Host stage preference, efficacy and fecundity of parasitoids attacking *Drosophila suzukii* in newly invaded areas

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HIGHLIGHTS

- Populations of two parasitoids of *Drosophila suzukii* were tested under laboratory conditions.
- Only the Italian population of *Leptopilina heterotoma* parasitized *D. suzukii*.
- Italian and North American populations of *Pachycrepoideus vindemiae* parasitized *D. suzukii*.
- Host pupal preference and lifetime fecundity of *P. vindemiae* were determined.
- A third parasitoid species, *Trichopria drosophilae*, showed the ability to parasitize *D. suzukii*.

GRAPHICAL ABSTRACT
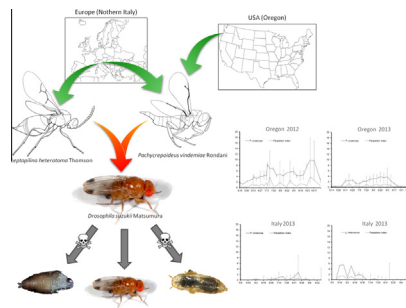
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ABSTRACT

Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) causes severe damage to certain fruit crops in both North America and Europe. This may be due, in part, to the absence of specialized natural enemies that suppress population outbreaks. We performed a series of experiments under controlled laboratory conditions in tandem with a field study to evaluate the presence and efficacy of natural enemies associated with this pest in Italian and western United States fruit production regions. Our study involved one larval parasitoid, *Leptopilina heterotoma* (Thomson) (Hymenoptera: Figitidae), and two pupal parasitoids, *Pachycrepoideus vindemiae* (Rondani) (Hymenoptera: Pteromalidae) and *Trichopria drosophilae* (Perkins) (Hymenoptera: Diapriidae). Three indices were used to describe host-parasitoid interactions: degree of infestation (DI), success rate of parasitism (SP) and total encapsulation rate (TER). Results confirmed that each of these parasitoid species can develop on certain populations of the pest. In addition, host stage

preferences of the tested parasitoid populations, developmental parameters and lifetime fecundity of North American *P. vindemiae* are provided. Results are discussed with respect to differences in potential utilization of *D. suzukii* among the tested parasitoid species and regional populations.

1. Introduction

The spotted-wing drosophila (SWD), *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), is a highly polyphagous invasive pest endemic to Southeast Asia. It was first documented in European and North American fruit production regions during 2008 and it has since had significant economic impact (Cini et al., 2012, 2014). *D. suzukii* females are able to lay eggs in healthy, ripening fruits using a serrated ovipositor. Developing larvae feed on fruit pulp; infested fruits turn soft and may eventually collapse, becoming unmarketable (Rota-Stabelli et al., 2013). The impact of *D. suzukii* in affected fruit production regions is substantial and is exacerbated by high female fecundity and a rapid lifecycle (Bolda et al., 2010; Cini et al., 2012; Goodhue et al., 2011); development from egg to adult requires about 8 days at 25 °C (Tochen et al., 2014). Larval feeding within the flesh of fruit causes direct damage and provides a pathway for secondary feeding by other insects as well as colonization by fungi and bacteria that may contribute to further fruit decay (CABI, 2014). Although no effective parasitoids of *D. suzukii* have been found in Europe or North America, several hymenopteran parasitoids of the genera *Ganaspis* and *Leptopilina* (Hymenoptera: Figitidae), *Trichopria* (Hymenoptera: Diapriidae), and *Asobara* (Hymenoptera: Braconidae) are reported from the endemic range (Ideo et al., 2008; Kasuya et al., 2013; Mitsui et al., 2007; Mitsui and Kimura, 2010; Nomano et al., 2014). The potential utility of these natural enemies as agents for the biological control of *D. suzukii*, and particularly some *D. suzukii*-associated Japanese species, has been assessed (Kasuya et al., 2013; Nomano et al., 2014).

Several approaches to reduce populations of *D. suzukii* have been attempted in different cropping systems. Although chemical controls can be inefficient (Bruck et al., 2011), insecticides continue to be the primary tool used by growers to manage *D. suzukii* (Bruck et al., 2011; Cini et al., 2012). Mass trapping techniques using food attractants or lures can have some effect at low population levels, but do not represent a viable solution in most crop systems (Cha et al., 2013; Landolt et al., 2012). The ability of local invertebrates and microbial communities to limit populations of *D. suzukii* has been investigated (Brown et al., 2011; Hamby et al., 2012; Siozios et al., 2013). Preliminary surveys of natural enemies parasitizing *D. suzukii* in Italy and Oregon showed that *D. suzukii* is attacked by the generalist pupal ectoparasitoid *Pachycrepoideus vindemiae* (Rondani) (Hymenoptera: Pteromalidae) (Brown et al., 2011; Rossi Stacconi et al., 2013) but the economic and ecological impact of this parasitoid is not fully understood.

Parasitoid species in newly invaded areas may gradually adapt and establish novel associations in response to recent invaders. In Europe, new parasitoid associations have been observed for economically damaging leaf miners including *Cameraria ohridella* Deschka and Dimic (Lepidoptera: Gracillariidae), *Phyllonorycter leucographella* Zeller (Lepidoptera: Lithocolletinae), and *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) on tree species in forests, gardens and orchards (Urbaneja et al., 2000). A better understanding of the physiological preadaptation of resident parasitoids may play an important role in guiding biological control strategies and contributing to future management of *D. suzukii* populations.

