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Cold tolerance of the predatory ladybird *Cryptolaemus montrouzieri*

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Abstract The effect of low temperature acclimation and diet on the supercooling point (SCP, the temperature at which the insect's body fluids freeze) and lethal time (LTime, time required to kill 50 % of the population at a temperature of 5 °C) of the mealybug destroyer, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), was assessed in the laboratory. The SCP of acclimated adult ladybirds which were allowed to complete development to adulthood at 18 °C and a 8:16(L:D)h photoperiod, or at 25 °C and a 16:8(L:D)h photoperiod, and which were subsequently kept at 10 °C and a 12:12(L:D)h photoperiod for seven days, was −17.4 and −16.8 °C, respectively. These SCP-values were approximately 7 °C lower than the value of −9.9 °C for non-acclimated ladybirds maintained at a temperature of 25 °C and a photoperiod of 16:8(L:D)h throughout development and in the first week of their adult life. Also food source had a significant effect on the freezing temperature of *C. montrouzieri*: the SCP of ladybirds fed the citrus mealybug, *Planococcus citri* (Risso)(Hemiptera: Pseudococcidae), was 1.6 °C higher than the value of −17.2 °C observed for ladybirds provided with eggs of the flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). However, neither cold acclimation nor diet had a significant effect on the lethal times of *C. montrouzieri*. Overall, the time required to kill 50 % of the population at a temperature of 5 °C ranged from 12.8 days for ladybirds fed *P. citri* mealybugs to 14.4 days for ladybirds fed *E. kuehniella* eggs. All individuals exposed to a constant 5 °C had died by day 24. Based on the results from this laboratory study, it is deemed unlikely that *C. montrouzieri* could establish outdoors in western Europe, and it is therefore expected to pose little risk to non-target species in this area when used as an augmentative biological control agent.

Keywords Biological control · Environmental risk assessment · Cold tolerance · Non-native species · Predator · Coleoptera · Coccinellidae

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Introduction

The predatory ladybird *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), also known as

the mealybug destroyer, is indigenous to eastern Australia (Queensland, New South Wales) and the South Pacific Islands (Clausen 1978; Booth and Pope 1986). Since its first introduction into California in 1891 to control the citrus mealybug *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) (DeBach and Hagen 1964), the coccinellid has been imported into over 40 countries throughout the warm temperate and tropical regions of the world to control mealybugs and scales (Booth and Pope 1986; Funasaki et al. 1988). In Europe, the first introduction of *C. montrouzieri* as a classical biological control agent was made in 1908 in Italy. Subsequent releases were made in Spain (1926), Corsica (1970), France (1974) and Portugal (1984) (Roy and Migeon 2010; Kairo et al. 2012). Since 1992, *C. montrouzieri* is also being released in Northwestern Europe as an augmentative biocontrol agent under protected cultivation and interior landscaping (Malais and Ravensberg 2002). Meanwhile, the ladybird has been reported outside greenhouses in natural environments in Sweden, Norway, Britain and Belgium (Ehnström and Lundberg 1997; Constantine and Majerus 1994; Moreau 2005; Hansen and Sagvolden 2007), but there is no unequivocal evidence of permanent establishment in those countries. Because *C. montrouzieri* is a (sub)tropical species, it is generally believed not to be sufficiently cold hardy to survive winters in cold and temperate climates, but observations from Britain indicate that the ladybird may survive shorter periods with frost (Halstead 1999). The overwintering potential of *C. montrouzieri* was never studied in detail and, to our knowledge, the abovementioned introductions of the predator in Europe were done without having been subjected to experimental risk assessment procedures.

