J. Crop Prot. 2015, 4 (2):145-156

Research Article

Variation of supercooling point in overwintering larvae of *Scrobipalpa ocellatella* (Lepidoptera: Gelechiidae)

Zahra Ganji and Saeid Moharramipour*

Department of Entomology, Faculty of Agriculture, Tarbiat Modares University, P.O. Box: 14115-336, Tehran, Iran.

Abstract: The beet moth, *Scrobipalpa ocellatella* (Boyd) (Lepidoptera: Gelechiidae) is one of the most destructive pests of beet. The insect overwinters as larvae of different instars in beets which are left in the field. Supercooling point (SCP) of individual larvae showed broad range variation from -6 to -25 °C. In the present study, factors affecting the broad range of SCP were investigated. Larvae showed a great increase in SCP when they were induced by exogenous ice nucleation. Defensive oral discharge (DOD) decreased SCP but it had no significant effect on supercooling ability of larvae. The presence of sufficient food in guts of field collected larvae induced an increase in SCP. Therefore it could be concluded that surface moisture and food particles in the gut may act as ice nucleating agents (INAs) that cause freezing of the body fluids at higher temperatures. Furthermore, second and third instar larvae, owing to their small size, had greater capacity to supercool in comparison to fifth feeding instars. Broad range in SCPs might be due to diversity in overwintering larvae which have different capacity for supercooling.

Keywords: beet moth, *Scrobipalpa ocellatella*, Supercooling point, Defensive oral discharge, Ice nucleating agents

Introduction

Insects must have the ability to survive at low temperatures, because for many ectotherm organisms, even brief exposure to subzero temperatures causes injury or death (Lee *et al*., 1991; Sømme, 1999). Many insect species must have physiological, biochemical and behavioral mechanisms for survival in the lethal subzero temperatures. Insects use two main strategies for winter survival: freeze avoidance and freeze tolerance (Salt, 1961; Zachariassen, 1985). Unlike the freeze tolerant species, freeze avoiding insects are not able to survive freezing (Sømme, 1999). The majority of investigated arthropods are freeze avoiding (Block, 1991; Sømme, 1999). They may enhance their capacity to supercool by elimination of the ice nucleators by some mechanisms such as gut clearing, synthesizing the antifreeze proteins and accumulating low molecular weight cryoprotectants (for example sugars and polyols) (Zachariassen, 1985; Bale, 1989; Lee, 1989; Zhao, 1997). Freeze tolerant insects employ exogenous or endogenous ice nucleating agents to stimulate ice growth, while, ice growth will increase the risk of death for freeze intolerant species. Ice nucleating agents (INAs) include bacteria (Strong-Gunderson *et al*., 1990; Lee *et al*., 1993), fungi (Tsumuki *et al*., 1992), proteins and lipoproteins (Zachariassen and Hammel, 1976;

Handling Editor: Dr. Nayereh Hamedi

* **Corresponding author**, e-mail: moharami@modares.ac.ir
Received: 7 June 2014, Accepted: 13 October 2014
Published online: 14 December 2014

Duman *et al*., 1991), crystalloid compounds (Mugnano *et al*., 1996), food particles (Cannon and Block, 1988) and surface moisture (Zachariassen, 1985). Some nucleators such as microorganisms or crystalloid compounds may be present in food particles in the gut. In many insect species, the cessation of feeding and gut purging are important preparations for winter survival (Sømme, 1999). Supercooling point (SCP) is the spontaneous nucleation temperature with desiccant present and the inoculative freezing point (IFP) refers to the temperature at which organism freeze when surrounded by ice (Sømme, 1982; Zachariassen and Kristiansen, 2000). INAs by promoting the SCPs and cold hardiness affect winter survival (Zachariassen and Hammel, 1976). Many temperate insects lower their SCPs through evacuation of gut to remove food particles that might initiate the freezing process (Salt, 1961; Baust and Rojas, 1985; Zachariassen, 1985; Duman, 2001).

Insects must sequester sufficient reserves prior to the onset of low temperatures that increase overwintering ability. Most insects are exposed to lack of food and are able to overwinter at a certain developmental stage during the winter; other stages often die during cold months. For instance, the elm leaf beetle, rice stem borer and the cabbage moth overwinter as adults, last instar larvae and pupae, respectively (Goto *et al*., 2001; Atapour and Moharramipour, 2009; Soudi and Moharramipour, 2011) that do not have any access to food during cold months. However, some insects overwinter at different developmental stages especially larval stage that feed all the seasons even throughout overwintering. The beet moth, *Scrobipalpa ocellatella* (Boyd) (Lepidoptera: Gelechiidae) causes serious damage in sugar beet fields. It has 3-6 generations through a year in Iran. *S. ocellatella* overwinters in different larval instars within vegetation residues and in non-harvested beet roots in the field. This pest has five larval instars (Kheyri *et al*., 1980). In Iran, sugar beet is harvested usually in late October and early November. After harvest, last instar larvae may overwinter in residues when no food is available. But the beet moth can overwinters as larvae of different ages in fresh leaves of winter beet crop over the winter. We argue that larvae of *S. ocellatella* are able to reserve sufficient nutrients before the harvest. It seems that larvae of the beet moth utilizes freeze avoidance strategy to survive subzero temperatures. Our previous investigations showed that the larvae of different ages have high tolerance to cold and 50% of individuals can tolerate -19 ºC for 2 hours in February (Ganji *et al*., unpublished data).

No study has been performed to assess SCP and cold hardiness of *S. ocellatella.* As the beet moth larvae die upon freezing, effective factors on SCP may play important role in overwintering success of the pest. In this study, the possible role of surface moisture and food particles has been investigated on supercooling capacity of *S. ocellatella* larvae during autumn and winter in Karaj, Iran.

