Biological traits of a *Pachycrepoideus vindemiae* Mexican population on the host *Drosophila suzukii*

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Abstract

Pachycrepoideus vindemiae (Rondani) (Hymenoptera Pteromalidae), a pupal ectoparasitoid, is considered a potential candidate for the biological control of *Drosophila suzukii* (Matsumura). To get a cost-effectively mass rearing under lab conditions, in this study, several life parameters of this parasitoid were assessed using *D. suzukii* pupae as host. In addition, the influence of *D. suzukii* pupal age on parasitism and sex ratio of *P. vindemiae* offspring was evaluated. Females started laying eggs when they were 0.12-d-old (= 3-h-old) and the number of eggs increased significantly when they were 0.5-d-old (= 12-h-old), with a pre-oviposition period less than 4 d after adult emergence. The maximum oviposition activity and parasitoid emergence were observed when females were very young (between 3-11-d-old for fecundity and 3-d-old for parasitoid emergence). The *P. vindemiae* survival rate and longevity were significantly higher in females than in males. Pupae of 48-h- and 96-h-old were significantly more parasitized than those of 24-h- and 72-h-old. The sex ratio of *P. vindemiae* adults was not affected by host age. We discuss the importance of the different life traits of *P. vindemiae* studied, especially its successful development in pupae of different ages of *D. suzukii*, to design a biological control strategy against this pest.

Key words: spotted wing drosophila, life parameters, no-choice test, biological control, parasitism.

Introduction

Spotted wing drosophila (SWD), *Drosophila suzukii* (Matsumura) (Diptera Drosophilidae), is endemic in East Asia and since 2008 it has spread rapidly through the temperate areas of other continents and got successfully established in Europe, North and South America (Asplen *et al*., 2015; de la Vega *et al*., 2020; CABI, 2020). SWD is considered one of the most important economic threats to berries and stone fruits worldwide (Kinjo *et al*., 2013; Yu *et al*., 2013; Stewart *et al*., 2014; Asplen *et al*., 2015; González-Cabrera *et al*., 2018) and it can also feed on more than 100 wild plants (Lee *et al*., 2015; Poyet *et al*., 2015; Kenis *et al*., 2016). Unlike most species of *Drosophila* genus that oviposit and feed on overripe fruits, SWD shows preference for ripening or ripe fruits (Walsh *et al*., 2011; Hamby *et al*., 2016; Entling *et al*., 2019).

To combat this pest, growers use broad-spectrum insecticides, but the control is not successful because of the insect´s high capacity to develop resistance (e.g., to spinosad in California; Gress and Zalom, 2019). In addition, pesticides are responsible for an extensive list of adverse effects on human health and environment, including the decline of beneficial arthropod populations and pollinators, secondary pests resurgence, and increase of the production costs in crops where SWD must be managed (Bruck *et al*., 2011; Cini *et al*., 2012; Wiman *et al*., 2014). Therefore, there is a recognized need to find alternatives for the control of SWD that are compatible with integrated pest management (IPM) practices. Biological

control, through the use parasitoids, has attracted particular interest because it is a very reliable tool that has been successfully implemented for many species of agricultural pests (Williams *et al*., 2013; Biondi *et al*., 2018).

Pachycrepoides vindemiae (Rondani) (= *dubius*; Hymenoptera Pteromalidae) and *Trichopria drosophilae* (Perkins) (Hymenoptera Diapriidae) are two cosmopolitan and generalist pupal parasitoids that have been frequently reported attacking SWD in several parts of the world (Gabarra *et al*., 2015; Moreno-Carrillo *et al*., 2015; Haye *et al*., 2016; Wang *et al*., 2016a; 2016b; Rossi Stacconi *et al*., 2018). Our study focused on *P. vindemiae*, a solitary idiobiont synovigenic ectoparasitoid, which preferably attacks pupae of almost 60 species of Diptera Cyclorrhapha, including SWD (Noyes, 2002; Marchiori and Barbaresco, 2007; Rossi Stacconi *et al*., 2013; Owens *et al*., 2015), even though it is also capable of attacking larvae (Rossi Stacconi *et al*., 2015). Unlike typical ectoparasitoids that attach their eggs to the outside surface of hosts, *P. vindemiae* typically lays an egg into the space between the host pupae and the puparium penetrating this protective layer with the serrated ovipositor (Wang and Messing, 2004). Furthermore, Nadel and Luck (1985) found that *P. vindemiae* could be considered as quasi-gregarious parasitoid. The use of this parasitoid against different species of dipteran pests (e.g., tephritids and muscids) has been evaluated in several parts of the world (Pickens *et al*., 1975; Petersen *et al*., 1992; Ovruski *et al*., 2000). At present, *P. vindemiae* is commercialized in Colombia and El Salvador for the control of *Anastrepha*