Knowledge of the cold tolerance of *C. montrouzieri*'s may contribute to the environmental risk assessment (ERA) at its release in areas where it is not indigenous. A non-indigenous biological control agent characterized by high cold tolerance may establish in a new area and cause undesired side-effects on local biodiversity (van Lenteren et al. 2003). An appropriate regulation concerning the import and use of natural enemies, based on a scientific risk assessment, is instrumental in preventing such non-target effects. The study of a candidate biocontrol agent's establishment potential is considered to be one of the key elements of an ERA procedure (van Lenteren et al. 2006; Bale 2011; Ehlers 2011). The methodology developed by Hart et al. (2002a) and used in several subsequent studies (Tullett et al. 2004; Hatherly et al. 2008; Hughes et al. 2009; Berkvens et al. 2010; Maes et al. 2012) to assess cold tolerance of arthropod biological control agents builds on several parameters, including the supercooling point (SCP, the temperature at which the insect's body fluids freeze) and lethal time at 5 °C (LTime, time required to kill 50 % of the population at a constant temperature of 5 °C).

Several factors may affect an insect's cold tolerance parameters and thus complicate the evaluation of its establishment potential in the framework of an ERA. In the present study, the effect of low temperature acclimation and diet on the supercooling capacity and lethal time of *C. montrouzieri* was assessed. In the laboratory, insects are usually cultured under continuous summer conditions (23–27 °C, long days). In the field, however, insects may become more cold tolerant in response to environmental cues such as decreasing temperature or photoperiod (Block 1990; Danks 2007). Exposing insects taken directly from laboratory cultures to low temperatures may thus lead to inaccurate predictions of their cold tolerance. Besides, diets used in commercial insectaries may have a strong impact on the physiological responses of the natural enemies produced. Unnatural (factitious) foods may change the fitness of a natural enemy (Grenier and De Clercq 2003) and may thus also influence its responses to climatic challenges. Therefore, we tested the cold tolerance of *C. montrouzieri* ladybirds fed on a natural prey (the citrus mealybug *P. citri*) or on a factitious food (frozen eggs of the Mediterranean flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae)).

Materials and methods

Insect cultures

Two laboratory colonies of *C. montrouzieri* were established in 2010 with larvae acquired from Katz Biotech AG (Baruth, Germany) and maintained in a climatic chamber set at 25 ± 1 °C, a relative humidity (RH) of 75 ± 5 % and a 16:8(L:D)h photoperiod. The first colony was maintained on frozen *E. kuehniella* eggs. Water was provided by way of a moist piece of cotton wadding fitted into a 1.5 cm (Ø) plastic dish. A larger piece of dry cotton wadding (5 × 5 cm) was offered to adult beetles and served as an artificial

Table 1 Overview of the different experimental treatments to assess the influence of climate and food source on the cold tolerance of *C. montrouzieri*

Treatment	Rearing conditions during development			Adults exposed to the acclimation regime?[a]
	Food source	Temperature (°C)	Photoperiod	
Climate				
Climatic regime 1	*E. kuehniella* eggs	25	16:8(L:D)h	No[b]
Climatic regime 2	*E. kuehniella* eggs	25	16:8(L:D)h	Yes
Climatic regime 3	*E. kuehniella* eggs	18	8:16(L:D)h	Yes
Food source				
Food source 1	*E. kuehniella* eggs	25	16:8(L:D)h	Yes
Food source 2	*P. citri* mealybugs	25	16:8(L:D)h	Yes

[a] Acclimation regime: first seven days of adult life at 10 ± 1 °C and 12:12(L:D)h, using the same diet as during development

[b] Maintained at 25 °C and 16:6(L:D)h during the first seven days of adult life

oviposition substrate for females. No mealybugs were used in this rearing system (Maes et al. 2014a). The second colony of the predator was reared on the mealybug *P. citri*. Mealybugs were cultured on potato sprouts and kept at ambient conditions. Potatoes infested with mealybugs and covered with ovisacs were transferred to the colony of *C. montrouzieri* to sustain its development and reproduction.