Materials and Methods

Sample collection

The sugar beet plants were collected in autumn and winter of 2012 from Karaj, Iran (35°83'96"N, 50°86'63"E, 1293 m from sea level) and transferred to laboratory. The larvae were carefully picked from sugar beet plants.

Supercooling point determination

The supercooling points (SCPs) were measured according to Saeidi *et al*. (2012). After drying the larval bodies by placing them on absorbent tissues, the SCP was recorded with a thermocouple (NiCr-Ni probe) attached with a sticky tape to the ventral body surface of the insects. The insects were cooled in a programmable thermal chamber (Binder, model MK53, Germany) with a cooling rate of 0.5 °C/min. The lowest temperature prior to temperature increase was taken as the SCP of the individuals.

SCP of fed and starved larvae

Field collected larvae in September 2012 were used in this experiment. Larvae of the beet moth were divided into three groups. 1- Fed last instar larvae: groups of 16 feeding individuals kept on food source to continue feeding before

SCP measurement, 2- wandering larvae: in the beet moth, the last instar turns reddish when it ceases feeding; This stage is referred to as wandering, and 3- small (mixture of second and third instar) larvae. SCP of three groups (ca 16 larvae in each group) was determined.

In other experiment, last instar feeding larvae and prepupae collected in January 2012 were used for SCP determination. 1- SCPs of 20 feeding individuals kept on food source were determined. 2- groups of 16 feeding larvae were held in Petri dishes and stored without any food for 24 h to allow excretion of the food particles prior the onset of SCP experiment (starved larvae). During this time, the larvae were held in outdoor conditions. 3- The prepupae, referred to as final instar, turn violet before pupation; in addition their bodies change to fusiform in shape.

SCP of defensive oral discharged larvae

Last instar larvae (12 larvae) were provoked by a fine soft brush to induce defensive oral discharge (DOD) prior to SCP determination. SCP of the intact larvae was measured as control. The experiment was conducted in December 2012.

SCP of the wet caterpillars

To investigate effect of surface moisture on SCP of last instars, larvae were wrapped in cotton soaked in distilled water. To immobilize the larvae, they were placed in wells of ELISA plate chambers. This experiment was carried out in January 2012 and compared with dried control larvae.

Statistical Analysis

Distribution of SCP is often bimodal (Spicer and Gaston, 1999). The separation of bimodal SCP distributions into high (with higher SCP) and low (with lower SCP) groups have been well discussed by Cannon and Block (1988). However, the break points in bimodal distributions are often determined arbitrarily; for example based on an obvious break in SCP (Worland and Convey, 2001; Sinclair *et al*., 2003; Chen and Kang, 2005). The data were analyzed by the *t*-test and one-way analysis of variance (ANOVA) using SPSS version 18.0 (SPSS Inc., 2009). Means were compared by post hoc Tukey's test at $P < 0.05$. SCPs of individuals were divided into two groups, upper and lower than median and percent frequency of each group was calculated.

Results

Effects of feeding condition and body size on SCP

Frequency distribution of SCPs in fed, wandering last instar and small (mixed 2nd and 3rd) larvae of *S. ocellatella* collected in September 2012 are shown in Fig. 1. There were significant differences between SCP of fed, wandering and small larvae ($F= 8.386$; $df= 2, 48$; $P < 0.01$). SCP (mean ± SE) of fed, wandering and small larvae was -15.9 ± 0.87, -20.4 ± 0.84 and -19.8 ± 1.21 ºC, respectively. The highest and the lowest SCP were observed in fed and small larvae, respectively. However, there were no significant differences between SCP of small and wandering larvae.

SCP of prepupae, fed and starved last instar was significantly different ($F = 10.015$; $df = 2, 53$; $P < 0.001$). So that prepupae, fed and starved last instar larvae had supercooling capacity of -21.1 ± 0.69, -15.7 ± 1.04, and -19.0 ± 0.89 ºC, respectively. SCP in fed larvae was significantly higher than prepupae and starved larvae. The SCPs of starved and prepupae individuals ranged from -13.0 to -23.4 °C and -17.9 to -26.1 °C, respectively. However, SCPs of fed larvae ranged from -5.8 to -21.8 ºC. Almost all prepupae and about 90% of starved larvae showed SCP below the break point, while it was 70% in fed larvae (Fig. 2).

Gut condition

The most gut fullness was observed in fed larvae. Starved larvae excreted most food particles after 24 h so it was sufficient time for gut depletion. Also no food particles were observed in guts of wandering larvae. Gut content in three groups are shown in Fig. 3.

