

# *Drosophila suzukii* response to augmentative releases of *Trichopria drosophilae* in Mexico

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## Abstract

Evaluations performed in agricultural fields of Mexico indicated that 4500 adults ha<sup>-1</sup> of the parasitoid *Trichopria drosophilae* (Perkins) reduced the population of the spotted wing drosophila, *Drosophila suzukii* (Matsumura), by 50%. To further the effectiveness that *T. drosophilae* exerted on *D. suzukii*, another field evaluation was executed, but now a 5-fold larger release rate was included. Thus, three release treatments were performed bi-monthly from January to December 2019 in commercial berry fields of Jalisco: 0 (no release control), 4500 and 27000 adults ha<sup>-1</sup>. These trials determined that the parasitization rate by *T. drosophilae* increased by 2.8 and 1.3 times in the low- and high-release sites, respectively. Moreover, the *D. suzukii* captures were reduced by 43.25% and 32.32% in the low- and high-release sites, respectively. These results indicate that between the tested release rates, the low rate is the best one to reduce *D. suzukii* populations, and that such activity contributes substantially to the phytosanitary management of this invasive pest.

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

## Introduction

The spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera Drosophilidae), is a high-risk pest of the worldwide berry industry (blackberries, blueberries, raspberries, among others) because it can cause losses up to 37% of crop value (DiGiacomo *et al.*, 2019); furthermore, as a pest of quarantine importance (EPPO, 2011), the detection of a single infested fruit at the loading docks may lead to rejection of the whole shipment (Mazzi *et al.*, 2017).

Prior to 2008, *D. suzukii* was present only in Southeast Asia (Cini *et al.*, 2012) and in the Hawaiian Islands (Hauser *et al.*, 2011); however, its current geographical distribution includes Africa, America, Europe, and other regions of Asia (Asplen *et al.*, 2015). Its control is based primarily on chemical products and cultural practices (Cini *et al.*, 2012; Haye *et al.*, 2016; Schetelig *et al.*, 2018), but these methods are not sustainable in the long term (Mazzi *et al.*, 2017; Gress and Zalom, 2019; DiGiacomo *et al.*, 2019), mainly because they are not applied in lands adjacent to the cultivation areas, and if these sites contain one of the 50 species of wild host plants of this invasive dipteran, they could be a constant source of inoculum toward agricultural areas (Asplen *et al.*, 2015; Haye *et al.*, 2016). Therefore, the use of parasitoids is considered a viable option to contribute to the phytosanitary management of *D. suzukii*, since its effect would reach both agricultural and surrounding areas.

*D. suzukii* was first detected in Mexico in 2011 (CABI, 2020). To protect the berry industry, the federal government implemented an overall strategy for its management and control in 2012 (DGSV, 2012), which included as an addendum, a program to explore and evaluate local parasitoids associated with this pest in 2013. So far, four stages of the program have been executed: (1) explorations were carried out in Cuauhtemoc Colima, and Zapotlan Jalisco, Mexico, and five species were collected (alphabetically ordered): *Ganaspis brasiliensis* (Ihering),

*Leptopilina boulardi* (Barbotin, Carton et Kelner-Pillault) (both, Hymenoptera Figitidae), *Pachycrepoides vindemiae* (Rondani), *Spalangia simplex* Perkins (both, Hymenoptera Pteromalidae) and *Trichopria drosophilae* (Perkins) (Hymenoptera Diapriidae) (Garcia-Cancino *et al.*, 2015; Moreno-Carrillo *et al.*, 2015; Gonzalez-Cabrera *et al.*, 2020); (2) laboratory evaluations of the collected species were executed at the Centro Nacional de Referencia de Control Biológico (CNRCB) (Gonzalez-Cabrera *et al.*, 2018; Sanchez-Gonzalez *et al.*, 2020), and it was concluded that *T. drosophilae* had the highest potential to reduce *D. suzukii* populations (Garcia-Cancino *et al.*, 2020); (3) *T. drosophilae* was released at different rates (0, 1500, and 3000 adults ha<sup>-1</sup>) from June 2015 to October 2016 in berry fields in Colima, Mexico, but at the end of the trials, there were no statistical significant differences among treatments in the population density of this invasive dipteran; and (4) 4500 adults ha<sup>-1</sup> of *T. drosophilae* were released from June 2017 to May 2018 in commercial berry fields of Colima and Jalisco, Mexico, and 50% reduction in *D. suzukii* population was registered (Gonzalez-Cabrera *et al.*, 2019).

