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## CHANGES IN MUSCULAR AND RESPIRATORY ACTIVITY PATTERNS IN YELLOW MEALWORM (*TENEBRIO MOLITOR*) AND GREATER WAX MOTH (*GALLERIA MELLONELLA*) PUPAE CAUSED BY SOME PLANT EXTRACTS, JUVENILE HORMONE ANALOGUES AND PYRETHROID

**Abstract.** Different periodical and regular stereotyped abdominal movements in *Tenebrio molitor* and *Galleria mellonella* pupae were described by visual observations and by recording patterns from a microrespirometer-actograph (MRA), which also registered simultaneously the oxygen consumption rate (OCR) and external gas exchange rhythms. After treatment with insecticidal plant extracts, juvenile hormone analogues (JHA) and permethrin, muscular and respiratory responses in the pupae were further studied with the use of the MRA, the infrared gas analyzer (IRGA) and the differential thermocouple calorimeter (DTC). Thereby, as a result of using subtoxic doses, it was found that the discontinuous respiration often turned into continuous one since the clear diffusional-convective (DC) cycles of the oxygen uptake and/or CO<sub>2</sub> release were abolished even without decreasing OCR.

Most plant extracts screened acted, first of all, as respiratory depressants and inhibitors of muscular contractions in the pupae. Yet, the fall of OCR was not related to their muscular inactivity, so it must have been their basal metabolism that had decreased. Following their recovery from a temporal paralysis, there usually occurred one or two moderate OCR rises. Maximum peaks of OCR elevations (3—4×) were observed during ataxia elicited by permethrin.

### Introduction

Very scanty data are available about the action of insecticidal plant extracts and juvenile hormone analogues (JHA) on the muscular activity and respiratory metabolism. Gas exchange has mostly been measured in intact insects as an index of toxication. It has been supposed that most contemporary insecticides affect the respiratory rate in an indirect way via neuromuscular system by stimulating or inhibiting muscular contractions which are maintained by oxidative metabolism.

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The problem of the relation between indirect and direct respiratory effects in an affected insect has often been discussed. It is clear that the part of muscular activity in respiratory responses should be trustworthily estimated only with a special equipment by means of which the muscular contractions expressed by body movements can simultaneously be registered together with the oxygen consumption rate (OCR) and carbon dioxide output rhythms; and for these investigations a special microrespirometer-actograph (MRA) was elaborated (Kuusik et al., 1991, 1992).

The pupal stage has more advantages for these researches than adults and larvae because of the lack of nutrition and excretion and due to a limited moving activity. Moreover, pupae require less time than adults and larvae to quiet down after experimental handling or mechanical stress which, inducing muscular contractions, can raise OCR.

Pupal movements have commonly been regarded as accidental and spontaneous, resulting from external excitation. There exist only few data concerning regular motions in coleopteran and lepidopteran pupae. The hemolymph pressure pulsation rhythm in pupae results from the contraction of certain abdominal muscles and the corresponding rhythmical movements of the abdomen (Slama, 1976; 1988; Farkas, 1984). Our preliminary data indicate that *Galleria mellonella* and *Tenebrio molitor* pupae are characterized by regular and rather stable periods of muscular activity which are expressed by the corresponding stereotyped abdominal movements (Tartes, 1990; Kuusik et al., 1991, 1992).

Scarce information is found about the influence of toxic substances on the gas exchange rhythm and on the pattern of muscular activity in pupae. Intermittent CO<sub>2</sub> emission was affected after DDT treatments in *Phormia regina* (Buck and Keister, 1949). Normal diffusive-convective (DC) cycles of the external gas exchange were abolished in *T. molitor* and *G. mellonella* pupae after applications of JHA (Куузик и др., 1983).

It is supposed that increased muscular activity including ventilatory movements as well as disturbances in cyclic gas exchange can raise the transpiratory water loss in insects (see Edney, 1957; Buck, 1962; Bursell, 1964). In the report, we describe the patterns of regular and periodical abdominal movements, including passive and active ventilation in the pupae of *T. molitor* and *G. mellonella*. The main features of muscular and respiratory responses caused by various toxic substances (insecticidal plant extracts, pyrethroid and JHA) are also studied. In addition, we give the first information on the metabolic cost of rhythmical pupal abdominal movements, including pumping motions.

