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Seasonal Change of Cold Hardiness in the Codling Moth, *Cydia pomonella* (Lepidoptera: Tortricidae)

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Abstract: To elucidate the relationship between diapause and cold hardiness, seasonal variations of supercooling point and low temperature survival of overwintering and non-diapausing larvae of codling moth were studied in two regions of Iran, Damavand and Karaj. In both regions supercooling point decreased gradually over the autumn. Supercooling point of field collected larvae in Damavand reduced from a mean value of -16.9°C in early September to -19.9°C in mid-October 2004. At the same time, supercooling point of overwintering larvae decreased from -19 to -21°C in Karaj. Supercooling point decreased gradually until mid-winter (January) in both regions and afterward increased gradually over the spring. Mean supercooling point for non-diapausing larvae was -13.4°C, which was significantly higher than that of overwintering larvae. A high coincidence was observed between decrease of supercooling point and increase of low temperature survival rate in the overwintering larvae. Survival rate at -20°C/24 h was lower than 20% in early autumn; that increased to upper than 50% during winter. Codling moth was shown to be a freeze susceptible and at the same time, a chill tolerant insect.

Key words: *Cydia pomonella*, overwintering, diapause, cold hardiness, survival, supercooling

INTRODUCTION

Cold hardiness and diapause are essential components of winter survival for most insects in the temperate zone (Denlinger, 1991; Sømme, 1999). Cold-tolerance strategies of insects have generally been divided into two major categories. One group tolerates the formation of extra-cellular ice within the body, whereas, other species are freeze susceptible or freezing intolerant and avoid the lethal effects of freezing by lowering the temperature at which the spontaneous freezing of body water occurs. This value is termed the Supercooling Point (SCP) and is experimentally determined by detecting the released latent heat of fusion as body water freezes (Sømme, 1999). The insects survive sub-zero temperatures by the use of either one of these strategies (Storey and Storey, 1996; Sinclair, 1999), although several species are known to have switched between them (e.g., Horwath and Duman, 1984; Gehrken *et al*., 1991; Baghdadi *et al*., 2001). In many insects, SCP has been used as an index of cold hardiness.

Different observations were expressed on diapause and cold hardiness relationships. Although, in some insects it is believed that cold hardiness adaptations become due to the induction of diapause (Denlinger, 1991; Hodkova and Hodek, 1994), but adaptations, in which cold hardiness and diapause are not related, are as well recorded (Danks, 1987, 2000; Bale, 1996).

The first widely study on *Cydia pomonella* L. in various areas of Iran, was carried out from 1975 through 1978. In this research, emergence times and flight of moth and generation numbers were reviewed (Rajabi *et al*., 1978). Although cold hardiness strategy of other insects (sunn pest, *Eurygaster integriceps* Put. and its parasitoid *Phasia subcoleopterata* L.) was studied in Iran (Baghdadi *et al*., 2001; Baghdadi, 2006), but no study exist about cold hardiness and low temperature survival and their relationships with diapause in the codling moth in Iran. However understanding the relationships between the level of winter survival in pest insects and their subsequent abundance and hence damage potential, in the following spring and summer is very important. This research enabled a better insight particularly into the relation between diapause development and cold hardiness increase in codling moth.

MATERIALS AND METHODS

Study sites and field survey: In this study, samples of diapausing and non-diapausing larvae were taken from

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two regions of Iran, Damavand (35°45′N, 51°53′E; alt. 2400 m) and Karaj (35°55′N, 50°54′E; alt. 1300 m). The samples were collected every month from August 2004 to March 2005 and simultaneous changes in SCP and low temperature survival were recorded. Feeding non-diapausing larvae (third through fifth instars) were obtained from infested apples in August and natural diapausing larvae were collected during autumn and winter seasons from cardboard bands which were placed around the trunks of apple trees in early September.

Determination of supercooling points: Supercooling Point (SCP) of individual larva (n = 8 larvae in every month) was measured using a thermocouple (NiCr-Ni probe) attached to an automatic recorder (Testo, model 177-T4) so that, cooling period could be recorded at 0.5 min intervals and data was read using Comsoft 3 software. The specimens were affixed to the thermocouple with plastic glue tape and placed into a programmable refrigerated test chamber (Binder, model MK 53, Germany), whose temperature was lowered at a rate of 0.5°C min^{-1}, starting at 20°C and ending at -30°C. The SCP was represented on the recorder by a sudden spike in the temperature of the thermocouple.

