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Thermal tolerance of the rosy apple aphid *Dysaphis plantaginea* and its parasitoids: Effect of low temperatures on some fitness activities of *Aphidius matricariae*

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ABSTRACT

Understanding the thermal tolerance of insect herbivores and their natural enemies is crucial for biological control programs. The rosy apple aphid *Dysaphis plantaginea* is one of the most problematic pests of apple orchards, causing economic losses of up to 30% due to damage to fruits. *Dysaphis plantaginea* is highly adapted to low temperature, enabling it to appear early in the season. This study aimed at evaluating the critical thermal minimum of *D. plantaginea* and of two parasitoid species: *Aphidius matricariae* and *Ephedrus cerasicola*. For the generalist parasitoid *A. matricariae* we also evaluated the fitness traits of flight, walking, and oviposition, at four temperatures: 20, 15, 10 and 8 °C. We found that both males and females did not fly at the two lowest temperatures. Walking, parasitism rate and sex ratio (proportion of female progeny) were reduced at 8 °C. In addition, the parasitism rate was significantly lower at 8 and 10 °C compared to 15 and 20 °C. The progeny emerging from the oviposition experiment at 8 °C were significantly larger compared with other temperatures, possibly attributed to longer development time. The fact that the parasitoids were unable to fly at 8 and 10 °C, in combination with a more male-biased sex ratio, could reduce their efficiency at low temperature, even though they may still be able to walk and parasitize aphids.

1. Introduction

A successful biological control (BC) program requires that the natural enemies can suppress or considerably reduce the targeted agricultural pests. Many successful programs have been established against various pests worldwide (Barratt et al., 2018), but on the other hand many have failed. The failure is often linked to a mismatch between the BC agent(s) and the pest because of differences in their low thermal tolerance (Furlong and Zalucki, 2017; Harms et al., 2021). Therefore, understanding the thermal tolerance of natural enemies and insect herbivores is important in BC programs (Harrington et al., 2001; Nanga et al., 2021).

The rosy apple aphid (RAA) *Dysaphis plantaginea* Passerini (Homoptera: Aphididae) is considered one of the most problematic pests in apple orchards worldwide, with economic losses of up to 30%, causing yellowish crumpled leaf galls, small and malformed fruits. The RAA may have several generations on apple before forming the winged adults that migrate to the alternative host, plantain *Plantago* spp. L. (Blommers et al., 2004). Previous studies have focused on the impact of many predators and parasitoids against RAA (Dib et al., 2010, 2017, 2020; Peusens et al., 2006). Apart from releases of natural enemies, various other approaches have been adopted in order to control the population of RAA, such as conservation BC, integrated pest management, and organic orchards (Brown and Mathews, 2007; Dib et al., 2012, 2016). However, the RAA remains uncontrolled and continues to cause damage.

The severity of the RAA in apple orchards is related to its early arrival in the season when it migrates from its alternative host plant (Dib et al., 2017) and to the high level of protection obtained inside the curled plant leaves (Blommers, 1999; Brown and Mathews, 2007). In addition, the RAA is adapted to low temperatures, since the lower temperature threshold for development was estimated to be 4.0 °C (Graf et al., 2006). Since most predators and parasitoids are inactive at such low temperatures, RAA could survive and reproduce without being impacted by their natural enemies. Recently, it has been found that a high number of aphid colonies on the host plant could manipulate the infested plants to reduce

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the emission of volatile organic compounds and thereby reduce attraction of parasitoids (Ismail et al., 2021b). This increases the need to release natural enemies earlier in the season when the aphid populations are still low in order to avoid the damage they would cause later in the season. Therefore, studies should focus on how the natural enemies of RAA, particularly parasitoids, respond to low temperatures. In this work, we compare two parasitoid species with respect to their tolerance to low temperature. Both species, *Aphidius matricariae* Haliday and *Ephedrus cerasicola* Stary (Hymenoptera: Aphidinae), are commercialized in Europe where they are extensively used in BC programs against many aphid species. The two parasitoid species are tiny insects and both are solitary koinobiont endoparastoids (Starý, 1970). While A. *matricariae* parasitizes many aphid species, *E. cerasicola* is more specialized. Both species attack the RAA in the field (Nicolas et al., 2013).