We expect that these preliminary data are necessary for further studies concerning relations between the transpiration rate and muscular hyperactivity in treated pupae.

### Material and methods

**Insects.** The pupae of greater wax moth (*G. mellonella*) were taken from stock cultures, bred on a modified semiartificial diet at 30°C and in constant darkness (King and Hartley, 1985). Yellow mealworm (*T. molitor*) was reared at 30°C and ca 70% RH in constant darkness. Flour, with the addition of 10% of yeast, or wheat was used as the diet. Male and female pupae of both species were sampled separately. Their age was determined with regard to larval-pupal ecdysis, except for the last 10—12 h during which we took into account cuticular pigmentation. The pupal age was known within 4 h. The day of larval-pupal ecdysis was designated as day-0, the next day as day-1 etc.

**Plants and extracts.** The extracts of the following plants were used for studying their physiological effects on pupae: *Heracleum sosnowskyi*, *Nicotiana rusticum*, *Artemisia absinthum*, *A. vulgaris*, *Tanacetum vulgare* and *Ledum palustre*, which were (except *N. rusticum*) collected from the vicinity of Tartu.

Shade-dried leaves or seeds were ground into fine powder and extracted for 24 h, using ethanol (80%) as a solvent. The extract was then filtrated and dried into paste under vacuum at 80°C, and dissolved in ethanol of various concentrations. All stock solutions were stored at 0°C.

For the dip method water solutions of ethanol-extracted paste (WSEE) were prepared at various concentrations. The water extract (WE) was also prepared from dried and powdered plant material, while various concentrations (calculated to powder weight) were used for dipping tests.

**Juvenile hormone analogues (JHA) and pyrethroid.** The JHA used in this study (further indicated as JHA-1, JHA-2 and JHA-3) were (3-methyl-5-alkoxy)-2-pentenyl ether (Лээтс и др., 1984); dodecyl-isobutyl-dialkoxy-methane (Когерман и др., 1991) and 3-methyl-5-isobutoxy-2-pentenyl-isobutylformal (Шмидт и др., 1991), respectively. The above publications also report our data on the morphogenetic activity of the tested JHA.

Pyrethroid used for submerging pupae (for 60 s) was water emulsions of the emulsifiable concentration of 10% ambush (permethrin with 2:3 *cis:trans* ratio).

**Treatments.** Plant substances were tested by dipping pupae in WE or in WSEE for 60 s; then the pupae were immersed into pure water, and allowed to dry on filter paper. Control pupae were also submerged into water for 60 s.

Injections of JHAs were performed on day-0 between two abdominal sternites with the use of a minute-calibrated glass-capillary micropipette with the point diameter 0.03 mm, while the needed pressure was induced by a syringe. The cuticula of a water-anaesthetized day-0 pupa was pierced with a sharp stainless steel needle at the point of the subsequent injection. The wound was not sealed. The injected pupae did not reveal any injury metabolism. JHA was injected at a dose of 10–30 µg per pupa in 0.5 µl of 10% ethanol. Control insects received injections of 0.5 µl 10% ethanol.

**Measurements.** The technical equipment for the present experiments consisted of the microrespirometer-actograph (MRA), the infrared gas analyzer (IRGA), the heat conductivity detector (catharometer) and the differential thermocouple calorimeter (DTC). These methods have already been described in detail in our papers referred to above.

Upward peaks of MRA records denote a decrease of the body volume and/or an air (oxygen) uptake gulp during DC gas exchange in *G. mellonella* pupae (Fig. 1). Active muscular contractions are also expressed by upward peaks (Fig. 2 and 3), abdominal rotation is represented by long fullscale up-down peaks, while every full turn (stroke) of the abdomen is reflected by one peak (Figs. 1 and 4).

A downward peak denotes a cyclic CO<sub>2</sub> burst from tracheae during DC respiration (Figs. 1, 2 and 3). Besides, a sharp and fast downward peak results from the body volume increase due to certain muscular contractions in an affected pupa (Fig. 4). When the increase in OCR results from muscular hyperactivity, body movement peaks due to muscular hyperactivity are also noted on the recording line of OCR (Fig. 5).