spp., *Ceratitis capitata* (Wiedemann) (Diptera Tephritidae), and *Dasiops* sp. (Diptera Lonchaeidae) fruit flies (Agro Activo, 2020; MAG-El Salvador, 2020).

The parasitism rate of *P. vindemiae* on SWD pupae can vary extraordinarily according to literature: 4 to 94% in blueberry and grape crops in Trento, Italy, and in Oregon, United States of America (Rossi Stacconi *et al*., 2013). In Mexico, the parasitism rate is not well known and Vázquez-González (2015) reported 27% in a blackberry crop using sentinel host pupa. The occurrence of this parasitoid in field suggests that, 12 years after SWD has expanded its distribution, it is already adapted to the new host and has the potential to succeed in biological control programs for this pest in the invaded areas (Bezerra Da Silva *et al*., 2019a).

Under laboratory conditions, different aspects of *P. vindemiae* biology have been elucidated in the past few years: its life history (Rossi Stacconi *et al*., 2015, 2017; Bezerra Da Silva *et al*., 2019a); host feeding behaviour (Bezerra Da Silva *et al*., 2019b); the effects of host age on the parasitization, development time, sex ratio, and the longevity of adult progeny (Wang and Messing, 2004; Zhao *et al*., 2013; Rossi Stacconi *et al*., 2015). However, more studies are needed to understand the *P. vindemiae* life traits on SWD to optimize its mass-rearing before can be included in an IPM program. This study was focused on several biological attributes (fecundity, preoviposition period, emergence, survival, and longevity) and on the influence of age of pupae on the parasitization and sex ratio progeny of this parasitoid.

Materials and methods

SWD and *P. vindemiae* rearing

SWD insects used in these tests came from a colony maintained for ~2 years in our laboratory with no history of insecticide exposure. SWD was collected from an open field of untreated blackberry in Caracha, municipality of Ziracuaretiro, in the state of Michoacán, Mexico (19°26'15"N 101°55'28"W and 1,421 m altitude). The blackberry fruits with SWD larvae and pupae were placed in thermal polystyrene containers, transported to the Instituto de Investigaciones Agropecuarias y Forestales (IIAF), Universidad Michoacana de San Nicolás de Hidalgo (UMSNH) and placed for 7 days in a ventilated plastic box (30 × 20 × 9 cm) under room conditions until adult emergence. The colony was infused three times per year with wild stock collected from the same site (between 80 and 150 adults each time) to help maintain genetic variability. Larvae and adults were reared on an artificial diet, developed for the fruit fly *Drosophila melanogaster* Meigen (Diptera Drosophilidae) (Guerrero, 2011), in a wooden frame (20 × 20 cm base and 30 cm in high) with the front wall and the ceiling covered with a gauze and the other three walls with transparent cellulose acetate sheets. The artificial diet, made of sugar (35 g), cornmeal (52.5 g), nutritional yeast (33 g), agar-agar (7.5 g), methylparaben (1 g), and water (700 ml), was placed into two glass jars (5 cm in diameter × 6 cm in high), and it also served as oviposition substrate. Water was also provided in two plastic cups (2 cm in diameter × 3 cm in high) covered by Parafilm with a piece of wipe sheet. After 24 hours, the glass jars with diet were removed from the frame box and covered with cotton wrapped in gauze. The entire rearing process was completed in a controlled environment chamber at 25 ± 2 °C, 60 ± 5% RH, and a photoperiod of 16L:8D that are the optimal conditions for SWD egg laying and immature development (Sánchez-Ramos *et al*., 2019).