Generally, insect species are ectotherms, which implies that their metabolism and activities and other physiological functions are related directly to temperature (Bale, 2002; Mellanby, 1939; Sinclair et al., 2003). Indeed, temperature is the principal factor in the environment that determines fitness traits of insect species (Hance et al., 2007; Ismail and Brooks, 2016; Moiroux et al., 2010; Tougeron et al., 2021). Every species is characterized by a thermal range, in which individual fitness increases gradually from the critical thermal minimum (CT_{min}), reaches the maximum fitness at the optimal temperature, and then decreases rapidly when approaching the critical thermal maximum (Huey and Kingsolver, 1989). The variation in thermo-tolerance is a main constituent in understanding the interaction among species with the environment (Kleynhans et al., 2014; Sinclair et al., 2012; Tougeron et al., 2016). Most insects can adapt to survive under suboptimal and supra-optimal temperatures, but this may affect survival (Ismail et al., 2013; McDonald et al., 1997; Renault et al., 2003), and other fitness traits (Hutchinson and Bale, 1994; Ismail and Brooks, 2016). In addition, this adaptation may lead to trade-offs between fitness traits (Ismail et al., 2010; Stearns, 1992; Tougeron et al., 2016). It may also alter development, reproduction, morphological and behavioural traits (Damien and Tougeron, 2019; Gols et al., 2021; Ismail et al., 2012, 2021a).

The objective of this study was to determine the CT_{min} of the RAA and its two parasitoid species *A. matricariae* and *E. cerasicola.* Since the RAA appears early in the season, we predicted that RAA is more cold tolerant than the two parasitoid species. We also investigated whether the two parasitoid species differ with respect to CT_{min}. The parasitoid species showing to be the most cold tolerant was then chosen to be used for quantifying three essential fitness traits: flight, walking and oviposition, at four different temperatures (8, 10, 15, and 20 °C).

2. Materials and methods

2.1. Plant production and maintenance of insect colonies

Apple seedlings used in this study were obtained from Walloon Agricultural research in Gembloux (CRA-W, Belgium), and cultured according to the *in vitro* vegetative propagation technique of *Malus x domestica* cv. Jonagold® (5–8 cm). The seedlings were then transferred individually to small plastic pots (10 cm diameter) containing the growing medium of soil and vermiculite (2:1) and kept under greenhouse conditions with a photoperiod of 16L:8D h and relative humidity of 70 ± 10%. The seedlings were placed in mesh net cages (100 × 100 × 100 cm) to exclude herbivorous insects.

The RAA individuals originated from a field population in the Walloon region (Gembloux 50°34′N 04°41′E) in Belgium in 2019. The aphid colony was reared continuously on apple plants in the Earth and Life Institute at the Université Catholique de Louvain. Aphids were kept inside cages with mesh nets (75 × 75 × 75 cm) in a growth chamber at 20 ± 2 °C under a photoperiod of 12L: 12D h and relative humidity of 55 ± 10%. The colony was provided with fresh plants regularly.

The two parasitoid species were obtained from Viridaxis SA in Belgium. *Aphidius matricariae* was maintained in the laboratory on the green peach aphid *Myzus persicae* feeding on turnip *Brassica rapa*. *Ephedrus cerasicola* was maintained on *M. persicae* feeding on broad bean *Vicia fabae.* The cultures were kept separately in growth chambers at 20 ± 2 °C under a photoperiod of 16L: 8D h and relative humidity of 50 ± 10%. The parasitoid species colonies were reared for two generations before starting the experiments.

2.2. Evaluation of CT_{min}

To measure potential genetic variation within the aphid population with respect to cold tolerance, we created six clones from six fundatrices of *D. plantaginea* collected from the mass rearing unit. Only new adult offspring of these fundatrices were used in the CT_{min} test. The number of adults used in this experiment ranged from 21 to 26 individuals per clone (in total 135 aphid adults).

For the parasitoid CT_{min} experiments, one-day-old females of both species were used. Females were collected from mummies obtained from the two parasitoid rearing units as explained above. 45 virgin females were used for each parasitoid species.

To determine CT_{min}, we used a procedure utilizing a double-walled glass column with ethylene glycol between the double-walls (Huey et al., 1992; Powell and Bale, 2006; Weber and Diggins, 1990). A MultiTemp III Pharmacia Biotech temperature controller (Pharmacia Biotech AB S-75182, Uppsala, Sweden) was used to cool the fluid with the column. Digital *remote sensors* (Hanna, Woonsocket, RI, USA) were placed in the top and the bottom of the column and used for controlling that the temperature was the same throughout the entire column.

The column had a lid on top and an opening at the bottom. Specimens of the three species were tested individually. An individual was inserted at the top of the column and kept for 5 min at 20 ± 1 °C before commencing the experiment. To avoid the potential rapid cold hardening response of tested individuals of the three species, a slow cooling rate (1.2 ± 0.5 °C min^{-1}) was chosen. The CT_{min} was defined as the temperature at which an individual was no longer able to cling to the wall and dropped through the opening at the bottom of the column.

