J. Crop Prot. 2013, 2 (2): 119-126

Long-term cold response in overwintering adults of ladybird *Hippodamia variegata* (Coleoptera: Coccinellidae)

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Abstract: *Hippodamia variegata* is an efficient and most abundant predatory coccinellid in many countries. Understanding the ability of long-term low temperature survival in beneficial insects can be used to make better predictions about subsequent abundance and hence the biological control potential in the next spring and summer. So in this study, effects of long-term low temperatures were investigated on mortality and supercooling point (SCP) of field collected (pre-diapausing) and overwintering aggregations of *H. variegata* adults. Unlike the pre-diapausing insects, aggregated coccinellids could easily survive at -3 and 0 °C for one month. One month acclimation at 10 °C caused more than 80% mortality in overwintering adults, indicating that higher temperatures were not appropriate for overwintering aggregated coccinellid. In December and January, when diapause was in its highest level, changing the habitat temperature did not affect SCP. Acclimatization at 5 and 0 °C for one month decreased SCP of pre-diapausing adults, collected from aphid infested plants in October. Our study revealed that long term exposure to temperatures below 0 °C that usually happen in natural conditions may be necessary for overwintering of the coccinellid.

Keywords: *Hippodamia variegata*, overwintering, acclimatization, supercooling point, low temperatures

[DOR: 20.1001.1.22519041.2013.2.2.1.1]

Introduction

The predatory ladybird beetle, *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae), is distributed widely and can be found in different parts of Iran. It is reported as the most abundant predatory coccinellid in some areas of Iran (Koohpayehzadeh and Mossadegh, 1991; Sadeghi, 1991; Bagheri and Mossadegh, 1995; Yaghmaee and Kharazi Pakdel, 1995). High numbers of active *H. variegata* feed on aphids from September until late October. It seems they are in pre-diapause phase in this period. Depending on the food supply and the ambient temperature, *H. variegata* populations migrate to the mountains from late September to late October where the aggregations are reformed, (Hamedi and Moharramipour, unpublished data). Sadeghi (1991) described an overwintering aggregation of 1 to 20 beetles of this species in Iran. These overwintering adults remain in the overwintering aggregations for up to 6 months before returning to the lowlands. From late November to late January, they are in diapause phase when the cold hardiness parameters are in their highest levels. It seems from February until their migration to the lowlands in April, they would be in post-diapause phase (Hamedi and Moharramipour, unpublished data).

Insects have evolved various adaptations to endure the adverse seasons in their life cycles. Low temperature affects insects differently based on the severity of the cold and the duration of

Handling Editor: Dr. Vahid Hossieninaveh

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Received: 25 October 2012; Accepted: 4 February 2013

exposure (Lee, 2010). Insects can be divided into two general groups for cold hardiness: freeze-intolerant, the most common, or freeze-tolerant (Baust and Rojas, 1985; Storey and Storey, 1988; Lee, 1991). Freeze-intolerant species cannot tolerate intracellular freezing, and the overwintering stages have usually evolved by decreasing supercooling points (SCP) of 10–40 °C lower than non-overwintering insects (Somme, 1982). Freeze-tolerant species usually have SCP above -10 °C throughout the year, and these high SCPs are maintained during the winter through the production of ice nucleators (Storey and Storey, 1988). Studies concentrated on the effect of low temperature are rare in coccinellids, although such data are of most importance especially if the species is introduced to a different climate for biological control (Hodek, 1973). Short-term thermal response (2.5 and 24 hours) has been investigated in *H. variegata* (Hamedi and Moharramipour, unpublished data). The authors indicated that this coccinellid is a freeze intolerant species that cannot survive the temperatures below their SCP. However, understanding long-term low temperature survival in beneficial insects can be used to make better predictions about subsequent abundance in the next spring. As far as the authors are aware, the literature contains no reference to the long-term cold response on this coccinellid. Therefore, the impact of long-term cold exposure effect was investigated on the SCP and mortality of ladybird beetle *H. variegata*.

Materials and Methods

Test insects

The feeding pre-diapausing adults were collected in October 2010 from the field (35° 44′N, 51° 10′E, and altitude of 1302 m). Overwintering coccinellids used in the tests were collected monthly from large aggregations beneath *Desmostachys bipinnata* (L.) (Gramineae) in the slopes of mountains in Hamadan, Iran (35°24′N, 48°83′E, and altitude of 1800-2200 m) from December 2010 until February 2011. They were transferred to the laboratory in plastic containers (length 24, width 17, height 10 cm) with a net cap. In the laboratory, plastic containers were kept in a refrigerated incubator at the same average temperature as the natural habitat of coccinellids and a photoperiod of 12:12 (D: L). To minimize physiological changes during transportation, SCP determination was made within 24 hours after returning from overwintering sites.

