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Studies on insect growth regulating (IGR) and toxic effects of *Ledum palustre* extracts on *Tenebrio molitor* pupae (*Coleoptera*, *Tenebrionidae*) using calorimetric recordings ☆

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Abstract

Insect growth regulating (IGR) and toxic effects evoked by *Ledum palustre* extracts on the metamorphosis stages of *Tenebrio molitor* were studied using differential thermocouple calorimeters. The timing of normal and failed ecdysis as well as the length of interecdysial periods were measured exactly from calorimetric recordings. Respiratory and muscular responses of poisoned insects were also reflected by characteristic patterns on the recordings.

Keywords: Calorimetry; Ecdysis; *Ledum*; Metabolism; Pupa; *Tenebrio*

1. Introduction

Calorimetric methods may prove quite useful in continuous monitoring of the several different developmental events that occur throughout the metamorphosis of individual insects. The moving activity pattern during this process in several insect

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species has been investigated using direct calorimetry [1,2]. Other studies have demonstrated a good coincidence between direct and indirect calorimetric measurements during the metamorphosis stages of holometabolous insects [3-6].

It was shown that even simple ("poor man's") calorimetric equipment can be applied to studying thermogenesis in small animals [7]. A simple differential calorimeter has been used to examine the periodicity of rhythmic abdominal muscular microcontractions that could barely be perceived by the naked eye [8]. Using the same method, the intermittent release of CO_2 was established for some small insects [6,8].

The aim of the present report is to study how insect growth regulating (IGR) and, at the same time, toxic effects are reflected on the recordings of differential thermocouple calorimeters. To induce morphogenetic failures and toxic effects, insects were treated with the extract of the plant *Ledum palustre* possessing both IGR activity and toxic properties.

2. Materials and methods

2.1. Plant extracts and procedures

Extracts of *Ledum palustre* were used for studying their physiological effects on pupae. The plants were collected from the vicinity of Tartu (Estonia).

Shade-dried leaves and flowers were ground into a fine powder and extracted for 24 h using ethanol (80%) as solvent. The extract was then filtered and dried to a paste under vacuum at 80°C. For use, it was diluted in water to various concentrations (weight/volume).

2.2. Animals and treatments

Yellow mealworm (*Tenebrio molitor* L.) was reared in Petri dishes on flour with the addition of 1% dried yeast, at $70 \pm 5\%$ RH. The population was kept at 25°C under 12 h light each day. Male and female pupae were sampled separately. Their age was determined with regard to larval-pupal ecdysis. Easily excitable individuals were eliminated from experiments by actographic pretreatment tests as previously described [8].

The treatments were made 12-16 h before larval-pupal ecdysis by dipping the pharate pupae in water solutions of extracts for 30 and/or 60 s. Reference insects were submerged in water for the same times.

2.3. Calorimetric measurements

Six differential thermocouple (copper-constantan) calorimeters with micronanovoltmeters and recorders were used, applying the principle of differential thermal analysis [9]. Two equal, cylindrical reference and animal boxes, made of copper foil (thickness 0.2 mm), were connected with a constantan wire (∅, 0.1 mm). The volume of each box was 0.25 ml. They were sufficiently spacious for the pupa to perform body movements. Calibration of the calorimeters was performed empirically [6] by means of a furnace spiral of known resistance (15–20 Ω) placed inside the boxes. During measurements, the calorimeter was exposed in a thermostat (30 ± 0.1°C) inside a 1 l thermos flask. Such a simple self-made calorimeter was sufficiently sensitive (0.02 μV μW^{-1}) for recording not only periods of muscular microcontractions but also peaks of intermittent CO_2 emission from the tracheae of *Tenebrio molitor* pupae. The reactiveness of the calorimeter was demonstrated by simultaneous recordings of cyclic CO_2 release from a respirometer-calorimeter [6].

3. Results

At the moment of breaking the old exoskeleton during the process of ecdysis, an amount of exuvial fluid is liberated and thus a "cooling" peak is noted (not due to exothermic reaction) on calorimetric recordings (Fig. 1). The moment of breaking the old cuticle along ecdysial sutures is regarded as the time of ecdysis. The whole process of shedding the old exoskeleton commonly lasts 5–10 min. Immediately before the cooling peak, a sharp upward peak (endothermic) is recorded, reflecting vigorous peristaltic body movements (Fig. 1) for breaking the old skeleton.