P. vindemiae colony was started with adults collected in the same blackberry field as the wild stock of SWD. Groups of 50 SWD pupae (24-h-old) were placed in four plastic cups (3 cm in diameter × 4 cm in high) using a small brush and provisioned with a layer of artificial diet, which helped to maintain the humidity. Plastic cups were covered with a mesh screen (hole size 1.3 mm^2 in diameter) and placed inside a yellow home-made wing trap, made of paperboard (15 × 15 × 15 cm). The trap was hung at a height of 1.5 m in the blackberry plants with a galvanized wire to allow parasitization. After 4 days, SWD pupae were transported to the IIAF, UMSNH, and placed for 20 days in a ventilated plastic box (30 × 20 × 9 cm) under the controlled conditions mentioned above, for emergence of adult wasps or flies.

After emerging, the *P. vindemiae* adults (n = 172) were released into two frame boxes similar to those described above for SWD and were fed *ad libitum* with small drops of honey (Great Value®, Xalapa, Veracruz, Mexico) deposited on the inner side of one of the three cellulose acetate walls. Water was also offered in plastic cups as described in *D. suzukii* rearing section. Every 4 days, <24-h-old pupae from the SWD rearing were used to sustain the parasitoid colony, which was maintained under the same conditions as the host colony.

P. vindemiae preoviposition period, fecundity, emergence, survival, and longevity

Twenty SWD pupae (<48-h-old), placed in a plastic cup (2.2 cm in diameter × 0.7 cm high), were exposed for 3 hours to 1 virgin female + 2 virgin males of *P. vindemiae* (3-h-old = 0.12-d-old) in a bigger plastic cup (5.7 cm in diameter × 7.5 cm high) (= one replicate). The SWD pupae were placed on a layer of artificial diet in order to maintain humidity. The upper part of the larger plastic cup was covered with a fine mesh screen (270 × 770 μm) to prevent insects from escaping while allowing ventilation. Any male that died before the female was replaced with a fresh male.

To assess *P. vindemiae* preoviposition period (time between female emergence and the start of egg laying) and egg laying peak, the plastic cup with SWD pupae was initially replaced when the parasitoids were 6, 9, 12, 24, 36, 48, 60, and 72-h-old (= 0.25, 0.37, 0.5, 1, 1.5, 2, 2.5, and 3-d-old, respectively). Afterwards, SWD pupae were replaced every 48 hours until the female died. Parasitoids were fed *ad libitum* with small drops of pure honey deposited on the cup walls. Forty replicates were used.

Fecundity of *P. vindemiae* female was determined using Carey's definition, as the number of eggs laid by a female in a specified age interval (Carey, 1993). It was difficult to determine whether a host pupa was parasitized or not by *P. vindemiae* by external examination. Therefore, the number of *P. vindemiae* eggs per female in each

exposition, as well as the cumulative mean number of eggs per female, was estimated by dissecting under a stereoscopic microscope the SWD pupae exposed to 20 of the 40 females of this parasitoid. Females that failed to produce eggs during the experiment were not included in the statistical analysis.

To determine the parasitoid emergence, the plastic cups with SWD pupae exposed to the remaining 20 *P. vindemiae* females were individually placed in higher plastic cups (3.7 cm in diameter × 4.2 cm high) closed with a gauze and a rubber band. The host pupae were kept undisturbed under the controlled environmental conditions mentioned above until either adult parasitoids or flies emerged. After 20 days we considered adult emergence, so all remaining SWD pupal cases that did not show emergence holes were dissected as previously described to determine the presence of fly or parasitoid cadavers (Rossi Stacconi *et al.*, 2015). When a parasitoid cadaver was found inside a pupa, it was considered parasitized. Parasitoid emergence was calculated as the quotient between the number of parasitoids (emerged + dead [those seen via dissection]) × 100 and the total number of pupae exposed for parasitization.

To determine survival and longevity, *P. vindemiae* adults (distinguishing between males and females) were assessed every 24 hours until they died.