Worldwide and in Mexico, multiple laboratory studies have concluded that *T. drosophilae* has potential as biological agent of *D. suzukii* because of its high fecundity, high host specificity, and high parasitization rates (Mazetto *et al.*, 2016; Wang *et al.*, 2016; Kacar *et al.*, 2017; Rossi-Stacconi *et al.*, 2017, Amiresmaeili *et al.*, 2018). Likewise, significant pest reduction has been reported in field studies as a direct consequence of augmentative releases of this parasitoid: 28 (Rossi-Stacconi *et al.*, 2018a) and 42% in Italy (Rossi-Stacconi *et al.*, 2018b), and 50% in Mexico (Gonzalez-Cabrera *et al.*, 2019); however, to the best of our knowledge, *T. drosophilae* has not been routinely released against this pest, except that it started to be marketed in Italy since 2017 (<http://bioplanet.eu/2152-2/>). Probably, more effective field releases are needed for their large-scale use.

To further the field effectiveness that *T. drosophilae* exerts on *D. suzukii* in Mexico, another field evaluation was executed, but with one important modification to the previous protocol: a 5-fold larger release rate was included, that is 27000 adults ha<sup>-1</sup>. This high-release rate was intended to reduce the pest far beyond the 50% reported by Gonzalez-Cabrera *et al.*, (2019), maybe close to 100%.

## Materials and methods

### Place of data processing and source of biological material

The data processing of this study was performed from January 2019 to January 2020, at the Centro Nacional de Referencia de Control Biológico (CNRCB), a governmental institution of the Dirección General de Sanidad Vegetal (DGSV) of SENASICA and located in Tecoman Colima, Mexico (18°55'37.73"N 103°53'0.41"W). The individuals of *D. suzukii* and *T. drosophilae* were obtained from colonies established at the CNRCB in March 2013 (~ 120 generations) and May 2015 (~ 60 generations), respectively (García-Cancino *et al.*, 2015; Moreno-Carrillo *et al.*, 2015). To maintain its genetic vigour, 300-400 wild adults are introduced per colony at the end of each year. The dipteran rearing system was described by García-Cancino *et al.*, (2015) and Moreno-Carrillo *et al.*, (2015), and the parasitoid reproduction by García-Cancino *et al.*, (2020); both species are reproduced under laboratory conditions, of 23 ± 1 °C and 40 ± 5% relative humidity for the dipterans, and 25 ± 1 °C and 60 ± 5% RH for the parasitoids.

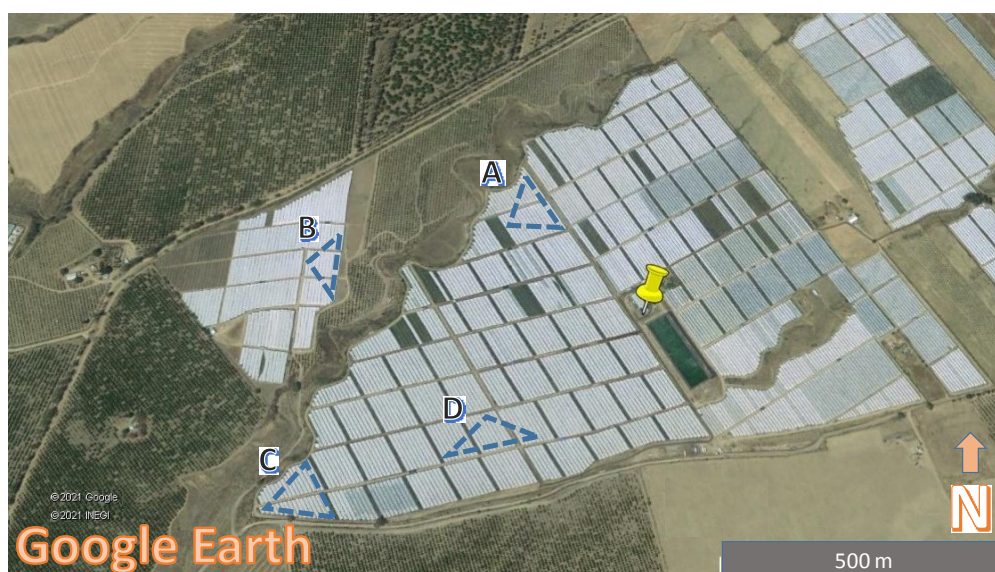
### Selection of berry fields and sampling sites

