Agronomy Research **7**(Special issue I), 328–334, 2009

Physiology of a carabid beetle *Platynus assimilis*

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Abstract. Predacious carabid beetle *Platynus assimilis* Paykull (Coleoptera: Carabidae) is a fast-moving insect which should be considered as an important component of biological control in organic farming. In this study we tested some factors of potentially dangerous influence of pesticide Fastac (synthetic pyrethroid) on overwintering physiology of adult ground beetle under laboratory conditions. Cold-hardiness (measured by supercooling point SCP) was determined 2 weeks after exposure to pesticide treated-food. Pesticide had decreased cold-hardiness of the ground beetles. Weak supercooling capacity could be harmful to overwintering insects in cold winters without snow cover.

Key words: *Platynus assimilis*, overwintering, respiration, alpha-cypermethrin

INTRODUCTION

Carabid beetles (Coleoptera: Carabidae) are species rich and abundant in arable habitats. Polyphagous carabid adults and larvae are important natural pest-control agents known to feed also on agricultural pests. Their food list contains a wide range of aphids, dipteran eggs, larvae and pupae, eggs and larval stages of the Colorado potato beetle *Leptinotarsa decemlineata*, slugs (Kromp, 1999). Carabids react sensitively to anthropogenic changes in habitat quality and are also affected by intensive agricultural cultivation. They can be affected by deep ploughing as well as by crop treatment with pesticides (Kromp, 1999). In organic farms it isn't allowed to use pesticides, but land-use in neighbouring conventional farms presents a probable risk. Carabid beetles may feed on chemically treated plants or pests after which they can move relatively fast to the organic fields because beetles long legs allow them to walk or run quickly on the soil surface (Kromp, 1999). So, the pesticide applications in conventional farms may cause ecological damage also in organic farms by killing beneficial organisms. Pyretroids are contact insecticides that have a broad and a long-lasting effect and are poisonous for almost all pollinating insects (Barten at al., 2006). Pyrethroids may bioconcentrate through the food web (Solomon et al., 2001). Pyrethroid Fastac (active substance alpha-cypermethrin) is a widely used insecticide in Estonian agriculture. The chemical compound is a structural analog of chrysanthemum plant pyrethrins, which are permitted for use in organic farming (Coats et al., 1989). This pesticide is highly toxic to insects and aquatic organisms (Muller-Beilschmith, 1990; Solomon et al.,

2001; Karise, 2007) but have relatively low toxicity to terrestrial vertebrates (Solomon et al., 2001; Yarkov et al., 2003). The influence of pyrethroids on carabids physiology (inclusively overwintering physiology) has not been studied yet.

Adults of the ground beetle *Platynus assimilis* (Paykull), is a night-active on soil surface dwelling beetle that is a well known common predator in agricultural fields (Thiele, 1977; Lövei & Sunderland, 1996), were chosen for test object in our experiment. The aim of current investigation was to assess the influence of Fastac on adult of *P. assimilis* under laboratory conditions. The poisoning effect assessment criteria were cold-hardiness evaluated by supercooling point (SCP), weight and respiration.

MATERIALS AND METHODS

Insects. Adults of *P. assimilis* were collected from the Tartu County, Estonia, in their hibernating sites – tree stumps. The tests were carried out in January 2009.

Chemical substance. Fastac 50 is a commercial formulation of alpha-cypermethrin (a.i. 50g l^{-1}). In the study, 0.15%, dose was used according to the recommendations for field spraying.

Laboratory tests. Test material collected from hibernating sites (52 individuals in total) was separated into two parts: treatment (24 individuals: 13 female and 11 male beetles) and control (28 individuals: 13 female and 15 male beetles). Beetles were weighed twice: before the treatment and at the end of study (14^{th} day). Alpha-cypermethrin was diluted in distilled water by dose recommendations. The beetles were fed with cat food (Friskies Junior-1 Purina) every fourth day. Feeding the laboratory-reared ground beetles by cat food is suggested also by Tréfás et al. (2001) and Ploomi (2004). For the insecticide exposure, food pieces were dipped in the emulsion of alpha-cypermethrin (treated) or in the distilled water (control group) for 10 seconds. The experiment lasted 14 days, while individual beetles were kept in plastic boxes (0.5 l) on wet tissue at room temperature (22±1°C).