P. vindemiae parasitism and sex ratio. No-choice tests

To determine the most suitable host age for *P. vindemiae* development, we tested SWD pupae of four different ages (24-, 48-, 72-, and 96-h-old) and used the host pupae/female parasitoid ratio (5/1) proposed by Wang *et al.* (2018). The test consisted of four replicates of 250 SWD pupae of every age, in total 1,000 SWD pupae per pupal age. These pupae were placed in five plastic cups (3.7 cm in diameter × 4.2 cm high; 50 pupae/cup) with a layer of artificial diet to maintain the humidity and exposed to 50 females + 50 males of *P. vindemiae* (72-120-h-old) in the frame boxes previously described in the "SWD rearing" section. According to data collected on the oviposition peak period, 72 to 120-h-old *P. vindemiae* individuals were selected for the tests. After 12 hours exposure (from 07:00 to 19:00 h), cups with SWD pupae were removed, covered with gauze and placed in ventilated plastic boxes (30 × 20 × 9 cm) under the controlled environmental conditions mentioned above for parasitoid or adult fly emergence. SWD pupae, from which there was no adult fly or parasitoid emergence, were dissected as previously described to determine the presence or absence of fly or parasitoid cadavers. The parasitism rate of *P. vindemiae* was evaluated using the formula previously described.

After emergence, the sex ratio of 100 adults randomly selected was calculated as the percentage of females in the population [(females/(females+males)]×100 for each age class of the SWD tested pupae.

Data analysis

All results are presented as the mean ± standard error (SE). Fecundity and parasitoid emergence data were subjected to a repeated-measure analysis of variance (ANOVA) test using the fixed-effects model (PROC MIXED). The parasitoid age was the repeating factor. Parasitism success was analysed with one-way ANOVA through a generalized linear model (PROC GLM). Means were separated by the LSD multiple range test ($P < 0.05$). The sex ratio was analysed using a binomial distribution model.

We used Gehan-Breslow Kaplan-Meier (K-M) survival analysis and the non-parametric procedure LIFETEST to compare *P. vindemiae* survival curves among sexes. A pairwise multi comparison procedure (Long-Rank test, $P < 0.05$) was used to detect significant differences. We took into account all the female or male survivors from the beginning to the end of the experiment. We used a Student's *t*-test to analyze male and female longevity data. All statistical analyses were performed using the SAS/STAT statistical software package (version 9.3; SAS Institute, Cary, NC).

Results

Fecundity and parasitoid emergence

Only one out of the 20 *P. vindemiae* females studied failed to produce eggs; the majority oviposited just one egg per pupa, and there were only four cases of superparasitism where females laid 2 eggs/pupa. The pre-oviposition period lasted 0.36 ± 0.03 days.

Fecundity of this parasitoid was significantly affected by female age ($F_{29, 277} = 21.69$, $P = 0.0001$) (figure 1). Females started laying eggs (2.48 ± 0.43 as an average) when they were 0.12-d-old and the number of eggs started increasing significantly after they were 0.5-d-old (5.70 ± 0.65 eggs per female). During the oviposition peak recorded from 3 to 11-d-old, the number of eggs per female (11.52 ± 0.54 to 12.63 ± 0.56) was significantly higher than at any other age. From this time onwards, the egg-laying decreased gradually and from 19 to 45-d-old, the number of eggs per female was 4.98 ± 0.50. The cumulative mean number of eggs per female during the lifetime was 129.9 ± 6.00.

Similar to fecundity, emergence of *P. vindemiae* was significantly affected by female age ($F_{26, 238} = 19.16$, $P = 0.0001$) (figure 1). The maximum peak of emergence was observed when 3-d-old, with an emergence percentage of $82.33 \pm 4.32\%$, which was significantly higher than those recorded for the rest of the days. Between 5 and 17-d-old, the values of parasitoid emergence oscillated between 49.50 ± 8.25 and $71.44 \pm 2.68\%$. From this moment onwards, the emergence gradually decreased; between 19 and 31-d-old, the parasitoid emergence rate oscillated between 18.33 ± 1.67 and $37.77 \pm 9.43\%$; and above 31-d-old it was $7.5 \pm 2.5\%$. There was not parasitoid emergence from eggs laid at 0.12, 0.25, and 0.37-d-old. The average emergence was $37.44 \pm 4.82\%$.