Using the infrared gas analyzer (IRGA), the CO<sub>2</sub> emission level and rhythms were registered in individual pupae. The movements of pupae were also visually observed through the thermostat window, especially in the case of great CO<sub>2</sub> output peaks.

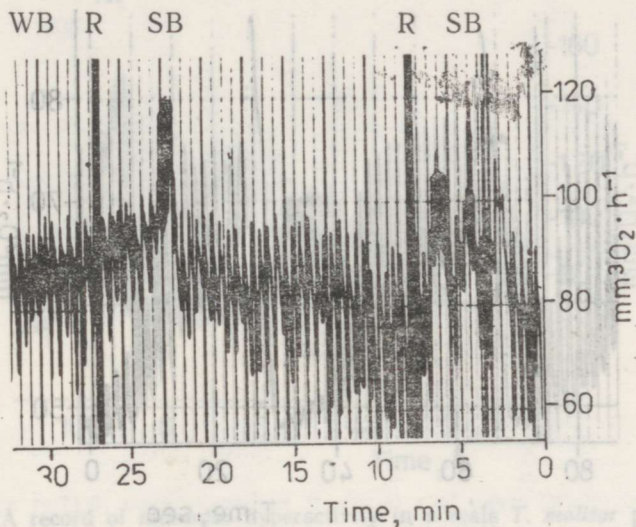


Fig. 1. An MRA record of gas-exchange level and cycles besides abdominal movements in a female (0.169 g) *G. mellonella* pupa. Sharp upward peaks indicate air uptake gulps, downward peaks result from  $\text{CO}_2$  intermittent release. Two bouts of SB movement (SB), two bouts of rotation (R) strokes and a period of WB activity are recognizable.

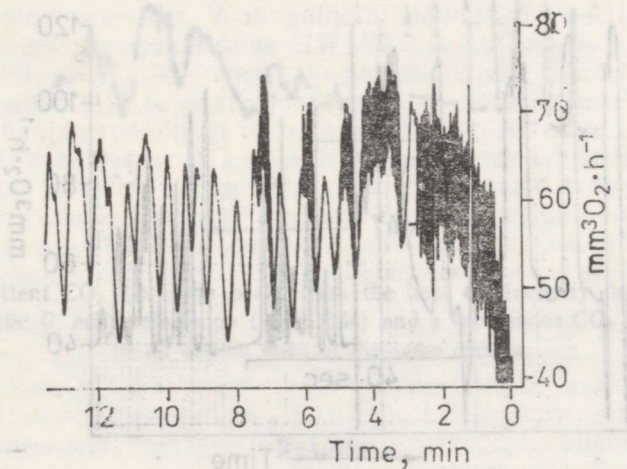


Fig. 2. An MRA record of a male (0.115 g) *T. molitor* pupa, with cyclic emission of  $\text{CO}_2$  (downward peaks). The period of SB activity due to handling stress is distinctly expressed on the right side of the record.

**Pretreatment tests.** Our tests made use of pupae weighing 0.10–0.17 g. Pupae with the mentioned weight — only about 60% — were suitable for present experiments, while the rest were considered physiologically labile. The latter were easily excitable individuals, who were not able to recover from handling stress during the first 30 minutes of respirometric measurements. Frequent irregular peaks of muscular contractions were characteristic of handling-disturbed pupae. “Normal” pupae quieted down rapidly, and showed a gas exchange pattern typical of undisturbed individuals (Fig. 2).

Using the records from catharometer, pupae with the clear gas exchange cyclicality were selected out of those who showed continuous respiration or irregular breathing rhythms (Fig. 6). Further, we indicate the individuals of the first group as “cyclic” pupae and the representatives of the second group as “acyclic” ones.

