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Comparative life history traits of indigenous Italian parasitoids of *Drosophila suzukii* and their effectiveness at different temperatures

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ABSTRACT

Drosophila suzukii, or spotted wing drosophila (SWD), is a highly polyphagous invasive pest which has recently invaded Europe and the Americas. Its huge economic impact is due in part to the lack of specialised natural enemies suppressing population outbreaks in newly invaded areas. By establishing new associations, endemic parasitoid species native to the invaded areas can play an important role in controlling the pest. This study aims to provide a range of baseline information on the developmental parameters and parasitisation efficacy of three Italian populations of *D. suzukii* parasitoids at different temperatures. The species tested were a larval parasitoid, *Leptopilina heterotoma* (Thomson), and two pupal parasitoids, *Pachycrepoideus vindemiae* (Rondani) and *Trichopria drosophilae* (Perkins). Two comparative experiments were set up: the first assessing longevity and the lifetime fecundity of each species at 23 °C, and the second investigating the effect of temperature on parasitisation efficacy, developmental time and sex-ratio. The results revealed different fecundity patterns for the three parasitoids, probably due to a different level of synovigeny. For *T. drosophilae* and *P. vindemiae*, the lifetime sex ratio was biased towards an increasing number of males, whereas *L. heterotoma* showed the opposite trend. Moreover, temperature markedly affected host-parasitoid interaction and was positively correlated with the parasitoid's developmental time, whereas the sex-ratio was not significantly influenced. On the basis of these experiments, *T. drosophilae* appears to be the best candidate for developing a biological control strategy.

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1. Introduction

Since it was first reported in Europe and America in 2008 (Hauser, 2011; Calabria et al., 2012), the spotted wing drosophila (SWD), *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), has been responsible for huge economic losses to the fruit production industry (Goodhue et al., 2011; Walsh et al., 2011). In eight years the pest has expanded its distribution area to include northern Europe, North and South Americas (Hauser, 2011; Walsh et al., 2011; Cini et al., 2014; Asplen et al., 2015). The fly is able to exploit ecological niches not filled by other Drosophilid species, mainly through the key adaptation of a serrated ovipositor, which allows the females to penetrate the skin of ripening fruits, specifically market-ready fruits (Rota-Stabelli et al., 2013; Atallah et al., 2014; Hamby et al., 2016). During the past eight years, many efforts have been made for developing effective control strategies, nonetheless chemicals remain the principal tools used by farmers for reducing the pest population (Bruck et al., 2011; Cini et al., 2012). The extensive and repeated applications of broad-spectrum insecticides result in a number of secondary problems such as chemical residues, pollinators and natural enemies' decline, pest resistance and pest resurgence originating from natural and unmanaged areas (Klick et al., 2014; Lee et al., 2015; Pelton et al., 2016). Alternative control strategies such as biocontrol, mass trapping, sterile male techniques, preventive and sanitation measures and exclusion netting are currently under investigation (Cha et al., 2012, 2013; Hampton et al., 2014; Cormier et al., 2015; Grassi et al., 2015; Daane et al., 2016). In particular, the use of parasitoids may help to reduce large reservoir populations from areas surrounding crop fields.