Collection of microhabitat temperature data

Microclimate temperatures in lowland farm and overwintering site were automatically recorded using two data loggers (Testo, model 175-H_2, Germany) from October 2010 until February 2011. Temperatures were recorded at hourly intervals beneath plants from which overwintering coccinellids were collected.

Long-term thermal response

For the thermal response test, samples of 20 to 30 adults in each month were acclimated to the temperatures of 10, 5, 0 and -3 ºC with cooling rate of 0.5 °C/min and then kept for 30 days under a short photoperiod of 12 L:12 D. The mortality was determined 30 days after exposure and the supercooling point was measured in the live adults thereafter. The coccinellids were considered dead if they failed to move.

Determination of supercooling point (SCP)

Supercooling points were measured in randomly selected individuals following each sampling and at the end of acclimation period. In each sampling, 4 to15 healthy adults were used for SCP measurement. The body surface of the individuals was attached to a thermocouple (flexible, 80 cm long, NiCr-Ni probe). Insect-thermocouple arrangements were placed into a programmable refrigerated test chamber (Cold/heat test chamber, model MK 53, Binder GmbH Bergstr., Germany) and cooled at a rate of 0.5 °C/min to the required temperature. Temperatures were recorded at 5 second intervals by a four-channel data logger (Testo, model 177-T4, Germany) and were read using Comsoft 3 software. The SCP of each

[DOR: 20.1001.1.22519041.2013.2.2.1.1]

insect was recorded as the lowest reading temperature reached before the release of a sudden temperature increase associated with formation of the ice in the tissue (Khani and Moharramipour, 2010).

Statistical analysis

Differences of SCP between treatments were compared by one-way analysis of variance (ANOVA) followed by LSD test at the 5% level. Values were reported as means ± SE. Statistical analysis of data were performed using the statistical package SPSS (PASW) version 18.0.

Results

Collection of microhabitat temperatures

The lowest recorded temperature, average of minimum temperature and the average temperature in each month are shown in Fig. 1. The average temperature in overwintering site was decreased from 0.63 ºC in December, 2010 to -1.12 ºC in January, 2011. Then it was increased to 1.89 ºC in February. The lowest recorded temperature was -14.72 ºC in January. January with an average minimum temperature of -6.41 ˚C was the coldest month in the winter (Fig. 1).

Long-term thermal response

The results obtained from long-term thermal effects on mortality and SCP of *H. variegata* are shown in Figs. 2 and 3. The pre-diapausing field collected adults in October had a low capacity to withstand -3 °C/30 days and led to 75% mortality. Overwintering adults gradually became cold hardy in autumn and a high level of chill tolerance was observed from December to February with less than 15% mortality in cold acclimated adults at -3 °C for 30 days. The least mortality at -3 °C/30 days was observed in the coldest month, January (Fig. 2).

Mortality at 0 °C for 30 days increased to 37% in October; however, no mortality was observed in the collected specimens in December, January and February. In contrast, acclimation at 10 °C for 30 days caused more than 80% mortality in overwintering coccinellids of December to February (Fig. 2).

