Performance of *Trichopria drosophilae* (Hymenoptera: Diapriidae), a Generalist Parasitoid of *Drosophila suzukii* (Diptera: Drosophilidae), at Low Temperature

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Abstract

Survival and parasitism activity of *Trichopria drosophilae* Perkins adults, a cosmopolitan parasitoid of *Drosophila* spp., were studied under laboratory conditions using five constant temperatures at the lower range known for this enemy, from 4 to 20°C in 4°C increments. *Drosophila suzukii* Matsumura, an invasive pest of small fruits, was used as a host. Commercially available adult parasitoids were provided with 1) food and *D. suzukii* pupae; 2) food and no *D. suzukii* pupae; 3) no food and no pupae. The results show that adult females of *T. drosophilae* lived longer than males, and both generally benefitted from food supply. The highest level of survival was observed between 8 and 12°C for fed insects, irrespective of whether they were offered host pupae or not. The absence of food led to the highest mortality, but the parasitoid demonstrated considerably resistance to prolonged starvation. Successful parasitism increased steadily with temperature and reached the highest value at 20°C. Conversely, *D. suzukii* emergence rate was high after exposure of pupae to parasitoids at 4°C, while pupal mortality increased strongly with temperature until 12°C. The findings indicate that *T. drosophilae* is well adapted to the relatively cold conditions experienced in early spring and in autumn or at high elevations, when the host pupae could be largely available. The long lifespan of the adults and the ability to parasitize the host at low temperature make *T. drosophilae* potentially useful for the biocontrol of *D. suzukii*.

Key words: longevity, parasitism, biocontrol, cold adaptation

To improve the knowledge on the biology of *T. drosophilae*, we investigate the longevity and survival of both sexes, and their performance, under different conditions at constant temperatures ranging from 4 to 20°C. Data obtained might clarify the potential of the biological control agent at colder sites or in cold seasons.

Materials and Methods

Adults of *T. drosophilae* used in the experiment were commercially available insects reared on *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) (Bioplanet s.r.l., Cesena, Italy) maintained at 15°C (±1°C) until the beginning of the experiments, whereas pupae of SWD were obtained from laboratory colonies reared according to Tonina et al. (2016).

Longevity of *T. drosophilae* adults and parasitism of SWD pupae were studied in a test tube (2.5 cm diameter, 8 cm length) made of transparent plastic and placed in five controlled cabinets set under long-day photoperiod (16 h light) at five constant temperatures: 4, 8, 12, 16, and 20°C (±1°C). Relative humidity (70–80%) was maintained in the test tubes using soaked cotton wool that provided also

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a constant source of water (Fisher and Andrés 1999) for the parasitoids of all treatments. Adult parasitoids were progressively taken from the commercial boxes as soon as they emerged from D. melanogaster pupae and were randomly inserted into each test tube (average 58, SD = 14, n = 30). Consequently, for mating purposes the sex ratio was male-biased (male:female average 2.68, SD = 0.95, n = 30) and slightly different in each tube, as we needed to test individuals of the same age (1–2 d-old female adults) with a high probability of being mated during the period of maximum egg load (Wang et al. 2016). Groups of adult parasitoids were assigned to three treatments: 1) with food; 2) with food and host; 3) without food and host. For food, ten droplets of honey diluted with water (80% honey) were provided on a plastic strip (0.8 × 3.0 cm) and inserted into each tube (Fisher and Andrés 1999). For host, 10 pupae of SWD (up to 4 d old; Wang et al. 2016) were provided for parasitoids on a filter paper strip (2.0 × 4.0 cm) sprayed with sterile water (Zhu et al. 2017). Two replicates of each treatment were carried out for a total of 30 test tubes (3 treatments × 5 temperature conditions × 2 replications).