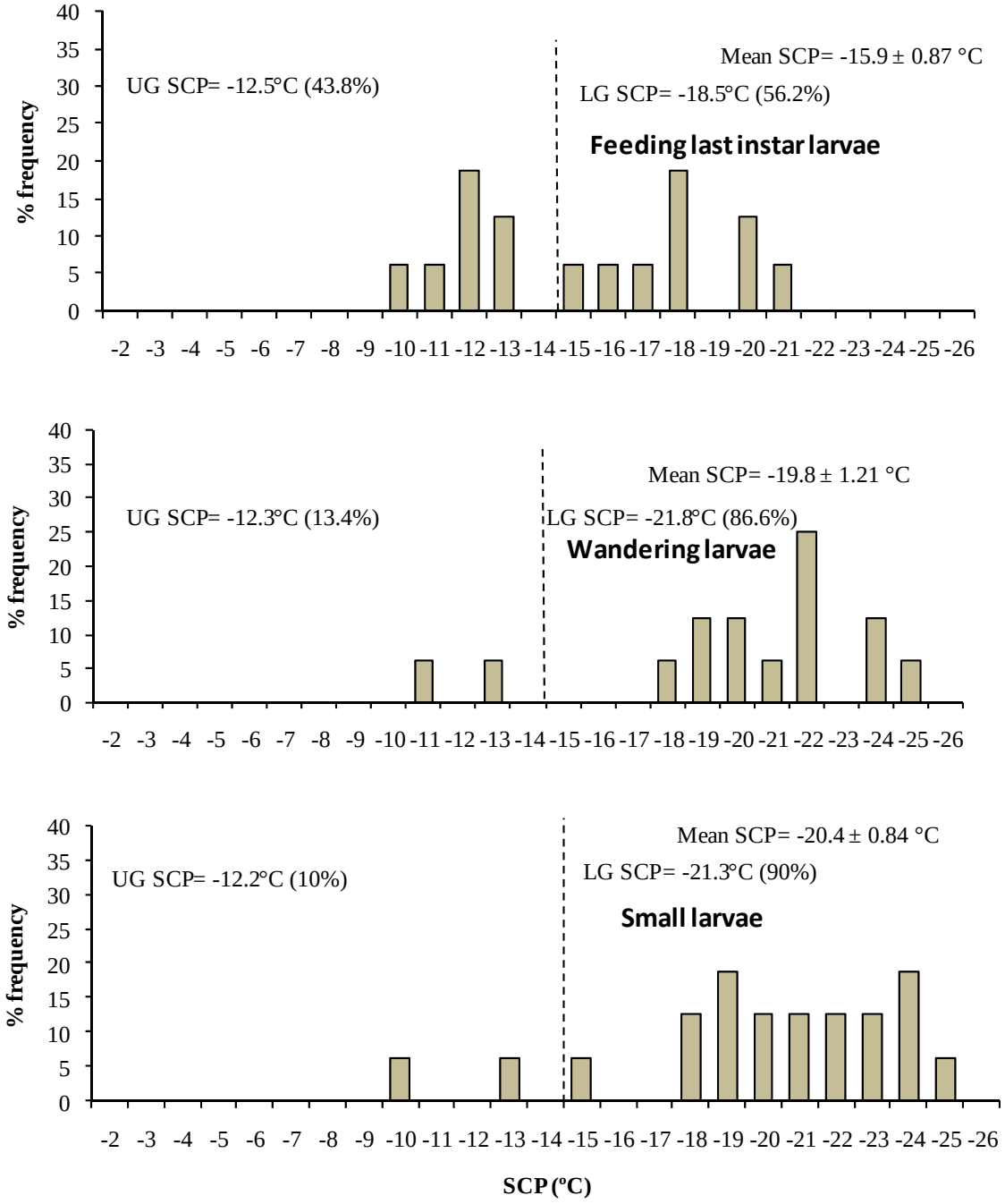

Figure 1 Frequency distribution of supercooling points (SCPs) in response to developmental stages. Fed, and wandering last instar and small (2nd and 3rd) instar larvae of *S. ocellatella* collected in September 2012. The break point is designated at –14 °C between lower group (LG; SCP < –14 °C) and upper group (UG; SCP > –14 °C). Mean SCP, the percent frequency in LG, UG and mean SCPs in each group are indicated in the figure (n = 51).