To select the sampling sites in which to perform the parasitoid-release treatments by quadruplicate (i.e., random-

ized complete block design), four berry areas [*Rubus fruticosus* L. (Rosales Rosaceae)] (i.e., A to D = adjacent land parcels) were selected from an organic farm of 30 ha, located in the Municipality of Zapotlan, Jalisco (figure 1); subsequently, in each area, three sampling sites of 0.1 ha were located the farthest possible from each other for random assignment of three treatments: low-release rate (4500 adults ha<sup>-1</sup>), high-release (27000 adults ha<sup>-1</sup>), and a no release control. The berry area selection was based on two criteria: just started to produce ripe fruit (i.e., newly harvesting section), and size of ~ 2 ha. Such size was intended to minimize environmental differences among treatments, and at the same time, to provide a 133.3 m of berry foliage, which was supposed to restrain the movement of parasitoids and dipterans among the experimental areas. Additionally, to allow comparison of treatments, the berry farmers were advised to apply normal agricultural practices, but with the caveat that such activity should be applied in the entire experimental field.

### Description of traps used per sampling site

The population density of *T. drosophilae* per sampling site was measured using sentinel traps, and the population of *D. suzukii* was monitored through both vinegar and fruit traps. Sentinel traps consisted of 12 pupae of *D. suzukii* (≤ 12 hours) placed on slices of fresh banana, in the bottom of plastic containers (8.5 cm in diameter by 7 cm in height) with a waterproof cap and holes of 1.3 mm in diameter for parasitoid access in its middle part. The vinegar traps were plastic cups (10 cm in diameter by 18 cm in height) with a waterproof lid, and 12 holes (0.8 cm in diameter at 16 cm from the bottom) to allow the entry of the dipteran, and 100 mL of apple vinegar for dipteran drowning. The fruit traps consisted of plastic cups (2.5 cm in diameter by 3 cm in height) with lid of organza fabric, in which ripe fruits were collected and placed within (procedure described below).



**Figure 1.** Berry parcels of Zapotlan Jalisco, Mexico, where were performed by quadruplicate (blocks = A to D), three release treatments of *T. drosophilae* during 2019: low and high density, and no release control. In each block, the treatments were randomly located at the vertices of the drawn triangle, and for geolocation purposes, the pin represents 103°33'16.94"W 19°41'38.86"N.

### Sampling procedure per site

Sampling per site was carried out bimonthly from January to December 2019, and each time it was performed in a four-step procedure, as follows: (1) 4 sentinel traps and 4 vinegar traps were placed in each site; (2) forty-eight hours later, the sentinel traps were retrieved and put into sealed Styrofoam coolers, the amount and type of dipterans present in the vinegar traps was visually counted, and 4 ripe fruits (grown on the same site, and of market quality) were randomly picked and individually placed into the empty plastic cups called “fruit traps” (previously described); after that, (3) the parasitoids contained in vials of 4 cm in diameter by 3.5 cm in height (randomly collected adults from the CNRCB colony,  $\leq 4$  days old) were released (opening the lid) at the centre of the site according to the treatment protocol. Finally, (4) the sentinel and fruit traps were transported at the CNRCB for incubation of parasitoid and dipteran eggs; after arrival, they were kept under laboratory conditions of  $25 \pm 1$  °C and  $60 \pm 5\%$  relative humidity. Twenty-five days later, when parasitoids and dipterans had emerged and died, the number of specimens per container was counted under a stereoscopic microscope SteREO-Discovery® V8 (ZEISS Group, DF, Mexico). Captured or emerged insects other than the species here evaluated were discarded because it was considered that their random effect was the same among treatments