Survival and longevity

When SWD pupae were continuously offered to *P. vindemiae* for parasitization, the parasitoid survival was significantly higher in females than males at 23 days (82% against 70%), and at 31 days (53% against 25%)
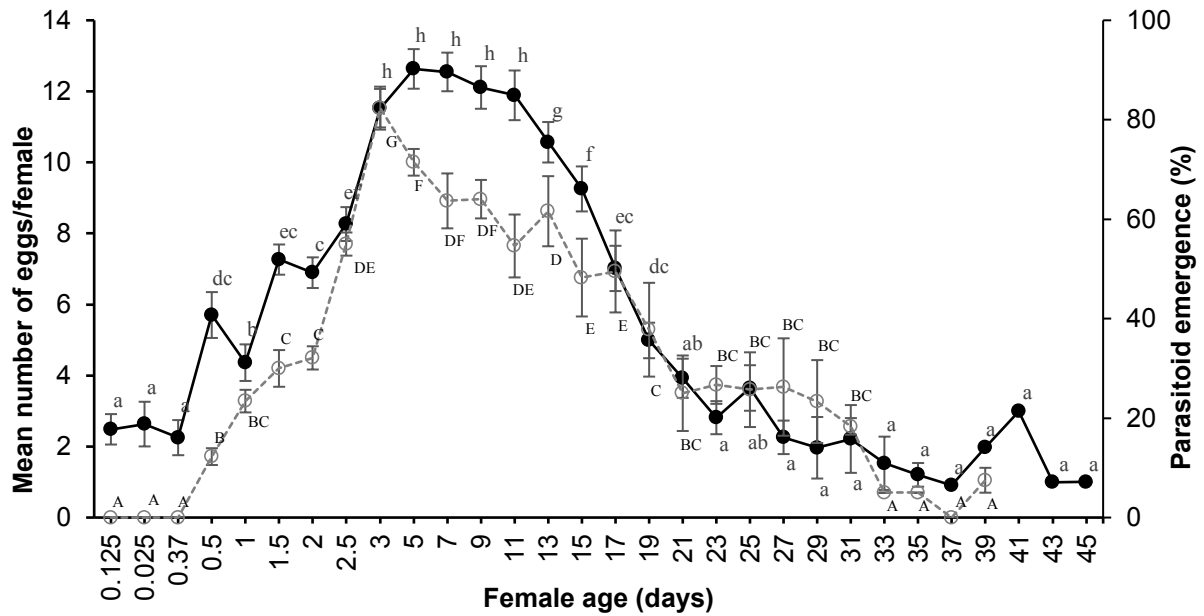
Figure 1. Evaluation of *P. vindemiae* fecundity (black solid line) and parasitoid emergence (grey dashed line) on *D. suzukii* pupa throughout lifetime. Data are mean ± SE; lower- and upper-case letters above the standard error bars indicate significant differences in fecundity and parasitoid emergence, respectively (LSD; *P* < 0.05).
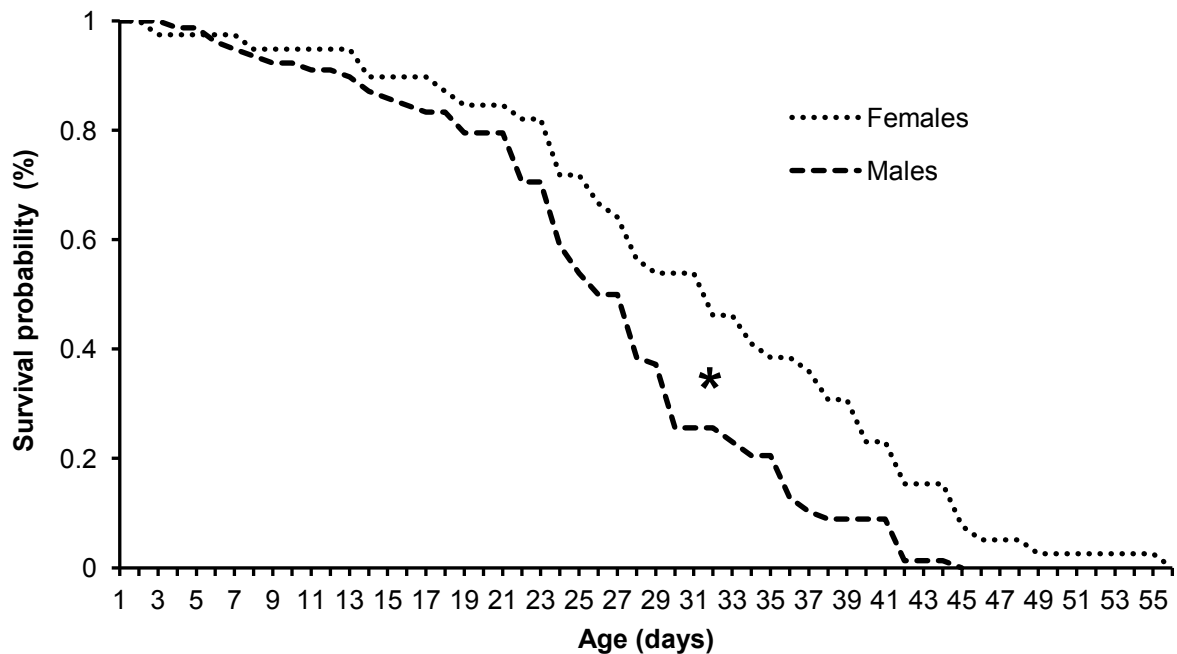