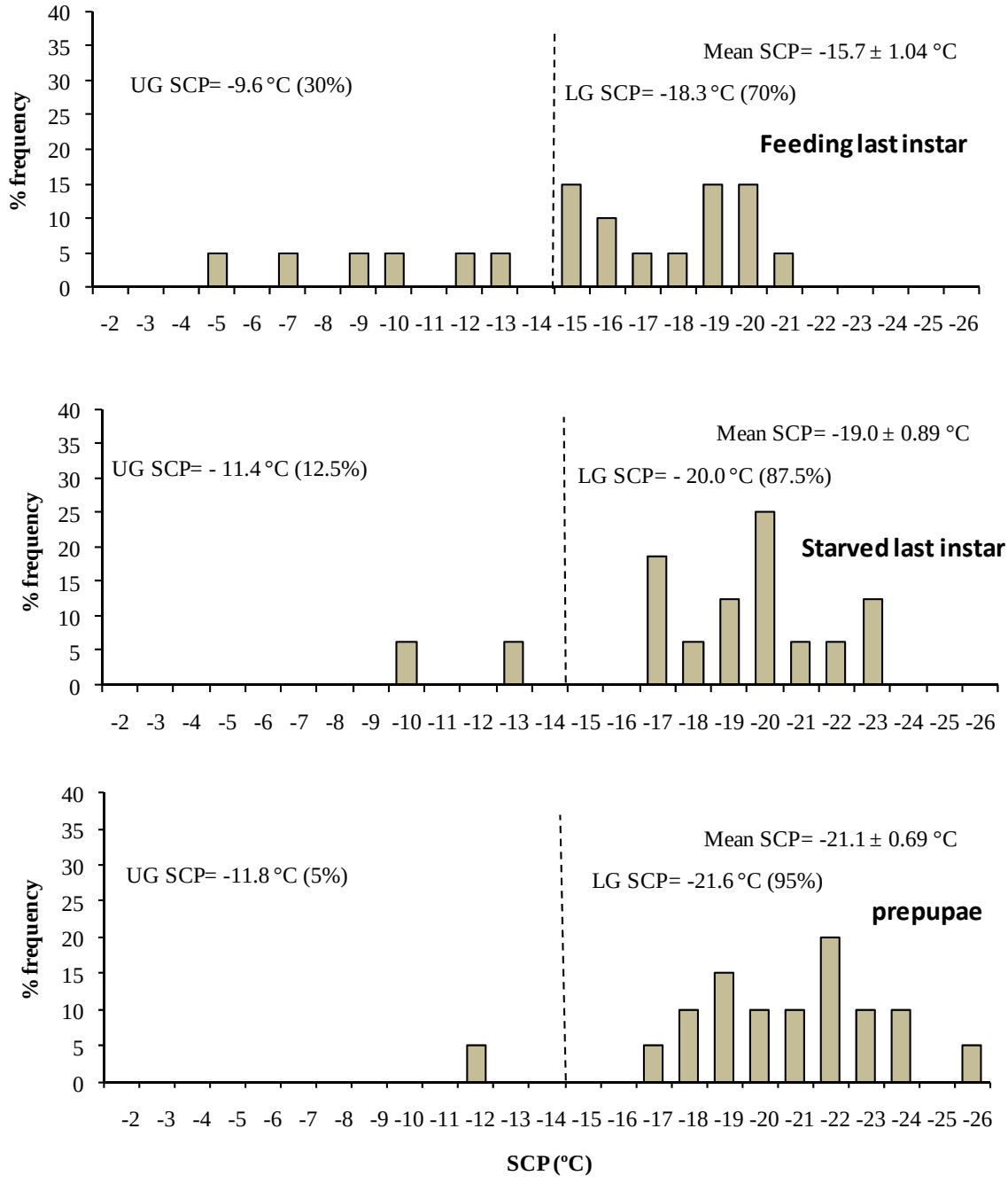

Figure 2 Effects of food particles in frequency distributions of supercooling point (SCP). Fed and starved last instar larvae and prepupae of *S. ocellatella*. All samples were collected in January 2012. The break point is designated at –14 °C between lower group (LG; SCP < –14 °C) and upper group (UG; SCP > –14 °C). Mean SCP, the percent frequency in LG, UG and mean SCPs in each group are indicated in the figure (n = 56).

Effects of defensive oral discharged larvae on SCP

The mean SCP of non-disturbed larvae was -13.1 ± 1.69 ºC (ranging from -6.7 to -24.4 ºC) but that of disturbed larvae was -17.0 ± 1.34 ºC (ranging from -7.5 to -23.8 ºC) (Figs. 4 and 5). However, despite the variation about 4 ºC between them, it was not significantly different ($P > 0.05$).

SCPs of wet caterpillars

Wet larvae had limited ability to supercool. Mean SCP and inoculative freezing points (IFP) were -8.0 ± 0.99 (-2 to -13 ºC) and -17.8 ± 0.92 ºC (-8 to -21 ºC), respectively. There was significant difference between SCPs and IFPs ($t = 6.908$; $df = 30$; $P < 0.001$). All of the wet individuals froze at temperatures above the median (break point) but 25% of the individuals showed higher SCP in control (Fig. 6).

Figure 3 (A) Fed (left), starved (middle) and wandering (right) last instar larvae of *S. ocellatella* and (B) following their gut condition. All samples were collected in January 2012. Food particles have been almost removed after 24 hours in starved larva and no food particle is observed in wandering larval gut.

Figure 4 Last instar larva of *S. ocellatella* before (left) and after (right) defensive oral discharge (DOD). Disturbed larvae discharge a defensive secretion from mouth. Gut darkness disappears after DOD induction.

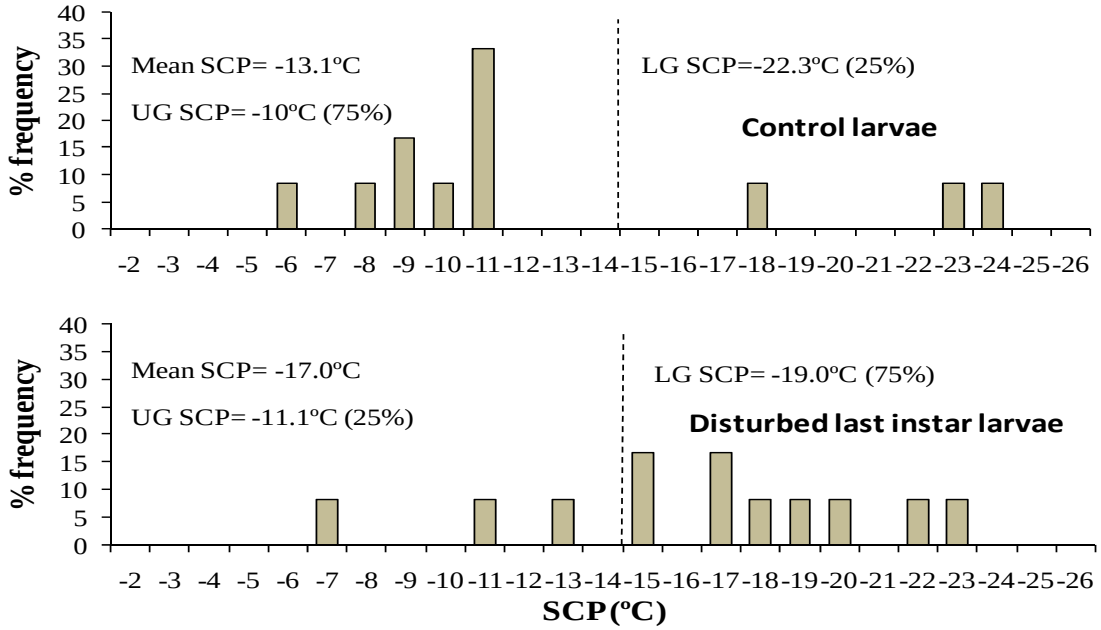

Figure 5 Effects of defensive oral discharge (DOD) on frequency distribution of supercooling points (SCPs). Non-disturbed, and disturbed last instar larvae of *S. ocellatella.* All samples were collected in December 2012. The break point is designated at –14 °C between lower group (LG; SCP < –14 °C) and upper group (UG; SCP > –14 °C). Mean SCP, the percent frequency in LG, UG and mean SCPs in each group are indicated in the figure (n = 24).