2.3. Evaluation of fitness responses of A. matricariae

Since *A. matricariae* was the parasitoid showing the lowest CT_{min}, this species was chosen for further investigations of how low temperature affects the following fitness traits: flying, walking and oviposition. Generally, aphid parasitoids prefer young aphid larvae (L2-L3). Thus, 20 RAA adults were placed on fresh apple plants to produce larvae. After 24 h, the adults were removed while the new larvae were kept on the plants until the L2 or L3 stages were reached and could serve as hosts for *A. matricariae* during experiments. Mummies of *A. matricariae* were collected and isolated individually in Eppendorf® tubes with a drop of honey to feed the emerging parasitoids. Four temperatures (treatments) were used in this study: 8, 10, 15, and 20 °C. These temperatures are above the development threshold of *A. matricariae*, which ranges between 1.4 and 7.9 °C, depending on the geographical distribution (Miller and Gerth, 1994). To avoid any potential thermal shock, all parasitoid individuals were acclimatized by gradually reducing the temperature from rearing conditions to the desired level, as described in Fig. 1. Indeed, the acclimation process has been proven to reduce the negative direct impact of low temperature on parasitoids (Ismail et al., 2010; Levie et al., 2005).

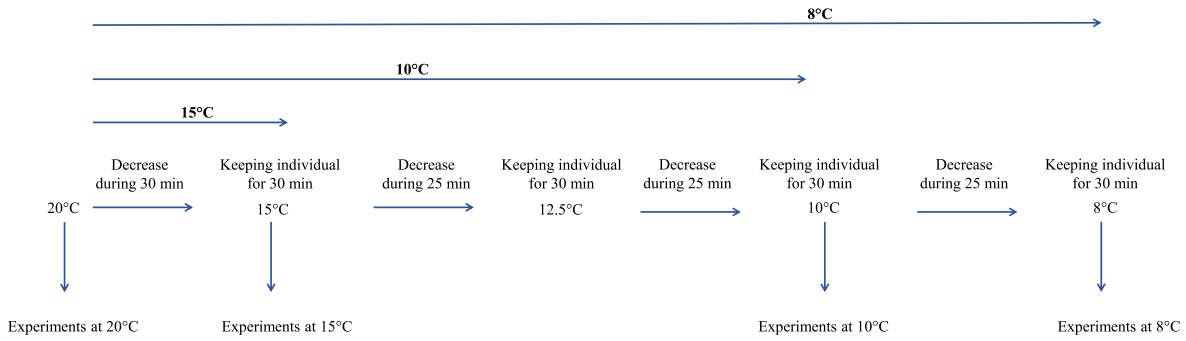

Fig. 1. Acclimatization design for *Aphidius matricariae* individuals before the experiments.

2.3.1. Flight capacity

To test the capacity of male and female parasitoids to fly at each of the four experimental temperatures, we used a protocol as described in Langer et al. (2004). 25 individuals of each sex were tested at each temperature (in total 200 individuals). For each test, 5 one-day-old individuals of the same sex were placed in a glass cylinder (20 cm height, 9 cm diameter). We used eight different glass cylinders, which were washed with 70% alcohol before each usage. The wall of each glass cylinder was painted black to make it dark and the top was covered by a transparent plastic lid with tiny holes. The inner side of the lid was covered with glue. Five glass cylinders were placed together in an incubator (Memmert UNB400) and exposed to white light. After 4 h, the number of individuals caught in the glue was counted. The flight activity at each temperature was calculated for each sex as the number of parasitoids found in the glue divided by the total number of individuals.

2.3.2. Walking capacity

The walking capacity at the four different temperatures was only evaluated for virgin females. 25 one-day-old females were tested at each temperature. The females were fed with honey before the experiments. The recording system was as follows: each female was placed in a glass Petri-dish (8 cm diameter) set on a light table (25 × 35 cm), which was then covered by a box of expanded polystyrene to exclude external light. The temperature inside the box was verified by means of a small *data logger (HOBO)*. After placing the box in an incubator SANYO MIR-554 set to the desired experimental temperature, the walking activity of the females was recorded for 30 min using a webcam Logitech (model C920 HD Pro Webcam 1080p) inserted through the polystyrene. The camera was connected to a computer, and the recordings were analyzed using a video tracking program EthoVision XT (Noldus Information Technology, Wageningen, The Netherlands). Walking velocity (cm/s) was assessed from the video recordings as the total distance moved divided by time (30 min).