The first aim of this study was to assess the life stage preferences of *Leptopilina heterotoma* (Thompson) (Hymenoptera:

Figitidae) and *P. vindemiae* on *D. suzukii* and to determine if these species successfully parasitize larvae and pupae on and within host fruits. Both of these species have been associated with wild populations of *D. suzukii* and the vinegar fly, *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) in Italy and Oregon. Therefore, life stage preferences and suitability of *D. melanogaster* as a host for both parasitoids were also determined for sake of comparison. Because *P. vindemiae* is generally considered a pupal parasitoid, we evaluated host suitability and parasitoid preference for different SWD pupal stages and its lifetime fecundity in order to determine its potential to control the target pest. A third parasitoid species, *Trichopria drosophilae* (Perkins) (Hymenoptera: Diapriidae), was observed to attack *D. suzukii* in Italy and California, but not in Oregon (Brown et al., 2011; Rossi Stacconi et al., 2013; Wang and Daane, unpubl. data).

2. Materials and methods

2.1. Insects

The ability of the parasitoids to successfully parasitize different life stages of *D. suzukii* was tested in the laboratory using a modification of the protocol of Gibert et al. (2010). We determined the preference for host medium, host stage preference and pupal age preferences of *L. heterotoma* and *P. vindemiae*. The *D. suzukii* and *D. melanogaster* populations used in this study were F_1 progeny of live adults collected from multiple locations in the Italian Province of Trento, the Willamette Valley, Oregon and the San Joaquin Valley, California during the summers of 2011–2013. Parasitoid populations of *L. heterotoma* and *P. vindemiae* were collected from sentinel bait traps placed in close proximity to fruiting host plant species in these same regions during June–October 2011–2013, as described in Rossi Stacconi et al. (2013). All flies were provided with a standard artificial diet (Dalton et al., 2011). All parasitoids were maintained with either *D. melanogaster* or *D. suzukii* host larvae or pupae and additionally provided with a 50% honey solution. Voucher specimens of the parasitoid species are deposited in the National Museum of Natural History, Washington DC, and Pisa University, Italy.

2.2. Preference for artificial medium and Drosophila host stage

Different life stages of Italian populations of *D. suzukii* and *D. melanogaster* served as hosts for parasitoids and were placed into vials containing either standard artificial diet or blueberries. Prior to experimental trials, newly eclosed adults of Italian populations of *L. heterotoma* and *P. vindemiae* were allowed to mate for 5 days. For each replicate, 50 individuals of each life stage (egg or larval instar) of either *D. suzukii* or *D. melanogaster*, were exposed to one mated female parasitoid for 24 h in a plastic vial (2.5 cm diam × 9.5 cm ht) and then maintained under the rearing conditions until either drosophilid flies or parasitoid wasps emerged. The numbers of emerged parasitoids and flies were counted as described in Chabert et al. (2012) and Gibert et al. (2010). Each treatment consisted of 20 vials, for a total of 1000 host eggs or larvae per treatment. *P. vindemiae* parasitoids were exposed to third instar *D. suzukii* within standard artificial diet (SWD_3); third instar *D. suzukii* within blueberries (SWD_{3b}); and third instar

D. melanogaster within standard artificial diet (DM$_3$). *L. heterotoma* were exposed to *D. suzukii* eggs within standard artificial diet (SWD$_e$); first instar *D. suzukii* within standard artificial diet (SWD$_1$); *D. suzukii* eggs within blueberries (SWD$_{eb}$); and *D. melanogaster* eggs within standard artificial diet (DM$_e$). Control treatments consisted of 20 unexposed vials containing the same substrate and life stages (C).

California and Oregon populations of *L. heterotoma* did not emerge as adults when SWD was offered as a host in these trials. The scope of this study did not allow for investigation of encapsulation, hence trials were conducted in these regions with North American strains of *P. vindemiae* only. North American populations of *D. suzukii* and *D. melanogaster* larvae were collected as first, second or third instars from standard artificial diet and 50 individuals of each life stage were placed into a plastic test vial (2.5 cm diam × 9.5 cm ht) containing standard artificial diet. For pupal stages, 50 newly sclerotized pupae were placed on moistened filter paper inside a plastic container measuring 10 × 10 × 6.5 cm (473 ml volume) with a modified lid, vented with a fine-meshed synthetic organza screen lid. Each replicate in all treatments was exposed to three mated *P. vindemiae* females for 72 h at 23 ± 1 °C, 60–70% RH under a 14L:10D photoperiod. The eight treatments were: first, second, third instar and pupal *D. suzukii* (SWD$_1$, SWD$_2$, SWD$_3$, SWD$_p$, respectively), and first, second, third instar and pupal *D. melanogaster* (DM$_1$, DM$_2$, DM$_3$, DM$_p$, respectively). Each treatment consisted of 10 replications for a total of 500 host individuals per treatment. Control treatments (C) were conducted simultaneously and consisted of 10 replications of each life stage without parasitoid exposure.

2.3. Host pupal stage preference and developmental parameters of P. vindemiae

Choice tests were conducted to determine host age preferences of western North American populations of *P. vindemiae* and assess the suitability of different host pupal stages under the same physical conditions as described above for preference tests. Four different ages of host pupae (1, 2, 3 and 4 days old) were collected over a 4 day period. For each replicate, five pupae of each age class were placed on a wet filter paper and exposed to a single female parasitoid for 24 h in a plastic Petri dish (8.5 cm diam), with a small streak of diluted honey provided as food for the parasitoid. Five concentric circles were marked on the filter paper with radii of 1.0, 1.5, 2.0, 2.5, 3.0 cm, respectively, and one point was marked on each circle at each of the four cardinal directions (north, south, east and west). The five host pupae of each age class were placed on the five points along one randomly-selected cardinal direction. The experiment consisted of 34 replicates, each employing a 5–7 days-old mated female that had no previous oviposition experience (naïve).