Respiration rate of *P. assimilis* was measured on the first day before treatment and 2 days after the last feeding (15^{th} day after the onset of the experiment). An infrared gas analyser or IRGA (Infralyt-4. VEB, Junkalor, Dessau) adapted for entomological research (Kuusik et al. 2001, 2004; Metspalu et al. 2001) was used to record the bursts of carbon dioxide release. By means of IRGA it has been proven, that the presumed CO_2 signals, i.e. the downward peaks on the recording of the electrolytic respirometer, were actually due to CO_2 bursts, and the instrument was used to measure the respiration level quantitatively. This flow-through respirometer was calibrated at different flow rates by means of calibration gases (Trägergase, VEB, Junkalor, Dessau), with gas injection. Air flow rate was commonly 3.6 l per h, by which the rate of carbon dioxide release was measured (VCO_2 ml h^{-1}). The flow-through respirometry combined with an infrared optical device referred to as infrared actograph has commonly been used' as an insect IR cardiograph (Hetz et al. 1999, Kuusik et al. 2001) or optocardiographic method (Slama 2001). Two IR-emitting diodes (TSA6203) were placed on one side of the insect chamber, and two IR sensor diodes (BP104) were placed on the opposite side. The light from the IR-diode was modulated by the abdominal contractions. The level of output voltage reflected the vigour of the

muscular contractions of the insect. CO_2 releases and abdominal movements were measured on *P. assimilis* before and after the treatment.

For measuring supercooling points (SCP) ground beetles were anesthezised by ether. The beetles were positioned so that its integument (thoracic tergit) was contacted with the copper-constantan thermocouple, placed in a glass vial, and then transferred to the circulator bath (Ministat 230w-2, Huber, –33°C to +200°C). SCP was determined using a 0.5 C min^{-1} cooling rate. The temperature was registered and saved by a data logger (Almemo 2890-9, Ahlborn).

Data acquisition and statistics. Computerised data acquisition and analysis were performed using an analog-to-digital converter and TestPoint software with 10 Hz sampling rate (DAS 1401, Keithley-Metrabyte). The CO_2 rate was automatically calculated by averaging data over a period involving at least 12 cycles of gas exchange.

Means, standard error and standard deviations are reported. Tests were performed using a statistic package StatSoft ver.8, Inc/USA. Means were compared by Student's *t*-test and Wilcoxon Matched Pairs Test.

RESULTS AND DISCUSSION

The results of the current study demonstrated the influence of the treatment by insecticide Fastac on the physiology of *P. assimilis*.

Body mass. The measured body masses showed the feeding activity during the study period. If the mean of the body mass of the control group in the beginning of study was 0.038 ± 0.002 g, then in the end of the study it was 0.042 ± 0.003 g (Fig. 1). The body mass of the control group of the beetles increased significantly ($P < 0.05$). On the other hand, the average body mass of carabid beetles of the treated group in the end of the test remained on the same rate like it was in the beginning of the experiment (0.040 ± 0.002 g).

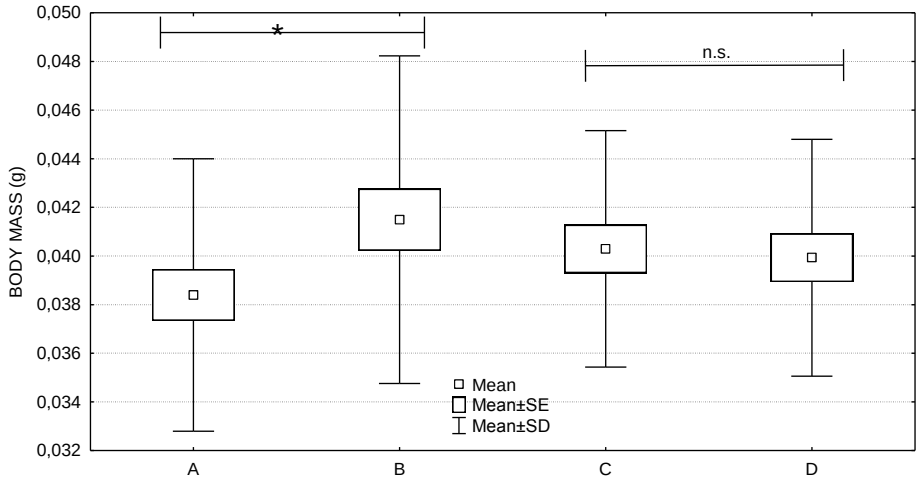

Fig. 1. The influence of treatment on body mass of the ground beetles *P. assimilis.* A - the body mass of control group before feeding; B- the body mass of control group after feeding; C- the body mass of treatment before feeding; D- the body mass of treatment after feeding. Asterisk indicates the significant difference (*Wilcoxon Matched Pairs Test*, $P < 0.05$, $n = 28$ (on control group); $n = 24$ (on treated group).

Although the voltage-sensitive sodium channel is likely to be the principal site of pyrethroid action, it's probably not the only target for insect-selective neurotoxins. Insect neurosecretory neurons are sensitive to very low concentrations of pyrethroids, and disruption of the neuroendocrine system has been involved as a factor contributing to the irreversible effects of pyrethroids in insects (Soderlund & Bloomquist, 1989). All organisms generate energy from the food they eat. Alfa-cypermetrin probably inhibits or disrupts energy production: while the beetle can eat and digest food after being poisoned, it cannot produce energy from food. Eventually, the insect stops eating and sometimes even moving (Brown, 2006). Lack of the increase of body mass on treated group is also explained with transpiration water loss on toxicated insects (Kuusik et al., 1995).