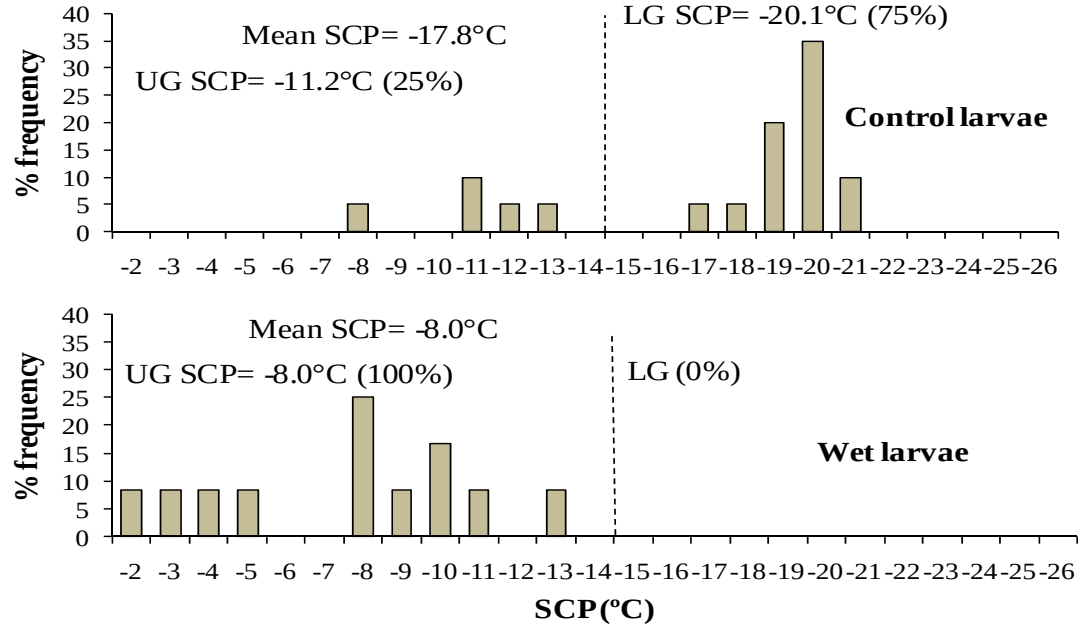

Figure 6 Frequency distribution of supercooling points (SCPs) in response to surface moisture. Dry and wet last instar larvae of *S. ocellatella.* All samples were collected in January 2012. The break point is designated at –14 °C between lower group (LG; SCP < –14 °C) and upper group (UG; SCP > –14 °C). Mean SCP, the percent frequency in LG, UG and mean SCPs in each group are indicated in the figure (n = 27).

Discussion

Our results showed that larvae of *S. ocellatella* overwinter as three developmental forms in Iran: 1) Prepupae and wandering (non feeding last instar) larvae with empty gut and low SCP. The final stage larva before changing to pupa usually is referred to prepupa. At prepupal stage, it creates gut purging. 2) Last instar larvae before emergence to prepupae with full gut, relatively high SCP and low cold tolerance. 3) Penultimate (second and third instar) larvae with full gut and lower SCP.

Freeze tolerant species survive the formation of internal ice, while freeze intolerant insects die upon freezing (Sømme, 1999). The *S. ocellatella* show a bimodal separation of SCP into upper (with higher SCPs) and lower (with lower SCPs) groups. There was large variation ranging from -6 to -25 ºC in SCPs among individuals during studied months. Similar to our results the supercooling values of field fresh, starved and wet caterpillars of *Embryonopsis halticella* Eaton were -17.6, -15.4 and -6.7 ºC, respectively (Klok and Chown, 2005).

SCPs of some insect species have been investigated in dry and moist condition (Humble and Ring, 1985; Shimada and Riihimaa, 1988; Gehrken *et al*. 1991; Larsen and Lee, 1994; Coyle *et al*., 2011; Boardman *et al*., 2012). Wet insects had limited ability to supercool, because of higher probability of lethal freezing in those insects. Surface moisture resulted in a significant elevation of freezing points. The beet moth caterpillars froze easily at -8.0 ± 1.44 °C when inoculated with ice. Humid microenvironment encouraged ice nucleating and caused freezing of the body fluids at relatively high temperatures (Zachariassen, 1985). Because of obvious effect of moisture on SCP, body surface of larvae were dried before experiments, so thatthe surface moisture could not affect the SCP and could not be the reason for high diversity in SCP. Interestingly, live beet moth larvae in contact with ice were observed inside the frozen sugar beets in the field.

The non-disturbed caterpillars had limited supercooling ability. They froze at higher temperatures (-13 ºC) compared to disturbed caterpillars (-17 ºC). But the difference was not significant. Unlike this pest, in *Hippodamia convergens* Guerin-Meneville stimulated adult has distinct effect on SCP due to reflex bleeding (Nedved, 1993).