After 24 h of exposure, the host pupae were placed on a moistened tissue paper in a plastic Petri dish (15 cm diam) and held for parasitoid or adult fly emergence. The number and sex of emerged parasitoids were recorded twice daily at ca. 0800 and 1700 h. After all parasitoids emerged, all dead pupae were reconstituted in water for 1–2 days, then dissected under a microscope to determine the presence or absence of fly or parasitoid cadavers. The number of parasitized hosts was estimated as the sum of emerged adult parasitoids and pupae containing a dead parasitoid. Sex ratio was estimated as the proportion of emerged adult parasitoids that were female. The number of parasitized hosts, sex ratio, offspring mortality (proportion of dead offspring among total parasitized hosts), and developmental times of both sexes were compared among the different host pupal stages.

2.4. Lifetime fecundity of P. vindemiae

The longevity and lifetime fecundity of female *P. vindemiae* were determined with *D. suzukii* pupae as hosts under the same physical conditions as described above. Pairs of newly emerged *P. vindemiae* (<24 h post-eclosion) were isolated in plastic Petri dishes (8.5 cm diam), and provided with either 10 pupae for 2 days, or 15 pupae for 3 days (*i.e.*, 5 pupae per day) until the female died. At each host replacement, the parasitoids were transferred to a new Petri dish with a small streak of diluted honey. The hosts were held in their original dishes until either parasitoids or adult flies emerged. Any male parasitoids that died before the female was replaced with a fresh male. The number, sex, and developmental time (egg to adult) of emerged parasitoids were recorded. These determinations were made on twenty-eight female *P. vindemiae* of similar size. All dead females were dissected within 24 h of their death to determine the number of mature eggs they contained. After adult emergence was complete, all remaining host pupae were dissected (as described above) to determine the presence or absence of fly or parasitoid cadavers. Female longevity, numbers of hosts parasitized and offspring developed, sex ratio, survival rate, and developmental time from egg to adult were calculated. Life table parameters were then estimated from these data, including net reproductive rate, intrinsic rate of increase, generation time, and doubling time. Mean number of offspring produced per day was estimated based on the total number of offspring produced during each 2 or 3 day exposure period.

2.5. Trichopria drosophilae

Three *T. drosophilae* females field-collected in Italy were placed in a plastic pot (200 ml container, Kartell S.p.a., Noviglio, Italy) containing a standard artificial diet layer infested with 100 third instar *D. suzukii*. The container was maintained under the same physical conditions as the other experiments until adult parasitoids emerged.

2.6. Host-parasitoid indices

Two indices were used to assess host-parasitoid interactions: the Degree of Infestation (DI); and the Success rate of Parasitism (SP) (Boulétreau and Fouillet, 1982; Delpuech et al., 1994; Eslin and Prévost, 1998). DI is the proportion of host larvae or pupae successfully parasitized and is estimated using DI = $(T - d_i)/T$. In cases where $T - d_i < 0$, we set $T - d_i = 0$. The index SP measures the probability that a host larva or pupa yields an adult parasitoid and is estimated as SP = $p_i/(T - d_i)$. In cases where $p_i > T - d_i$, we set SP = 1 and where $T - d_i = 0$, we set SP = 0. The total number of unemerged pupae (up), adults of *Drosophila* (d_i) and parasitoids (p_i) emerging from each vial was counted as described in Chabert et al. (2012) and Gibert et al. (2010). In Oregon, the parameter (up) was replaced by (nd) and included larvae that did not develop into pupae. The developmental success of the *D. suzukii* and *D. melanogaster* populations in the absence of parasitism (T) was estimated from control treatments. In Italian trials, an evaluation of the ability of drosophilids to immunosuppress successful parasitism was determined by counting the number of adult flies containing a melanized capsule (d_c). This parameter, the Total Encapsulation Rate (TER), is estimated using TER = $d_c/(T - d_i + d_c)$ to express the number of *Drosophila* with a capsule as a proportion of the number parasitized.

2.7. Statistical analysis

Data from efficacy trials were analyzed using the Kruskal–Wallis test followed by the Bonferroni-Dunn post hoc multiple

comparison test to separate means (Siegel and Castellan, 1988). Data from *P. vindemiae* host stage preference and fecundity trials were compared using ANOVA and are presented as means (± SE); these means were separated by the Tukey HSD test when F values were significant. Prior to analyses, all percentage data were logit transformed to normalize the variance. All analyses were performed using JMP V10 (SAS 2008, Cary, North Carolina, USA).

3. Results

3.1. Performance of resident parasitoids

Results demonstrated that *P. vindemiae* populations from both countries and *L. heterotoma* from Italy were able to parasitize *D. suzukii* successfully (Figs. 1a, 2a and 3a). The Oregon population exhibited a higher DI on *D. suzukii* compared to the Italian population ($H = 17.1$, $P < 0.001$, Figs. 2b and 3b), while difference in SP was not significant between the two populations ($H = 3.254$, $P = 0.071$, Figs. 2c and 3c). For Italian parasitoids, DI and SP were higher on *D. melanogaster* than on *D. suzukii* (Figs. 1b, c and 2b, c). In Italy, the TER of *L. heterotoma* was considerably lower in *D. melanogaster* than in *D. suzukii* (Fig. 1d). For both Italian species, SWD_{3b} and SWD_{eb} resulted in lower parasitism indices, likely due to the higher levels of desiccation in fruit, compared to standard artificial diet, and *L. heterotoma* was affected to a larger extent than *P. vindemiae* (Fig. 1a and 2a).