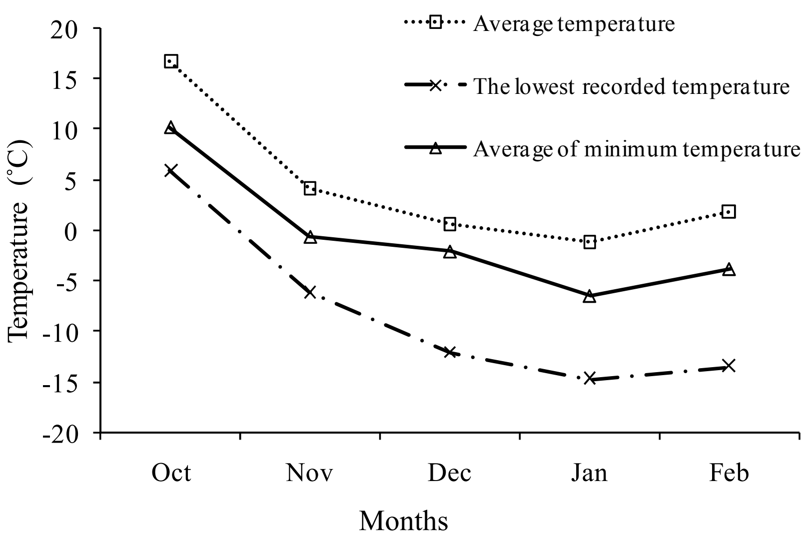

Figure 1 Temperature parameters of lowland farm and overwintering site located in the vicinity of Hamadan, Iran from October 2010 to February 2011.

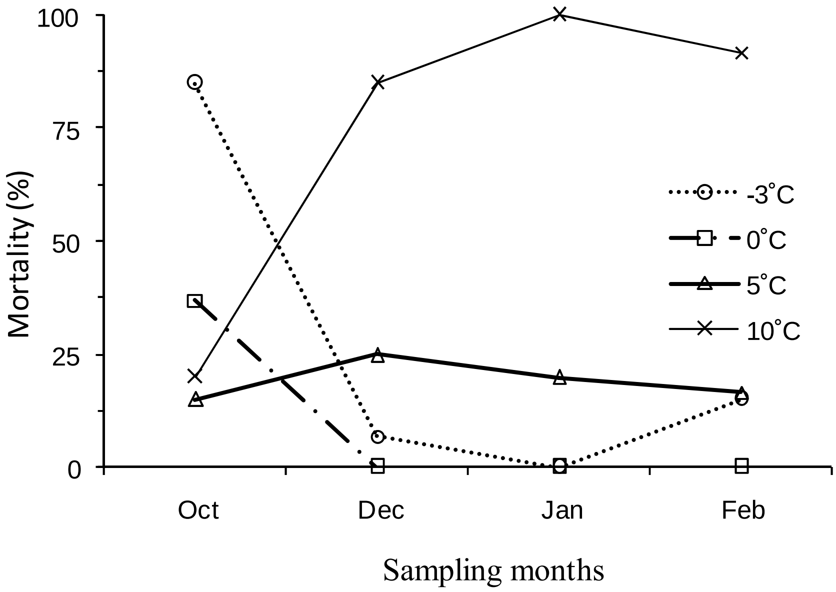

Figure 2 Effects of thermal acclimation at 10, 5, 0 and -3 ºC for 30 days on mortality of field collected adults (pre-diapausing in October 2010) and overwintering aggregations of *H. variegata* (diapausing and post-diapausing from December to February 2010-2011).

