}	82.17 ± 0.64^{ab}
Peach	3.49 ± 0.02^{ab}	1.12 ± 0.06^{a}	3.92 ± 0.19^{a}	81.42 ± 0.64^{b}
Banana	3.36 ± 0.02^{a}	1.23 ± 0.14^{a}	4.15 ± 0.06^{a}	85.40 ± 0.01^{a}
Artificial diet	3.31 ± 0.02^{a}	1.27 ± 0.14^{a}	4.22 ± 0.06^{a}	86.20 ± 0.01^{a}

Means ± SE (same column) followed by the same letter are not statistically different, within each subgroup of comparisons (separated by a thin dark line), by ANOVA and Tukey's multiple comparison tests ($p \leq 0.05$).

22 °C by Emiljanowicz *et al*. (2014) and Tochen *et al*. (2014): 12.8 and 14 days for a Standard Artificial Diet of Drosophila (SADD) and wild cherry [*Prunus avium* L. (Rosales Rosaceae)], respectively.

The total pupae size (length by width) of *D. suzukii* was not statistically different among hosts, but the hind tibia of the females reared in banana and blackberry were statistically larger than the hind tibia of the females reared in the other hosts (i.e., larger adults were produced on the former group) (Tukey's multiple comparison tests: $f \leq 79.52$, $df \leq 4$, $p \leq 0.001$) (table 2). In insects, the size of the adult is positively correlated with its fecundity, longevity and search capacity, among other characteristics (Slansky, 1982), consequently, it is expected a greater population growth of this fly when reproduced in banana and blackberry.

The sex ratio of *D. suzukii* was not statistically different among hosts (Tukey's multiple comparison tests: $f \leq 1.63$, $df \leq 4$, $p \geq 0.089$) (figure 1A). This result indicates that the sex ratio is not influenced by the type of ingested food. On a related issue, the females ovipositing in banana produced more adults than females on the other evaluated hosts (Tukey's multiple comparison tests: $f \leq 76.4$, $df \leq 4$, $p \leq 0.001$) (figure 1B), i.e., they had 90.01, 64.74, 80.21, 65.85 and 67.53% more specimens than in apple, blackberry, artificial diet, grape and peach, respectively. Among insect populations that do not show differences in their sex ratio, a higher offspring production is positively correlated with higher population growth (Russell *et al*., 2008). Thus, this higher productivity in banana indicates, under the premise that the others biological parameters have similar numerical values, that *D. suzukii* reared in this fruit should have more larvae and pupae than when reared in the other evaluated hosts.

Compared with the other hosts, the banana produced the largest number of adults of *D. suzukii*, and these adults had the largest size (except specimens in blackberry); additionally, the eggs deposited on this substrate registered the shortest development time to reach the adult stage. Consequently, these results indicate that the alternative host (i.e., banana) has a greater potential to increase the productivity of the colony of this fly. To date, no studies have been reported where banana has been used in the rearing of this fly, although it is commonly used in sentinel traps to capture this pest (Rossi-Stacconi *et al*., 2015; Mazzetto *et al*., 2016) and its parasitoids (Chabert *et al*., 2012). Perhaps, this fruit has not been evaluated as a host because, globally, *D. suzukii* colonies are commonly reared on SADD (Rossi-Stacconi *et al*., 2006; Emiljanovich *et al*., 2014; Lee *et al*., 2015) or in SADD enriched with some fresh fruit (Chabert *et al*., 2012; Kacsoh *et al*., 2012).

The result that the banana has the greatest potential to rear *D. suzukii* will facilitate its mass rearing at the CNRCB, for the following reasons: it is cheaper than the grape (0.77-0.88 USD/kg and 3.33-3.88 USD/ kg, respectively) (SNIIM, 2017), its price is stable over time, and being a local crop (6,057 ha planted and 195,169 ton/year of harvested fruit in the state of Colima during 2016) (SIAP , 2016), there is abundant availability of fresh fruit throughout the year. Moreover, the