3.2. Host medium and stage preference of Leptopilina heterotoma in Italy

The DI of *L. heterotoma* on *D. suzukii* differed significantly among the four treatments (Kruskal–Wallis test: $H = 38.74$, $df = 3$, $P < 0.001$; Fig. 1b), as did SP ($H = 41.4$, $P < 0.001$; Fig. 1c). On standard artificial diet, the number of emerged *L. heterotoma* was almost twofold higher when attacking larvae, compared to eggs (Fig. 1a). There were no differences in TER between eggs and larvae (SWD_e vs SWD_1) or between standard artificial diet and fresh fruit (SWD_e, SWD_1 vs SWD_{eb}) treatments ($H = 35.02$, $P < 0.001$; Fig. 1d).

For Italian *P. vindemiae*, no differences in DI were significant between SWD_3 and SWD_{3b} ($H = 23.51$, $P < 0.001$, Fig. 2b) and these life stages demonstrated similar suitability for successful development ($H = 11.81$, $P < 0.005$).

North American *P. vindemiae* showed significant differences in DI and SP among treatments ($H = 59.20$, $df = 7$, $P < 0.001$; and $H = 49.80$, $df = 7$, $P < 0.001$, respectively). For *D. suzukii*, DI was significantly less in first and second instar, with no difference between third instars and pupae. For *D. melanogaster*, DI was significantly lower in first, second and third instar than in pupae. For first and second instar SWD and first instar DM, SP was zero, and there was no significant difference in SP between third instars and pupae for either host (Fig. 3a–c).