Determination of low temperature survival: Overwintering larvae were monthly collected over the autumn and winter and were transferred (n = 20-25 in every temperature) to a programmable refrigerated test chamber, whose temperature was lowered at a rate of 0.5°C min^{-1}, starting at 20°C and ending at the desired treatment temperature (-15, -20 or -25°C). The larvae were held at these temperatures for 24 h, then slowly (0.5°C min^{-1}) raised to 25°C and held at that temperature for 24 h. The numbers of live and dead larvae were counted. The larvae showing no movement were judged to be dead.

Statistical analysis: Statistical analysis of the data was performed by the independent samples t-test or one-way analysis of variance (ANOVA) with Tukey's post-test using SPSS version 13.00 for Windows. SCP data were normalized by removing almost 5% of distant records. Normality of distribution was tested by Kolmogorov-Smirnov test, a nonparametric test for testing goodness-of-fit of data. For investigating of a supposed linear relationship between two variables, Pearson correlation test was used. The results were expressed as mean±SE and considered significantly different at $p<0.05$.

RESULTS

Seasonal variations of supercooling point and low temperature survival rate: Variations in supercooling points (SCPs) and low temperature survival of overwintering larvae collected in two regions, Damavand and Karaj, are shown in Fig. 1.

Damavand region: Feeding non-diapausing larvae were collected in August had SCPs from -8.2 to -16.9°C (mean = -13.43±0.9°C), regardless of the instars, that was significantly higher than that of overwintering larvae collected during autumn and winter seasons (t = -6.15, df = 39, $p<0.001$). Whole body SCPs of overwintering larvae of codling moth altered significantly from September through March (F6,42 = 3.87, $p<0.01$). The

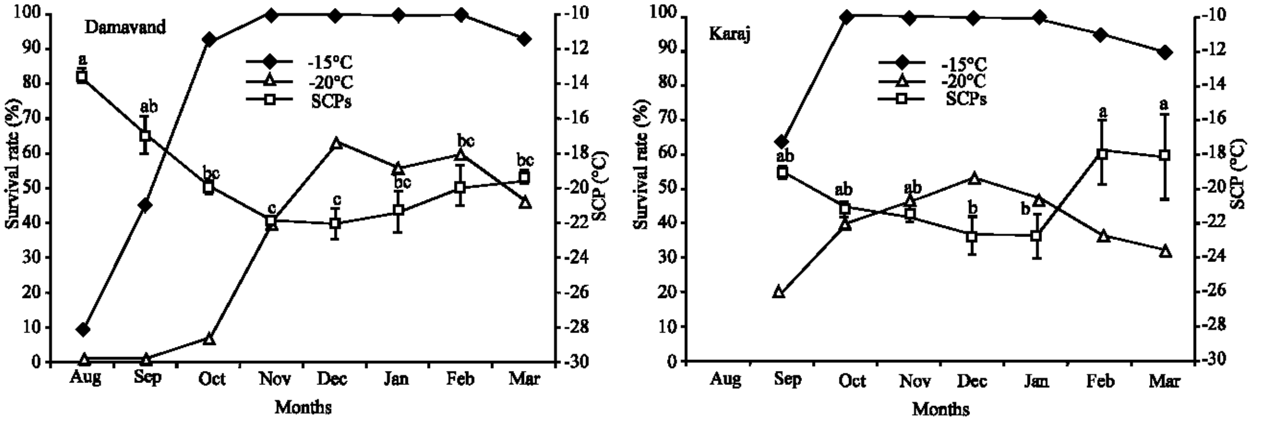

Fig. 1: Seasonal changes of supercooling point and low temperature survival rate of non-diapausing (August) and overwintering larvae of codling moth (September through March) in two regions of Iran, Damavand and Karaj in 2004-2005. Each value of spercooling points and survival rate represents the mean (±SE) of eight and twenty five samples, respectively. Different letters above the mean SCPs, indicate a significant difference ($p<0.05$) with the Tukey test after analysis of variance

mean (±SE) supercooling point of larvae were collected from apple fruits just before beginning of diapause was -16.9±1.1°C in early September 2004 that decreased to -19.9±0.35°C in overwintering larvae collected from cocoons in mid-October. Then supercooling point reduced during autumn and finally arrived to a minimum of -22.03±0.9°C in December. Afterward SCPs increased gradually during spring.