Small larvae showed good supercooling ability and supercooled to -20.4 ºC in comparison to feeding last instar larvae, that may be related to small body size (Zachariassen and Kristiansen, 2003) and therefore lower water content (Vali, 1995). Also, lower food quantity in gut of small larvae could enhance supercooling capacity and permit body fluid to remain unfrozen at subzero temperatures. As gut nucleators play an important role in freezing, feeding cessation and then gut evacuation is a reflex to subzero temperatures that provide the possibility of decreased freezing and therefore result in a reduction of the mean individual SCPs (Block and Sømme, 1982). In regions with low temperatures, feed cessation allows organisms to be physiologically inactive for long periods (Worland and Convey, 2001; Sinclair and Chown, 2005). Insects stop feeding due to many factors including molting and low temperature. Enforced starvation acts as natural feed cessation mechanism to reduce SCP (Worland and Convey, 2001). Gut fullness during autumn and winter indicated that larvae were continuously feeding. Larvae with food in their alimentary canal froze at higher temperatures compared to gut evacuated larvae. Feeding larvae of *S. ocellatella* were found in sugar beets throughout the cold months. As shown in Fig. 3, insects (*S. ocellatella*) that were kept away from the food evacuated their guts and starvation greatly affected their SCP. The effects of feeding on SCP have been studied in several insects (Parish and Bale, 1990; Worland and Convey, 2008; Hiiesaar *et al*., 2011; Boardman *et al*., 2012). In nature many insect species stop feeding to prepare for cold months (Denlinger, 1991; Fields *et al*., 1998). Therefore in many experiments of cold hardiness studies, similar to field conditions, insects were held without any food before the onset of experiments preventing the induction of ice nucleation (Milonas and Savopoulou-Soultani, 1999; Hiiesaar *et al*., 2011). While in some insects such as *S. ocellatella* which overwinter in food source (beets) feeding in suitable condition, gut content plays fundamental role in SCP and successful overwintering.

The results showed that the SCP of fed larvae was higher than starved larvae and prepupae. Therefore larvae with more food particles in their guts induce freezing at higher temperature. After feeding cessation naturally or due to laboratory starvation, the SCP of *S. ocellatella* larvae dropped about 5 degrees. This improved capacity to withstand supercooling is explained by the absence of food particles in alimentary canal which induce ice formation at low temperatures (Carrillo *et al*., 2005; Zachariassen *et al*., 2008). Susceptibility to inoculative freezing varies depending on intrinsic factors. The effect of inoculative nucleation is depending on the INAs used and the anatomic site of application (Steigerwald *et al*., 1995).

This study indicates that variation in SCP may be related to several factors, such as gut fullness and surface moisture that induce freezing. The majority of overwintering larvae were wandering larvae that stopped feeding. Very low SCPs were observed in wandering larvae and prepupae. These larvae survived at low temperatures due to empty gut. Smaller portion of the overwintering population were the penultimate larvae. In spite of their continuous feeding they were observed until late winter. The sugar beet root heads remain in field and larvae of different ages pass the autumn and winter on them. Along with decrease in air temperature and root head drying, one group of last instar larvae complete their development, cease feeding and empty their gut but do not leave sugar beets, referred to wandering larvae. If the plants become dry the larvae seek refuge in soil and wet shelters.

Wandering larvae and prepupae were found to be the most tolerant individuals to cold in overwintering populations of the beet moth (Ganji *et al.*, unpublished data). Mild winter may not be favorable for wandering larvae, because it may affects metabolic rate, increase respiration and thereby cause mortality and reduce population, while such condition may be in favor for younger larvae by providing situation for feeding and energy reserve. Wandering larvae are the majority of population, so mild winters could reduce overwintering population of *S. ocellatella*. According to our observations, wandering larvae of *S. ocellatella* are the most important forms of developmental stages during the winter and are responsible for maintenance of pest population until the next growing season.

References

Atapour M. and Moharramipour S. 2009. Changes of cold hardiness, supercooling capacity, and major cryoprotectants in overwintering larvae of *Chilo suppressalis* (Lepidoptera: Pyralidae). Environmental Entomology, 38: 260-265.

Bale, J. S. 1989. Cold hardiness and overwintering survival of insects. Agricultural Zoology Reviews, 3: 157-192.

Baust, J. G. and Rojas, R. R. 1985. Insect cold hardiness: facts and fancy. Journal of Insect Physiology, 31: 755-759.

Block, W. 1991. To freeze or not to freeze? invertebrate survival of sub-zero temperatures. Functional Ecology, 5: 284-290.

Block, W. and Sømme, L. 1982. Cold hardiness of terrestrial mites at Signy Island, maritime Antarctic. Oikos, 38: 157-167.

Boardman, L., Grout, T. G., and Terblanche, J. S. 2012. False codling moth *Thaumatotibia leucotreta* (Lepidoptera, Tortricidae) larvae are chill-susceptible. Insect Science, 19 (3): 315-328.

Cannon, R. J. C. and Block, W. 1988. Cold tolerance of microarthropods. Biological Reviews, 63: 23-77.

Carrillo, M. A., Heimpel, G. E., Moon, R. D., Cannon, C. A. and Hutchison, W. D. 2005. Cold hardiness of *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae), a parasitoid of pyralid moths. Journal of Insect Physiology, 51: 759-768.

Chen, B. and Kang, L. 2005. Implication of pupal cold tolerance for the northern overwintering range limit of the leafminer *Liriomyza sativae* (Diptera: Agromyzidae) in China. Applied Entomology and Zoology, 40 (3): 437-446.

Coyle, D. R., Duman, J. G. and Raffa, K. F. 2011. Temporal and species variation in cold hardiness among invasive Rhizophagous

weevils (Coleoptera: Curculionidae) in a Northern Hardwood Forest. Annals of the Entomological Society of America, 104 (1): 59-67.