Respiration. *P. assimilis* respiration rate measured before (Fig. 2) and after the treatment (Fig. 3) demonstrate clearly the differences between treatments by alpha-cypermethrin. The simultaneous recording of the flow-through CO_2 respirometer and infrared actograph showed in insects (Fig. 2) regular muscular contractions due to active continuous ventilation (upper trace) and the sinusoidal weak cyclic release of CO_2 (lower trace) (lower trace) in the resting state before the treatment (Fig. 2). After the treatment by alpha-cypermethrin insects had greater mean rate of carbon dioxide outbursts (Fig. 3) and constant cyclic CO_2.release was disappeared. Treatment with alpha-cypermethrin caused irregular muscular contractions of *P. assimilis*.The respirometry and IR actography revelated that adult *P. assimilis* has relatively high expenditure of metabolic energy during the the irregular muscular contractions.

After the treatment with alfa-cypermetrin, a typical symptom of influence of this pyrethroid on carabide beetles was paralyse after 2–3 days. Pyrethroids are nerve poisons that effect nerve axon. The main target site is neuronal sodium channels and it increases sodium entry into the nerve cell and induces depolarization of the nerve membrane and blocks of nerve conduction. The normal function of the nervous system is affected, stimulating repetitive nerve discharges leading to paralysis. The paralysis is often preceded by spastic activity of the organism due to the hyper-activity of nerve endings. The spastic activity is caused by sodium channels repeatedly polarizing and depolarizing, mimicking neuro-transmission where none is actually taking place (Vijverberg & van den Bercken, 1990).

Supercooling point (SCP). The study demonstrated that the mean SCP on treated group was higher than control group (see Fig. 4). Control group mean SCP was –5.5°C and treated group mean SCP was –5°C, which is statistically significant (Student *t*-test; t = -2.63253, $df = 50$; $P < 0.05$). The first toxic effect on the treatment group by neurotoxicants is the dehydration on their bodies. The neurotoxicant caused faster excretion of body fluids from digestive system or water transpiration which increases SCP (Kuusik et al., 1995). By comparing treated group versus control, insects in treated group displayed larger variance in SCP. Its shows that some insects from treated group are more vulnerable than others, for some reasons (illness, certain winter damages etc.). On the other hand, pyrethroids cause extensive damage to haemolymph (Saleem et al., 1998), which may increase the SCP. Pyrethroids can affect other biochemical changes, for instance level of glucose and trehalose (M'diaye & Bounias, 1993).

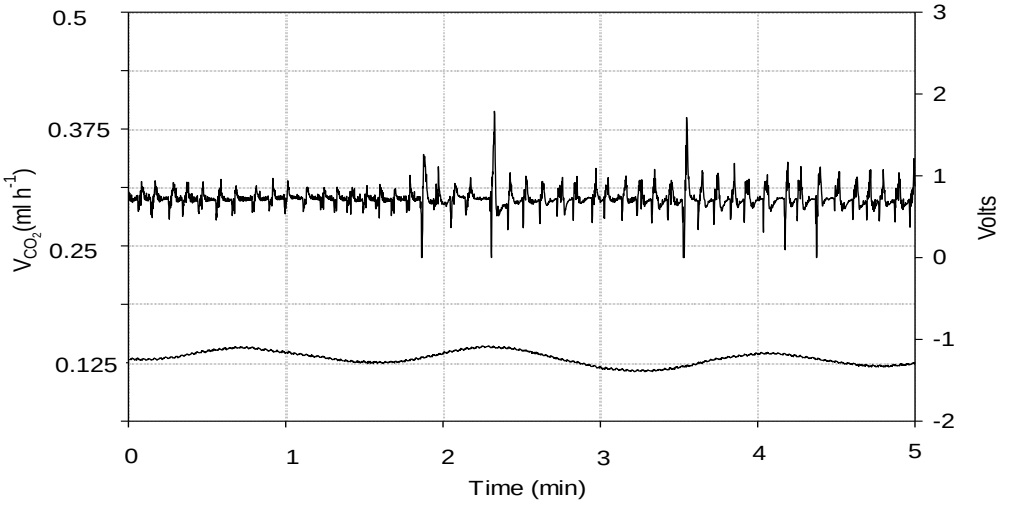

Fig. 2. A simultaneous recording of infrared actograph (upper trace, Volts) and infrared gas analyser (lower trace) representing active and regular tracheal ventilation (upper trace) and weak sinusoidal curve due to cyclic release of CO_2 in adults *P. assimilis* before treatment with alpha-cypermethrin.