3.3. Host pupal preference and developmental parameters of P. vindemiae

In choice tests, *P. vindemiae* did not prefer one *D. suzukii* pupal age class over others in terms of the number parasitized ($F_{3,132} = 0.675$, $P = 0.569$, Fig. 4a). Offspring mortality generally decreased with host age and was significantly lower on 3- and 4 days-old pupae than on one day-old pupae, but similar within those two age classes ($F_{3,125} = 5.2114$, $P = 0.002$, Fig. 4b). There was no difference in sex ratio among host classes ($F_{3,124} = 0.998$, $P = 0.396$, Fig. 4c). Developmental time (egg to adult) was not affected by host age, but males developed faster than females in all host age classes and the interaction between host age and sex was not significant (host stage: $F_{3,457} = 0.853$, $P = 0.465$; sex: $F_{1,457} = 137.0$, $P < 0.001$; host stage × sex: $F_{3,457} = 1.453$, $P = 0.227$).
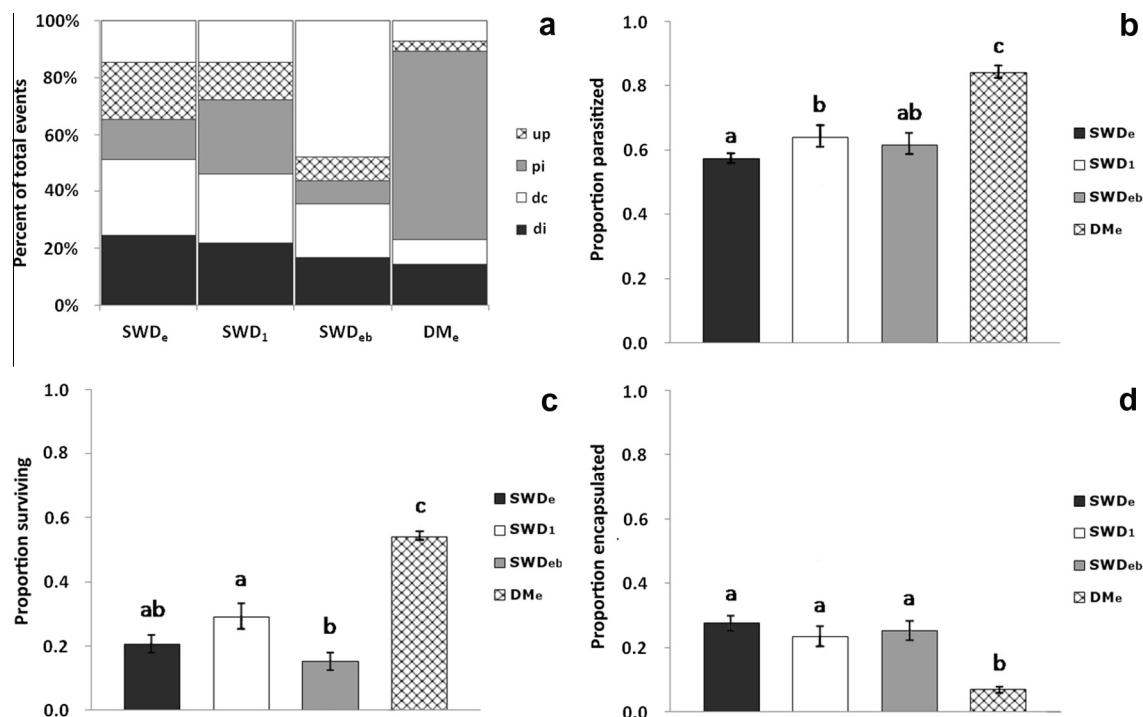
Fig. 1. Parasitism indices for the Italian strain of *L. heterotoma*. (a) Mean percentage of outcomes (up = unemerged pupae; pi = emerged parasitoids; dc = emerged *Drosophila* carrying a melanized capsule; di = emerged Drosophila); (b) mean (± SE) percent parasitism; (c) mean (± SE) offspring survival, and (d) mean ± (SE) encapsulation rate when female wasps were provided different hosts (SWD_e = *D. suzukii* eggs in diet, SWD_1 = *D. suzukii* first instar larvae in diet, SWD_{eb} = *D. suzukii* eggs in blueberries, DM_e = *D. melanogaster* eggs in diet).
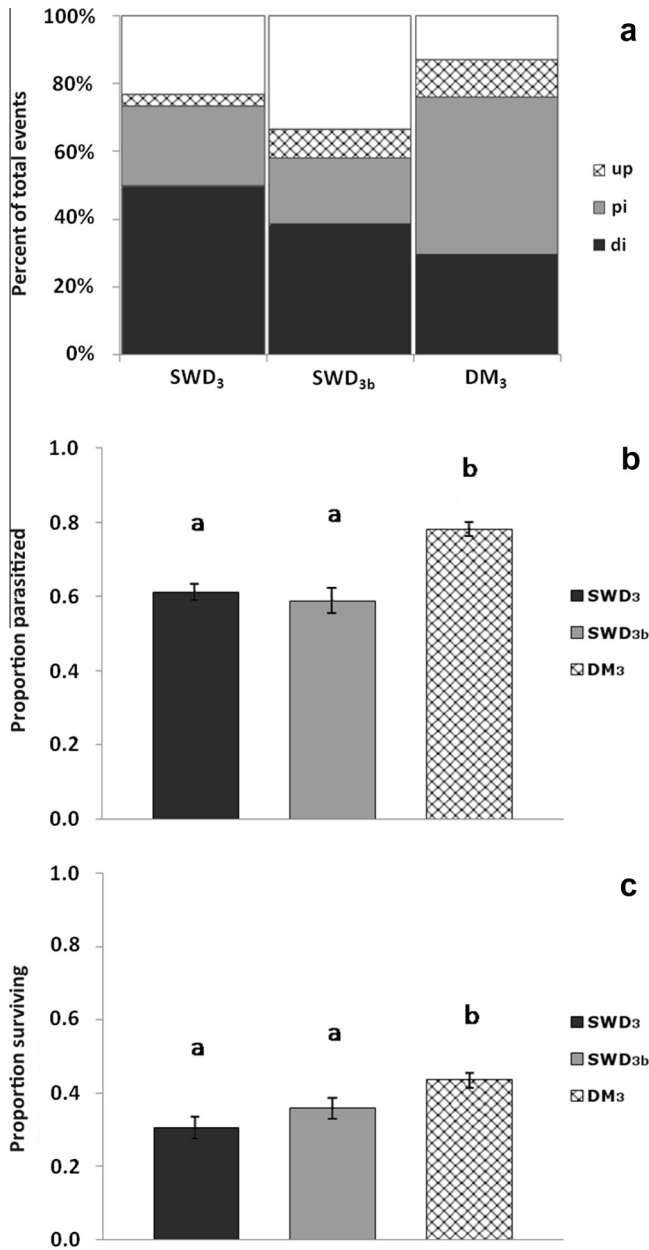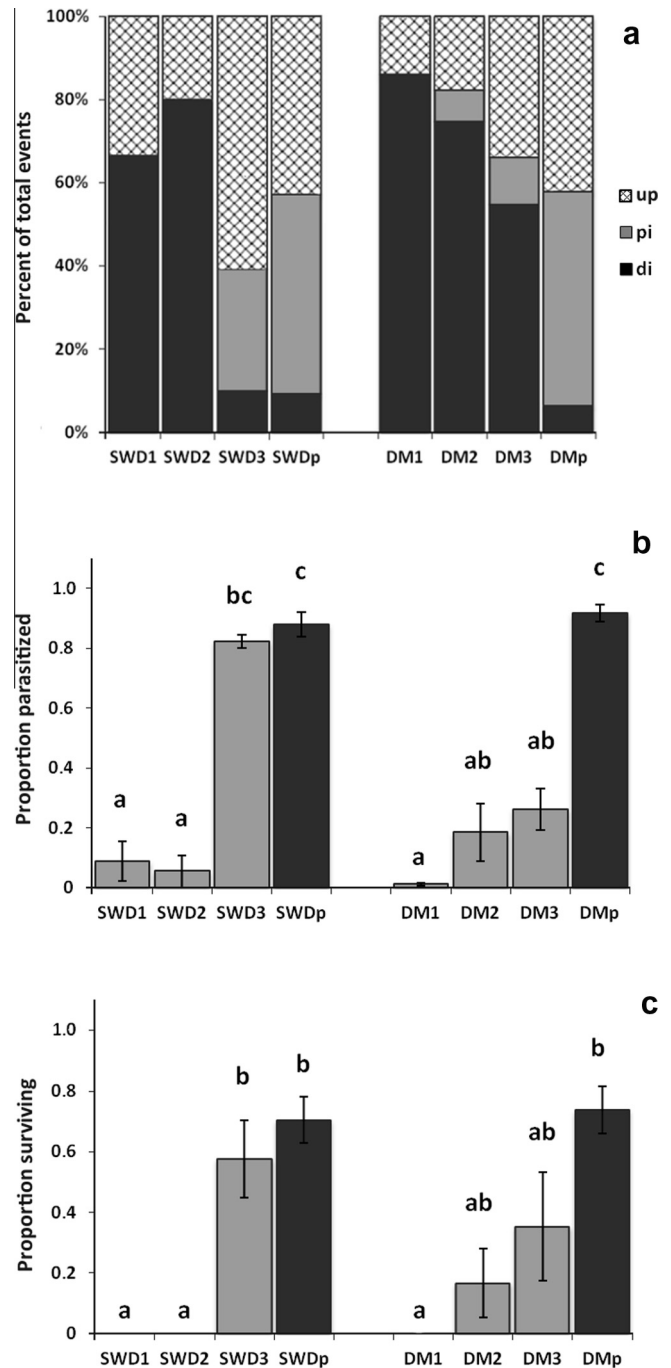
Fig. 2. Parasitism indices for the Italian strain of *P. vindemiae*. (a) Mean percentage of outcomes (up = unemerged pupae; pi = emerged parasitoids; di = emerged Drosophila); (b) mean (± SE) percent parasitism and (c) mean (± SE) offspring survival when female wasps were provided different hosts (SWD_3 = *D. suzukii* third instar larvae in diet, SWD_{3b} = *D. suzukii* third instar larvae in blueberries, DM_3 = *D. melanogaster* third instar larvae in diet).