Test tubes were checked and pupae replaced twice a week for adult parasitoid survival and performance in the first 3 mo, and then weekly until the end of the experiment, i.e., when no live T. drosophilae adults initially put inside the test tubes were still present (total number of checks = 26). Dead parasitoids were not replaced during the experiment. At each check, the cotton wool ball was sprayed with sterile water, and the plastic strip with honey droplets and the pupae were replaced. SWD pupae were transferred in new tubes kept at 20°C (±2°C) under the same photoperiod used in the experiment until SWD or parasitoid emergence. All individuals were then identified, counted, and sexed, and the pupal cases examined. If no exit holes had been observed, SWD pupal cases were carefully dissected and the content analyzed and classified as: 1) not fully developed or emerged T. drosophilae; 2) not fully developed/ emerging SWD; 3) pupae dead for unknown reasons (i.e., pupae parasitized or not, as the parasitoid or the fly died in the early stages of development; Wang et al. 2016).

The variables considered in the study were: 1) lifespan of T. drosophilae; 2) mortality of SWD pupae caused by T. drosophilae; 3) unparasitized healthy pupae of SWD; 4) dead pupae of SWD for unknown reasons. The lifespan of T. drosophilae was obtained from periodical check of the number of live T. drosophilae. Pupal mortality of SWD caused by T. drosophilae was based on the sum of T. drosophilae adults emerged (or SWD pupal cases with a parasitoid emergence hole when adult parasitoids were not easily countable) and pupal cases containing undeveloped T. drosophilae. Unparasitized healthy pupae of SWD was based on the sum of SWD adults emerged or pupal cases with a SWD emergence hole and of pupal cases containing undeveloped SWD. Lastly, pupae dead for unknown reasons were obtained from the pupal cases without emergence holes and pupal cases with a content not identifiable.

As our experimental design considered weekly cumulative mortality, nonlinear regressions with a 95% confidence interval including the two replicates of each trial were used. A three-parameter sigmoidal equation \( y = a/(1 + e^{-(x - x_0)/b}) \) was run to calculate the 50% of survival and to plot the temperature-dependent survival curves. Regressions and related statistical analyses were performed and plotted with the software Sigmaplot 3.1 and SigmaPlot 11.0, respectively (SSPS, Inc., Point Richmond, CA). Linear models was used: 1) to test the differences in the temperature-dependent survival curves, with temperature, treatment and sex as fixed factors, and all possible interactions among factors; 2) to examine the effects of temperature (categorical fixed factor), T. drosophilae survival (continuous fixed factor) and their possible interactions on the outcome of the pupal development. The models were performed using the packages ‘lme4’ for general mixed-effects models (Pinheiro et al. 2018) implemented in R (version 3.0.2—R Core Team 2014). The assumptions of the models were evaluated by visual inspection of the diagnostic plots of the model residuals.

### Results

Both male and female T. drosophilae generally lived longer when supplied with food than when offered food and host pupae or when they were deprived of both food and host (Table 1, Fig. 1). The highest level of survival was observed between 8 and 12°C for fed parasitoids irrespective of the sex and host presence. Females lived longer than males, especially at low temperature (from 4 to 12°C). The low and high temperatures used in the experiment (4 and 20°C) produced similar results for the parasitoids provided with food and with food and host. Insects deprived of food and host survived longer at 4°C and then survival decreased to a stable level between 12 and 16°C.

The outcome of pupal development (emergence of T. drosophilae, emergence of SWD, dead pupae of SWD) was significantly affected only by temperature, regardless of the parental age of the parasitoid (Table 2). Successful parasitism started at 8°C and then increased steadily with temperature. Conversely, SWD emergence rate was higher after exposure at 4°C and then decreased rapidly, reaching almost 0 values at temperatures between 16 and 20°C. Pupal mortality increased strongly with temperature until 12°C and then remained almost constant (Fig. 2).