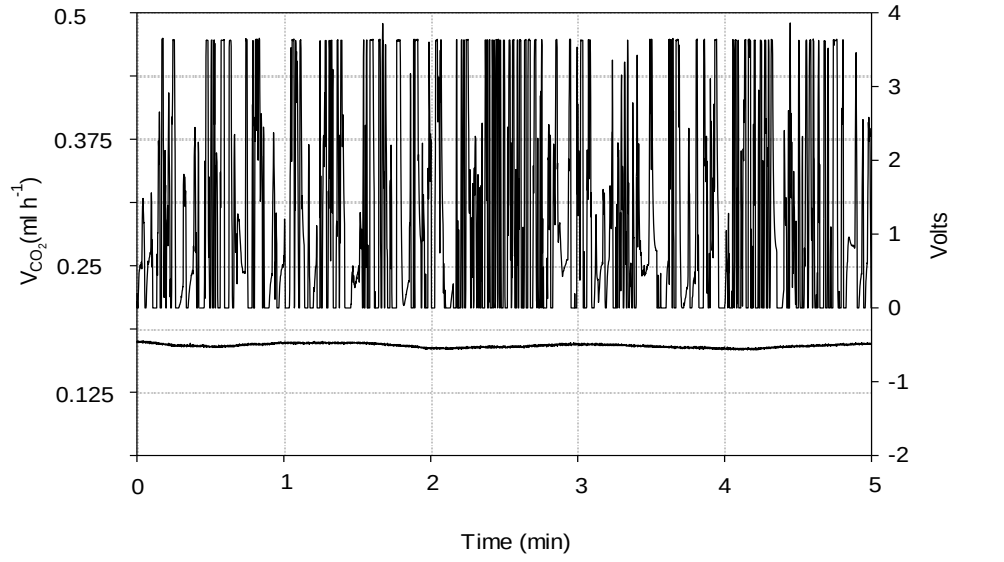

Fig. 3. A simultaneous recording of infrared actograph (upper trace, Volts) and infrared gas analyser representing a continuous release of CO_2 without cyclicity (lower trace) in adults *P. assimilis* after treatment with alpha-cypermethrin. Note that the irregular pattern of muscular contractions due to locomotor activity (upper trace).

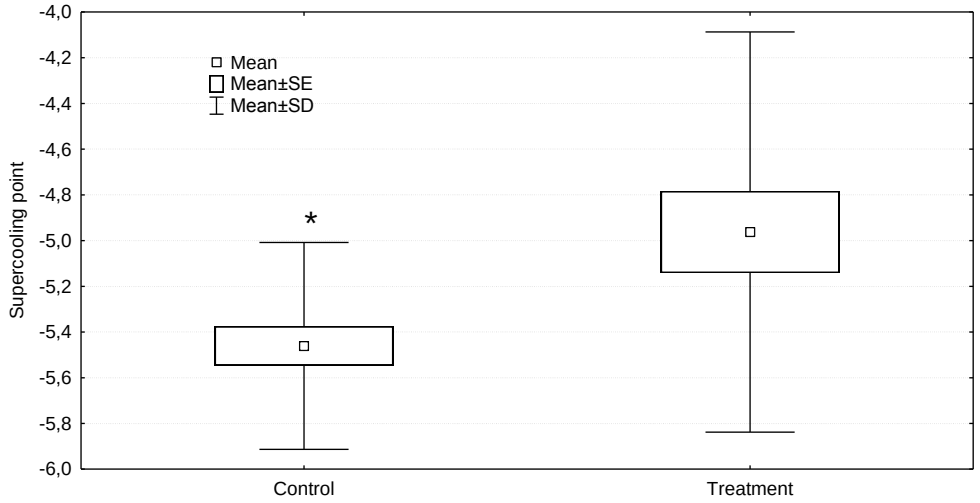

Fig. 4. The influence of the treatment on supercooling points of *P. assimilis.* Asterisk indicates the significant difference (Student *t*-test; $P < 0.05$, $n = 28$ (on control group); $n = 24$ (on treated group).

CONCLUSIONS

Alpha-cypermethrin affected normal function of the nervous system and caused increasing metabolic rate. The carabid beetle respond to the treatment with alpha-cypermethrin, showing paralyze on the second and third day after the treatment. Reduced mobility means that the beetles are less able to migrate from pesticide-treated fields and thus are exposed to potentially lethal effects for longer periods. The respiration and transpiration systems are the vulnerable targets of the alpha-cypermethrin used on the exposed carabids.

ACKNOWLEDGEMENTS. This research was supported by the Estonian Science Foundation grants No. 7130, 6722, 6781 and Estonian Ministry of Education and Research targeted financing projects No. SF170057s09, SF0170014s08 and SF0170021s08.

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