2.3.3. Oviposition

One-day-old female parasitoids were used to measure how many eggs a female could lay during one day. Before the experiment, the females were allowed to mate by placing one female together with one adult male in an Eppendorf® tube for 2 h and they supplied with a drop of honey. Females were then transferred individually to a Petri-dish (5 cm diameter), containing an apple leaf placed upside-down on a damp filter paper (5 cm diameter), onto which 50 (L2 and L3) aphids had been carefully transferred, using a fine brush. Fifteen female parasitoids were tested at each temperature. The Petri-dishes were placed inside an incubator (Memmert UNB400) and exposed to 8, 10, 15 or 20 °C for one day, whereupon the aphids were transferred to apple seedlings at 20 °C. The individuals from experiments conducted at 8, 10 and 15 °C were gradually acclimatized to 20 °C. Fecundity rate (eggs/female/day) at a given temperature was calculated as the number of mummies (empty cuticle of the dead aphid containing a developing parasitoid larva) divided by the total number of aphids offered.

Development time was calculated from the first day of each experiment until the emergence of individuals. Emergence rate corresponded to the number of emerging individuals divided by the number of mummies. The sex ratio of progeny was expressed as the proportion of females and calculated as the number of females divided by the total number of emerged individuals. The size of females was represented by the measurement of the hind tibia of each female. The length of hind tibia was measured under a binocular microscope (Leica LED3000 NVI) linked to a video camera (JVC KYF50), and using the numeric image analysis software *ImageJ*® (v. 1.52). The length of the hind tibia is generally considered as a proxy for the size of parasitoids (Godfray, 1994; Ismail et al., 2010). Seventy-five females were measured at each tested temperature.

2.4. Statistical analysis

All data were analyzed using the statistical software R version 4.0.5 (2021-03-31) (R. Core Team, 2021). The homogeneity and normality tests of most of data rejected the null hypothesis ($p < 0.05$). The CT_{min} values of the six clones of aphids were analyzed by mixed effects models using the package *lme4* and function *lmer* (Bates et al., 2015), with aphid clone as a random effect and individuals nested within clones. A Kruskal-Wallis (KW) test was used to test for differences in CT_{min} between RAA (the six clones grouped in one species) and the two parasitoid species. The Mann–Whitney *U* test was then used for multiple comparisons with a Bonferroni adjustment. CT_{min} data were presented in the text by the median with interquartile range (IQR = upper quartile – lower quartile). Generalized Linear Models (GLMs) were used to analyze flight capacity (proportion data with quasi-binomial distribution and logit link) with temperature as a covariate and sex as a fixed factor and interaction between temperature and sex. The GLMs with Gamma distribution and inverse were used to analyze the walking velocity with temperature as a covariate. Parasitism rate, emergence rate and sex ratio were analyzed by means of GLMs with quasi-binomial distribution and logit link, treating temperature as a covariate. For all analyses, significant results at $p < 0.05$ were followed by the use of Tukey's honest significant difference test for multiple comparison using the *multcomp* package and *glht* function (Hothorn et al., 2008). The Cox Proportional Hazard Model was used to analyze the development time

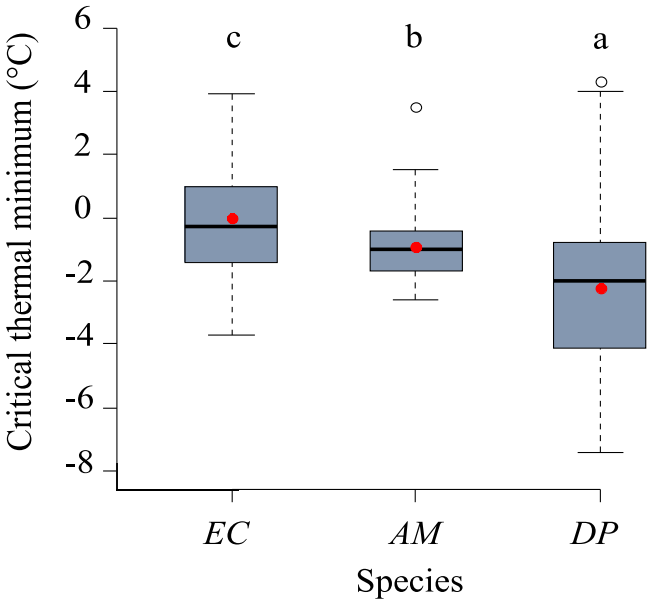

Fig. 2. Box plot of critical thermal minimum of three species: *Dysaphis plantaginea* (*DP*), *Aphidius matricariae* (*AM*) and *Ephedrus cerasicola* (*EC*). Small letters represent significant differences among species. The boxplot summarized: minimum (first quartile −1.5 * interquartile range of the data), maximum (third quartile + 1.5 * interquartile range of the data), boxes: 25th and 75th percentiles, median (thick line), mean (closed circle), whiskers (continuous dash line) and outliers (data point in open circle).

