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# Calorimetric investigations on physiological stress in *Tenebrio molitor* (Coleoptera, Tenebrionidae) pupae[1]

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**Abstract**

Individual variation in the susceptibility to handling stress (mechanical irritation) in laboratory-reared pupae of yellow mealworm, *Tenebrio molitor*, was studied by means of simple thermocouple twin set-up calorimeters.

The stress condition was characterised by disturbances in normal rhythms of gas exchange and body stereotyped movements well distinguishable from calorimetric recordings. Recovering time from stress, induced by the device, was 10–15 min.

In every newly established population, a portion of pupal individuals (13–15%) was highly sensitive to handling and these pupae lost significantly more water than the pupae exhibiting no symptoms of stress. © 1998 Elsevier Science B.V.

*Keywords:* Direct calorimetry; Gas exchange; Insects; Respirometry; Standard metabolic rate; *Tenebrio*; Water loss

## 1. Introduction

A common and often unavoidable event during physiological experiments with insects is handling stress due to mechanical excitation [1,2]. The stress condition is well observable as disturbances in gas exchange cycles [3] or as the hyperactivity of body skeletal muscles [4]. An individual may recover from handling stress well within 5–10 min after the beginning of physiological measurements, another may need many hours for recovering. Thus, an individual variation may be expected in the neuromuscular susceptibility to the stress evoked by a laboratory device.

The variation between individuals for certain characteristics may be accepted as normal in most biological studies [5]. Nevertheless, if laboratory-reared insects are used in certain toxicological studies then it is necessary to group individuals according to their irritability during pre-treatment tests or according to quality assessment [6].

To our knowledge no special study exists for the estimation of individual differences in the susceptibility to handling stress, in a laboratory-reared insect often used in physiological and toxicological studies, with special reference to body water loss.

Evidently, the action of stressors should be studied by a simultaneous registration of standard metabolic rate (SMR), respiration rhythms and patterns of body movements.

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For precise measurements of $O_2$, $CO_2$ and $H_2O$, special sensors are available but these do not allow any direct actographic recordings of body movements [2,7].

It was earlier demonstrated that direct calorimetry is suitable for describing the development and ageing in living organisms [8,9] including insects [10–12]. Simultaneous recordings of calorimeter and other signals, such as audial [13], optical [14], respirometric [12] and actographic [15], were used.

In the present paper, we study the susceptibility to handling in the case of the pupae of yellow mealworm, *Tenebrio molitor* with the use of twin set-up calorimeters combined with automatic respirometer-actographs. In addition, we estimate the body water loss in pupae taking into consideration individual characteristics revealed during respiration and muscular activity.

## 2. Materials and methods

### 2.1. Animals and weighing

Yellow mealworms (*T. molitor*) were reared at 27°C in constant darkness as described earlier [16].

The emerging time of pupae was verified (±1 h) and the pupae were then held separately at 25±0.2°C and at 70±3% RH.

For the experiments we used only female individuals weighing 100–130 mg.

We weighed individual pupae by using a micro-analytical balance (Meopta A4-20) with an accuracy of ±0.02 mg. During weighing the previous exposing conditions were maintained.

### 2.2. Calorimetric and respirometric measurements

The construction of simple twin set-up thermocouple calorimeters has been described elsewhere [15–17]. We inserted the animal and reference vessels into an automatic electrolytic microrespirometer, also functioning as an actograph [18,19].

The calibration of our calorimeter–respirometer was carried out by empirical calorimetric curves [20] by means of a heating spiral of a known resistance (15–20 Ω) placed into the vessels.

During the measurements, the calorimeter–respirometer was placed in a thermostat (25±0.1°C) inside a 1 l Dewar flask.

The small time-constant (<5 s) of the calorimeter was demonstrated by the simultaneous recordings of exothermic peaks due to discontinuous $CO_2$ release with adequate respirometric peaks [17].

## 3. Results

In earlier studies [15–17], the main events reflected in the calorimetric recordings were analysed. The small upward, i.e. exothermic peaks, as shown in Fig. 1, denoted the discrete bursts of $CO_2$ release. The periodically occurring weak abdominal movements (pulsations) resulted in higher exothermic peaks usually lasting 5–6 min (Fig. 1). Body wriggling movements, made mostly in 2–3 strokes, were marked on recordings as larger amplitudes (Fig. 2, bottom).

There exists a great diversity in the individual patterns of muscular activity and gas-exchange rhythms. We differentiated at least four characteristic types by patterns of respiration and body stereotyped movements in pupae. Accordingly, the pupae were divided into four groups.

