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Food Baits and Traps for Monitoring Drosophila Suzukii and Zapronius Indianus "Drosophilidae" in Fig Orchards

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ABSTRACT

Drosophila suzukii Matsumura and *Zaprionus indianus* Gupta (Diptera: Drosophilidae) are invasive pests that attack fig crops in Mexico. Monitoring fruit flies ' populations' density is the main tool for taking control action choices. In the case of fruit flies, the use of food baits and traps is a regular practice in fruit and vegetable production. However, local fig growers are characterized by a low technological level, with a constant search for economical and practical options to improve their quality and incomes. Here we tested three traps (two from formal manufacturers and one plastic handmade) and three food baits for collecting adults of *D. suzukii* and *Z. indianus*. Two trials were conducted in different periods, November-December 2018 and July-August 2019 in local fig orchards. Handmade plastic jar traps baited with commercial food baits showed good performance against commercial traps, for the two fruitflies in both evaluation periods. The role of the handmade trap design in the attraction efficiency for fruitflies collection is discussed. The region of study is considered with a high population level of *D. suzukii* and *Z. indianus*.

Keywords: Fig flies, Drosophilid flies, Trapping, Handmade trap.

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INTRODUCTION

The fig tree (*Ficus carica* L., Moraceae) is a native species from the south-western Mediterranean area and is considered the first plant domesticated by human beings [1]. As a crop, is cultivated in temperate, tropical, and subtropical regions, its rusticity has allowed being grown in great soils diversity [2]. Worldwide, the cultivated area exceeds 376,100 hectares, with an estimated production of 1,064,400 tons; Turkey is considered as the main fig producer [3]. In Mexico, the cultivated surface reported in 2018 was 1,357 ha, a production volume of 7,700 tons, and an economic value of 8 million dollars [4]. The most important production areas are concentrated in the central Morelos state, characterized by their low technological level [5]. The principal tool for pest management is the periodical chemical pesticides application.

Recently, two Drosophilidae (Insecta: Diptera) have been affecting quality and phytosanitary fruit status: *Drosophila suzukii* Matsumura and *Zaprionus indianus* Gupta [6, 7], considered devastating pests of strawberry, peach, blackberries, figs, and small fruits with thin epicarp [5, 8, 9].

Drosophila suzukii is original from southeast Asia [10], described from Japan in 1931 [11]. In Mexico, *D. suzukii* was reported as an invasive species in 2011 and was considered as present in only one municipality: Los Reyes (Michoacan) [12]. Is presently considered as Mexico and under governmental control, although only in some areas with cultivated hosts meets the formal quarantine pest definition [7, 13]. Unlike other drosophilid flies, *D. suzukii* is characterized by a serrated ovipositor, used to easily cut the fruit epicarp to oviposit into ripened and unripened fruits; this species feeds on overripe or decaying fruits [14, 15].

Zaprionus indianus was reported for the first time in America in fig fruits from Brazil, causing losses of 40 a 50% in commercial figs, and where it was called the Africa fig fly [16]. It was detected in Mexico for the first time in Chiapas in 2002 [17], despite the fact it has received little attention, its distribution includes crops from seven federal entities [6, 7, 17]. In fig is considered a major pest [18]. *Zaprionus indianus* unlike *D. suzukii* does not have a serrated ovipositor, this fly penetrates the ostiole to oviposit when the fig is reaching its maturity [19].

As part of the strategies implemented for these drosophilid management, trapping for monitoring adult populations in fig production areas and regions with invasion risk has been implemented [20]. Monitoring with traps allows to determine the drosophilid adult presence or absence and to calculate population fluctuations. Traps used by local growers are plastic hand man jars, baited with apple vinegar, disposed of in quadrants separated at 1 km [5] but mainly the typical Multilure© baited for other fruitflies (personal observation). Different types of commercial and homemade baits based on apple cider vinegar, wine, and yeast have been evaluated for the monitoring of these flies [21]. However, apple vinegar has the potential to attract a wide diversity of Drosophilidae flies, included not target species, and other Diptera, Lepidoptera, Hymenoptera, and Coleoptera species [22, 23].