Experimental set-up

The effect of acclimation on the cold tolerance of *C. montrouzieri* was evaluated by examining the supercooling point and lethal time at 5 °C of ladybirds undergoing one of three climatic regimes (Table 1). Larvae subjected to climatic regimes 1 and 2 were reared from first instar to adulthood in an incubator set at a temperature of 25 ± 1 °C, 75 ± 5 % RH and a 16:8(L:D)h photoperiod, whereas larvae of treatment group 3 were exposed to autumn conditions and maintained in an incubator kept at 18 ± 1 °C, 75 ± 5 % RH and a 8:16(L:D)h photoperiod. Newly emerged larvae (<24 h) were taken out of the stock colony reared on *E. kuehniella* eggs (generation 10), placed in polystyrene Petri dishes (Ø: 9 cm, height: 1.3 cm) at a density of one (SCP) or ten (LTime; three replicates) individuals per Petri dish and transferred to their respective climatic regime. After they reached adulthood, ladybirds undergoing climatic regime 1 were maintained at 25 °C for a further seven days. In contrast, individuals subjected to treatments 2 and 3 were allowed to acclimatize to lower temperatures by keeping them in an incubator set at 10 ± 1 °C and a 12:12(L:D)h photoperiod for seven days (humidity was not controlled during acclimation). The ladybirds were supplied with *E. kuehniella* eggs and water during their entire lifespan. Non-acclimated adults subjected to the SCP measurements were starved for 24 h before being exposed to freezing temperatures.

The effect of food source (during larval development and early adult stage) on the cold tolerance of *C. montrouzieri* was assessed by measuring the supercooling point and lethal time at 5 °C of two populations (Table 1). The first *C. montrouzieri* population (Food source 1) was reared on *E. kuehniella* eggs for 12 generations, whereas the second population (Food source 2) was reared on mealybugs for eight generations. Newly emerged larvae (<24 h) were taken out of their respective stock colony and placed individually in polystyrene Petri dishes at a density of one (SCP) or ten (LTime; three replicates) individuals per Petri dish. The ladybirds were provided with their respective diet and water throughout the experiments and were maintained at 25 ± 1 °C, 75 ± 5 % RH and a 16:8(L:D)h photoperiod. Before the cold tolerance of the adult beetles was assessed, they were allowed to acclimate to lower temperatures in an incubator set at 10 ± 1 °C and a 12:12(L:D)h photoperiod for seven days, during which they were also fed. Non-acclimated adults subjected to the SCP measurements were starved for 24 h before being exposed to freezing temperatures.

Measurement of SCP

For each diet and acclimation regime, ca. 50 adult *C. montrouzieri* (both males and females in a 1:1

proportion) were subjected to the supercooling experiment, except for ladybirds reared on mealybugs where only 40 individuals (20 males and 20 females) were tested. The supercooling point was measured using a Picotech TC-08 thermocouple datalogger and a low temperature programmable Haake Phoenix II CP30 alcohol bath. Each thermocouple was led individually through the lid of a 1.5 ml Eppendorf tube, which was sealed with Pritt Poster Buddy (adhesive, synthetic rubber). The Eppendorf tubes were placed individually in glass tubes and subsequently immersed in the alcohol bath (Berkvens et al. 2010). The starting temperature was set at 25 °C (rearing temperature) or 10 °C (acclimation temperature). Insects were cooled at 0.5 °C min^{-1} until the thermocouple registered the release of exothermal heat, at which point the supercooling temperature was reached. Insects were weighed using a semi-microbalance Sartorius Genius ME215P (Sartorius AG, Goettingen, Germany) (±0.01 mg) when they became adult and before being subjected to the experiments to determine whether supercooling ability was correlated to body weight and weight loss or gain between adult emergence and the time of SCP measurement.

Measurement of lethal time

For each diet and acclimation regime, 60 Petri dishes each containing ten adult *C. montrouzieri* (five males and five females) were set up at room temperature. Food and moisture were provided ad libitum throughout the experiment. Predators from treatment groups at 25 and 18 °C were subsequently transferred to incubators set at 15 and 10 °C, and held there for 30 min each time to avoid possible mortality due to cold shock, before being finally transferred to an incubator set at 5 ± 1 °C. Individuals undergoing the acclimation treatment at 10 °C for seven days were directly transferred to 5 °C. Throughout exposure to 5 °C, the insects were kept in total darkness and RH was not controlled. Three Petri dishes (in total 30 individuals) were taken from the incubator set at 5 °C at regular time intervals and transferred subsequently to incubators set at 10 and 15 °C, where they were held for 30 min each time. The insects were then transferred to 25 °C and survival was determined after 24 h.