Group A included individuals with clear and almost regular cycles of $CO_2$ emission. In addition, these pupae exhibited regular periods of abdominal pulsa-

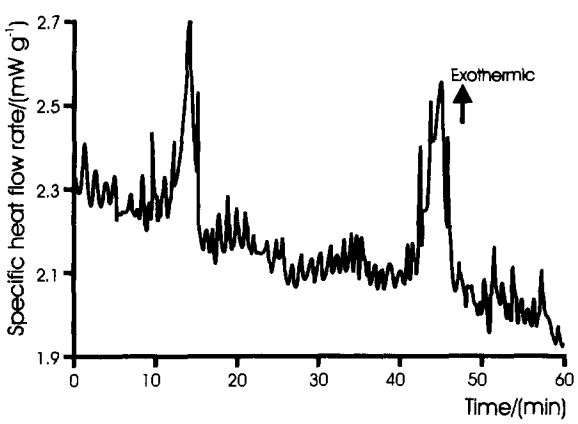


Fig. 1. Calorimetric recording of *T. molitor* pupae expressing the almost regular rhythm of discontinuous $CO_2$ release (short upward peaks) and two periods of abdominal pulsations. Note that during abdominal movements the $CO_2$ peaks persisted.

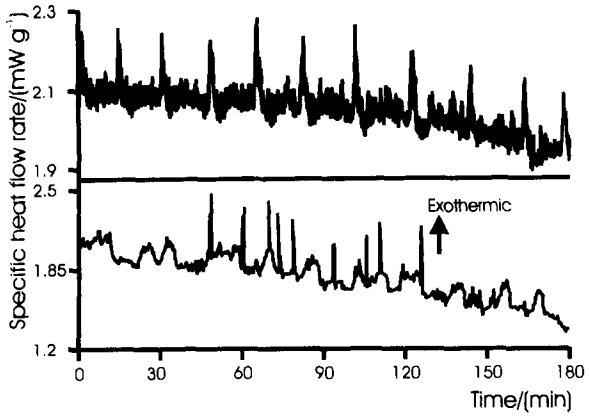


Fig. 2. A typical calorimetric recording (above) of *T. molitor* pupae possessing a clear $CO_2$ release and the periodicity of abdominal pulsations (higher peaks). The record below belongs to *T. molitor* pupae displaying the symptoms of handling stress: the clear periods of abdominal pulsations are abolished and the wriggling strokes are clearly seen as exothermic (upward) peaks.

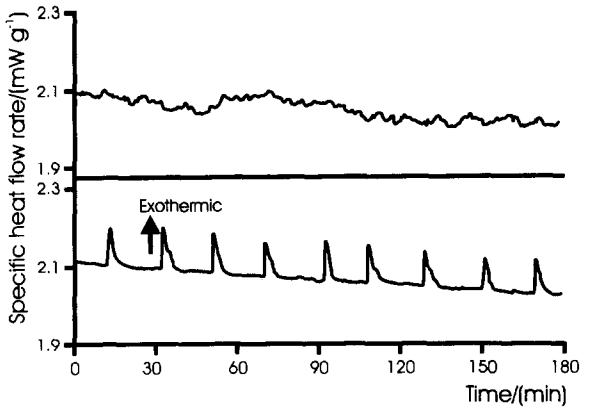


Fig. 4. Calorimetric recordings of *T. molitor* pupae. Top: Record of a pupa lacking both the $CO_2$ cycles and the periods of abdominal pulsations. Bottom: Typical record for *T. molitor* pupae with regular periods of abdominal pulsations but lacking cyclic $CO_2$ release.

tion activity. No wriggling occurred in these individuals (Figs. 1 and 2 (top) and Fig. 3).

Group B was characterised by the missing $CO_2$ peaks. No muscular hyperactivity was to be seen. Abdominal pulsations may or may not be displayed (Fig. 4).

Group C individuals exhibited the clear rhythms of discontinuous $CO_2$ emission, but instead of periodic abdominal pulsations only steady, continuous, weak body pulsations were seen on the recordings of respirometric actograph. On the calorimetric recording, only the almost regular upward peaks $CO_2$ were noted (as represented in middle parts of Figs. 1 and 3).

Group D included the pupae with chaotic rhythms of $CO_2$ release, irregular abdominal pulsation periods and, at times, wriggling movements (Fig. 2 bottom). Thus, a permanent condition of excitation was proper in this case. Pupae of this group showed a body mass loss significantly higher than in other groups (Table 1).