There are commercial traps (Multilure© and Pherocon SWD©) and food attractants (Pherocom SWD© Dual-Lure, Suzukii Trap©) recommended for monitoring and even for mass trapping for these and other fruitflies, but they never have been tested under local grower conditions in Morelos. The purpose of this work was to evaluate the efficacy of commercial baits and traps for monitoring adults of two drosophilid adults (Insecta: Diptera) with economic importance for cultivated fig in Morelos, Mexico.

MATERIALS AND METHODS

Experiment location

The research was carried out in commercial fig orchards, variety Black Mission, grown in Ayala, Morelos, Mexico (18.734206° -98.915858). The climate in the region is warm subhumid with an average annual temperature of 24°C [24].

Traps and food baits

Two commercial, Multilure© (Ferommis, Mexico) and Pherocon SWD© (Trécé, Inc., United States) and one handmade jar trap were used. Handmade traps were made with one-liter plastic jars, with 10 holes of 4 mm around the upper container, and a red base [5, 25]. As food baits, Suzukii Trap© (Bioiberica, Spain), Pherocon SWD© Dual-Lure© (Ferommis, Mexico), and Cera Trap© (Agrotecnologia Alternativa, S.A. de C.V., Mexico) were analyzed. 250 ml of liquid baits (Suzukii Trap© and Cera Trap©) was placed by trap, for Pherocon SWD© Dual-Lure, one dispenser was attached in the interior of the traps, and soapy water (5%) was used for insect retention.

Experimental design

Two factors were analyzed, trap (with three levels) and food bait (three levels), combination of both factors produced nine treatments 1) Handmade plastic jar-Cera trap©; 2) Handmade plastic jar-Suzukii Trap©; 3) Handmade plastic jar-Pherocom SWD© Dual-Lure©; 4) Multilure©- Suzukii Trap©; 5) Multilure©- Suzukii Trap©; 6) Multilure©- Pherocom SWD© Dual-Lure©; 7) Pherocon©- Suzukii Trap©; 8) Pherocon©- Suzukii Trap©; and 9) Pherocon©- Pherocom SWD© Dual-Lure©.

The experimental design was completely randomized in blocks, each treatment had four repetitions, and all treatments were arranged in four rows. Traps were checked weekly, and at each sampling date, treatments were randomized to avoid any positional bias. The separation distance between traps was 20 m and between rows was 30 m, traps were placed at ¾ height of trees, avoiding direct exposure to the sun. The experiment was carried out on two occasions, with a duration of six weeks. The first period was from 2018 November 19th to December 24th, and the second from 2019 July 12th to August 16th. On both dates, fruits were in ¾ of their physiological maturity. Pherocom SWD® Dual-Lure© dispenser was changed every four weeks, and the soapy water weekly. Suzukii trap® and Cera trap® baits were filled when necessary.

Entomological determination

All specimens captured were preserved in alcohol 70% and determined with taxonomic keys [26].

Data analysis

Data was tested for normality and homogeneity of variance by species, then treatment means were compared (ANOVA), and when significant differences were detected the Fischer test was applied (α= 0.05). In the second period, data was transformed ($\sqrt{X}$ +0.5) to normalize the distribution and homogenize the variations before analysis.

Day Trap Flies index (DTF)

The Day Trap Flies index is used to calculate the relative fruiflies adult presence in a particular area and time, commonly used in native fruit flies of the genus *Anastrepha* (Diptera: Tephritidae), used to choose management strategies for tephritid populations [27]. The index was calculated by species by treatment [5]:

$$DTF = F/(TxD) \quad (1)$$

Donde:

F= Number of flies collected

T = Total number of traps revised

D = Number of days those traps were exposed in the field

DTF is expressed in the format 0.0000 [28], level prevalence is categorized as high (≥0.0100), low (≤0.0100), and absent (0.0000).