Statistical analysis

All data were analyzed using SPSS 21.0 (SPSS Inc. 2009). For the supercooling experiment, immature survival rates were compared by means of a logistic regression. This regression is a generalized linear model using a probit (log odds) link and a binomial error function (McCullagh and Nelder 1989). P-values below 0.05 were considered significant. Developmental times, body weights, changes in adult body weight between adult emergence and SCP measurement and SCP-values of ladybirds undergoing different climatic regimes were analyzed using a one-way analysis of variance (ANOVA). Means were separated using Tukey or Tamhane post-hoc tests when a Levene test indicated homoscedasticity or heteroscedasticity, respectively. The developmental parameters, SCP-values and weight loss during acclimation of ladybirds receiving different food sources were compared using homoscedastic or heteroscedastic Student's *t* tests. The relationship between SCP on the one hand and sex, developmental time, adult body weight, weight loss or gain between adult emergence and the time of SCP measurement, and body weight prior to testing on the other hand, was assessed with a Pearson's correlation test (SPSS Inc. 2009).

The results from the lethal time experiments were analyzed using probit analysis in order to estimate the time required to kill 10, 50 and 90 % of the population at a temperature of 5 °C. Significant differences were identified by non-overlapping fiducial limits (Hart et al. 2002a).

Results

The supercooling ability of *C. montrouzieri* was affected by the climatic regimes the ladybird was exposed to before testing ($F = 156.72$, $df = 2, 148$, $P < 0.001$) (Table 2). Ladybirds reared under climatic regime 2 and 3 during their immature stages had an average SCP which was 7.5 and 6.9 °C lower, respectively, than that of their counterparts maintained under climatic regime 1 (both $P < 0.001$; Tamhane post-hoc tests). There was no significant difference in SCP between individuals exposed to climatic regime 2 and 3 ($P = 0.377$; Tamhane post-hoc test). The food offered to *C. montrouzieri* during its immature development and early adult stage had a

Table 2 Survival and developmental time from first instar to adulthood, adult body weight and supercooling point of *C. montrouzieri* ladybirds reared under different climatic regimes and offered different foods

Population	N	Survival (%)	Developmental time (days)	Adult body weight (mg)	SCP (°C)	Range of SCP (°C)
Climate						
Climatic regime 1	53	94.3 ± 3.2a*	23.4 ± 0.2a	11.1 ± 0.2a	−9.9 ± 0.4a	−5.6 to −16.2
Climatic regime 2	52	92.3 ± 3.7a	23.2 ± 0.2a	11.0 ± 0.2a	−16.8 ± 0.5b	−11.6 to −19.6
Climatic regime 3	64	79.7 ± 5.0b	54.2 ± 0.2b	8.8 ± 0.3b	−17.4 ± 0.2b	−12.0 to −20.7
Food source						
Food source 1	55	90.9 ± 3.9a	23.7 ± 0.2a	10.8 ± 0.2a	−17.2 ± 0.2b	−13.1 to −20.4
Food source 2	42	92.9 ± 4.0a	24.4 ± 0.1b	10.0 ± 0.2b	−15.6 ± 0.3a	−11.7 to −20.3

* Mean ± SE within a column followed by the same letter are not significantly different [$P > 0.05$; Climatic regime: probit (Wald-χ^2)(survival), Tukey test (developmental time), Tamhane test (body weight, SCP); Food source: probit (Wald-χ^2)(survival), *t* test (developmental time, body weight, SCP)]

significant effect on its supercooling ability ($t = 3.99$, $df = 87$, $P < 0.001$): the SCP of ladybirds fed food source 2 was 1.6 °C higher than that of ladybirds provided with food source 1. None of the tested adult ladybirds survived the freezing treatment.