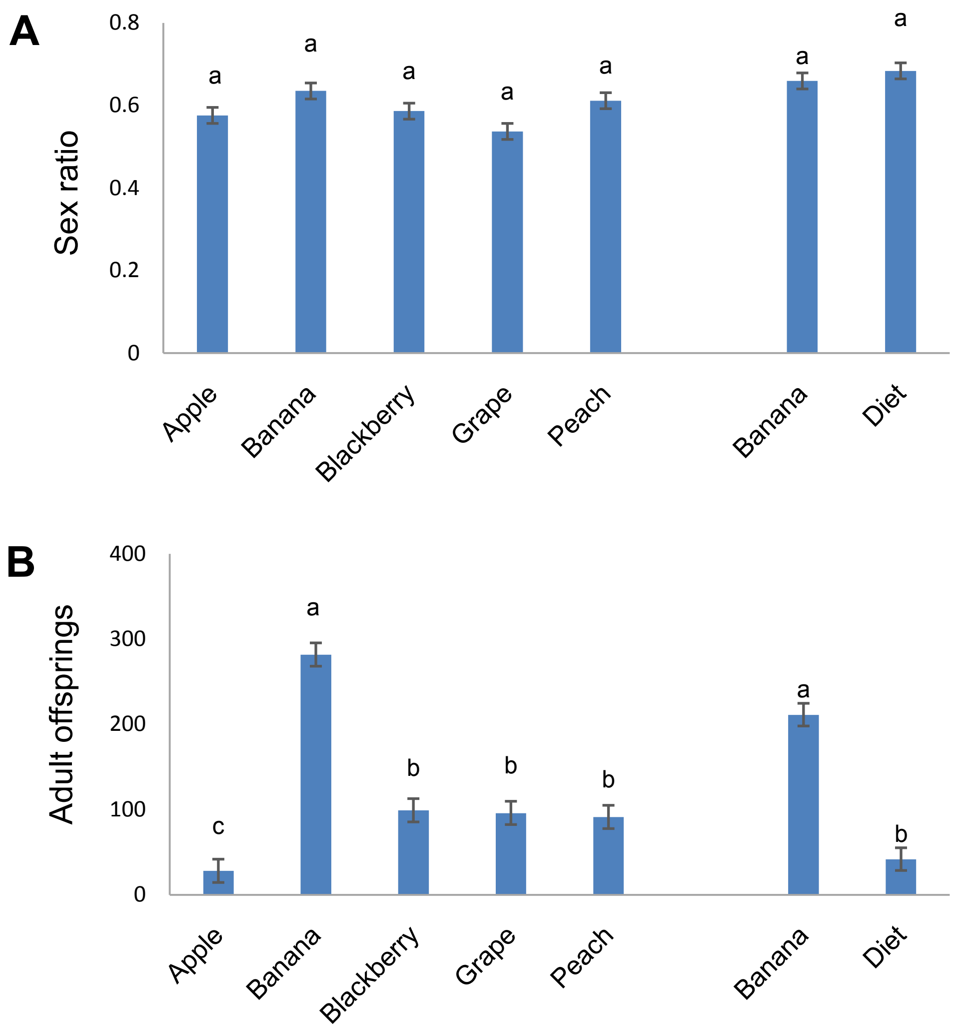

Figure 1. Sex ratio (A) and adult offspring (B) of *D. suzukii* developed in five fresh fruits and an artificial diet. Means ± SE followed by the same letter are not statistically different, within each subgroup of comparisons (separated by a blank space), by ANOVA and Tukey's multiple comparison tests ($p \leq 0.05$).

banana could be a better option to rear this fly than SADD or enriched SADD, for two reasons: (a) in both types of artificial diets are used high-cost ingredients, such as the solidifying agar (44.44 USD/kg) and the antimicrobial preservative methyl paraben (77.77 USD/kg), but these products are not used on banana rearings; consequently, this fruit could be a cheaper substrate for fly production; and (b) to date, no diet has been shown to be better host of *D. suzukii* than banana, on the contrary, Chabert *et al*. (2012) enriched SADD with this fruit because relatively few eggs were deposited.

This study was intended to increase the productivity of *D. suzukii* and to subsequently implement the mass rearing of the native parasitoids. Consequently, it was evaluated the diet reported by Bautista-Martinez (2004) because it uses low-cost ingredients, such as the solidifying corn flour (0.72 USD/kg) and the antimicrobial preservative sodium benzoate (2.22 USD/kg). Although the diet registered lower performance than banana, further research will be done to improve it. An optimized diet could be better than banana in two ways: (a) *D. suzukii* larvae burrow deeper in this fruit than in diet, so, potentially *L. boulardi* could parasitize them more easily on diet; and (b) parasitized pupae of *D. suzukii* in diet (i.e., Petri dishes) could be transported to the field easier than parasitized pupae in banana (i.e., trays R10®), so, the reared parasitoids could emerge in the field, rather than emerging at the CNRCB (current production system).

The spotted wing drosophila is present in ten states of Mexico (Lasa and Tadeo, 2015; García-Avila *et al*., 2016) but mathematical models based on climatic, demographic and physiological data indicate that its potential niche is the entire country (Damus, 2009; Gutierrez *et al*., 2016). In this likely scenario of wide distribution and its inherent economic losses, it is necessary to reinforce the methods of prevention and control, and the use of native parasitoids represents a good option that can be performed immediately. The results (here obtained) will make possible to increase the production of both larvae and pupae of *D. suzukii* at the CNRCB, and thus they will facilitate the mass rearing of the parasitoids collected by Moreno-Carrillo *et al*. (2015) and Garcia-Cancino *et al*. (2015).

Conclusions

The adults of *D. suzukii* developed faster in the fresh banana than in apple, blackberry, diet, grape and peach.

The fresh banana and blackberry originated larger *D. suzukii* females than the other evaluated hosts.

The females ovipositing in banana produced 90.01, 64.74, 80.21, 65.85 and 67.53% more specimens than in apple, blackberry, diet, grape and peach, respectively.

The sex ratio (females/offspring) of *D. suzukii* was not influenced by the host type.

Overall, the aforementioned results indicate that the banana has a greater potential to increase the productivity of the spotted wing drosophila.

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