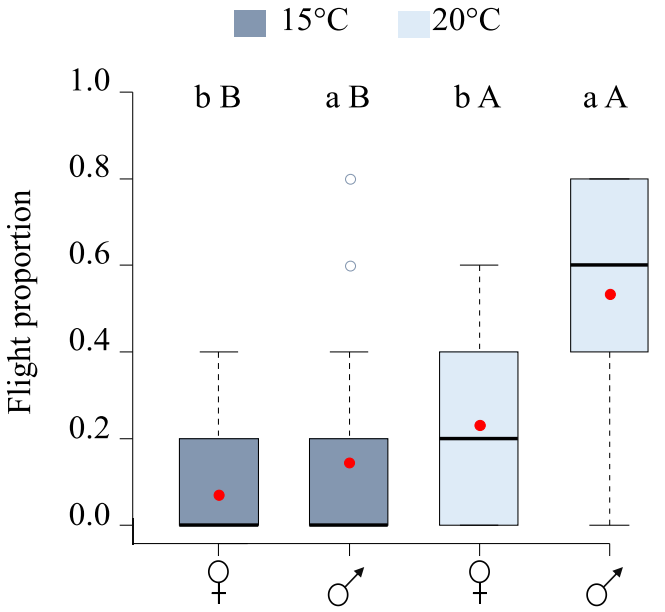

Fig. 3. Box plot of flight capacity of males and females of *Aphidius matricariae* at various temperatures (20 and 15 °C). No flights were recorded at 10 and 8 °C for both sexes. Flight was expressed as the number of individuals that flew divided by the total number individuals exposed to a given temperature. Small letters represent significant difference between males and females, and capital letters represent significant difference between 20 and 15 °C.

with temperature as a covariate, using the *Survival* package and *coxph* function (Collett, 1994; Cox, 1972). When a significant effect was found $p < 0.05$, multiple comparisons were made using the *pairwise_survdiff* function and the *survminer* package (Kassambara et al., 2021). As tibia length was normally distributed ($p > 0.05$), data were analyzed by means of a one-way Anova with temperature as a covariate, followed by Tukey's honest significant difference test for multiple comparisons using the *TukeyHSD* function. Finally, exponential models, given as $y(T) = y_0 e^{\beta T}$, were used to model the effect of temperature (T) on velocity, parasitism rate and tibia length. Thus, y_0 denotes the predicted value of y at temperature 0 °C, while β determines how strongly temperature affects a given trait value.

3. Results

3.1. Minimum critical temperature (CT_{min})

No difference in CT_{min} among the six clones of RAA (ANOVA: LRT = 0.28, df = 1, $p = 0.59$). When data from the six aphid clones were pooled in the same analysis that also included the two parasitoid species, the analysis showed a significant difference in CT_{min} among all species (KW: $\chi^2 = 29.15$, df = 2, $p < 0.001$; Fig. 2). The RAA showed the highest tolerance to cold (CT_{min} = - 2 °C (IQR = 3.3)), followed by *A. matricariae* (CT_{min} = - 1 °C (IQR = 1.30)) and *E. cerasicola* (CT_{min} = - 0.3 °C (IQR = 1.95); Fig. 2).

3.2. Flight capacity of Aphidius matricariae

Neither sex of *A. matricariae* showed any flight capacity at the two low temperatures (8 and 10 °C). Thus, the statistical analysis was only conducted for 15 and 20 °C. Flight capacity of individuals was significantly greater at 20 °C than at 15 °C (GLM: $F_{1,98} = 39.00$, $p < 0.001$; Fig. 3). The flight capacity was significantly higher in males than in females (GLM: $F_{1,97} = 19.71$, $p < 0.001$; Fig. 3). No interaction was found between sex and temperature (GLM: $F_{1,96} = 0.87$, $p = 0.35$).

3.3. Walking capacity of Aphidius matricariae

The walking velocity of females decreased significantly with temperatures (GLM: $F_{1,98} = 124.16$, $p < 0.001$; Fig. 4A). The relationship between temperature and walking velocity of *A. matricariae* females was described by a simple exponential model (Appendix 1).