Immature survival was affected by temperature and photoperiod during development ($\chi^2 = 6.80$, $df = 2$, $P = 0.033$): the survival rate of larvae and pupae reared at under climatic treatment 3 was approximately 13 % lower than that of those maintained under climatic regime 2. In contrast, the food offered to the larvae had no influence on immature survival ($\chi^2 = 0.12$, $df = 1$, $P = 0.729$). Developmental time was significantly affected by both climatic regime ($F = 8063.65$, $df = 2, 148$, $P < 0.001$) and diet ($t = -2.77$, $df = 70.86$, $P = 0.007$). Larvae and pupae reared under climatic regime 1 or 2 developed approximately 31 days faster than those maintained under climatic treatment 3 (both $P < 0.001$; Tukey post-hoc tests), whereas predators fed food source 2 developed ca. one day slower than those given food source 1 ($P = 0.007$; Tukey post-hoc test). Climatic regime during development ($F = 43.60$, $df = 2, 148$, $P < 0.001$) and food source ($t = 3.48$, $df = 87, P < 0.001$) both influenced adult body weight. Ladybirds exposed to climatic treatment 3 weighed approximately 20 % less than those exposed to climatic treatment 1 and 2 (both $P < 0.001$; Tamhane post-hoc tests) and predators reared on diet 1 gained a 7 % higher body weight than their counterparts reared on diet 2. The body weight of adults undergoing an acclimation period of seven days decreased, whereas adults maintained at rearing conditions gained weight (Fig. 1). Weight loss in adults during acclimation was more pronounced in ladybirds reared at 18 °C than in those reared at 25 °C ($F = 128.94$, $df = 2, 148$, $P < 0.001$), and in ladybirds fed *E. kuehniella* eggs versus those fed mealybugs ($t = 4.96$, $df = 87$, $P < 0.001$).

There was no significant correlation between either SCP and sex ($r = 0.104$, $P = 0.207$, $n = 149$ for climate; $r = -0.119$, $P = 0.269$, $n = 89$ for food source), adult body weight ($r = -0.125$, $P = 0.128$, $n = 149$ for climate; $r = 0.163$, $P = 0.127$, $n = 89$ for food source), weight loss during acclimation ($r = 0.035$, $P = 0.730$, $n = 99$ for climate; $r = 0.094$, $P = 0.380$, $n = 89$ for food source), weight gain for non-acclimated ladybirds ($r = 0.155$, $P = 0.282$, $n = 50$) or body weight just before testing ($r = -0.188$, $P = 0.107$, $n = 149$ for climate; $r = 0.139$, $P = 0.193$, $n = 89$ for food source).

The lower lethal times for 10, 50 and 90 % mortality ($LTime_{10,50,90}$) at a temperature of 5 °C for *C. montrouzieri* reared under different climatic regimes and with different food sources are presented in Table 3. Neither climate nor diet had a significant effect on the $LTime_{50}$ of *C. montrouzieri*. Overall, the time required to kill 50 % of the population ranged from 12.8 to 14.4 days. All individuals died by day 24.

Discussion

Low temperature acclimation had a positive effect on the supercooling ability of *C. montrouzieri*. Ladybirds reared under continuous summer