The extract of *L. palustre* evoked various morphogenetic effects depending on the time of treatment. When the pharate pupae of *T. molitor* (with lateral abdominal spines becoming visible through the cuticle) were treated, perfect larval-pupal ecdysis (Fig. 1) and late partial ecdysis (with only the anterior part of the body being shed) into extra-pupal instars were common events (Fig. 2). Usually these partial ecdyses were recorded as downward and relatively short peaks.

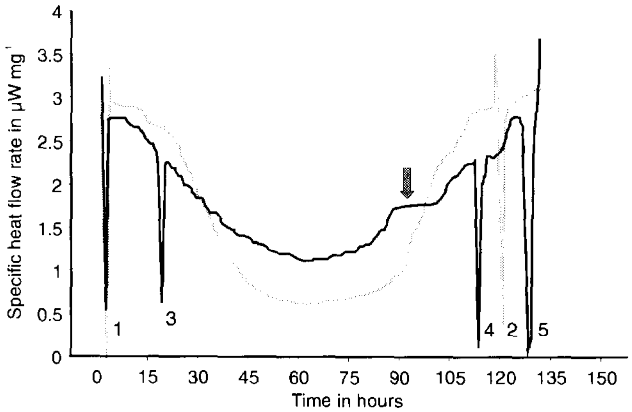

Fig. 1. Metabolic curves (DTA measurements) of normal pupal development of *Tenebrio molitor* (dotted line) and of inhibited metamorphosis (solid line) due to treatments (dipping pharate pupa for 30 s in WE of *Ledum palustre*). Downward peaks result from liberated exuvial fluid: 1, perfect larval-pupal ecdysis (E); 2, pupal-adult ecdysis (E); 3, leaking of exuvial fluid; 4 and 5, partial ecdysis into extra-pupal instars. Upward peaks denote muscular hyperactivity prior to ecdysis.

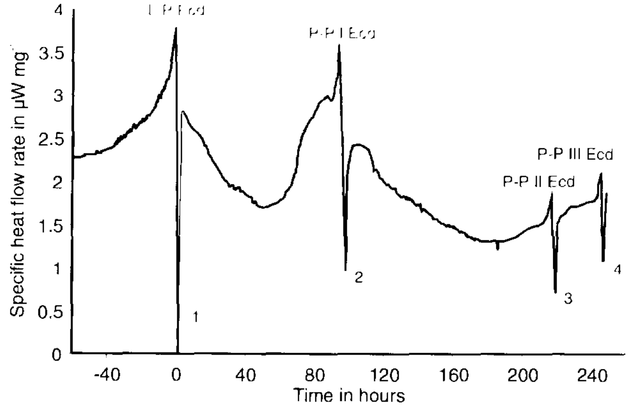

Fig. 2. Metabolic curves (DTA measurements) of *Tenebrio molitor* metamorphosis affected by treatment: 1, normal larval-pupal ecdysis (E); 2, perfect pupal-pupal E; 3 and 4, partial E into extra-pupal instars. For the significance of other peaks, see Fig. 1.

A part (approx. 10%) of the treated pharate pupae underwent a perfect larval-pupal ecdysis and a subsequent perfect pupal-pupal ecdysis. As seen from Figs. 1 and 2, a U-shaped metabolic curve is represented in the case of normal pharate adult development as well as during transformation into extra-pupal instars. Yet the lowest level of basal metabolism during affected transformation remained on a considerably higher level when compared with the normal metabolic curve (Fig. 1). The interecdysial period between larval-pupal and subsequent pupal-pupal ecdysis was always 1–1.5 days shorter than normal. A characteristic metabolic plateau (Fig. 1) existed before ecdysis into the extra-pupal instar (Fig. 2).

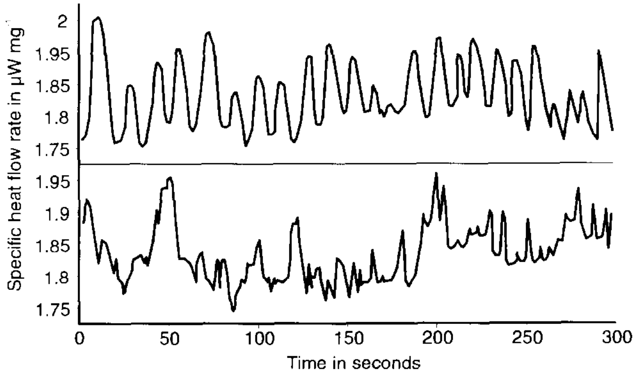

Fig. 3. Above, calorimetric recordings: upward (endothermic) peaks are due to bouts of rhythmic pupal abdominal movements of *Tenebrio molitor* (bendings or coelopulses); on the baseline, the sharp peaks of CO_2 bursts are seen. Below, lengthening of muscular activity periods (bouts) and shortening of interbout periods; cyclic emission of CO_2 from tracheae is irregular.