3.4. Oviposition of Aphidius matricariae

The parasitism rate of *A. matricariae* on aphids decreased significantly at 10 and 8 °C compared to 15 and 20 °C (GLM: $F_{1,58} = 71.95$, $p < 0.001$; Fig. 4B). The relationship between temperature and parasitism rate was described by a simple exponential model (Appendix 2A). Development time from eggs to adults decreased significantly with temperature (Cox proportional hazard: $\chi^2_1 = 36.16$, $p < 0.001$; Fig. 5A). The emergence rate from mummies did not vary with temperature

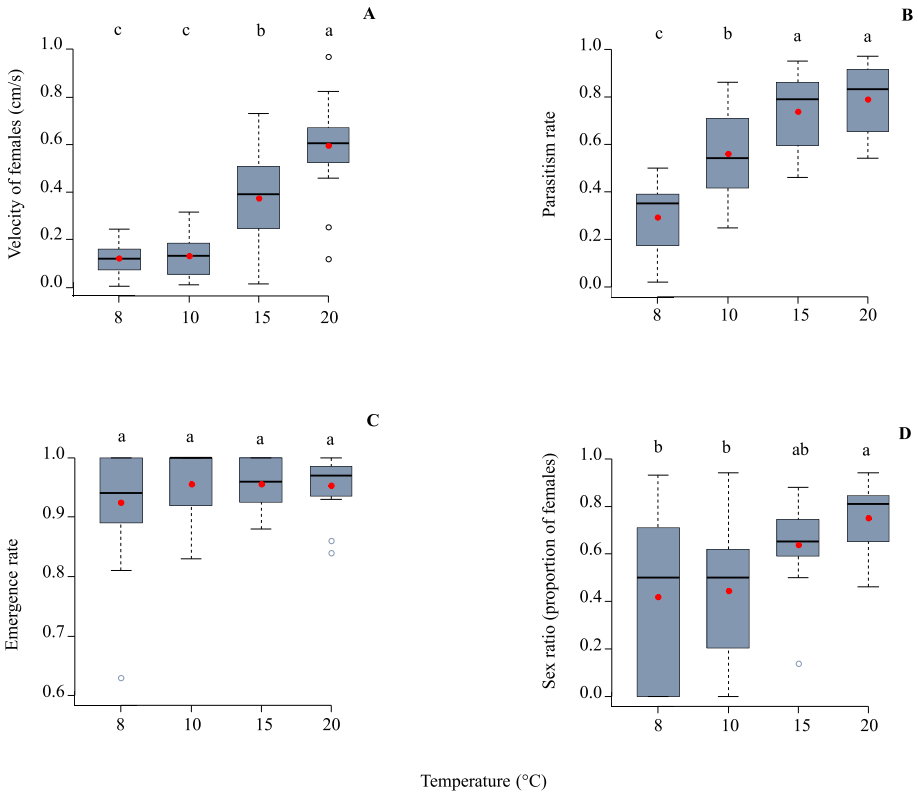

Fig. 4. Box plot of (A) walking velocity (B) parasitism rate, (C) emergence rate, and (D) sex ratio of the parasitoid *Aphidius matricariae* at different temperatures (20, 15, 10 and 8 °C). Small letters represent significant differences among treatments.

(GLM: $F_{1,58} = 1.36$, $p = 0.25$; Fig. 4C). Low temperatures (8 and 10 °C) had a negative effect on the sex ratio, leading to a lower proportion of females (GLM: $F_{1,58} = 17.39$, $p < 0.001$; Fig. 4D). The size of emerging progeny females increased notably at 8 °C compared to the other temperatures (ANOVA: $F_{1,298} = 15.22$, $p < 0.001$; Fig. 5B). The relationship between temperature and tibia length was described by an exponential model (Appendix 2B).

4. Discussion

Evaluating the physiological and behavioural responses of natural enemies to temperature under laboratory conditions is crucial for understanding their efficacy as biological control agents. Indeed, such studies can save costs and time, help to avoid negative impacts in the field, and even help to predict population growth (Ismail et al., 2014). In point of fact, the lack of success of one BC program against the RAA may be due to the early arrival of the aphids in early spring to apple branches, when temperature is still too low for the natural enemies to impact their hosts, thereby enabling the aphids to achieve high population densities later in the season (Dib et al., 2017).

The results for CT_{min} confirmed our prediction that RAA has a higher capacity for cold tolerance than the two parasitoid species investigated. This may, at least partly, explain why RAA is capable of occurring early in the season in contrast to the parasitoids, which tend to arrive later in the season. This time gap between RAA and its parasitoids may allow aphids to increase rapidly, to feed and cause leaves to curl, and ultimately result in losses of fruits. However, RAA in this study was reared under laboratory conditions, and the *A. matricariae* and *E. cerasicola* used originated from commercialized populations. Consequently, in order to estimate the real value of the CT_{min}, it would be relevant to test individuals collected in the field. Indeed, location could significantly impact cold tolerance in insects (Alford et al., 2017; Foray et al., 2013). For instance, Tougeron et al. (2016) found that parasitoids sampled in open areas tolerated low temperatures better than parasitoids from closed areas, due to acclimation effects.