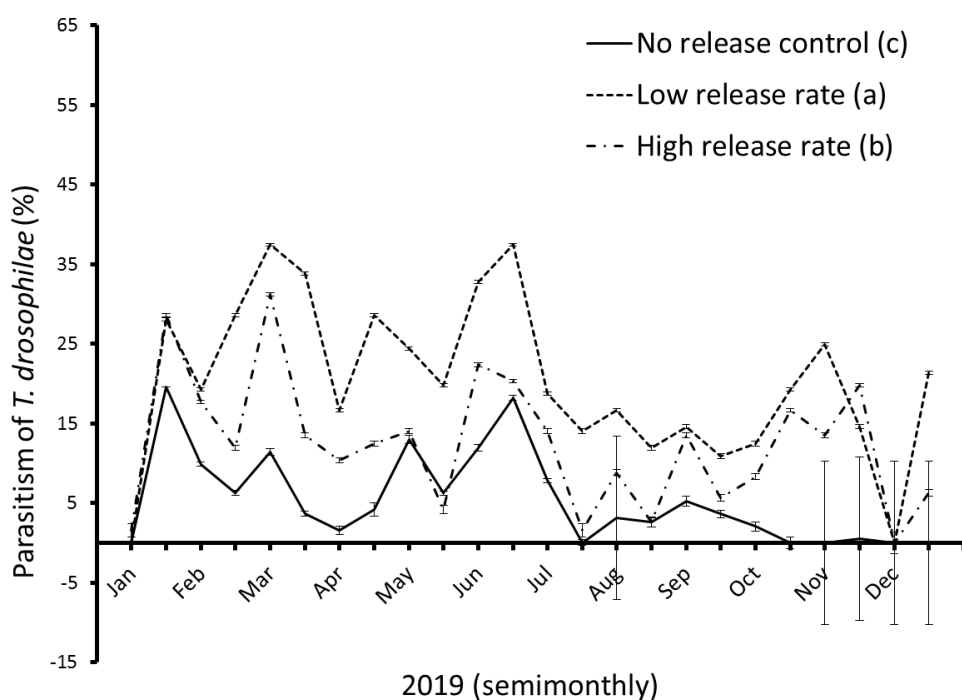
### Statistical analysis

The statistical differences among treatments in the population density of *D. suzukii* and *T. drosophilae* were calculated through a generalized linear model using the GENMOD Procedure with a Poisson probability

distribution, scale option specified at 1.0 and a log-link function fit by maximum likelihood estimation (GENMOD Proc. SAS/ STAT®. SAS Institute Inc. Cary, NC, USA), in which the explanatory variables were the parasitoid-release treatments (low and high density, and no release control), sampling dates (bimonthly, from January to December 2019) and berry fields (four experimental blocks), and the response variables were the number of dipterans trapped in the vinegar traps, dipterans emerged in the fruit traps, and the percentage of parasitism of *T. drosophilae* in the sentinel traps (measurement used as a proxy of population density). Post hoc comparisons for the explanatory factors among treatments were based on Wald  $\chi^2$  tests (LS MEANS statement with “pdiff” option in GENMOD Proc). All data were analysed using the statistical package SAS/STAT® v.9.2 and  $P \leq 0.05$ .