### Discussion

Adults of both sexes of T. drosophilae coped well with low temperature if constantly supplied with honey and water, surviving more

### Table 1. Results of the linear model used to test the effects of temperature, sex, treatment, and all possible interactions among these factors on Trichopria drosophilae survival

| Factor and interaction | df | Sum sq. | Mean sq. | F value | Pr(>F) |
|------------------------|----|---------|----------|---------|--------|
| Temperature            | 4  | 35,274  | 8,818.4  | 525.3551| <2.2e-16*** |
| Sex                    | 1  | 5,089.1 | 5,089.1  | 303.1798| <2.2e-16*** |
| Treatment              | 2  | 35,374  | 17,687.1 | 1053.7037| <2.2e-16*** |
| Temperature × Sex      | 4  | 704     | 175.9    | 10.4795 | 1.96e-05*** |
| Temperature × Treatment| 8  | 5,979   | 747.4    | 44.5272 | 1.47e-14*** |
| Sex × Treatment        | 2  | 307     | 153.7    | 9.1582  | 0.000786*** |
| Temperature × Sex × Treatment | 8 | 747   | 93.4     | 5.5619  | 0.0002349*** |

***P < 0.001.
than 4 mo and thus almost doubling the value found by Amiresmaeili et al. (2018) with sugar-fed parasitoids. Furthermore, depending on factors such as increasing temperature and/or energy-consuming activities (i.e., mating and reproduction), life expectancy declined for both males and females, although at a different rate since males lived shorter than females. As in females, a trade-off between reproduction and survival is expected in males, but little is known about balancing the costs for sperm production and finding females (Hoogendoorn et al. 2002). In our experiment, there were no costs involved in finding females: thus, we can only hypothesize the occurrence of different metabolic rates and different temperature-induced shift in resource allocation in the two sexes. This was also demonstrated by the fact that at the lowest temperature tested (4°C), when no successful parasitism occurred, there were no differences in the lifespan recorded within each sex between the treatments ‘food’ and ‘food and host’. The same result emerged at the highest tested temperature (20°C) suggesting that, if food is available, T. drosophilae is able to easily replace the resources allocated to reproduction before approaching the thermal optimum for its performance (i.e., 20–25°C; Rossi-Stacconi et al. 2017). Above 25°C, metabolic costs likely become too high, especially if the host is abundant, as female parasitoids can survive only 27 d at 23°C (Wang et al. 2016). As expected, the absence of food led to the highest mortality, but adults were able to cope with food deprivation as they were able to survive up to 3 mo at 4°C and up to 30 d at 20°C. As far as we know, there are no similar data on T. drosophilae or other parasitoids associated to Drosophila spp. to be used for comparison. Only Da Silva et al. (2019) assessed that adults of Pachycrepoideus vindemmiae (Rondani) (Hymenoptera: Pteromalidae), kept without food but with a constant supply of water, lived for about 15 d at 24°C. Nonetheless, the ability of fasting T. drosophilae adults to survive at low temperature might be an important trait, allowing the biocontrol agent to cope with host absence and long cold periods.

Interestingly, we observed that T. drosophilae can successfully parasitize SWD pupae when temperature is above 8°C, a result that lowers the threshold of 12.6 and 15°C recently demonstrated

### Table 2. Results of the linear model employed to test the effects of temperature and parental age of Trichopria drosophilae, and their interaction, on the pupal development of SWD

| Factor and interaction                          | df  | Sum sq. | Mean sq. | F value | Pr(> F) |
|------------------------------------------------|-----|---------|----------|---------|---------|
| Outcome: % of pupae producing T. drosophilae adults |     |         |          |         |         |
| Temperature                                      | 4   | 1.04147 | 0.260367 | 28.0579 | 6.75e−13 *** |
| Parental age                                     | 1   | 0.00001 | 0.000007 | 0.0008  | 0.9781  |
| Temperature × Parental age                       | 4   | 0.02419 | 0.006047 | 0.6516  | 0.6281  |
| Residuals                                        | 57  | 0.52894 | 0.00928  |         |         |
| Outcome: % of pupae producing SWD adults         |     |         |          |         |         |
| Temperature                                      | 4   | 9.9966  | 2.49916  | 421.0564| <2e−16 *** |
| Parental age                                     | 1   | 0.0032  | 0.00321  | 0.5415  | 0.4648  |
| Temperature × Parental age                       | 4   | 0.0313  | 0.00782  | 1.3179  | 0.2743  |
| Residuals                                        | 57  | 0.3383  | 0.00594  |         |         |
| Outcome: % of SWD dead pupae (content not identifiable) |     |         |          |         |         |
| Temperature                                      | 4   | 5.0397  | 1.25993  | 92.4229 | <2e−16 *** |
| Parental age                                     | 1   | 0.0157  | 0.01566  | 1.1485  | 0.2884  |
| Temperature × Parental age                       | 4   | 0.0648  | 0.01621  | 1.1889  | 0.3255  |
| Residuals                                        | 57  | 0.777   | 0.01363  |         |         |