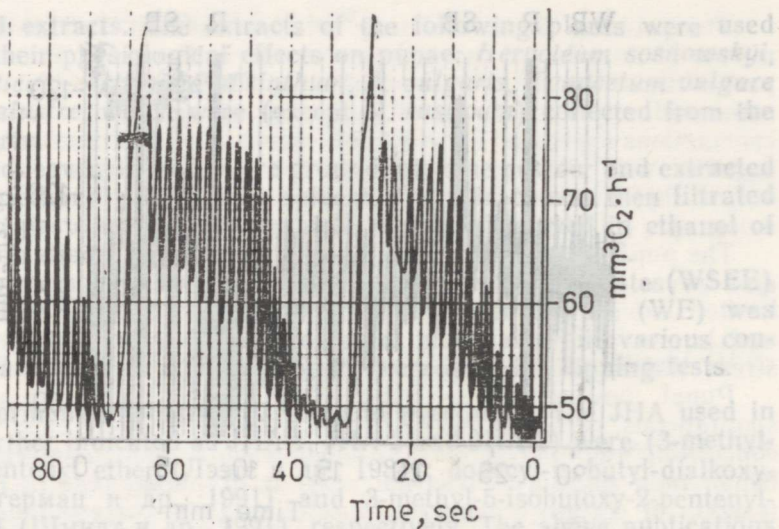


Fig. 3. Cycles of DC gas exchange with frequent peaks of WB movements in a *G. mellonella* female pupa (0.168 g) from MRA records. Air suction into tracheae ( $O_2$ ) and carbon dioxide release ( $CO_2$ ) are indicated.

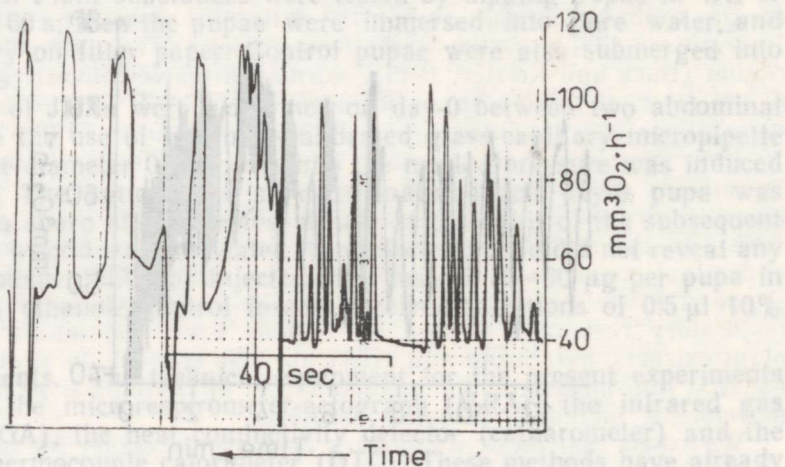


Fig. 4. Abdominal rotation strokes from an MRA record of an affected (dipping in 1% permethrin WSEE for 60 s) male *G. mellonella* pupa (0.135 g). On the right side of the record rotation strokes are presented soon after treatment, on the left side 5 h later when every stroke is prolonged due to the lasting contractions of dorsal intersegmental muscles.

**Anoxia tests.** Respiratory effects after dipping in water for 60 s were determined in special experiments. In *G. mellonella* and *T. molitor* pupae no appreciable increase in the oxygen uptake following the anoxic period could be discovered. However, there exists a possibility that oxygen debt repayment had occurred rapidly already before our respiratory measurements had started, after the 10-minute stabilizing period of MRA. The carbon dioxide post-anaerobic output always took place during 5 min after the anoxia, and therefore it was easy to recognize the postanoxic  $CO_2$  peak in immobile pupae on the recordings of the infrared gas analyzer (IRGA).

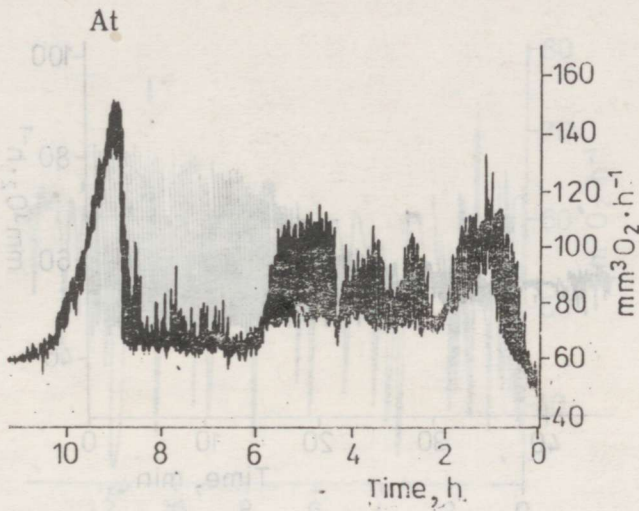


Fig. 5. An MRA record of muscular hyperactivity in a male *T. molitor* pupa (0.140 g) after treatment (injection of 10  $\mu\text{g/pupa}$  JHA-3). Irregular wriggle together with SB movements causes ca 2-fold OCR increase (right side), while the higher peak of OCR (At) is due to an attack of ataxia.