RESULTS AND DISCUSSION

Monitoring is an important tool to determine and know the present/absence of pests in a crop and allows making correct decisions for their integrated management [21]. For fruit flies, trap design and food baits influence capturing adults [29, 30]. The trap design must be attractive for fruit flies, with a capacity for temporal storage of the food bait, and with space for the release of the bait compounds; at the same time, food bait needs to be the correct lure and could act as the retention mechanism [31].

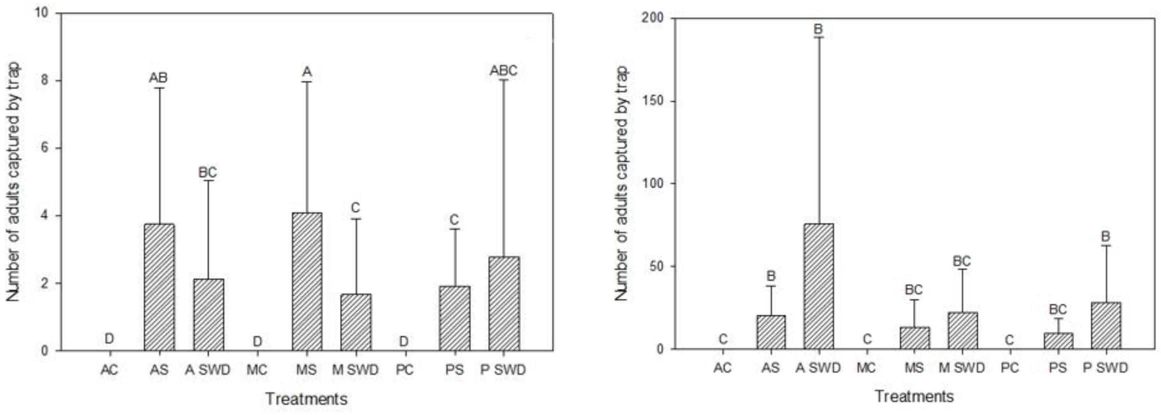
First trial

Traps and food baits

4,458 specimens were captured, 8.0 % (373) were determined as *D. suzukii* and 92% (4,285) were classified as *Z. indianus*. The type of food bait affected the drosophilid species captured. Suzukii trap© was more effective to capture *D. suzukii*, but only when is used in combination with the handmade or Multilure® traps; its attraction is reduced with Pherocom© trap **(Figure 1a)**. However, this food bait captured not target Drosophilidae (data not shown). Pherocom© trap plus Pherocom SWD© Dual-Lure© food bait showed similar captures in comparison with Suzukii trap© in handmade or Multilure® traps.

For *Z. indianus*, handmade plastic traps baited with Pherocom SWD© were the best combination, collecting three or five times the rest of the treatments **(Figure 1b)**.

For both drosophild species, Cera Trap® did not capture specimens, independently of the trap used **(Figures 1a and 1b)**.



a) b)

Figure 1. *Drosophila suzukii* (a) and *Zapronius indianus* (b) captures in a combination of three traps and food baits in ficus, Mexico (November-December 2018). AC: Handmade trap + Cera Trap©; AS: Handmade trap+ Suzukii© trap; ASWD: Handmade trap + Pherocom SWD© Dual-Lure©; MC: Multilure© trap + Cera Trap©; MS: Multilure© trap + Suzukii© trap; MSWD: Multilure© trap + Pherocom SWD© Dual-Lure©; PC: Pherocom© trap + Cera Trap©; PS: Pherocom© trap + Suzukii© trap; PSWD: Pherocom© trap + Pherocom SWD© Dual-Lure©.