In the beginning of calorimetric and respirometric measurements, the handling always induced a stress condition in pupae characterised by irregular body

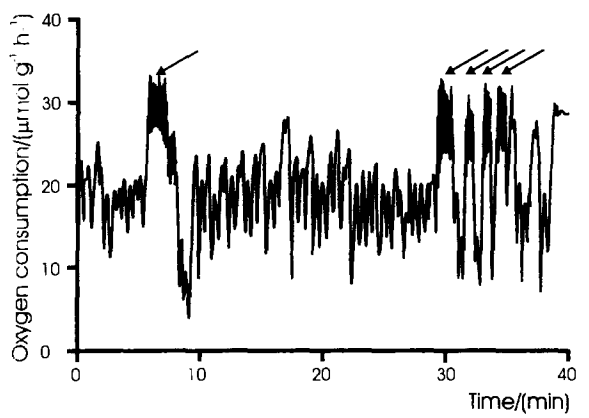


Fig. 3. Respirometric recording representing the bouts of abdominal pulsations (arrows) in *T. molitor* pupae, and the sharp downward peaks due to discrete $CO_2$ releases.

Table 1
Body mass loss in four *T. molitor* pupal groups [a]

| Pupal group | *N* | Initial body mass (mg) mean±SE | Body mass loss per day mg $g^{-1}$ $h^{-1}$ |
|---|---|---|---|
| A | 60 | 105.4±1.72 | 11.8±0.073 a [b] |
| B | 50 | 123.6±2.81 | 10.2±0.091 a [b] |
| C | 35 | 115.6±3.54 | 12.8±0.065 a [b] |
| D | 25 | 105±2.83 | 22.6±0.38 b [b] |

[a] First three pupal groups showed regular $CO_2$ cycles (or those were absent) while no muscular hyperactivity occurred. Group D was characterised by high excitability resulting in muscular hyperactivity.

[b] Different letters (a and b) denote statistically significant differences, *t*-test ($p<0.05$).

wrigglings and accidental contractions of certain skeletal muscles as represented in Fig. 2. During handling stress, the usual rhythm of $CO_2$ emission was abolished. In addition, abdominal pulsations occurred at irregular intervals.

By use of a respirometer–actograph, it was established that the recovery from handling stress usually occurred within 10–15 min after the insertion of the pupa into the device. This time was shorter than the equilibration time of the twin calorimeters.

A number of individuals (group D) exhibited the symptoms of handling stress persistently, and during the 6 h of calorimetric measurements no signs of calming down was revealed. We could not definitively establish the time needed for recovery from the excited state. It was likely that these pupae were very susceptible to any kind of environmental changes (i.e. temperature, substrate, shaking).

The pupae without any signs of mechanical irritation on calorimetric recordings were divided into the first three groups according to their pattern of respiration and body movements.

We examined the body mass loss in all the pupal groups. The first weighing was carried out in 24±2 h after larval–pupal ecdysis, the subsequent weighings followed after intervals of 24 h (Table 1).

Immediately after the second weighing, we determined the metabolic level in all pupae. The respirometric measurements gave evidence that, between the four examined pupal groups, no significant differences in the level of SMR existed. The oxygen consumption level ranged from 19.2 to 25 $\mu mol\ g^{-1}\ h^{-1}$.

## 4. Discussion

We did not find any significant differences in the water losses between pupal groups A, B and C (Table 1). Thus, the water loss did not depend either on the pattern of $CO_2$ release or on the periodicity of abdominal pulsations.

Cyclic $CO_2$ release (discontinuous ventilation) in many insect species is regarded as a water-conserving mechanism [1,21,22]. We could not distinguish significant differences in the water losses between the pupae displaying clear and discrete $CO_2$ bursts and those who showed a rather smooth line on recordings instead of $CO_2$ peaks (Fig. 4).

These results suggest a partially opened configuration of spiracles resulting in patterns looking like continuous respiration. It is likely that such a mode of spiracular control may conserve water no less effectively than it is suggested for the release of $CO_2$ in discrete bursts [23].

In pupal group D, characterised by the extraordinary contractions of certain skeletal muscles, the rate of body mass loss was significantly higher than in the other groups (Table 1). This faster body mass loss was not due to the metabolic rate. More water in these pupae was obviously lost by reason of disturbances in spiracular control being a result of muscular hyperactivity.

The regular periods of abdominal pulsations were characteristic of groups A and B. These pulsation periods were independent of the $CO_2$ release.

The main cause of the disturbances in cyclic $CO_2$ release and in the irregularity of abdominal pulsations was the hyperactivity of skeletal muscles.

The abnormal changes in body rhythmic movements due to hemolymph pressure pulsations were also seen as the earlier symptoms of insecticide poisoning in insects [24].

The individual variation in neuromuscular irritability may partially originate from environmental factors, such as the rearing-medium. Nongenetic, environmentally induced variations of this type between individuals are common in the laboratory-reared insects [5,6].

According to our (unpublished) data, the pupae of group D, characterised by high irritability and greater water losses, were at the same time the most susceptible group to some toxicants or to chemical stress.

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