Denlinger, D. L. 1991. Relationship between cold hardiness and diapause. In: Lee, R. E. Jr. and Denlinger, D. L. (Eds.), Insects at Low Temperature. Chapman & Hall, New York, pp. 174-198.

Duman, J. G. 2001. Antifreeze and ice nucleator proteins in terrestrial arthropods. Annual Review of Physiology, 63: 327-357.

Duman, J. G., Wu, D. W., Xu, L., Tursman, D. and Olsen, T. M. 1991. Adaptations of insects to subzero temperatures. Quarterly Review of Biology, 66: 387-410.

Fields, P. G., Fleurat-Lessard, F., Lavenseau, L., Gérard, F., Peypelut, L. and Bonnot, G. 1998. The effect of cold acclimation and deacclimation on cold tolerance, trehalose and free amino acid levels in *Sitophilus granarius* and *Cryptolestes ferrugineus* (Coleoptera). Journal of Insect Physiology, 44: 955-965.

Gehrken, U., Stromme, A., Lundheim, R. and Zachariassen, K. E. 1991. Inoculative freezing in overwintering tenebrionid beetle, *Bolitophagus reticulatus* Panz. Journal of Insect Physiology, 37: 683-687.

Goto, M., Li, Y. P, Outani, S. and Suzuki, K. 2001. Cold hardiness in summer and winter diapause and post-diapause pupae of the cabbage armyworm, *Mamestra brassicae* L. under temperature acclimation. Journal of Insect Physiology, 47: 709-714.

Hiiesaar, K., Williams, I. H., Mänd, M., Luik, A., Jõgar, K., Metspalu, L., Švilponis, E., Ploomi, A. and Kivimägi, I. 2011. Supercooling ability and cold hardiness of the pollen beetle *Meligethes aeneus*. Entomologia Experimentalis et Applicata, 138 (2): 117-127.

Humble, L. M. and Ring, R. A. 1985. Inoculative freezing of a larval parasitoid within its host. Cryo-Letters, 6: 59-66.

Kheyri, M., Naiim, A., Fazeli, M., Djavan-Moghaddam, H. and Eghtedar, E. 1980. Some studies on *Scrobipalpa ocellatella* Boyd in Iran. Applied Entomology and Phytopathology, 48: 1-39 (In persian).

Klok, C. J. and Chown, S. L. 2005. Inertia in physiological traits: *Embryonopsis halticella* caterpillars (Yponomeutidae) across the Antarctic Polar Frontal Zone, Journal of Insect Physiology, 51 (1): 87-97.

Larsen, K. J. and Lee, R. E. Jr. 1994. Cold tolerance including rapid cold-hardening and inoculative freezing of fall migrant monarch butterflies in Ohio. Journal of Insect Physiology, 40: 859-864.

Lee, R. E. Jr. 1989. Insect cold-hardiness: to freeze or not to freeze. Bioscience, 39: 308-313.

Lee, R. E. Jr., Lee, M. R. and Strong-Gunderson, J. M. 1991. Biological control of insect pests using ice nucleating microorganism. In: Lee, R. E. Jr. and Denlinger, D. L. (Eds.), Insects at Low Temperature. Chapman & Hall, New York, pp. 257-269.

Lee, R. E. Jr., Lee, M. R. and Strong-Gunderson, J. M. 1993. Insect cold-hardiness and ice nucleating active microorganisms including their potential use for biological control. Journal of Insect Physiology, 39: 1-12.

Milonas, P. G. and Savopoulou-Soultani, M. 1999. Cold hardiness of diapause and non-diapause larvae of the summer fruit tortix, *Adoxophyes orana* (Lepidoptera: Tortricidae). European Journal of Entomology, 96: 183-187.

Mugnano, J. A., Lee, R. E. Jr. and Taylor, R. T. 1996. Fat body cells and calcium phosphate spherules induce ice nucleation in the freeze-tolerant larvae of the gall fly *Eurosta solidaginis* (Diptera, Tephritidae). Journal of Experimental Biology, 199: 465-471.

Nedved, O., 1993. Comparison of cold-hardiness in two ladybird beetles (Coleoptera, Coccinellidae) with contrasting hibernation behavior. European Journal of Entomology, 90: 465-470.

Parish, W. E. G. and Bale, J. S. 1990. The effect of feeding and gut contents on supercooling in larvae of *Pieris brassicae*. Cryo-letters, 11 (1): 67-74.

Saeidi, F., Moharramipour, S. and Barzegar, M. 2012. Seasonal patterns of cold hardiness and cryoprotectant profiles in *Brevicoryne brassicae* (Hemiptera: Aphididae). Environmental Entomology, 41: 1638-1643.

Salt, R. W. 1961. Principles of insect cold-hardiness. Annual Review of Entomology, 6: 55-74.

Shimada, K. and Riihimaa, A. 1988. Cold acclimation, inoculative freezing and slow cooling: essential factors contributing to the freeze tolerance in diapause larvae of *Chymomyza costata* (Diptera: Drosophilidae). Cryo Letters, 9: 5-10.

Sinclair, B. J., Klok, C. J., Scott, M. B., Terblanche, J. S. and Chown, S. L. 2003. Diurnal variation in supercoling points of three species of Collembola from Cape Hallett, Antarctica. Journal of Insect Physiology, 49: 1049.

Sinclair, B. J. and Chown, S. L. 2005. Deleterious effects of repeated cold exposure in a freeze-tolerant sub-Antarctic caterpillar. Journal of Experimental Biology, 208 (5): 869-879.

Soudi, S. and Moharramipour, S. 2011. Cold tolerance and supercooling capacity in overwintering adults of elm leaf beetle *Xanthogaleruca luteola* (Coleoptera: Chrysomelidae). Environmental Entomology, 40: 1546-1553.