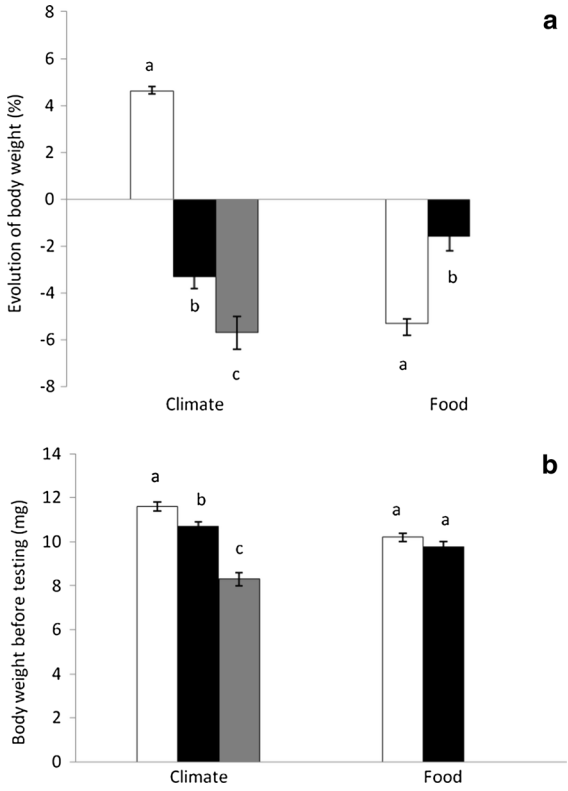

Fig. 1 Relative change in adult body weight between adult emergence and SCP measurement (**a**) and adult body weight before SCP measurement (**b**) of *C. montrouzieri* reared under different climatic regimes (*white* = 25 °C and 16:8(L:D)h without acclimation; *black* = 25 °C and 16:8(L:D)h with acclimation, *grey* = 18 °C and 8:16(L:D)h with acclimation) or offered different foods (*white* = *E. kuehniella* eggs, *black* = *P. citri* mealybugs). Within each factor (climate or food), *graph bars* (mean ± SE) with the same *letter* are not significantly different ($P > 0.05$; climate: Tamhane tests, food: *t* tests)

conditions (25 °C, long days) froze at a temperature which was approximately 7 °C higher than the freezing temperature of ladybirds undergoing a short acclimation period at 10 °C before testing. Within acclimated ladybirds, no significant difference in SCP was observed between ladybirds reared at 18 °C under short day conditions and those reared at 25 °C under long day conditions. This finding suggests that exposing *C. montrouzieri* taken directly from the laboratory culture to a short acclimation period is sufficient to detect a possible acclimation response. Maes et al. (2012) showed that a similarly short acclimation period had a significant impact on the SCP of the predatory bug *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae): bugs acclimated to lower temperatures froze at a lower temperature than their counterparts maintained at rearing conditions.

An increase in cold tolerance after acclimation might indicate an insect's potential to gradually adjust to decreasing temperatures. Although cold acclimation had a positive effect on the supercooling ability of *C. montrouzieri*, no adjustive response could be detected in terms of its lethal time. This is in contrast with earlier studies which either demonstrated an acclimation response for both SCP and lethal time or an acclimation effect for lethal time but not for SCP. For example, both SCP and lethal time measurements indicated that the parasitoid *Spathius agrili* Yang (Hymenoptera: Braconidae) was less tolerant to low temperatures when reared under high temperature and long photoperiod conditions than when reared under low temperature and

Table 3 Lethal time$_{10,50,90}$ [± 95 % fiducial limits] at 5 °C for *C. montrouzieri* ladybirds reared under different climatic regimes and offered different foods

Population	LTime$_{10}$ (days)	LTime$_{50}$ (days)	LTime$_{90}$ (days)
Climate			
Climatic regime 1	9.3a [8.3–10.2]	13.3a [12.5–14.1]	17.3a [16.4–18.3]
Climatic regime 2	10.4a [9.4–11.3]	14.4a [13.6–15.2]	18.4a [17.5–19.4]
Climatic regime 3	9.0a [8.0–9.8]	13.0a [12.2–13.6]	16.9a [16.1–17.9]
Food source			
Food source 1	7.9a [6.4–9.1]	14.0a [13.0–14.9]	20.0a [18.8–21.4]
Food source 2	6.8a [5.2–8.0]	12.8a [11.8–13.7]	18.8a [17.7–20.1]

LTime$_{10,50,90}$ within a column followed by the same letter are not significantly different (based on overlapping fiducial limits)

short photoperiod (Hanson et al. 2013). Further, acclimated individuals of the predatory bug *Macrolophus caliginosus* Wagner (Hemiptera: Miridae), the parasitoid *Eretmocerus eremicus* (Rose & Zolnerowich)(Hymenoptera: Aphelinidae) and the predatory mites *Amblyseius californicus* McGregor (Acari: Phytoseiidae) and *Typhlodromips montdorensis* (Schicha)(Acari: Phytoseiidae) were more cold hardy than non-acclimated individuals based on their lethal time in exposures at −5, 0 or 5 °C, whereas acclimation had no effect on their supercooling abilities (Hart et al. 2002a, b; Hatherly et al. 2004; Tullett et al. 2004).