Figure 2. *P. vindemiae* survival probability when parasitizing *D. suzukii* pupae. Asterisk indicates significant differences between sexes (*P* < 0.05). (N = 35 females and 66 males from the beginning the experiment).

(Log-rank test, $\chi^2 = 8.52$, $P = 0.003$) (figure 2). The 10% survival threshold was reached at 38 days for males and 45 days for females. Two females survived until 48 days and one up to 55 days, while only one male survived up to 44 days (figure 2).

The average longevity of *P. vindemiae* females (n = 38; 32.2 ± 1.83 days) was significantly higher ($t = -2.60$, $P = 0.010$) than that of males (n = 78; 28.2 ± 1.06 days).

Parasitism and sex ratio

P. vindemiae parasitism was affected by the age of SWD pupae, in no-choice tests ($F_{3,11} = 113.11$, $P = 0.001$). Unfortunately, results did not show a systematic pattern, with parasitism rate higher on 48- and 96-h-old pupae than on 24- and 72 h-old pupae (table 1).

The host age did not significantly affect the sex ratio of *P. vindemiae* adults ($F_{3,16} = 0.21$, $P = 0.89$). The per-

Table 1. Influence of *D. suzukii* pupa age on the parasitism success and sex ratio of *P. vindemiae* offspring. No-choice tests.

Drosophila suzukii pupa age h-old	*Pachycrepoideus vindemiae* parasitism rate (%)	*Pachycrepoideus vindemiae* sex ratio (% females)
24	50.40 ± 1.87b	61.64 ± 2.51a
48	71.60 ± 1.87c	59.40 ± 2.11a
72	27.60 ± 1.87a	61.83 ± 3.39a
96	66.93 ± 1.87c	60.55 ± 2.18a

Data are mean ± SE. Within the same column, data followed by the same letter are not significantly different (ANOVA and LSD mean separation; $P > 0.05$).

centage of parasitoid females oscillated between 59 and 62% irrespective the SWD pupal age offered for parasitization (table 1).

Discussion

Like other synovigenic ectoparasitoids, after emergence females of *P. vindemiae* continue to mature eggs through part or all of their adult life (Quicke, 1997; Giron *et al*., 2002). Under our conditions (25 °C), the pre-oviposition period of *P. vindemiae* was very short (0.36 ± 0.03 days), in contrast to Rossi Stacconi *et al*. (2015; 2017) who reported that females laid eggs within 2 days after emergence when reared at 23 °C. A short pre-oviposition period is a good feature if *P. vindemiae* is used in the future in augmentative biological control programs against SWD because shortly after release the parasitoid adults can start searching for the host and laying eggs.