### Results and discussion

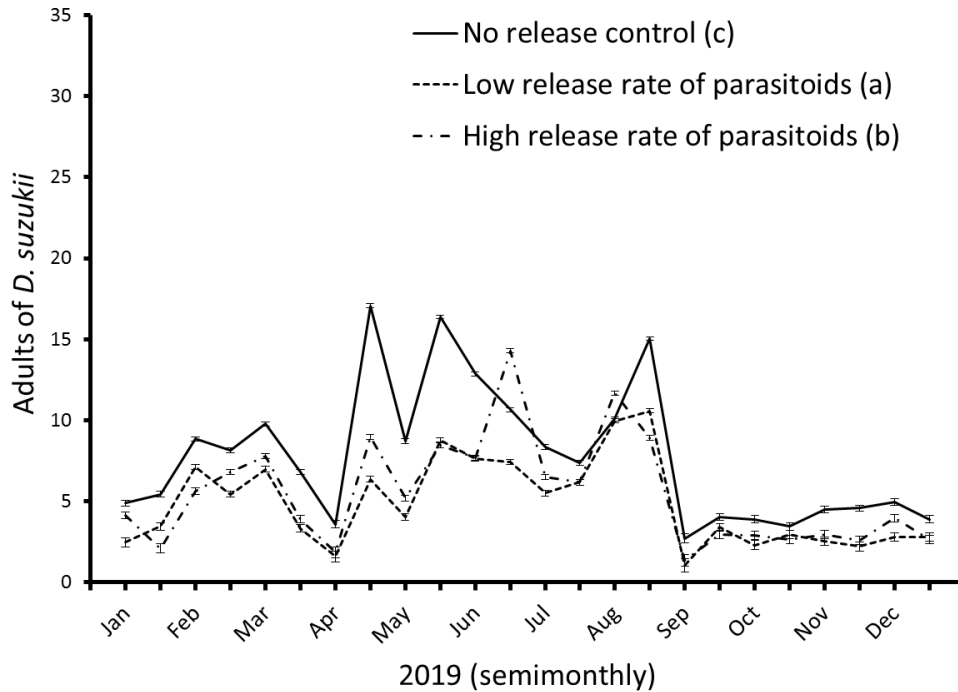
Based on the percentage of parasitism (used as a proxy of population density) obtained from the sentinel traps during January to December 2019, the population density of *T. drosophilae* was statistically significantly more abundant in the low-release sites than in the high-release sites (Wald's  $\chi^2 = 160.20$ ,  $df = 2$ ,  $P < 0.001$ ) (figure 2). The parasitoid population density was  $12.98 \pm 1.89$ ,  $7.98 \pm 1.41$  and  $3.49 \pm 0.58$  adults, respectively, for the sites with low-release, high-release, and without releases, that is, in relation to the control group the parasitoid population increased 2.8 and 1.3-fold at the sites with low and high release rate, respectively.



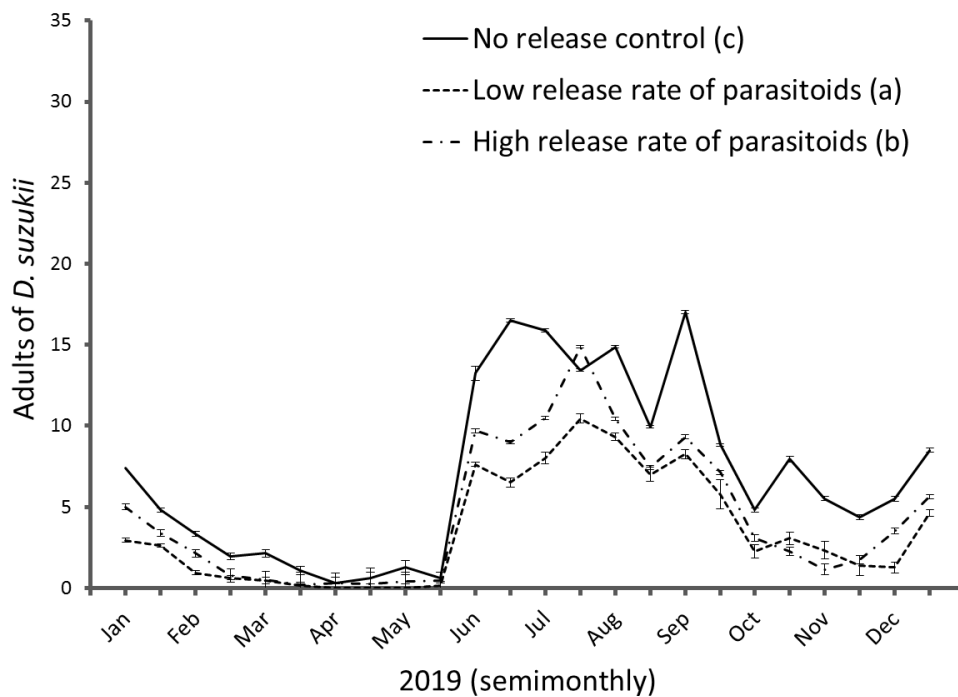
**Figure 2.** Percentage of parasitism in sentinel traps (used as a proxy of population density) of *T. drosophilae*, in three release treatments carried out in blackberry crops of Zapotlan Jalisco, Mexico. Treatments (upper right corner) with different letters indicate significant differences by Wald  $\chi^2$  tests ( $P \leq 0.05$ ).