As the earliest indicators of subsequent ecdysial failures, one or two short cooling peaks were often observed soon after perfect larval-pupal ecdysis (Fig. 3).

As well as the morphogenetic failures, non-lethal mild toxic effects were induced by *L. palustre* extracts. Weak bending (WB) movements in pupae were transformed into vigorous bending movements while the bouts of movements lengthened (Fig. 3).

4. Discussion

The extracts of *L. palustre* produced a wide spectrum of morphogenetic effects in *T. molitor* depending on the time of treatments. The treated pharate pupae transformed into extra-pupal instars, which is a symptom of a juvenilizing effect. When treatments were performed at the beginning of ocellar retraction then prothetelic intermediates often resulted (our unpublished data). At the present time it is well known that there are several mechanisms of inducing morphogenetic failures in insects. The moulting programme may be affected via the neuroendocrine system which controls morphogenetic hormones, as was suggested in case of IGR action of extracts from the neem tree (*Azadirachta indica*) [10].

By means of calorimetric recordings, the course of basal metabolism can easily be compared in normal and affected pupae (Fig. 4). The flattening of the U-shaped metabolic curve reflects adequate morphological changes during transformation [11]. The significance of a clear plateau level prior to the extra-pupal moults has yet to be explained.

The main features of the mild poisoning in pupae was the lengthening of WB periods and strengthening of these movements. The bending movements, caused by microcontractions of abdominal intersegmental longitudinal muscles, at the same time cause pulsations in hemolymph pressure. The hemolymph pressure pulsations were described by Slama [12] and Slama et al. [13] as autonomic extracardiac

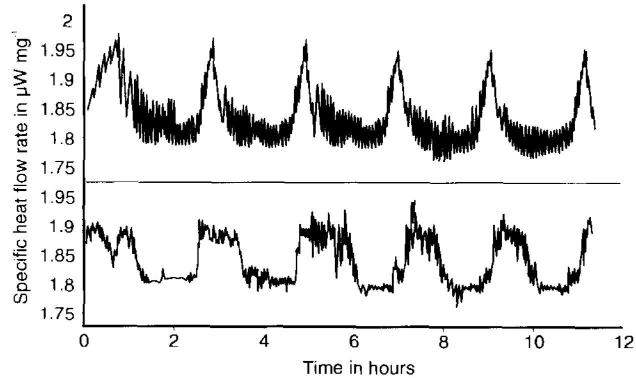

Fig. 4. A section of the interbout period (see Fig. 3) in an untreated pupa (above) and a mildly poisoned one (below). Clear endothermic peaks (above) result from CO_2 burst from trachea.

pulsations (coelopulses) in several insect species. The frequency and periodicity of coelopulses are widely influenced by toxic and hormonally active substances [14–16]. It is remarkable that despite the lengthening of bending bouts (a series of muscular microcontractions 40–80 per min) the general periodicity of bouts remained almost unchanged (Fig. 4), while interbout periods had shortened. The persistence of normal periodicity of coelopulses in poisoned insects obviously indicates the involvement of a slow oscillator that regulates the periodicity of bending bouts. According to Miller [17] slow oscillators may act in a variety of behaviour patterns to gate repetitive activity.

A typical symptom of mild poisoning in *T. molitor* pupae was the disappearance of the clear periods of intermittent CO_2 release. The same symptom of weak toxicosis was revealed in the pupae of *Galleria mellonella* [8]. It seems very likely that the gas exchange of both species is closely related to the so-called Prague cycles which are autonomically controlled by nervous centres located in the thoracic ganglia [18]. These cycles are characterized by short intervals (less than 5 min) between CO_2 bursts. The simultaneous disturbances in bending rhythms (coelopulses) and gas exchange rhythms in poisoned pupae of *T. molitor* and *G. mellonella* indicate a peculiarity of the autonomic nervous control of these rhythms.

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