3.4. Lifetime fecundity of P. vindemiae

At 23 °C, female *P. vindemiae* started oviposition within 2 days after emergence and produced a similar number of offspring throughout life until a sharp decline before death (Fig. 5). Adult female *P. vindemiae* survived 21.5 ± 1.7 days (range: 5–44 days) when provided with food and *D. suzukii* pupae. Females parasitized a mean lifetime total of 78.4 ± 7.5 host pupae with 68.4 ± 6.8 offspring successfully emerging as adults, of which 80.0 ± 3.0% were female. Offspring survival was high (86.8 ± 1.2%), but the sex ratio (% female offspring) decreased with maternal age (Fig. 5). Net reproductive rate was 50.5 and the intrinsic rate of increase was 0.1385. Mean generation time and double time were 28.3 and 5.0 days, respectively. Each female contained 1.0 ± 0.3 mature eggs
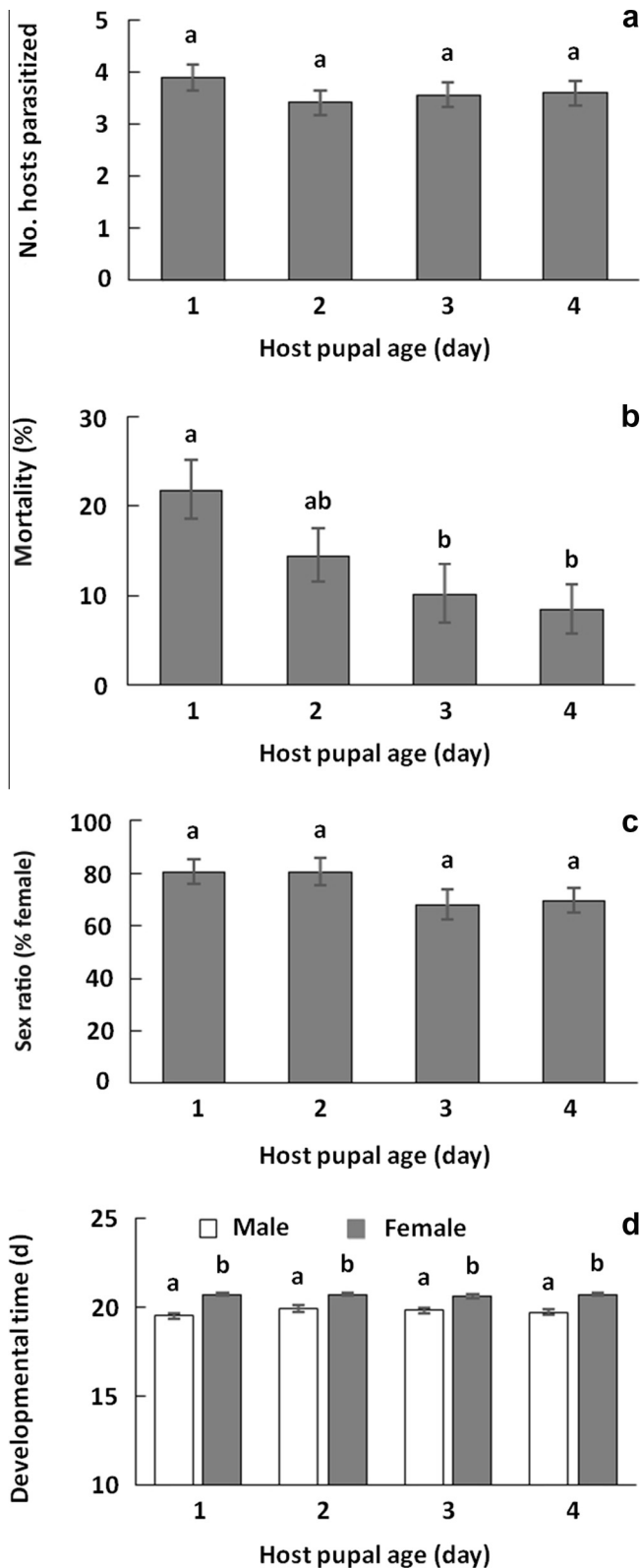

Fig. 3. Parasitism indices for the Oregon strain of *P. vindemiae*. (a) Mean percentage of outcomes (up = unemerged pupae; pi = emerged parasitoids; di = emerged Drosophila); (b) mean (± SE) percent parasitism and (c) mean (± SE) offspring survival when female wasps were provided different hosts (SWD_1 = *D. suzukii* first instar larvae in diet, SWD_2 = *D. suzukii* second instar larvae in diet, SWD_3 = *D. suzukii* third instar larvae in diet, SWD_p = *D. suzukii* pupae in diet, DM_1 = *D. melanogaster* first instar larvae in diet, DM_2 = *D. melanogaster* second instar larvae in diet, DM_3 = *D. melanogaster* third instar larvae in diet, DM_p = *D. melanogaster* pupae in diet).