Cold hardiness (the ability to survive exposure to -15 and -20°C/24 h) increased with diapause development from September to December accompanied by decrease of SCPs. The survival rate at -15°C/24 h increased gradually from 45 to 93.1% during autumn. Survival at -15°C/24 h was lower than 10% during transition to diapause but increased to 63.3% in December. One day exposure to -20°C was successfully endured by diapause larvae (40-60% mortality), whilst this treatment caused 100% mortality of feeding non-diapausing larvae. No overwintering larvae were alive at -25°C/24 h (the temperature lower than SCPs) and in all cases insects were found dead after measuring of supercooling point. Although, a negative relationship was considerably existed between variations of SCP and survival rate at -20°C, but this correlation was not statistically significant ($r = -0.698$, p = NS).

Karaj region: Similar to Damavand region, whole body SCPs of overwintering larvae changed significantly from September through March ($F_{6,57} = 4.4$, $p<0.01$). The mean (±SE) supercooling point of overwintering larvae were collected from cocoons formed in cardboard bands, was -19.06±0.3°C at the beginning of diapause in early September 2004. Then supercooling point reduced during autumn and finally arrived to a minimum of -22.68±1.12°C in December. Afterward SCPs increased gradually during spring.

Similar to Damavand region, cold hardiness increased with diapause development from September to December. The survival rate at -15°C/24 h increased gradually from 64 to 100% during autumn. Survival at -20°C/24 h increased from 20% in September to a maximum of 63.3% in December. A significant negative correlation was observed between variations of SCP and survival rate at -20°C ($r = -0.957$, $p<0.05$; Fig. 1). Also a significant correlation was existed between variations of monthly mean SCPs in two regions ($r = 0.777$, $p<0.05$). Similar to Damavand region, no larvae stayed alive at -25°C/24 h during autumn and winter.

DISCUSSION

Instability of various parameters such as SCP and low temperature survival rate during the progressing cold season (September through December) indicated that overwintering larvae of codling moth could be able to increase their cold hardiness in the natural conditions. Gradual decreasing of temperatures in autumn resulted in continuing reduction of SCP and enhancement of cold hardiness that is evidenced by the survival at low temperatures.

Many freezing-intolerant insects may die during long or even brief exposures to temperatures above their SCP. Consequently, the real ecological value of the SCP remains ambiguous, but due to existing a good correlation between short-term (24 h) survival at subzero temperatures and the SCP in overwintering larvae of codling moth (Fig. 1), SCP may then serve as a good estimate of cold hardiness in this species.

The codling moth found to be a freezing intolerant insect since, the overwintering larvae, could not tolerate the temperatures lower than SCP (e.g., -25°C used in this study) and also died after measuring of SCP. Therefore *C. pomonella* does not survive freezing of its body fluids, i.e., it is freeze avoiding (freezing intolerant) according to the classes by Bale (1996). Death after measuring of SCP was reported in other freezing intolerant insects (Coulson and Bale, 1996; Jones and Kunz, 1997).

Food may be the source of ice nucleating agents (Lee *et al*., 1996) in codling moth, because SCPs of feeding non-diapausing larvae was significantly higher than diapausing larvae. Overwintering larvae of codling moth had a mean SCP about 9°C lower than non-diapausing larvae (-22 vs. -13°C), indicating a relation between cold hardiness and diapause. It seems development of cold hardiness in codling moth coincide with progress of diapause and accumulate of appreciable amounts of polyols (Minder *et al*., 1984; Neven, 1999; Khani *et al*., 2005) to decrease of SCP and avoiding of lethal freezing in the winter. This physiological event is similar to other freezing intolerant insects (Lee, 1991).

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