The cumulative number of eggs laid by each *P. vindemiae* female (130 eggs) during the lifetime on SWD pupae under our conditions, was about 1.5 to 2.5 times higher than that recorded by other authors in the same host (75 or 52 eggs/female; Rossi Stacconi *et al*., 2015; 2017) or in pupae of *Anastrepha ludens* (Loew) (89 eggs/female; Cancino *et al*., 2004), but 3.4 times lower than the value reported by Bezerra Da Silva *et al*. (2019a), also on SWD pupae. Rossi Stacconi *et al*. (2015; 2017) recorded oviposition for a similar time (females lived up to 42-45 days), but offered 10 1-d-old SWD pupae to the parasitoid instead of 20 2-d-old pupae in the present case and performed tests under different climatic conditions [23 ± 1 °C, 60-70% RH and 14L:10D photoperiod against 25 ± 2 °C, 60 ± 5% RH, and 16L:8D photoperiod]. They also maintained the parasitoid on *D. melanogaster* pupae for many generations in the lab before performing the tests, which could affect the body size of female wasps and consequently the fecundity. On the other hand, the higher fecundity observed by Bezerra Da Silva *et al*. (2019a) may be due to a better survival of *P. vindemiae* females in their study.

In our trials, the oviposition peak of *P. vindemiae* females was between 3- and 11-d-old against between 5- and 9-d-old in Bezerra Da Silva *et al*. (2019a). As expected, because the oocyte quantity and/or quality in the ovaries decrease as females get older (Hofsvang and Hagvar, 1975), the number of eggs laid by this parasitoid decreased with the increase in female age in our trials, especially from the age of 19-d-old onwards. On the contrary, Rossi Stacconi *et al*. (2015; 2017) did not report

any obvious peak, even though they registered a drastic fecundity dropping from 35-d-old onwards and the oviposition behaviour was present until females died because of their initial egg load and egg maturation rate.

The survival probability of *P. vindemiae* females under our conditions (25 °C) dropped below 50% from the age of 32-d-old onwards. In contrast, Bezerra Da Silva *et al*. (2019a) found that 50% of females survived until 40 days when experiments were performed at a lower temperature (23 °C). This long survival had a clear influence on the reproductive parameters of this parasitoid. In our case, the highest peaks of both fecundity and parasitoid emergence were observed when females were very young (between 3-11-d-old for fecundity and 3-d-old for parasitoid emergence) and had a high survival rate between 95-97%. From this moment onwards, both parameters decreased with the increase in female age, as previously reported in this parasitoid (Bezerra Da Silva *et al.*, 2019a). In the current study, *P. vindemiae* females significantly lived, on average, 4 days more than males when they were provided with water + honey and SWD pupae for parasitization. Females of *P. vindemiae* can also practice host feeding (Bezerra Da Silva *et al*., 2019a; 2019b) and feed on the haemolymph that exits the host pupae after puncturing the skin epidermis for parasitization in order to acquire nutrients for somatic maintenance and continued egg production (Quicke, 1997; Jervis and Kidd, 1999). Although host feeding was not quantified in the present study, we observed this behaviour in some *P. vindemiae* females and we can suppose that the haemolymph obtained from the host, which contains a significant amount of proteins and sugars (Giron *et al*., 2002; 2004) could have contributed to the prolonged longevity in *P. vindemiae* females compared to males. This statement is also supported by the studies of Cancino *et al*. (2004), Rossi Stacconi *et al*. (2015), and Bezerra Da Silva *et al*. (2019a). They found that *P. vindemiae* females provided with *D. suzukii* or *A. ludens* pupae and, in the absence of water or honey, lived significantly more that host-deprived females, which did have not the opportunity of obtaining these nutrients. However, the shorter lifespan of males compared to females is a common feature among parasitic hymenopterans, and has been observed in other parasitoid species which do not practice host feeding, e.g. *Phymastichus coffea* LaSalle (Portilla and Grodowitz, 2018) and *T. drosophilae* (Colombari *et al*., 2020). Therefore, our hypothesis about influence of host feeding behaviour on longevity differences between males and females in our studied species should be tested in the future.