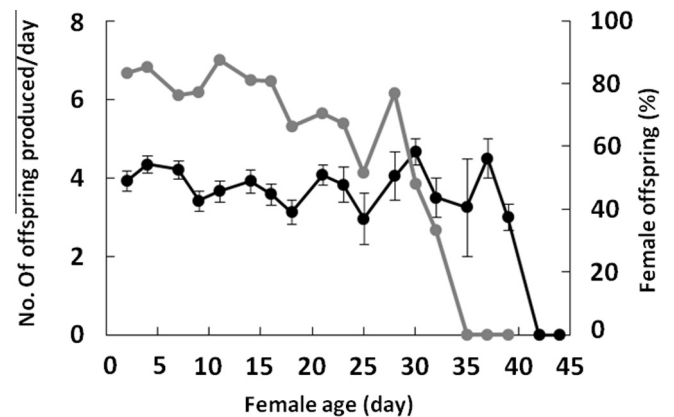(range: 0–7 eggs); *i.e.*, most females laid almost all their eggs before death.

3.5. Trichopria drosophilae in Italy

Trials with Italian *T. drosophilae* indicated that this pupal parasitoid can successfully attack *D. suzukii*. The three specimens

Fig. 5. Mean (± SE) daily fecundity (black line) and sex ratio (% female offspring, gray line) for female *P. vindemiae* parasitizing *D. suzukii* pupae at 23 °C.

Fig. 4. *P. vindemiae* preference for, and suitability of, different age lasses of *D. suzukii* pupae. Mean (± SE) (a) No. hosts parasitized, (b) offspring mortality, (c) sex ratio (% female), and (d) developmental time (egg–adult). Columns bearing different letters were significantly different (Tukey's HSD, *P* < 0.05).

collected in the field gave rise to a progeny of 72 individuals that, in the absence of superparasitism, would correspond to 72% parasitism of *D. suzukii* larvae. No additional experiments could be

performed with this species because the females obtained from the field were unmated and produced only male offspring.

4. Discussion

Assessing the capacity of local natural enemies to attack a newly introduced pest is one of the earliest and most important steps in a biological control program. Typically, the main questions to be addressed in this kind of evaluation are whether the natural enemies have the ability to attack a new pest as a potential host, and whether these natural enemies can overcome host defenses. In this study we showed that *P. vindemiae*, *T. drosophilae* and *L. heterotoma* are able to attack Italian populations of *D. suzukii*. In the California and Oregon studies *P. vindemiae*, but not *L. heterotoma*, were able to successfully parasitize *D. suzukii* under standard laboratory conditions. Data collected from field studies in both regions strongly support this work (Miller et al., unpublished data). In reference to additional recent studies (Brown et al., 2011; Chabert et al., 2012; Gabarra et al., 2014; Rossi Stacconi et al., 2013), our findings confirm the ability of the pupal parasitoids, *P. vindemiae* and *T. drosophilae*, to attack *D. suzukii*. In Italy, and in contrast with previous studies conducted both in the U.S.A. and Europe (Chabert et al., 2012; Kacsoh and Schlenke, 2012), we illustrated that Italian populations of *L. heterotoma* are able to overcome immunological response by *D. suzukii* under standard laboratory conditions.

The two main mechanisms adopted by parasitoids to avoid the encapsulation process by their hosts include the presence of a non-reactive coating on their eggs, or a suppression of the host immune system by injecting venom into hosts along with their eggs (Kacsoh and Schlenke, 2012; Lee et al., 2009). For both strategies, genetic intra-specific variations are present, and a diverse immuno-suppressive effect has been observed among different populations (Dubuffet et al., 2009, 2007; Dupas and Carton, 1999; Dupas et al., 1996). In the case of Italian populations of *L. heterotoma*, this may be due to a particularly high virulence of the wild parasitoid population collected in Trento Province, or due to the fact that in previous experiments (Chabert et al., 2012; Kacsoh and Schlenke, 2012) the tested parasitoid populations had been reared on *D. melanogaster* for many generations, possibly lowering their virulence level against *D. suzukii*. The Oregon populations of *L. heterotoma* were collected from traps baited with lab-reared *D. melanogaster*, which provides additional support of this hypothesis. On the other hand, since the same genetic intra-specific fluctuations exist in flies for resistance against wasps (Carton and Nappi, 2001), it is also possible that the

Italian *D. suzukii* population used in these experiments has a reduced immune response against parasitoids, as suggested by the low values of TER found in all the treatments, compared to other populations tested so far. This alternate hypothesis could be further examined by testing Oregon populations of *D. suzukii* for TER or by conducting genetic analysis of both fly and parasitoid populations from both regions.