Regarding the fitness activities of *A. matricariae* in response to low temperatures, we found that both males and females were unable to fly at low temperatures. Moreover, significant reductions in walking speed, parasitism rate and sex ratio of females were found. Similar responses to low temperatures have been found in other parasitoid species (Langer et al., 2004). It seems likely that at low temperatures females allocate energy to walking and oviposition at the expense of flight. Indeed, flight activity is more energy expensive than walking and oviposition (Chapman, 1998; Fischbein et al., 2011). Thus, a trade-off between these traits may reflect an adaptation to minimize energy expenditures during cold weather, thereby allowing the parasitoids to survive until environmental conditions improve.

It is evident that individuals keep consuming fat at low temperatures (Colinet et al., 2006a; Ismail et al., 2010). The inability to fly would directly hamper biological control. Generally, the stimulus for males to fly is the female sexual pheromones (McClure et al., 2007), while for females it is the emission by plants of volatile organic compounds. This stands to reason, as parasitoid females need to locate host-insect infested plants over long distances, before walking and attacking the host in order to lay their eggs (Guo and Wang, 2019).

As demonstrated by Visser and Ellers (2008), most parasitoid species emerge with a high quantity of lipids, which decreases with age due to the inability to synthesize lipids, whilst having a lower quantity of glycogen that increases with age (Olson et al., 2000; Rivero and West, 2002). While lipid is the main resource for egg production (Ellers et al., 2000; Visser and Ellers, 2008), glycogen is the main resource for flight (Amat et al., 2012; Zera et al., 1999). Another explanation for the incapacity for flight and reduction in walking velocity might be related to changes, at low temperature, in the biogenic amines responsible for the functionality of neuromuscular junctions (Lubawy et al., 2020). Dysfunctionality of the junctions would ultimately result in paralysis

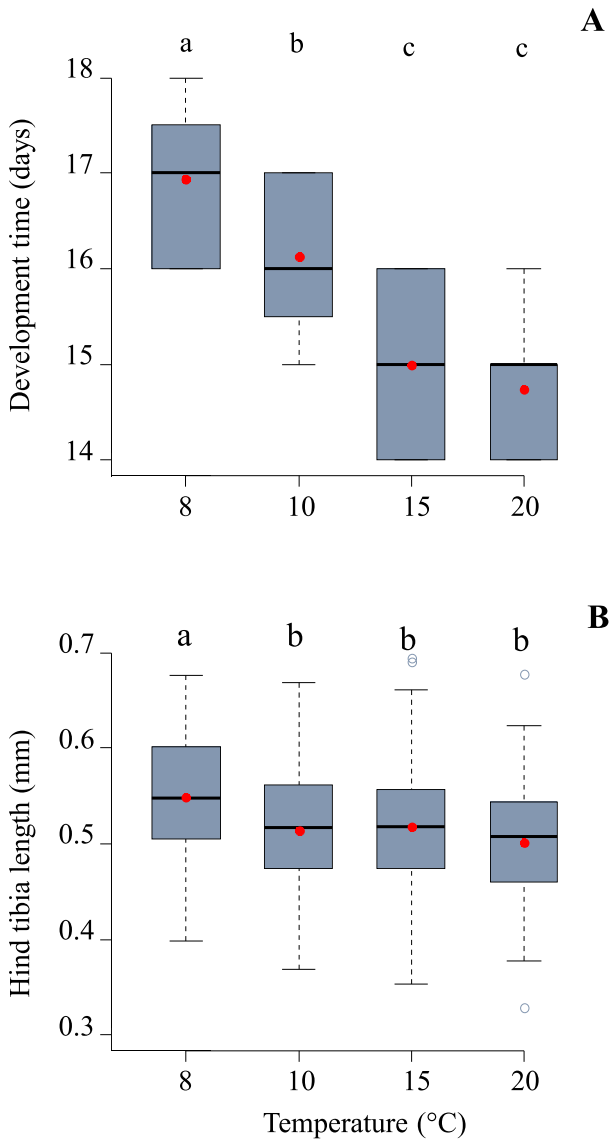

Fig. 5. Box plot of (A) development time in days and (B) hind tibia length of females of *Aphidius matricariae* at various temperatures (20, 15, 10 and 8 °C). Small letters represent significant differences among treatments.