Host age is one of the most important factors that determine the survival of *P. vindemiae* offspring (Wang and Messing, 2004). From a nutritional point of view, pupal age is important because the nutrition quality that is offered to pupal parasitoids may change as long as they do metamorphosis (histolysis, histogenesis and differentiation to form adult internal organs and sclerotized appendages; Quicke, 1997; Rossi Stacconi *et al.*, 2015). Besides, parasitism on not fully formed pupae can be unsuccessful (Wang and Messing, 2004). In choice tests, females of this parasitoid did not show a preference for SWD pupae of 24-, 48-, 72-, or 96-h-old (the parasitism rate oscillated between 70-80%; Rossi Stacconi *et al.*, 2015). In contrast, in our no-choice experiments, the parasitism of *P. vindemiae* on SWD was significantly higher in medium- (72%, 48-h-old) and older- (67%, 96-h-old) pupae than in the young ones (50%, 24-h-old). Besides, the lowest parasitism was observed on the pupae of 72-h-old (28%). In this respect, we have not a clear explanation for this result because recently a similar parasitism in both 72- (67%) and 96-h-old (74%) was recorded using the same host-parasitoid complex (S. Pineda, unpublished data). However, in agreement with our results, the parasitism rate of this parasitoid was also higher on medium- than in young-pupae of other tephritids: 31-34% on medium- (72-96-h-old) *vs* 22% on young pupae (48-h-old) of *Bactrocera cucurbitae* (Coquillet) (Zhao *et al.*, 2013) and 50% on medium- (between 120-192-h-old) *vs* 33% on young pupae (between 24-96-h-old) of *A. ludens* (Cancino *et al.*, 2004); and 70% on medium- (2-3-d-old) *vs* 28% on young pupae (<1-d-old) of *C. capitata* (Wang and Messing, 2004). In the present study, the parasitism caused by *P. vindemiae* on SWD pupae of different ages is very advantageous because the pest has overlapping generations in the field (Emiljanowicz *et al.*, 2014; Tochen *et al.*, 2014) and the availability of pupae of different ages could assure the parasitoid survival at any time, as shown by Zhao *et al.* (2013) for *B. cucurbitae.*

In parasitic wasps, reproduction is under maternal control because it is based on arrhenotoky (females are heterozygous and males can be homozygous and haploid or heterozygous and diploid; Schneider and Viñuela, 2007). Quite often the sex ratio, which can be modified by several environmental and biological factors such as the host species or the host age (Cancino *et al.,* 2004; Uckan and Gulel, 2002), is biased towards males, causing a crash of cultures because the single locus complementary sex determination seems to be widely distributed (Beukeboom, 2001). In our assays, however, *P. vindemiae* female percentages were always high irrespective of the SWD pupa age offered for parasitization (about 60% in any case). In contrast, female percentages higher than ours (between 70-80%) were reported by Rossi Stacconi *et al.* (2015) using the same host, pupal ages and parasitoid species as us, which could be explained by different temperatures used in the trails (23 °C *vs* 25 °C). The host species also influenced *P. vindemiae* female percentage, which was lower in the host *B. cucurbitae* for any of the six pupal ages studied (2-, 3-, 4-, 5-, 6, and 7-d-old; 48-56% of females; Zhao *et al.*, 2013). In contrast, in the host *A. ludens*, a highest female rate (72%) was registered when young pupae of different ages were offered (between 1-4- and 5-8-d-old), compared to older pupae (between 9-12- and 13-16-d-old; 61% females), but the lack of statistical analysis prevents to conclude whether the two groups of pupae were different or not (Cancino *et al.*, 2004). Variability in *P. vindemiae* female percentage reported in studies of Cancino *et al.* (2004), Zhao *et al.* (2013), Rossi Stacconi *et al.* (2015), and ours are not surprising because it is widely known that one of the most important factors influencing the offspring sex ratio in parasitoids is the host/parasitoid ratio, which in turn can be affected by the host size.

Summarizing, our study has shown several important features of *P. vindemiae* when parasitizes SWD pupae, which can be indicative of its success in the field. The pre-oviposition period is very short, the female rate is rather high and it is not modified with the host age, females longevity is longer than that of males and this is very advantageous because the former are the responsible for attacking the host and increasing the parasitoid population numbers, which finally led to successfully controlling the pest (Bueno *et al.*, 2017). However, before practical use of *P. vindemiae* for the control of SWD, more studies aiming at understanding its functional response to different host densities and its population performance under different climatic conditions need to be conducted. Also, depending on origin, as shown by Wang *et al.* (2018) and at unrevealing, the possible competition of *P. vindemiae* with other parasitoids under more realistic conditions are needed because they could affect its capacity to regulate the populations of this important pest.

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