DTF index

Interaction between traps and food baits affected the prevalence population-level calculated for *D. suzukii*: the biggest DTF index value was calculated with Multilure© trap and Suzukii trap© as food bait and similar when this food bait is used with handmade plastic jar traps. Pherocom© trap plus Pherocom SWD© Dual-Lure© bait had a similar value as Multilure© trap and Suzukii trap© combination **(Table 1)**. The rest of the treatments showed minor sensibility to calculate the DTF index, and even those traps baited with Cera trap© did not allow to calculate it. Food baits Suzukii trap© and Pherocom SWD© Dual-Lure© used with all three traps calculate a high population prevalence for *D. suzukii*.

Table 1. Day Trap Flies (DTF) population prevalence index, maximum and minimum values calculated for *Drosophila suzukii* with different traps and food baits in figs from Morelos, Mexico (November-December 2018).

Treatment (trap plus food bait)	Maximum prevalence level	Minimum prevalence level	Weeks with prevalence values 0.0000
Handmade + Cera trap©	0.0000	0.0000	6
Handmade + Suzukii trap©	0.9200	0.2500	0
Handmade + Pherocom SWD© Dual-Lure©	0.8500	0.0700	0
Multilure© + Cera trap©	0.0000	0.0000	6
Multilure© + Suzukii trap©	1.1700	0.3200	0
Multilure© + Pherocom SWD© Dual-Lure©	0.5700	0.0300	0
Pherocom© + Cera trap©	0.0000	0.0000	6
Pherocom© + Suzukii trap©	0.4600	0.1700	0
Pherocom© + Pherocom SWD© Dual-Lure©	1.0000	0.0700	0

Table 2. Day Trap Flies (DTF) population prevalence index, maximum and minimum values calculated for *Zaprionis indianus* with different traps and food baits in figs from Morelos, Mexico (November-December 2018).

Treatment (trap plus food bait)	Maximum prevalence level	Minimum prevalence level	Weeks with prevalence values 0.0000
Handmade + Cera trap©	0.0000	0.0000	6
Handmade + Suzukii trap©	8.8500	1.0700	0
Handmade + Pherocom SWD© Dual-Lure©	33.4000	2.2800	0
Multilure© + Cera trap©	0.0000	0.0000	6
Multilure© + Suzukii trap©	2.8900	0.1740	0
Multilure© + Pherocom SWD© Dual-Lure©	7.4200	1.2800	0

Pherocom© + Cera trap©	0.0000	0.0000	6
Pherocom© + Suzukii trap©	2.3500	0.7500	0
Pherocom© + Pherocom SWD© Dual-Lure©	8.8500	0.7800	0

A similar response for the DTF index was recorded for *Z. indianus*, high population prevalence was calculated with the food baits Suzukii trap© and Pherocom SWD© Dual-Lure©, in all the traps tested **(Table 2)**. With the higher DTF index calculated by Handmade plastic jar handmade plus Pherocom SWD© Dual-Lure© bait. Cera Trap© as a food bait did not allow the calculation of DTF index independently of the trap analyzed **(Table 2)**.