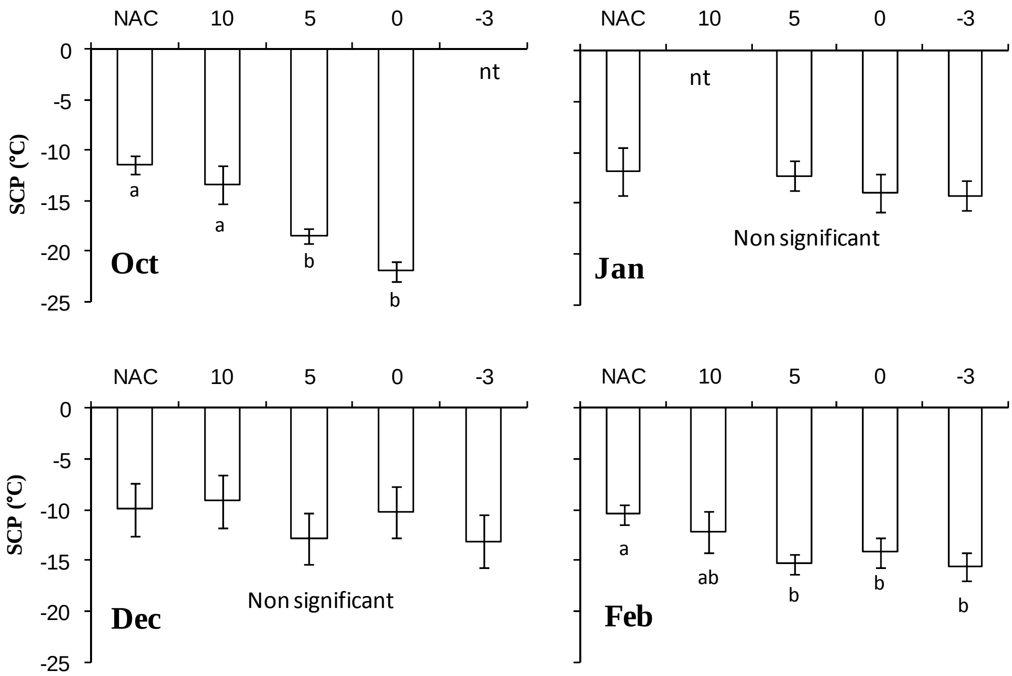

Figure 3 Supercooling point (SCP) of *H. variegata* in non-acclimated (NAC) and acclimation at 10, 5, 0 and -3 ºC for 30 days in field collected (pre-diapausing in October 2010) and overwintering aggregation adults (diapausing and post-diapausing from December to February 2010-2011).

Means followed by different letters are significantly different (LSD, $P < 0.05$). Vertical bars indicate standard error of mean.

nt: not tested

[DOR: 20.1001.1.22519041.2013.2.2.1.1]

The SCP decreased significantly in October (F = 22.17; df = 3, 14; P < 0.001) and February (F = 8.36; df =4, 35; P < 0.001) when coccinellids were acclimated at various low temperatures (Fig. 3). Acclimation at 5 and 0 °C/30 days decreased the SCP of insects in October from -11.4 ± 0.83 in non-acclimated to -18.45 ± 1.92 and -21.95 ± 0.70, respectively. Moreover, same trend was observed in February, so that a reduction in SCP occurred from -10.44 ± 1.01 in non-acclimated to -15.23 ± 0.98, -14.18 ± 1.42 and -15.54 ± 2.05 °C when acclimated at 5 and 0 and -3 °C/30 days, respectively. However, 30 days cold acclimation did not affect SCP of insects collected in December and January (Fig. 3).

Discussion

In this study, changes of cold tolerance and supercooling capacity were assessed in *H. variegata* in response to long-term thermal acclimation. The overwintering beetles always had a very high capacity to survive at 0 and -3 °C (0-10% mortality) but not able to withstand 10 °C for 30 days. In contrast, field collected insects could not tolerate -3 °C/30 days and caused over 85% mortality. It could be concluded that sub-zero temperatures were not favorable for overwintering preparation of pre-diapausing insects and natural gradual decrease of temperature in habitat was necessary to enhance cold hardiness of insects (Somme, 1982; Bale, 1987; Slachta *et al.*, 2002; Wang *et al.*, 2006).

At diapause stage, insect cold tolerance to temperatures below zero is rather high, due to gradual temperature decrease resulting in cold hardening. However, early or late frosts can be very damaging (Hodek, 1973). In contrast, 10 °C was not favorable for overwintering coccinellids (Fig. 2). In addition, desiccation at high temperature (10 °C), might be a reason for mortality. Watanabe (2002) demonstrated that *H. axyridis* individuals survived more at 0 °C than at 5 °C, suggesting that the mortality at higher temperature could be caused by depletion of energy reserves or water rather than by chilling injury. In other study, evaluation of lipid proportion of *H. axyridis* demonstrated a higher depletion of lipids during winter at 10 °C than at -5 °C, suggesting that energy reserves are important for survival of *H. axyridis* in heated houses (Labrie *et al.*, 2008). Also, cryoprotectants, which act as membrane protectants and stabilize a membrane's bilayer structure, were accumulated in temperatures below 0 °C. Moreover, our previous study showed that exposure to high temperatures resulted in a rapid catabolism of cryoprotectants in *H. variegata* (Hamedi and Moharramipour, unpublished data). Cold acclimated adults at -3 °C/30 days in December and January exhibited high levels of tolerance. The increase in capacity to survive long-term exposure at 5 °C has been reported in overwintering freeze-intolerant adults of leaf elm beetle, *Xanthogaleruca luteola* (Muller), during December and January (Soudi and Moharramipour, 2011)**.** Such data has also been demonstrated in codling moth, *Cydia pomonella* (L.) (Khani *et al.*, 2007). They showed that one-month exposure to 5 °C led to a 23% mortality of larvae in early October. Overwintering larvae gradually became cold hardy in autumn and a high level of chill tolerance was observed in late November. Chill tolerance then decreased in March so that 14% of cold acclimated larvae died during this period.