Two different approaches have been taken into consideration so far. The first relies on the introduction and permanent establishment of natural enemies hailing from the *D. suzukii* native range, namely “classic biological control” (Cini et al., 2012; Daane et al., 2016). However, this approach is complicated by very strict laws regulating the importation of alien species, even as biocontrol agents. The second option, which appears to be a simpler strategy, is based on the enhancement of parasitoid populations already present in newly invaded areas. Indeed, according to the Enemy Release Hypothesis (Keane and Crawley, 2002), these populations may gradually adapt to the new host, establishing and strengthening new associations with the pest, thus contributing to its demographic control (Chabert et al., 2012; Rossi Stacconi et al., 2013, 2015; Miller et al., 2015). However, the adaptation process of local natural enemies requires a variable amount of time, depending on the parasitoid plasticity level. In general, larval endo-parasitoids (koinobionts) show narrower host ranges, since they coevolve and establish close physiological relations with their hosts (Dubuffet et al., 2007; Eslin et al., 2009; Fleury et al., 2009; Moreau et al., 2009; Desneux et al., 2012), whereas egg and pupal parasitoids (idiobionts) usually develop as saprophytic consumers, killing their hosts within a short while after oviposition (Strand and Obrycki, 1996). Accordingly, several recent studies have reported the resistance of *D. suzukii* to the attack of most indigenous larval parasitoids, mainly due to an immune-based defense, whereas pupal parasitoids were not observed to suffer the pest immune response (Chabert et al., 2012; Kacsoh and Schlenke, 2012; Poyet et al., 2013; Rossi Stacconi et al., 2015; Mazzetto et al., 2016; Wang et al., 2016a).

Our research therefore focused on providing data concerning the biology and reproductive strategies of parasitoids endemic to the invaded areas that have been proven to successfully attack *D. suzukii*, with different levels of efficacy (Chabert et al., 2012; Rossi Stacconi et al., 2015; Wang et al., 2016a). The chosen species were two pupal parasitoids, *Pachycrepoideus vindemiae* Rondani (Hymenoptera; Pteromalidae) and *Trichopria drosophilae* Perkins (Hymenoptera; Diapriidae), and a larval parasitoid *Leptopilina heterotoma* Thomson (Hymenoptera; Figitidae).

The study had two main objectives. Firstly, we aimed to assess the basic biological parameters of northern Italian populations of these

parasitoids by assessing their effectiveness against *D. suzukii* in laboratory conditions. Secondly, we investigated the influence of different temperatures on their parasitisation activity and developmental period. Our findings will help to establish strategies for biocontrol of *D. suzukii* by means of resident hymenopteran parasitoids. In particular, knowledge of the influence of temperature on their natural enemies' biology is crucial for establishing the release strategy in the context of *D. suzukii* biocontrol in the early season, when the pest population is still low after the winter bottleneck (Rossi Stacconi et al., 2016).

2. Material and methods

2.1. Insects

The *D. suzukii* and *Drosophila melanogaster* populations used in this study derive from live adults collected from multiple locations in the Italian Province of Trento during summer 2015. Parasitoid populations of *L. heterotoma* and *P. vindemiae* were collected from sentinel traps placed in close proximity to fruiting *D. suzukii* host plants in the same locations and period. For further details on traps preparation refer to Miller et al. (2015). Colonies of *T. drosophilae* were initiated from commercially available insects (Bioplanet s.c.a., Cesena, Italy). All flies were provided with standard artificial medium and maintained in plastic cages under laboratory conditions ($23 \pm 1^\circ\text{C}$, 16L:8D, $70 \pm 10\%$ RH). All parasitoids were maintained with *D. melanogaster* host larvae or pupae and also provided with honey droplets.