P. vindemiae is a cosmopolitan species (Carton et al., 1986) with a wide host range comprising several dipteran groups (Machiorri and Barbaresco, 2007; Wang and Messing, 2004a; Zhao et al., 2013) and even other parasitic hymenopterans (Goubault et al., 2003; Wang and Messing, 2004b; Wharton, 1989). It is therefore no surprise that this species is found to attack *D. suzukii*. Although it is widely considered a pupal parasitoid, Oregon trials indicate that *P. vindemiae* is capable of attacking larvae. The high variation associated with the infestation of earlier instars suggests that this capability is uncommon in natural environments. As expected, the DI exhibited by the Italian population of this species was consistent with the ones observed by Chabert et al. (2012), whereas the SP was lower but still considerable. The Oregon population of this species exhibited a considerably higher DI and comparable SP. This evidence would pave the way for the use of *P. vindemiae* as a *D. suzukii* control agent. Another consideration is the fact that this parasitoid has already been introduced into Hawaii and Costa Rica for control of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) and to various localities in the New World against *Anastrepha* spp. (Ovruski et al., 2000; Purcell, 1998).

For *T. drosophilae* we conducted only limited trials with the three field-collected females; however, the outcomes of colony rearing attempts provided a strong indication that *D. suzukii* is an acceptable host for this pupal parasitoid. Three wild females gave rise to an arrhenotokous generation, successfully parasitizing almost 75% of the total available hosts. This result is corroborated in a similar scenario with a French population (Lyon) of *T. drosophilae* (Chabert et al., 2012).

Age-related developmental changes, both physiological and morphological, can result in variable host quality and influence a parasitoid's host acceptance for oviposition and suitability for offspring (Quicke, 1997). This is particularly true for parasitoids attacking static developmental stages such as pupae. As the pupa develops, its tissues undergo histolysis, histogenesis, and differentiation to form adult internal organs and sclerotized appendages; therefore, less host resources may be available to the developing parasitoid. It would thus be expected that *P. vindemiae* prefer to attack young host pupae. However, female *P. vindemiae* did not show a preference among differently aged *D. suzukii* pupae. *P. vindemiae* attacks puparia of various cyclorrhaphous flies (Wang and Messing, 2004a), and a female parasitoid typically lays an egg into the space between the host pupa and the puparium (Wang and Messing, 2004b). Oviposition into young puparia where pupae have not fully formed may result in the parasitoid's eggs being placed into the host hemolymph, thus preventing their development (Wang and Messing, 2004b). Indeed, in the current study, offspring mortality decreased with increasing host pupal age. Younger host pupae might offer nutrition of higher quality for parasitoid development and easy penetration of the parasitoid's ovipositor, but they are associated with a potential cost in terms of offspring survival. The trade-off between host nutrition, host handling time and offspring survival among differently aged host pupae may preclude the parasitoid's preference for young hosts as supported by our novel discovery that this pupal parasitoid can utilize third instar *D. suzukii*.

We did not find an obvious oviposition peak throughout the lifetime of female *P. vindemiae*. Not all hosts were parasitized when each female was provided 10 or 15 hosts every 2 or 3 days in the fecundity test. However, in the host stage preference test, when a naïve female was provided a total of 20 pupae (5 pupae of each of the four pupal stages), the parasitoid produced about 12.4 offspring during the 24 h exposure. Each mature female *P. vindemiae* contained a mean of 15 mature eggs (Wang et al., unpublished data). This suggests that this parasitoid's strategy is to lay all mature eggs once hosts are available. Like other synovigenic ectoparasitoids (Quicke, 1997), female *P. vindemiae* produce only a few large eggs at a time, and egg-limitation likely occurred in this parasitoid when they were consistently provided with hosts during the fecundity test. Dissection of dead females from the fecundity test further confirms that most dead females had exhausted their eggs before they died. This may explain why the number of offspring produced was similar during different periods of the female lifetime.

The current study shows some potential of these resident parasitoids to impact *D. suzukii*. In the scope of IPM programs, new sanitation strategies against fruit flies based on the concept of "Augmentoria" have been studied (Deguine et al., 2011; Jang et al., 2007; Klungness et al., 2005). An augmentorium is a container, or a series of containers, placed adjacent to a cultivated field, where farmers can regularly deposit infested fruits. The augmentorium employs a net of a certain diameter mesh, which sequesters adult flies that emerge from infested fruit while allowing parasitoids to escape, thus "augmenting" their population. This approach has already been taken into account for the management of *D. suzukii* populations with *T. drosophilae* in protected crops with promising results (Trottin et al., 2014) and, hence, would deserve to be evaluated with other indigenous parasitoids and in open field conditions.

Our work indicates that the current parasitoid complex in newly invaded areas provides management of some *D. suzukii* life stages, but also highlights the lack of adequate control currently provided by these agents in each of these production regions. Classical biological control should help alleviate this shortage of biological resources in these regions and needs further investigation. Our studies were conducted under laboratory conditions and do not provide a picture of how effective the respective biological control agents will be in the field, where many factors may contribute to parasitoid performance. Our experiments do not take into account environmental variables that may affect parasitoid activity, the field abundance of alternative hosts that may distract the parasitoid from the target pest search, access to *D. suzukii* larvae within the ripe fruits, and the synchronization between the pest and the parasitoid in the field. Nonetheless, our studies provide an indication of the current status of biological control in the respective regions. Future activities should include continued monitoring of native parasitoids, and investigation of promising imported parasitoids to assess their potential to control *D. suzukii*.

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