The food source offered to *C. montrouzieri* during its immature development and early adult stage affected its supercooling ability. Predators reared on *E. kuehniella* eggs had a lower SCP than their counterparts offered *P. citri* mealybugs. Likewise, *M. pygmaeus* bugs reared on *E. kuehniella* eggs were found to be more cold tolerant than those fed an artificial diet based on egg yolk (Maes et al. 2012). Specty et al. (2003) reported that *E. kuehniella* eggs are rich in fatty acids and amino acids and pointed out that these nutrients may protect a predator fed on this factitious food against extreme temperatures by delivering components that promote winter survival. As SCP measurements evaluate an insect's resistance to a brief cold exposure, whereas lethal time measurements assess its cold hardiness when faced with a long-term cold exposure (Chown and Terblanche 2006), a more pronounced effect of the lepidopteran eggs with their higher caloric value could be expected on lethal time than on SCP. However, no significant difference in lethal times between ladybirds fed *E. kuehniella* eggs and those offered *P. citri* mealybugs could be detected. Our experiments indicate the complexity of predicting the cold hardiness of a candidate biological control agent, more specifically as to which factors should be taken into consideration when standardizing an experimental protocol for assessing its establishment potential in the framework of an ERA. In a previous study, the cold tolerance of the predatory bug *M. pygmaeus* was not only affected by acclimation and diet, but also by its infection status with endosymbionts (Maes et al. 2012). Hence, several factors related to the rearing or origin of the population under study might influence the outcome of cold tolerance experiments performed in the laboratory.

Based on the results of this study and information in the literature, it is deemed unlikely that *C. montrouzieri* could establish outdoors in northwestern Europe. Optimal temperatures for development and reproduction of this ladybird are 25 °C and above (Fisher 1963; Babu and Azam 1987). In the present study, exposing the immatures to a temperature of 18 °C and short day conditions led to a drastic increase in developmental time (from 23 to 54 days) and a substantial decrease in both immature survival (13 %) and adult body weight (20 %). Iperti (1999) showed that *C. montrouzieri* does not possess any diapause trait but resists drastic changes in climate by reducing its speed of development. The predator's developmental threshold is relatively high and estimated at 14 °C (Fisher 1963; Malais and Ravensberg 2002). Further, our study showed that *C. montrouzieri* did not survive temperatures below its supercooling point and can therefore be classified as freeze intolerant (Sømme 1982). Bartlett (1974) reported that only 50 % of *C. montrouzieri* adults survived an exposure of −5.5 °C for 12 h. Besides, all *C. montrouzieri* adults in our study had died by day 24 when exposed to 5 °C, indicating its susceptibility to chilling injury due to above-zero cold temperatures. Hatherley et al. (2005) reported a strong positive correlation between maximum field survival and survival at 5 °C in the laboratory for several arthropod biological control agents and this trend has been confirmed by subsequent studies (Hatherly et al. 2008; Hughes et al. 2009). When applying the relationship between $LTime_{50}$ at 5 °C and field survival calculated by Hatherly et al. (2005) to our dataset, it can be predicted that *C. montrouzieri* would not persist longer than 40 days in the field in western European winters and that the ladybird can be classified in the low risk category (Bale et al. 2009). Thus, the data obtained suggest that *C. montrouzieri* is unlikely to permanently establish in the cooler temperate climate of western Europe and, together with its relatively narrow host range (Ślipiński 2007; Finlay-Doney and Walter 2012; Maes et al. 2014b), is therefore expected to pose little risk to non-target species in this area.

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