2.2. Longevity and life fecundity

A test was performed in order to assess the reproductive performance of *T. drosophilae*, *P. vindemiae* and *L. heterotoma*. For the pupal parasitoids, the longevity and lifetime fecundity of females were determined using one-day old *D. suzukii* pupae, since it has been demonstrated that pupae age doesn't affect the rate of parasitization (Rossi Stacconi et al., 2015; Wang et al., 2016a). For the larval parasitoid, the same parameters were determined using second instar larvae of *D. suzukii* as hosts (Thiel and Hoffmeister, 2009). Parasitoid couples were isolated in plastic vials within 6 h from their eclosion. The vials contained 10 g of standard medium, a honey droplet and a small cotton ball soaked with water. For each parasitoid species, twenty couples were used. A subset of ten couples was host-deprived, while the remaining ten couples were constantly provided with pupae or larvae, depending on the parasitoid species. Any male parasitoid dying before the female was replaced with a newly emerged male. All dead females were dissected to determine the number of residual mature eggs they contained. In the host-provided treatment, 10 *D. suzukii* individuals were offered to each female parasitoid every two days (Wang et al., 2016a). On each host replacement, the parasitoid couples were transferred to a new vial containing fresh hosts. All the hosts were kept in their original vials and maintained in an incubation chamber (23°C , 65% RH, 16:8 L:D) until either parasitoids or adult flies emerged. After thirty days we considered adult emergence to be complete, so all remaining host pupae were dissected under a microscope to determine the presence of fly or parasitoid cadavers. Similarly to the host-provided treatment, the plastic vials of the host-deprived parasitoids were renewed every two days to ensure good conditions and prevent the diet from going mouldy. Two additional sets of 10 control vials, containing either one-day-old pupae or second instar larvae, were placed alongside the parasitisation vials, in order to assess the *D. suzukii* survival rate in the absence of parasitism. All vials were checked daily and the female longevity, the number of parasitised hosts and offspring, the survival rate, the residual egg load and the developmental time from egg to adult were considered. Sex ratio, expressed as proportion male, was also calculated by considering the sex of both the emerged individuals and the unclosed pupae (when recognisable). Cases of single and multiple emergences were treated the same.

Fig. 1. Lifespan parasitisation activity of *P. vindemiae* (A), *T. drosophilae* (B) and *L. heterotoma* (C) on *D. suzukii* at 23 °C. For each species, the daily fecundity of both the parasitoid (light grey bars) and the host (black dotted line) is showed. Unclosed parasitized pupae are reported as dark grey bars, while the grey dashed line indicates the *D. suzukii* survival level in the absence of parasitism. Data are mean values.

For each parasitoid species, demographical parameters were calculated. Given the age-specific survival rate (l_x) and the number of female offspring produced per living female at age x (m_x), the intrinsic rate of natural increase (r) was estimated according to the equation from Birch (1948): $\sum e^{-rx} l_x m_x = 1$

The net reproductive rate (R_0) was calculated as $R_0 = \sum l_x m_x$, the mean generation time in days (T) as $T = \ln R_0 / r$, and the doubling time in days (DT) as $DT = \ln(2) / r$ (Biondi et al., 2013; Wang et al., 2016a).

2.3. Influence of temperature on performance and development

A second experiment was performed to evaluate the effect of temperature on parasitisation activity and the developmental period of the three parasitoids. In order to guarantee optimal experimental conditions, newly-eclosed female parasitoids were kept in mating vials along with a male for 48 h and provided with water and honey. After this period, the 2-day-old females were transferred individually into parasitisation vials containing 10 g of standard medium and provided with 20 *D. suzukii* hosts, either one-day-old pupae or second instar larvae, depending on the parasitoid species. These vials were placed in temperature-controlled growth chambers at 15°, 20°, 25°, 30° and 35 °C (65% RH; 16L:8D photoperiod), in order to test the Italian average maximum temperature range from April to October. A set of 20 parasitisation vials was prepared for each temperature treatment and parasitoid species. After 24 h all the parasitoids were removed and the vials were kept at the five temperature regimes until the complete emergence of the adults. In each growth chamber, two additional sets of 20 control vials, one containing one-day-old pupae and one containing second instar larvae, were placed alongside the parasitisation vials, in order to evaluate the developmental success of the *D. suzukii* population

in the absence of parasitism (T) (Chabert et al., 2012).

2.4. Data analysis

To determine whether the longevity of females is affected by the oviposition activity, data sets were analysed by means of Cox's proportional hazard model (Cox, 1972; Kalbfleisch and Prentice, 1980). The host availability (absence/presence) was used as covariate for testing the null hypothesis ($\beta = 0$).

Trends in the offspring sex-ratio throughout the parasitoid's lifespan were analysed using linear regression. In this case, only vials in which adult eclosion occurred or in which the sex of the dead parasitoid within the host pupae was recognisable were considered, whereas the other vials were not included in the analysis.