(MacMillan and Sinclair, 2011). Consequently, a possible solution to stimulate mobility and to keep the functionality of junctions would be subjecting the parasitoids to cold treatment prior to releases. Indeed, Boersma et al. (2019) found that cold treatments of field and laboratory populations of false codling moth *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) would enhance flight performance under low-temperature conditions.

Our results showed that the development time of parasitoids from eggs to emergence was significantly longer at 8 and 10 °C, despite the ovipositing females and their hosts were exposed to low temperatures for only one day whereupon the parasitized aphids were transferred to 20 °C. This short time of exposure to low temperature was enough to slow down the later development of individuals. We also observed that emerged females at 8 °C had a larger size compared to those at other temperature treatments, likely associated with the prolonged development time. Our results are consistent with the temperature-size rule, a common pattern in biology which states that individuals reared at low temperatures have longer development time and reach larger body size than individuals developing at higher temperatures (Atkinson, 1994; Atkinson and Sibly, 1997). Since aphids continue to feed after they had been parasitized, the plant host may also affect the longevity, daily survival, size, and progeny of the parasitoids (Ayelo et al., 2017; Eben et al., 2000; Ero et al., 2011). In addition, plant quality could influence the relationship between development time and the temperature-size rule (Ismail et al., 2021a). However, in the present study, the plant host provided was of the same quality in all experiments.

We did not evaluate the consequences of low temperatures on progeny, where potential impact could be anticipated. Ismail et al. (2012) demonstrated that smaller females of the parasitoid *Aphidius ervi* switched their reproductive strategy towards early reproduction (synovigenic to more pro-ovigenic) in response to low temperatures. Consistent with many studies (Colinet et al., 2006b; Ismail et al., 2010, 2014; Zamani et al., 2007), the proportion of female offspring decreased at low temperatures. On the other hand, other studies showed that the sex ratio was not affected by low temperature (Augustin et al., 2021; Kidane et al., 2020; Rezaei et al., 2020). The differences among studies could be related to variation among parasitoid species in response to low temperature, and their capacity to tolerate low temperature (Colinet and Hance, 2010). A male-biased sex ratio would reduce the efficiency of a BC program, since hosts are only attacked by female parasitoids. In addition, producing more males in a population would lead to an increase in local male competition for females (Hamilton, 1967).

Besides, King (1987) asserted that female parasitoids, as haplodiploid species, could control the sex ratio of offspring due to their capacity to adjust the proportion of fertilized eggs. It has been suggested that females can adjust the sex ratio according to the expected mortality of one sex (Werren and Charnov, 1978), or to the sex that would have a higher capacity to survive and disperse (Frank, 1986). Zamani et al. (2007) proposed that male or female-biased sex ratios could be related to differences between the sexes with respect to sensibility to low temperature during larval development. However, since the emergence rate in our study was almost the same at the four temperatures, we may exclude that differential temperature sensibility was responsible for the more male-biased sex ratio at the low temperatures. The mechanisms determining the sex of a female's offspring are still unclear, but stressful conditions, such as low and high temperatures, certainly affect the sex ratio of parasitoids. Moiroux et al. (2014) speculated that low and high temperatures could affect the neural system of parasitoid females and consequently affect the sex ratio. Another explanation could be that low temperatures cause a degradation of the quality of sperm stored in the spermathecae, but this needs further investigations.

In conclusion, the cycle of the RAA starts earlier in the season compared to the two parasitoid species due to the aphid's tolerance to low temperatures. The fact that the parasitoids are unable to fly at 8° and 10 °C, and the decrease in the number of female progeny at low temperatures, could hamper the efficiency of parasitoids released against the RAA at low temperatures, even though they can walk and parasitize aphids. In this regard, it would be interesting to study the mechanism of muscle movement in relation to glycogen at low temperatures. Since the results from this study are based on commercialized species, it would be essential to conduct studies on various geographic populations obtained directly from the field, as they may be better adapted to the local climate. However, this study highlights the vital importance of evaluating the tolerance of chosen species for low temperature in order to select the best-adapted strains to be used in BC programs.

Author statement

All persons who meet authorship criteria are listed as authors. MI, KT, TH and LA: conceived and designed the research. TH and LA: Funding acquisition and Supervision. AV and LA: performed the experiments. MI and LA: analyzed the data and prepared the figures. MI and LA: interpreted the results and wrote the manuscript. MI, KT, TH and LA: revised and approved the final version.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Data availability

The data that support the findings of this study is available in the Mendeley Data repository: http://dx.doi.org/10.17632/hxvc85jdwb.1.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2022.103377.

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