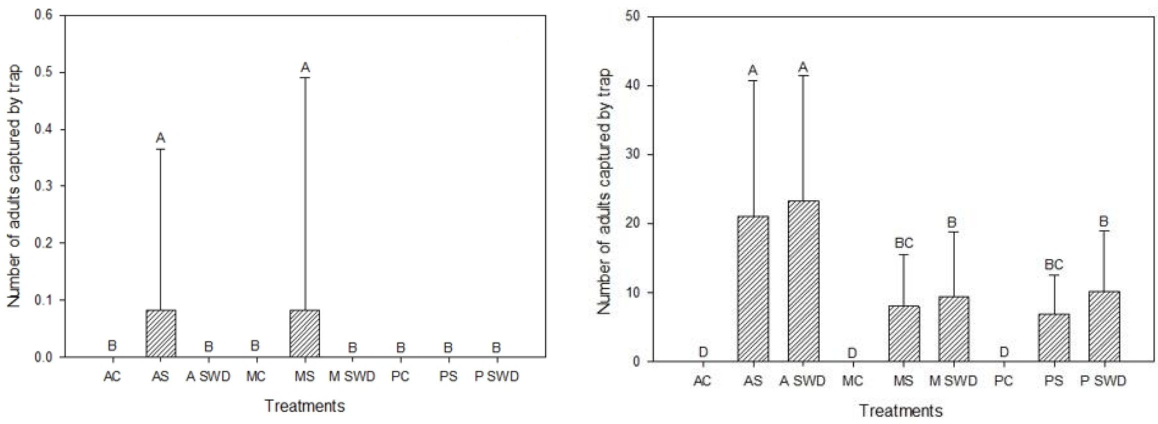
Second trial

Traps and food baits

In the second trial, captures for both species were lower than the first trial. 1,899 were collected, just four of *D. suzukii* (0.2 %), the rest of the specimens were determined as *Z. indianus* (1,895). All *D. suzukii* individuals were captured in the handmade plastic jar or Multilure© traps baited with Suzukii© trap, without statistical differences **(Figure 2a)**.

In *Z. indianus*, type of trap affected the number of specimens captured, handmade plastic jar trap baited with Suzukii trap© or Pherocom SWD© Dual-Lure© collected more than 100% in comparison with the rest of treatments **(Figure 2b)**.

Similar to the recorded in the first trial, Cera Trap® food bait did not capture specimens.



a) b)

Figure 2. Drosophila suzukii (A) and Zapronius indianus (B) captures in a combination of three traps and food baits in ficus, Mexico (July-August 2019). AC: Handmade trap + Cera Trap©; AS: Handmade trap+ Suzukii© trap; ASWD: Handmade trap + Pherocom SWD© Dual-Lure©; MC: Multilure© trap + Cera Trap©; MS: Multilure© trap + Suzukii© trap; MSWD: Multilure© trap + Pherocom SWD© Dual-Lure©; PC: Pherocom© trap + Cera Trap©; PS: Pherocom© trap + Suzukii© trap; PSWD: Pherocom© trap + Pherocom SWD© Dual-Lure©.

The performance achieved by the handmade plastic jar trap is probably influenced by the combination of two factors: perforations and color. The number and diameter of the holes (4 mm) around the plastic jar walls facility dispersion of the food bait to the environment and the same time the entry of the fruitflies. In contrast, the Pherocom© trap has two big lateral holes covered with a plastic mesh with a 2 mm diameter opening, which is possibly difficult for direct entry to the trap. Finally, the Multilure© trap is characterized by a wide-open mouth at the bottom and a yellow base; is possible that hole dimension allows flies easy entry, but at the same time may also facilitate its exit if the retention media is not adequate. As was pointed before [32], increasing the entry trap areas decrease their capture capacity and its performance [29]. An additional issue is the possibility to increase captures of not Drosophilidae target, even Diptera or another bigger arthropod [32]. Handmade plastic jar traps showed the minor entry space, and this could be their first advantage in comparison to the other traps, being more selective to drosophilid flies [29].

Regarding the color, although it has been considered that red is attractive for the capture of *D. suzukii* adults [29], coinciding partially with the handmade red base trap, some authors consider that other colors could generate a greater attraction response [33, 34]. It seems that more options for this variable still need to be explored.

The drosophilid species responded differently to the bait tested, *D. suzukii* was attracted mainly to Suzukii trap® and *Z. indianus* to Pherocom© SWD Dual-Lure©. *Drosophila suzukii* is attracted mainly by fermented substances such as wine and vinegar, as well as some yeasts [29]. Suzukii trap® is composed of enriched protein substances, with a good attraction response of *D. suzukii*, proposed to be used in mass trapping for this fruitfly [35]. On the other side, *Z. indianus* has a preference for juices, vinegar, and wines [34, 36], with a god response to Pherocom© SWD Dual-Lure© [37] because is composed of wine and vinegar volatile substances (acetic acid, ethanol, acetone, and methanol) [38]. The null response of both drosophila to Cera trap ® is related to its origin. It was designed and tested for *Ceratitis capitata* Wiedeman, however is now widely used for many *Anastrepha* species [39]. Results derived from this work suggests the use of Suzukii trap® and Pherocom© SWD Dual-Lure©, but Cera trap ® must be discarded immediately as bait food for these two drosophilid flies. The optimal selection of trap and food bait for fruiflies is a continuous task [40], as new designs and baits are being offered constantly, the options for local growers will increase their capacity for deploying better trapping systems.