The effect of temperature on the developmental period, as well as trends in the offspring sex-ratio at different temperatures, were analysed using the Kruskal-Wallis test (non-parametric ANOVA). The latter parameter is reported using a skew index for better visualisation. In particular, for each temperature the sex skew index was calculated as the difference between female and male offspring, divided by the total number of offspring. This value varies between 1 (100% females) and -1 (100% males).

The parasitoid's effectiveness at different temperatures was assessed by scoring two indices: the Degree of Infestation (DI) and the Success rate of Parasitism (SP) (Boulétreau and Fouillet, 1982; Delpuech et al., 1994; Eslin and Prévost, 1998). The first indicates the proportion of host pupae that are successfully parasitised. This was estimated as the $DI = (T - di) / T$. If $T - di < 0$, we set $T - di = 0$. The second indicates the probability of an infested pupa giving rise to an adult wasp. This was estimated as the $SP = pi / (T - di)$. If $pi > T - di$, we set

SP = 1 and if $T - di = 0$, we set SP = 0. For the larval parasitoid *L. heterotoma*, evaluation of the ability of the drosophilid host to immunosuppress parasitism was determined by counting the number of adult flies containing a melanized capsule (dc). This parameter, the Total Encapsulation Rate (TER), was estimated as $TER = dc / (T - di + dc)$ and expresses the number of *Drosophila* with a capsule as a proportion of the total parasitised hosts. To compare the indices' trends at different temperatures, we performed the Friedman test (nonparametric repeated measures ANOVA) without replications, followed by Steel-Dwass pairwise multiple comparisons (Siegel and Castellan, 1988). All statistical analysis were performed using KyPlot version 2.0 beta 15 (1997–2001 Koichi Yoshioka). All mean values are presented as averages $\pm$ standard deviation (SD).

3. Results

3.1. Longevity and life fecundity

T. drosophilae females started to oviposit within 24 h of emergence, while *L. heterotoma* and *P. vindemiae* commenced after 48 h (Fig. 1). The number of offspring produced by *T. drosophilae* decreased with increasing female age and stopped after 28 days, whereas for *P. vindemiae* the production of offspring was spread out gradually over time, and continued until the parasitoid's death (Fig. 1a, b). For *L. heterotoma*, the offspring number was extremely irregular and low, to the extent that it was not possible to describe any clear temporal trend (Fig. 1c). The average daily eclosures of *D. suzukii* were 1.5 ± 0.14 , 2.2 ± 0.21 and 2.4 ± 0.19 (mean $\pm$ SE) for *P. vindemiae*, *T. drosophilae* and *L. heterotoma* treatment respectively. The lifetime number of parasitised hosts, corresponding to the sum of total emerged parasitoids and un-emerged pupae containing a parasitoid, was 56.7 ± 6.54 and 41.7 ± 4.99 (mean $\pm$ SE) for *P. vindemiae* and *T. drosophilae* respectively, whereas for *L. heterotoma* only 5.3 ± 1.16 offspring were produced by each female. The residual mature egg load was determined for each species at the end of the experiment. It was low for both *T. drosophilae* and *P. vindemiae* populations (0.9 ± 0.23 and 1.9 ± 0.61 mature eggs/female respectively). *L. heterotoma* also laid most of its eggs before death and each female contained 4.7 ± 1.52 mature eggs on average. No difference in longevity between host-provided and host-deprived individuals was observed for *L. heterotoma* (Fig. 2). In contrast, *P. vindemiae* showed a longer life when allowed to oviposit ($\chi^2 = 8.00$; df = 1; $p < 0.005$; $\beta = 0.87$), whereas *T. drosophilae* lived longer when host deprived ($\chi^2 = 4.17$; df = 1; $p < 0.05$; $\beta = -0.58$) (Fig. 2). The overall offspring sex-ratio, was 0.498 and 0.199 in *T. drosophilae* and *P. vindemiae* respectively. For both species a significant decreasing trend with maternal age was observed (*T. drosophilae*: $R^2 = 0.43$; df = 22; $P < 0.005$ and *P. vindemiae*: $R^2 = 0.67$; df = 39; $P < 0.005$) (Fig. 3a–b). In *L. heterotoma* the offspring sex-ratio was 0.293 and in contrast to the other two species, a significant pattern of increasing female offspring with maternal age was observed ($R^2 = 0.37$; df = 12; $P < 0.025$) (Fig. 3c). For each parasitoid species, life table fertility parameters are reported in table 1. For *L. heterotoma* r , T and DT are not reported since the parasitoid's success of parasitism on *D. suzukii* did not support any population increase ($\Sigma e^{-rx_{i,m_x}} < 1$ for any estimated value).