Spicer, J. I. and Gaston, K. J. 1999. Physiological Diversity and Its Ecological Implications. Blackwell, Oxford, 241 pp.

SPSS Inc. 2009. PASW Statistics for Windows, Version 18.0. Chicago: SPSS Inc.

Sømme, L. 1982. Supercooling and winter survival in terrestrial arthropods. Comparative Biochemistry and Physiology A, 73: 519-543.

Sømme, L. 1999. The physiology of cold hardiness in terrestrial arthropods. European Journal of Entomology, 96: 1-10.

Steigerwald, K. A., Lee, M. R., Lee, R. E. Jr. and Marshall, J. C. 1995. Effect of biological ice nucleators on insect supercooling capacity varies with anatomic site of application. Journal of Insect Physiology, 41: 603-608.

Strong-Gunderson, J. M., Lee, R. E. Jr., Lee, M. R. and Tammy, J. R. 1990. Ingestion of ice-nucleating active bacteria increases the supercooling point of the lady beetle *Hippodamia convergens*. Journal of Insect Physiology, 36: 153-157.

Tsumuki, H., Konno, H., Maeda, T. and Okamoto, Y. 1992. An ice-nucleating active fungus isolated from the gut of the rice stem borer, Chilo suppressalis Walker (Lepidoptera: Pyralidae). Journal of Insect Physiology, 38: 119-125.

Vali, G. 1995. Principles of ice nucleation. In: Lee, R. E. Jr., Warren, G. J. and Gusta, L. V. (Eds.), Biological Ice Nucleation and Its Applications. American Phytopathological Society, St. Paul, Minnesota, pp. 1-28.

Worland, M. R. and Convey, P. 2001. Rapid cold hardening in antarctic microarthropods. Functional Ecology, 15: 515-524.

Worland, M. R. and Convey, P. 2008. The significance of the moult cycle to cold tolerance in the Antarctic collembolan *Cryptopygus antarcticus*. Journal of Insect Physiology, 54 (8): 1281-1285.

Zachariassen, K. E. 1985. Physiology of cold tolerance in insects. Physiological Review, 65: 799-832.

Zachariassen, K. E. and Hammel, H. T. 1976. Nucleating agents in the haemolymph of insects tolerant to freezing. Nature, 262: 285-287.

Zachariassen, K. E. and Kristiansen, E. 2000. Ice nucleation and antinucleation in nature. Cryobiology, 41: 257-279.

Zachariassen, K. E. and Kristiansen, E. 2003. What determines the strategy of cold-hardiness? Acta Societatis Zoologicae Bohemicae, 67: 51-58.

Zachariassen, K. E., Li, N. G., Laugsand, A. E., Kristiansen, E. and Pedersen, S. A. 2008. Is the strategy for cold hardiness in insects determined by their water balance? A study on two closely related families of beetles: Cerambycidae and Chrysomelidae. Journal of Comparative Physiology B, 178: 977-984.

Zhao, Z. 1997. Progress in the research on mechanism of insect cold-hardiness. Insect Science, 4: 265-276.

تغییرات نقطه انجماد در لاروهای زمستان‌گذران بید چغندر قند *Scrobipalpa ocellatella* (Lepidoptera: Gelechiidae)

زهرا گنجی و سعید محرمی‌پور

گروه حشره‌شناسی کشاورزی، دانشکده کشاورزی، دانشگاه تربیت مدرس، تهران، ایران
*پست الکترونیکی نویسنده مسئول مکاتبه: moharami@modares.ac.ir
دریافت: ۱۷ خرداد ۱۳۹۳؛ پذیرش: ۲۱ مهر ۱۳۹۳

چکیده: بید چغندر قند، *Scrobipalpa ocellatella* (Boyd) (Lepidoptera: Gelechiidae) یکی از مهم‌ترین آفات چغندر می‌باشد. این حشره زمستان را به‌صورت سنین مختلف لاروی در چغندرهای بجا مانده در مزرعه سپری می‌نماید. نقطه انجماد لاروها در محدوده گسترده‌ای از ۶- تا ۲۵- درجه سلسیوس قرار داشت. در این مطالعه، عوامل مؤثر بر نقطه انجماد لاروها مورد بررسی قرار گرفت. با القاء هسته یخ خارجی در لاروها، افزایش چشم‌گیری در نقطه انجماد آن‌ها مشاهده شد. تخلیه مایع دفاعی از دهان، موجب کاهش نقطه انجماد شد اما تأثیر معنی‌داری بر نقطه انجماد نداشت. وجود مقدار کافی غذا در دستگاه گوارش لاروهای جمع‌آوری شده از مزرعه، موجب افزایش مقدار نقطه انجماد شد. بنابراین، می‌توان نتیجه گرفت که وجود رطوبت در سطح بدن و ذرات غذایی در دستگاه گوارش، به‌عنوان عوامل مولد هسته یخ عمل نموده و موجب یخ زدن مایعات بدن در دماهای بالاتر می‌شود. علاوه بر این، لاروهای سن دوم و سوم به‌دلیل اندازه کوچکتر بدن در مقایسه با لاروهای سن پنجم، توانایی قابل ملاحظه‌ای در کاهش نقطه انجماد دارند. در نتیجه، تنوع زیاد در نقطه انجماد را می‌توان به تفاوت بین لاروهای زمستان‌گذران مربوط دانست که قابلیت فوق سرد شدن متفاوتی دارند.

واژگان کلیدی: بید چغندر قند، *Scrobipalpa ocellatella*، نقطه انجماد، ترشحات دفاعی از دهان، عوامل هسته یخ