The dipterans caught and emerged (used as a proxy of population density), respectively, that were registered from vinegar and fruit traps throughout the experimental period indicated that the population size of *D. suzukii* was statistically significantly larger in the high-release sites

than in the low-release sites (Wald's  $\chi^2 \geq 82.43$ ,  $df = 2$ ,  $P < 0.001$ ) (figures 3 and 4). The vinegar traps had  $4.21 \pm 0.04$ ,  $4.76 \pm 0.03$  and  $6.7 \pm 0.03$  adults for the sites with low-release, high-release and no-release sites, respectively, and following the aforementioned order of sites,



**Figure 3.** *D. suzukii* adults caught in vinegar traps, in three release treatments of the parasitoid *T. drosophilae* carried out bimonthly in blackberry crops of Zapotlan Jalisco, Mexico. Treatments (upper right corner) with different letters indicate significant differences by Wald  $\chi^2$  tests ( $P \leq 0.05$ ).



**Figure 4.** *D. suzukii* adults emerged in fruit traps, in three release treatments of the parasitoid *T. drosophilae* carried out bimonthly in blackberry crops of Zapotlan Jalisco, Mexico. Treatments (upper right corner) with different letters indicate significant differences by Wald  $\chi^2$  tests ( $P \leq 0.05$ ).

the fruit traps had  $2.01 \pm 0.04$ ,  $2.55 \pm 0.03$  and  $3.97 \pm 0.03$  adults, respectively, that is, in relation to the control group and considering both types of traps, it was quantified 43.25 and 32.34% fewer adults at the sites with low and high-release rate, respectively.

In this work, it was expected that the high-release rate of *T. drosophilae* would decrease the *D. suzukii* population close to 100%, but such goal was not accomplished. On the contrary, a greater reduction of the pest was recorded at the sites with low-release rate. A better performance in low-release rate of entomophagous insects rather than in high rates was theorized by Collier *et al.*, (2004) and Crowder *et al.*, (2007), and it was registered in *Encarsia formosa* Gahan (Hymenoptera Aphelinidae) (Hoddle *et al.*, 1997), *Spalangia cameroni* Perkins (Hymenoptera Pteromalidae) (Skovgard *et al.*, 2015), and *Tamarixia radiata* (Waterston) (Hymenoptera Eulophidae) (Razmjou *et al.*, 2019).

The worse performance of *T. drosophilae* at the sites with high-release rate can be explained by three reasons: (1) many females fed on the same pupa. While there are no records that this parasitoid behaves in such a manner, synovigenic parasitoids [i.e., females need to feed on the host to express their full reproductive capacity (Jervis *et al.*, 2001)], as *T. drosophilae* has been called by many authors (Wang *et al.*, 2016; Kacar *et al.*, 2017; Chen *et al.*, 2018), can decrease the quality of the pupae because of their multiple host feeding (i.e., several females feed on the same host), and with it, they can affect negatively the amount of their emerged offspring (Jervis and Kidd, 1986), as demonstrated in *Pimpla nipponica* Uchida (Hymenoptera Ichneumonidae) and *S. cameroni*, where the amount of adults decreased 60 and 80%, respectively (Ueno, 1997; Bockmann *et al.*, 2012); (2) many females oviposited on a single host. Although there are no records of this parasitoid behaving in such a manner, solitary organisms like *T. drosophilae* (Wang *et al.*, 2016; Kacar *et al.*, 2017; Boycheva *et al.*, 2019) can lay many eggs on a single pupa when the ratio of parasitoid to host is high (Carton *et al.*, 1986), for example, Michel Bouletreae found up to 19 eggs of *Trichopria* sp. in a single pupa (mentioned by Carton *et al.*, 1986), and if multiple oviposition occurs, subsequently, the larvae could kill each other by direct competition, until just one survives or they all die; and (3) mutual interference among the released adults. A high ratio of released parasitoids to hosts can reduce the number of parasitized pupae because of antagonistic interactions among conspecifics (Collier *et al.*, 2004), as demonstrated by Skovgard *et al.*, (2015) in *S. cameroni*.