3.2. The effect of temperature on parasitisation activity

At 15 °C, only *T. drosophilae* was able to successfully develop on *D. suzukii*, whereas the other two parasitoids only showed a certain rate of parasitism starting from 20 °C (Fig. 4a, c). The best performance by *T. drosophilae* was observed at 20° and 25 °C, with significant DI reductions at the other temperatures ($\chi^2 = 46.79$; df = 4; $P < 0.001$) (Fig. 4a, c). *P. vindemiae* showed the highest DI level between 25° and 30 °C, and significantly decreased host parasitisation at both lower and higher temperatures ($\chi^2 = 43.91$; df = 4; $P < 0.001$) (Fig. 4c). For

Fig. 2. Proportion of adult female parasitoid survival in host-provided and host-deprived conditions. Asterisk indicate significant difference between the treatments ($p < 0.05$).

both pupal parasitoids, the SP was significantly reduced only at the lowest temperature (*T. drosophilae*: $\chi^2 = 15.59$; df = 4; $P < 0.001$ and *P. vindemiae*: $\chi^2 = 43.59$; df = 4; $P < 0.001$) (Fig. 4c). *L. heterotoma* attacked *D. suzukii* at temperatures from 20° to 35 °C ($\chi^2 = 38.64$; df = 4; $P < 0.001$), whereas its successful development was observed only at 20° and 25 °C ($\chi^2 = 32.81$; df = 4; $P < 0.001$) (Fig. 4c). The TER was significantly lower at 15° and 20 °C ($\chi^2 = 39.16$; df = 4; $P < 0.001$) and increased with temperature (Fig. 4b). For all parasitoids, the developmental time showed a significant negative relationship with temperature (*T. drosophilae*: $H = 38.93$, df = 4, $P < 0.001$; *P. vindemiae*: $H = 21.65$, df = 4, $P < 0.001$; *L. heterotoma*: $H = 11.39$, df = 4, $P < 0.001$) (table 2). For *T. drosophilae* and *L. heterotoma*, the rate of development was always positively correlated with temperature, whereas for *P. vindemiae* a negative trend was observed between 30° and 35 °C (table 1).

For all parasitoids, no significant differences were observed in the sex skew index at different temperatures (Fig. 5a). In the case of *P. vindemiae* and *L. heterotoma*, the offspring sex-ratio was always biased towards more females, whereas for *T. drosophilae*, it switched towards more males between 25° and 30 °C (Fig. 5a).

The developmental time from egg to adult significantly decreased with increasing temperatures, for both males (*T. drosophilae*: $H = 66.65$, df = 4, $P < 0.001$; *P. vindemiae*: $H = 48.36$, df = 3, $P < 0.001$; *L. heterotoma*: $U = 17.94$, $P < 0.001$) and females (*T.*

Fig. 3. Trends in offspring sex ratio (% female) for *P. vindemiae* (A), *T. drosophilae* (B) and *L. heterotoma* (C); values refer to the average percentage of offspring per female. Only vials in which adult eclosion occurred or in which the sex of the dead parasitoid within the host pupae was recognisable were considered.

Table 1

Life table demographic fertility parameters of the tree parasitoids.