DTF index

The population density in the second trial affected calculating the DTF index (**n n**). Just two treatments can calculate the index, Handmade plastic jar plus Suzukii trap© and Multilure© trap plus Suzukii trap© bait, with a high prevalence level (0.0300-0.0700). According to the rest of the treatments, *D. suzukii* is absent in the study region in this period **(Table 3)**.

Table 3. Day Trap Flies (DTF) population prevalence index, maximum and minimum values calculated for *Drosophila suzukii* with different traps and food baits in figs from Morelos, Mexico (July-August 2019).

Treatment (trap plus food bait)	Maximum prevalence level	Minimum prevalence level	Weeks with prevalence values 0.0000
Handmade + Cera trap©	0.0000	0.0000	6
Handmade + Suzukii trap©	0.0300	0.0000	4
Handmade + Pherocom SWD© Dual-Lure©	0.0000	0.0000	6
Multilure© + Cera trap©	0.0000	0.0000	6
Multilure© + Suzukii trap©	0.0700	0.0000	5
Multilure© + Pherocom SWD© Dual-Lure©	0.0000	0.0000	6
Pherocom© + Cera trap©	0.0000	0.0000	6
Pherocom© + Suzukii trap©	0.0000	0.0000	6
Pherocom© + Pherocom SWD© Dual-Lure©	0.0000	0.0000	6

Table 4. Day Trap Flies (DTF) population prevalence index, maximum and minimum values calculated for *Zaprionis indianus* with different traps and food baits in figs from Morelos, Mexico (July-August 2019).

Treatment (trap plus food bait)	Maximum prevalence level	Minimum prevalence level	Weeks with prevalence values 0.0000
Handmade + Cera trap©	0.0000	0.0000	6
Handmade + Suzukii trap©	5.4600	1.8200	0
Handmade + Pherocom SWD© Dual-Lure©	5.3900	1.0350	0
Multilure© + Cera trap©	0.0000	0.0000	6
Multilure© + Suzukii trap©	1.6700	0.4600	0
Multilure© + Pherocom SWD© Dual-Lure©	2.2500	0.8500	0
Pherocom© + Cera trap©	0.0000	0.0000	6
Pherocom© + Suzukii trap©	1.7800	0.4600	0
Pherocom© + Pherocom SWD© Dual-Lure©	2.4600	0.0000	1

A high prevalence level was calculated for *Z. indianus* in all the traps and food baited used, except when Cera Trap© was involved. Handmade jar trap showed the higher index values, with similar results using Suzukii trap© or Pherocom SWD© Dual-Lure© **(Table 4)**. In this trap, the DTF index was two or three times higher than other treatments.

DTF index pointed for a high prevalence level for *D. suzukii* and *Z. indianus*, confirming that fig production area has the environmental conditions for the continuous reproduction of this species [7] and calling for the impulse of regional and coordinate activities to reduce populations and potential damage from this fruitflies.

CONCLUSION

Drosophila suzukii Matsumura and *Zaprionus indianus* Gupta, are two fruit flies of economic importance for fig production in Morelos, Mexico, where environmental conditions provide an opportunity for their population to increase. For the optimal implementation of a monitoring program for drosophild adults, results derived from this study indicate that *D. suzukii* and *Z. indianus* could be trapped deploying handmade plastic jar traps baited with Suzukii trap® **or** Pherocom© SWD Dual-Lure©.

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