Effect of thermal acclimation on SCP depends on the temperature and the time of sampling. Researchers have also demonstrated that response to temperature, changes during the diapause periods (Tauber *et al.*, 1986). Feeding adults obtained from aphid infested plants in October that were probably in a preparation phase of diapause, needed low temperature exposures, as happen in natural conditions, to decrease their SCP. Temperatures below 5 °C caused SCP reduction in pre-diapausing and post-diapausing adults collected in October and February, respectively. Some cold hardiness mechanisms such as elevation of cryoprotectants in the insect body is also stimulated at low temperatures usually between 0 to 5 °C (Storey and Storey, 1991; Soudi and Moharramipour, 2012). In contrast, in December and January, when diapause progressed to its highest level, changing the habitat temperature did not affect the SCP. Such data were demonstrated in *Xanthogaleruca luteola* (Muller) (Soudi and Moharramipour, 2011) and codling moth, *C. pomonella*, (Khani *et al.*, 2007). The high value of SCP was observed in this coccinellid because of evoking reflex bleeding, which might cause inoculative freezing at higher temperatures (Hamedi and Moharramipour unpublished data). This phenomenon has been proved previously in two other coccinellid species, *Coccinella septempunctata* L. and *Semiadalia undecimnotata* (Schneider), (Nedved, 1993). So caution should be taken when measuring SCP of the samples.

Only adults that have previously entered diapause stage were able to show complementary response to declining temperatures and survive at sub-zero temperatures. Furthermore, the given coccinellids owing to a protective microclimate within their aggregation (Koch *et al.*, 2004), can possibly survive in environments with even lower than sub-zero temperatures when overwintering. The current study focused on the seasonal long-term thermal effect on survival and SCP of *H. variegata* over winter, while the insect undergoes a series of biochemical changes to maximize its cold hardiness.

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[DOR: 20.1001.1.22519041.2013.2.2.1.1]

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پاسخ حشرات کامل کفشدوزک‌های زمستان‌گذران *Hippodamia variegata* به سرماهای بلندمدت

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دریافت: ۴ آبان ۱۳۹۱؛ پذیرش ۱۶ بهمن ۱۳۹۱

چکیده: کفشدوزک *Hippodamia variegata* یک شکارگر مؤثر و فراوان‌ترین کفشدوزک شکارگر در بسیاری از کشورها می‌باشد. فهم توانایی زنده ماندن حشرات مفید در دماهای پایین به‌مدت طولانی می‌تواند در پیش‌بینی جمعیت و در نتیجه پتانسیل کنترل بیولوژیک در بهار و تابستان سال بعد مفید باشد. لذا در این مطالعه اثرات دماهای پایین بر مرگ و میر و نقطه انجماد بدن (SCP) حشرات کامل جمع‌آوری شده از مزرعه در مهر ماه (مرحله پیش دیاپوز) و حشرات کامل جمع‌آوری شده از تجمعات زمستان‌گذرانی آنها در کوه طی ماه‌های آبان تا بهمن ۱۳۸۹ بررسی شد. کفشدوزک‌های زمستان‌گذران، بر خلاف حشرات کامل در حال تغذیه در مزرعه، به راحتی به‌مدت یک ماه در دماهای ۳- و صفر درجه سلسیوس زنده ماندند. بر عکس یک ماه نگهداری حشرات کامل زمستان‌گذران در دمای ۱۰ درجه سلسیوس سبب مرگ و میر بیش از ۸۰ درصد از جمعیت شد که نشان می‌دهد دماهای بالا برای زمستان‌گذرانی این جمعیت مناسب نمی‌باشد. در آذر و دی ماه، هنگامی که دیاپوز به اوج خود رسیده بود، تغییر دمای زیستگاه اثری روی میزان نقطه انجماد بدن نداشت. اما برعکس نگهداری حشرات کامل جمع‌آوری شده از روی گیاهان در مهر ماه در دمای ۵ و ۰ درجه سلسیوس، موجب پایین آمدن نقطه انجماد بدن آنها گردید. به‌طور کلی این مطالعه نشان داد که رویارویی با دماهای زیر صفر درجه سلسیوس که در شرایط طبیعی به‌مدت طولانی در طبیعت رخ می‌دهد، ممکن است برای زمستان-گذرانی این کفشدوزک ضروری باشد.

واژگان کلیدی: *Hippodamia variegata*، زمستان‌گذرانی، سرمادهی، نقطه انجماد، دماهای پایین