	Net reproduction rate (R_0)	Intrinsic rate of natural increase (r)	Mean generation time (T)	Doubling time (DT)
<i>T. drosophilae</i>	17.65	0.107	26.83	6.47
<i>P. vindemiae</i>	24.56	0.098	32.66	7.07
<i>L. heterotoma</i>	0.57	–	–	–

drosophilae: $H = 38.93$, $df = 4$, $P < 0.001$; *P. vindemiae*: $H = 21.65$, $df = 4$, $P < 0.001$; *L. heterotoma*: $U = 18.46$, $P < 0.001$ (Fig. 5b). In the case of *T. drosophilae*, the only species that was able to develop at all temperatures, the mean developmental time ranged between 48.57 ± 3.56 days at 15°C and 16.09 ± 0.85 days at 35°C , and from 52.99 ± 3.45 days at 15°C and 17.16 ± 0.91 days at 30°C , for males and females respectively (Fig. 5b).

4. Discussion

Our experiments showed that parasitoid activity on *D. suzukii* was

substantially different according to the species taken into account. *T. drosophilae* started parasitisation a few hours after eclosion. This rapid and highly effective parasitisation was balanced by the limited duration of the action. The effectiveness of *P. vindemiae* was more progressive and showed major variations over time. Females of this species oviposited throughout their entire lifetime, constantly lowering the daily emergence of the flies. Finally, *L. heterotoma* had a lower impact on the host population than the other two species and mostly during the first 18 days of experiment. For this species, parasitisation activity did not necessarily imply offspring production, since in most cases host-parasitoid interaction led to the death of both of them.

Although the oviposition patterns are not necessarily indicative of the ovigeny index, these findings may suggest a different level of synovigeny for the two pupal parasitoids (Jervis et al., 2001; Jervis and Ferns, 2004). In particular, the tested *T. drosophilae* population revealed a similar daily fecundity pattern to that of the South Korean *T. drosophilae* population tested by Wang et al. (2016a). Both populations showed a peak at day 10, followed by a decreasing trend and the suspension of any parasitisation activity halfway through their lifespan. This was probably due to a progressive reduction in the egg maturation rate, which does not compensate for egg-load depletion (Jervis and Copland, 1996). Female dissections at the end of the test confirmed the low residual mature egg load. In contrast, *P. vindemiae* oviposition activity was supported throughout its entire lifespan by either the initial egg load or by the egg maturation rate. This difference in fecundity patterns between the two species may have a variable impact on their actual fecundity in nature. In particular, when hosts are abundant should be more likely for *T. drosophilae* to become egg-limited. This may be favorable in case of *T. drosophilae* releases in environments with elevated *D. suzukii* presence. In fact, according to Driessen and Hemerik (1992) egg limitation should lead the parasitoid to be more selective in the host choice and it was recently reported that *T. drosophilae* shows a parasitization preference for *D. suzukii*, likely due to the difference in size compared to *D. melanogaster* (Wang et al., 2016a; Mazzetto et al., 2016). The larger dimensions of *D. suzukii* in comparison with most local *Drosophila* species, could be then a preferential feature driving the host selection of *T. drosophilae*.

Host-provided females of *P. vindemiae* lived significantly longer than host-deprived females, whereas *T. drosophilae* showed an higher longevity of host-deprived females, and no differences between the two treatments were observed for *L. heterotoma*. Host-feeding is an important source for egg maturation, especially in species that are strongly synovigenic, such as *P. vindemiae* (Jervis and Kidd, 1986). This pupal parasitoid is known to feed on its hosts (Phillips, 1993; personal observation), thus in our experiment host-deprived females may have suffered from a lack of specific nutrients deriving from this activity (Giron et al., 2002, 2004). A plausible hypothesis explaining the longer life of host-deprived *T. drosophilae* females, a synovigenic parasitoid (Wang et al., 2016a), may be egg-resorption (Jervis et al., 2001). Indeed, each ovariole has a limited egg storing capability at any one time, and mature eggs can only be retained for a brief period in the lateral oviducts. Therefore, when a female is host deprived for a period longer than the egg's storage life, the mature eggs begin to be reabsorbed (Jervis, 2005). Through this mechanism, eggs are returned to the body of the wasps with only a partial loss of energy and materials (Jervis and Kidd, 1986), thus prolonging the parasitoid's life.