***P < 0.001.
by Wang et al. (2018) and Amiresmaeili et al. (2018), respectively. At 4°C, *T. drosophilae* was not able to exert any action against the host, as demonstrated by the high percentage of pupae (almost 100%) giving rise to SWD adults. As host-feeding has not been observed for *T. drosophilae* (Carton et al. 1986), the high rate of pupal mortality (starting at 8°C, peaking at 12°C, and then showing no significant variations at 16 and 20°C) without a corresponding/proportional number of parasitoid offspring, according to Abram et al. (2019) could be explained by nonreproductive effects (e.g., pseudoparasitism). Despite the fact that, for parasitoids and for biocontrol practitioners, nonreproductive effects could be costly from many points of view, they can sometimes be the principal cause of host mortality and thus useful in the short-term. This study also showed the absence of any variation in the parasitism rate of females during their lifespan, a result that complements the study of Chen et al. (2018). In their experiment, the authors took into consideration females of *T. drosophilae* maintained without hosts but with food from 1 to 40 d. After mating, females of different ages were offered pupae of *D. melanogaster* and demonstrated an extremely high parasitism rate at all time points. In this study, we obtained the same outcome, extended up to 100 d for females kept at 12°C.

In conclusion, compared with the other parasitoids known to attack SWD in the field, our study demonstrates that *T. drosophilae* is the only species able to be active during these low temperature periods. At constant 15°C, the pupal parasitoid *P. vindemmiae* does not show any parasitization activity as well as the larval parasitoid *Leptopilina heterotoma* (Thomson) (Hymenoptera: Figitidae) (Rossi-Stacconi et al. 2017), whereas for the most part of the congeneric *Leptopilina boulardi* (Barbotin, Carton & Kelner-Pillault) (Hymenoptera: Figitidae), alongside *Asobara tabida* (Nees) (Hymenoptera: Braconidae), enter a diapause (Carton et al. 1986). However, in *A. tabida* the mechanism is more complex, being associated with other factors such as photoperiod and host species (Fleury et al. 2009). As successful parasitism has been observed at low temperature under seminatural conditions (unpublished), availability of field data is needed to support and confirm the importance of the seasonal role of the parasitoid in the population suppression of SWD.

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**References Cited**

Abram, P. K., J. Brodeur, A. Urbaneja, and A. Tena. 2019. Nonreproductive effects of insect parasitoids on their hosts. Annu. Rev. Entomol. 64: 239–276.

Amiresmaeili, N., C. Jucker, S. Savoldelli, and D. Lupi. 2018. Understanding *Trichopria drosophilae* performance in laboratory conditions. Bull. Insectol. 71: 231–256.

Bezerra Da Silva, C. S., B. E. Price, and V. M. Walton. 2019. Water-deprived parasitic wasps (*Fasbrycroydipes vindemmiae*) kill more pupae of a pest (*Drosophila suzukii*) as a water-intake strategy. Sci. Rep. 9: 3592.

Carton, Y., M. Bouletreau, J. J. M. van Alphen, and J. C. van Lenteren. 1986. The *Drosophila parasitic* wasps, pp. 347–394. In M. Ashburner, H. L. Carson, and J. N. Thompson (eds.), The genetics and biology of *Drosophila*, vol. 3. Academic Press, London, United Kingdom.