At 25 °C, *T. drosophilae* has an intrinsic rate of increase ( $r_m$ ) of 0.18 and mean generation time (T) of 21.29 (Zhu *et al.*, 2017); therefore, with the caveat that at least some hosts were available (in this study  $\geq 56\%$  of *D. suzukii* pupae were not parasitized), the parasitoid population should increase over time, but such increase was not observed throughout the study (figure 1). This lack of increase can be attributed to multiple factors, such as negative environmental conditions (cold temperature or heavy rain), presence of natural enemies (ants or Coleoptera Staphylinidae), and the various tasks performed in the cultivation of blackberry. In these agricultural areas

of Jalisco, the production of blackberry follows the agronomic management known as forced production that is, the intense application of cultural practices, chemical promoters and bioregulators to produce berries almost the entire year (<https://www.intagri.com/articulos/frutillas/produccion-forzada-de-berries>); likewise, to protect the plant against pests and diseases it is required to apply an extensive number of measures, including total fruit removal, mass trapping (vinegar or yeast + sugar traps), detergents and soaps, and botanical extracts (garlic, onion, or neem). These agronomic practices contributed to keep a low population density of *D. suzukii* (figures 3 and 4), but perhaps they negatively affected the survival of *T. drosophilae*.

As previously stated, blackberry production in Jalisco requires the intense application of multiple management practices, and such strategy can be effective in controlling pest and diseases, as well as in obtaining ripe fruit all year round, but at the same time, it can affect the survival of the beneficial insects (Welch and Harwood, 2014), as demonstrated by Norton and Welter (1996) in *Anaphes iole* Girault (Hymenoptera Mymaridae), where after 96 hours of field release, the parasitoid population decreased 93%. Based on this theoretical short survival time, the positive effect that *T. drosophilae* registered in these trials could be improved by executing smaller but more frequent releases, for example, one-third portions every 5 days instead of 100% every 15 days. Likewise, because there are parasitoids that attacks the larval stage of *D. suzukii*, the effect that *T. drosophilae* exerts on this pest could also be improved by the combined release of this parasitoid and one larval species, and *G. brasiliensis* has been mentioned several times as a promising candidate to provide such additive effect (Nomano *et al.*, 2017; Giorgini *et al.*, 2018; Girod *et al.*, 2018).

The result that the experimental fields with high-release rate had lower pest control than the low-release sites is useful because it delimits the optimal release rate of *T. drosophilae* within the range of 4500 to 27000 parasitoids  $ha^{-1}$ . Theoretically, in biological control by augmentation there is an optimal point between the amount of released organisms and pest reduction (Collier *et al.*, 2004; Crowder *et al.*, 2007).

A caveat of this study is that it was not possible to increase the field effectiveness of *T. drosophilae* beyond the 50% pest reduction achieved by Gonzalez-Cabrera *et al.*, (2019). However, the current *D. suzukii* control methods (insecticides and cultural practices) are capital intense, high labour input (DiGiacomo *et al.*, 2019; Gress and Zalom, 2019) and not sustainable in the long term (as previously stated), and additionally, they also have the disadvantage that are not always efficient, i.e., even though all the management practices are applied, the pest still causes a mean yield loss of 19% in raspberries and other soft fruits (DiGiacomo *et al.*, 2019).

The results here obtained show that a significant pest reduction (i.e., 43.25%) is obtained by releasing 4500 parasitoids  $ha^{-1}$ , and based on the fact that the evaluation was carried out in agricultural areas in which the farmers were allowed to follow normal agricultural practices, these results also indicate that *T. drosophilae* is a viable option for use in an integrated management of *D. suzukii*.

## Conclusions

The high-release rate of *T. drosophilae* had a detrimental effect among conspecifics, as indicated by the fact that the parasitoid population was more abundant in the low-release sites than in the high-release sites.

The augmentative releases of *T. drosophilae* against the spotted-wing drosophila, *D. suzukii*, had a higher major efficacy (i.e., pest reduction) in the low-release sites than in the high-release sites.

There is an optimal release rate of *T. drosophilae* as a biological agent against *D. suzukii*, which should fall within the range of 4500 to 27000 parasitoids ha<sup>-1</sup>.

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