For both pupal parasitoids, the percentage of female progeny showed a decreasing trend with maternal age. On the other hand, for *L. heterotoma* the trend was towards an increase in female offspring over time. Indeed, in most parasitic wasps, regardless of host species, sex ratio decreases with parental age, and unfertilised eggs (males) can be oviposited for several days after the oviposition of fertilised eggs (females) has ceased (Kopelman and Chabora, 1986). Although several variables contribute to the determination of sex ratios, the principal internal causes of shifts towards male-biased sex ratios in parasitoids exposed to high-density hosts for long periods are sperm depletion or

Fig. 4. Effect of temperature on parasitisation activity. A) Average parasitoid fecundity at five constant temperatures. Bars refer to the mean ($\pm$ SE). B) Total encapsulation rate on *L. heterotoma*. Bars refer to the mean ($\pm$ SE) and different letters indicate significant differences. C) Degree of infestation (black lines) and success of parasitism (grey lines) at different temperatures for the three parasitoid species. Values with different letters are significantly different.

depletion of spermathecal gland secretions (Flanders, 1939; King, 1987). It may be possible that *L. heterotoma* has adopted certain strategies to compensate for this mechanism, such as bigger spermathecae or multiple mating for example (Chevrier and Bressac, 2002). Actually, it was reported that parasitoid females caged in small environments may be incline to accept higher number of copulations (Biondi et al., 2013).

In both *T. drosophilae* and *L. heterotoma*, the best parasitisation performance occurred between 20° and 25 °C, whereas *P. vindemiae* was most effective at 30 °C. Moreover, *T. drosophilae* was the only species showing successful parasitism at the lower temperature. This suggests a difference in the optimal temperature range for the three parasitoids, which may result in different time windows for parasitisation activity during the season. Indeed, different thermal tolerance may be an adaptive strategy allowing parasitoids sharing hosts to occupy different temporal niches within the same habitat (Le Lann et al., 2011). In our case, *T. drosophilae* and *L. heterotoma* may benefit from a time offset with *P. vindemiae* in order to avoid both interspecific host competition and parasitism, since the latter is known to be a facultative hyperparasitoid (Chen et al., 2015). According to Ris et al. (2004), temperature and host quality interact with each other and affect the parasitoid's

performance and phenotype. In our case, the effect of thermal variation was stronger on the degree of infestation, whereas developmental success was affected to a lesser extent. This was particularly evident for the two pupal parasitoids, suggesting that temperature mostly influenced their activity in terms of host search and acceptance, rather than altering host-parasitoid physiological interaction. For *L. heterotoma*, both the DI and SP, as well as the TER, were significantly influenced by temperature changes. Indeed, koinobiont parasitoids establish strong interaction with their hosts, within which both partners are living organisms and are conditioned by the environment (Godfray, 1994). As a consequence, external factors such as temperature can markedly influence the outcome of parasitism.

Identification of efficient biological control agents requires multi-level investigations, including both inter- and intra-specific screening processes (Benvenuto et al., 2012). On comparing our work with similar studies testing the same host-parasite interaction, intraspecific differences in terms of developmental success are clear. For example, North American populations of both *T. drosophilae* and *P. vindemiae* (Rossi Stacconi et al., 2015; Wang et al., 2016a), as well as the South Korean population of *T. drosophilae* (Wang et al., 2016a) were reported to parasitise a larger number of hosts during their life in comparison with

Table 2
Effect of temperature on the developmental time of the three parasitoids (Mean $\pm$ SE).

