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Cold Hardiness Strategy of *Phasia subcoleopterata* L.

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Abstract: To investigate cold hardiness of *Phasia subcoleopterata* L., the supercooling point of pupa during November to March 2005-2006 measured on samples collected from altitudes of Ghara-ghaj Varamin-Iran. The supercooling point during February was lowest, being about -15.3±1.38°C. With considering to minimum temperature in natural condition (-7°C in February), pupa use Freeze intolerant strategy for cold hardiness. Furthermore, supercooling points of different weight and size were not significantly different.

Key words: *Phasia subcoleopterata* L., cold hardiness, supercooling point, over wintering

INTRODUCTION

To survive extremely low temperature in winter, insects may use one of two strategies :1) The ability to avoid of freezing on exposure to low temperatures by supercooling to considerable degree (Freeze intolerant or freeze avoidance) and 2) survival with actual ice formation within the body (freeze tolerant) (Salt, 1961; Asahina, 1969; Voituron *et al*., 2002). But possible dual cold hardiness strategies reported in *cisseps fulvicollis* (Lepidoptera: Arctiidae) (Fields and McNeil, 1986) and in *Eurygaster integriceps* put (Baghdadi *et al*., 2001).

Phasia subcoleopterata is one of important adult endoparasitoids of sunn pest (*E. integriceps*), as most important pest of wheat and barley in Iran. This species leave adult sunn pest in September and pupate in microhabitat of host under different plant especially *Astragalus* sp. Also they spend the autumn and winter in mountains as pupa. Adults emerge in the late March and begin to migrate to fields. The female lay eggs on margins of pronotum of adult host and newly hatch larvae penetrate and feed on throughout larval development. Last instars larvae pupate in soil and then adult emerge in late May. The female of new generation laid eggs on new adult host. Therefore they have 2 generations per year.

The present study was conducted to elucidate the cold hardiness mechanism of *P. subcoleopterata.*

MATERIALS AND METHODS

Pupa was collected in mountains of Ghara-ghaj located in the central part of Iran from November to March 2005-2006. In mountain, pupa located under different plant species was sampled from altitude 2100 and 2250 m from sea level at November to March. Recording of daily temperature and relative humidity was carried out regularly at all months of the late autumn and winter in mountain by placing a thermohygrograph under *Asteragalus* sp. in altitude 2250 m where specimens collected.

Determination of supercooling point: The measurement of supercooling point method (Somme, 1964). Each insect was placed in contact with a thermocouple which connected to two-channel temperature recorder (Model Testo 650). The tip of thermocouple was kept in a fixed position on surface body of insect located inside a small glass tube. The tube was placed inside another glass tube and then cooled in a freezer (-40°C). Specimens were cooled gradually at 1°C min^{-1}. In supercooling point determining the thermocouple showed a rapid increase temperature which caused of releasing fusion latent heat.

RESULTS

Minimum temperature under natural condition from November to next March 2005-2006 is shown in Fig. 1. Minimum temperature was observed -7°C in February 2006. Average relative humidity being around 50% and relatively constant.

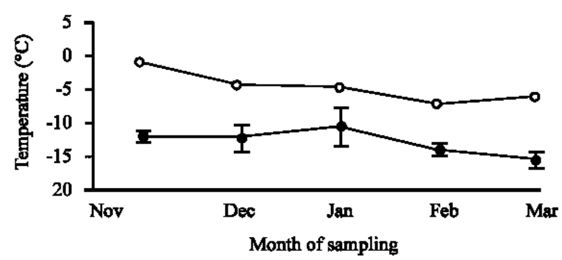

Fig.1: Change of the supercooling point (solid circle) of pupa *P. subcolopterata* from November to March and minimum temperature (open circle) of natural condition in altitude of 2250 m Gahra-ghaj Varamin Iran. Bars represent SE of mean

Table 1: The supercooling point of pupa *P. subcoleopterata* in two altitudes during January 2006, Ghara-ghaj Varamin-Iran

Altitude (m)	Crystallization temperature (°C)	Weight (g)	Size (cm)
2100	-11.62±0.962	0.032±0.002	0.63±0.044
2250	-10.45±2.89	0.036±0.002	0.063±0.047

All values are means±SE

Table 2: The supercooling point of pupa *P. subcoleoptera* in altitude 2250 m and different weight and size November to March of 2005-2006. Ghara-ghaj Varamin- Iran

Altitude (m)	Month	Supercooling point (°C)	Weight (g)	Size (cm)
2250	Dec	-12.2±1.91(12)	0.033±0.0017(12)	0.65±0.057(9)
2250	Jan	-10.45±2.89(14)	0.036±0.002(14)	0.063±0.047(13)
2250	Feb	-13.72±0.88(11)	0.032±0.0031(11)	0.62±0.047(11)
2250	Mar	-15.36±1.38(16)	0.036±0.005(16)	0.64±0.516(8)

All values are means±SE

The supercooling point of pupa during November to March 2005-2006 in 2250 m (from sea level) is shown in Fig. 1. The supercooling point was -15.3±1.38°C which observed at lowest degree during February 2006.

The supercooling point of pupa *P. subcoleopterata* in two altitudes (2100 and 2250 m) from January was studied (Table 1). There was no difference between 2250 m (-10.45±2.89°C) and 2100 m (-11.62±0.962°C).

The supercooling point of different weight and size of pupa were measured during late autumn and winter in altitude of 2250 m (Table 2). Weight and size showed no significant correlation with supercooling point ($r = 0.07$ and $r = -0.02$, respectively in 100 sample). Therefore results indicate that these factors may not affected on pupa supercooling point.

DISCUSSION

This is the first report about cold hardiness on *P. subcoleopterata*. Since supercooling point about 10°C lower from minimum temperature of natural conditions (Fig. 1). Then cold hardiness of *P. subcoleopterata* described as a freeze-intolerant. In terms of energetic cost and benefits, the two strategies are strongly different from each others (Block, 1991; Joanisse and Storey, 1996; Voituron *et al*., 2002). For instance, the maintenance of metabolic functions is more costly in supercooled state than in living frozen tissues. Probably, it appears pupa can be better survival in natural conditions.

We design experiment with considering population size of pupa of *P. subcoleopterata* in different altitudes and increasing with altitude of Ghara-ghaj mountains. The results showed no differences between two altitudes (Table 1). Therefore altitude could not affect on supercooling point.

With respect to size trends in supercooling capacity among insects are consistent with the effect of varying water volume in physical studies. In small arthropods, such as collembola, mites and ants as well as insect eggs, supercool to lower temperatures than large ones (Somme, 1982). This trend also holds true intraspecifically. In larvae of mealworm *Tenebrio molitor*, the supercooling point is significantly correlated with weight (Johnston and Lee, 1990). For investigation of effect weight and size, we select pupa of *P. subcoleopterata* with different weight and size and measure supercooling point. Since Table 1 show no difference is occurred and probably some internal events be equal in different weights and size.

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