	<i>T. drosophilae</i>		<i>P. vindemiae</i>		<i>L. heterotoma</i>	
	Females	Males	Females	Males	Females	Males
15 °C	52.99 $\pm$ 0.38 A (20)	48.57 $\pm$ 0.35 A (14)	–	–	–	–
20 °C	28.60 $\pm$ 0.22 B (112)	26.41 $\pm$ 0.21 B (77)	29.01 $\pm$ 0.18 A (70)	28.23 $\pm$ 0.18 A (36)	26.76 $\pm$ 0.16 A (16)	25.28 $\pm$ 0.15 A (13)
25 °C	20.89 $\pm$ 0.12 C (105)	19.07 $\pm$ 0.1 C (82)	19.81 $\pm$ 0.15 B (92)	19.17 $\pm$ 0.12 B (72)	22.31 $\pm$ 0.15 B (16)	19.76 $\pm$ 0.1 B (8)
30 °C	17.48 $\pm$ 0.1 D (43)	16.27 $\pm$ 0.1 D (64)	16.57 $\pm$ 0.1 C (85)	15.82 $\pm$ 0.1 C (61)	–	–
35 °C	17.16 $\pm$ 0.1 D (8)	16.09 $\pm$ 0.1 D (17)	17.48 $\pm$ 0.1 D (30)	16.59 $\pm$ 0.1 D (23)	–	–

Means followed by the same letter in the same column are not significantly different ($P > 0.05$). Figures in brackets are the number of individual wasps.

Fig. 5. Effect of temperature on parasitoids' biological parameters: A) Offspring sex ratio (% female) at different temperatures. B) Mean developmental time at different temperatures. Bars refer to the mean ($\pm$ SE) and different letters indicate significant differences.

the population tested in this work. Chabert et al. (2012) and, more recently, Mazzetto et al. (2016) showed the inability of both French and north-western Italian *L. heterotoma* populations to successfully develop on *D. suzukii*, whereas in other cases a certain success in terms of parasitism was shown (Rossi Stacconi et al., 2015). The origin of this variability probably lies in either parasite virulence or *D. suzukii* immunological resistance (Henter and Via, 1995; Kraaijeveld et al., 2002; Colinet and Hance, 2010; Poyet et al., 2013) but also the physiological nature of the host with regard to parasitoid requirements (Boulétreau and Wajnberg, 1986). *D. suzukii* has expanded its geographical range to North America and Europe in the last decade (Cini et al., 2012), and hence the interaction between the fly and the resident parasitoid populations in these invaded areas is very recent. Although some of these communities are made up of generalist and ubiquitous parasitoid species also present in the pest's native range (Daane et al., 2016; Novković et al., 2011), the short co-adaptation time may explain the very few and unspecific pest-parasitoid associations reported by the numerous studies carried out in several invaded areas so far (Rossi Stacconi et al., 2013, 2015; Gabarra et al., 2015; Miller et al., 2015; Cancino et al., 2015; Mazzetto et al., 2016).

5. Conclusion

The current study shows that of Italian resident parasitoids able to successfully attack *D. suzukii*, *T. drosophilae* is the most promising candidate for use in biological and integrated *D. suzukii* control programmes, by its conservation and/or augmentation. Our tests revealed that this pupal parasitoid is the most effective, has the longest life and parasitises the pest at the lowest temperature. This last feature in particular may be of great importance, if we consider that a biocontrol strategy aimed at reducing *D. suzukii* infestation should be applied early in the season, when the pest population is reduced (Rossi Stacconi et al., 2016; Wang et al., 2016b; Wiman et al., 2016). However, extensive open field studies taking into account all the environmental, ecological (e.g. interspecific competition; Wang et al., 2016c) and agronomic (notably insecticide side effects; Biondi et al., 2012) variables that may affect parasitoid activity are needed to confirm the potential beneficial impact of *T. drosophilae* as a control agent of *D. suzukii*.

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