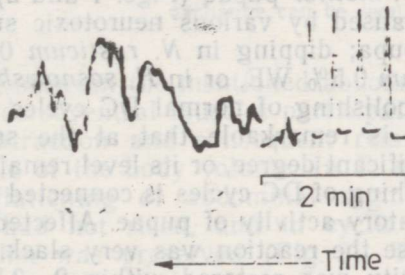


Fig. 6. Intermittent  $\text{CO}_2$  discharge peaks from the heat conductivity detector (catharometer) in a cyclic *G. mellonella* pupa (right side) and a continuous  $\text{CO}_2$  emission pattern in an acyclic pupa of the same species.

## Results

We distinguished at least four types of periodical and regular abdominal movements in *G. mellonella* and *T. molitor* pupae with the use of MRA. From abdominal movements we differentiate passive ones occurring due to passive suction ventilation during cyclic gas exchange, and active body movements due to perceivable muscular contractions. The role of muscular contractions in DC gas exchange cycles is not yet clear, and the part of these contractions in the DC cycles of *G. mellonella* and *T. molitor* cyclic pupae has been demonstrated in a rather indirect way (Kuusik et al., 1991, 1992).

**Abolishing of diffusional-convictional (DC) gas exchange cycles.** In the cyclic pupae of *G. mellonella* and *T. molitor*, the DC cycles of  $\text{CO}_2$  are noted on MRA records as downward peaks. The length and duration of a  $\text{CO}_2$  emission peak depends on the amount of this gas and on the speed of the absorption of  $\text{CO}_2$  in the respiratory chamber of MRA. The DC cycles of *G. mellonella* are characterized by a clearer pattern than those of *T. molitor*. Moreover, at the beginning of every following gas-exchange

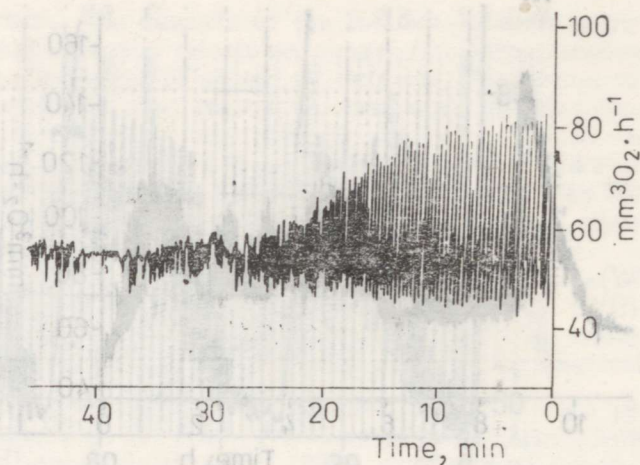


Fig. 7. Vanishing of DC cycles in a female *G. mellonella* pupa (0.17 g) soon after treatment (injection of 20  $\mu\text{g}$  JHA-3).

cycle of a *G. mellonella* pupa, a rapid and sharp upward peak is drawn on the respirogram due to an abrupt gulp of air uptake, which is always absent in the DC cycle of *T. molitor* pupae (Figs. 1 and 2).

A typical toxic effect caused by various neurotoxic substances (e.g. injection of JHA-2 10  $\mu\text{g}$ /pupa; dipping in *N. rusticum* 0.3% WE, or in *A. vulgaris* and *A. absinthum* 0.5% WE, or in *H. sosnowskyi* 0.1% WSEE; permethrin 0.1%) is the abolishing of normal DC cycles (Fig. 7) in the pupae of both species. It is remarkable that at the same time OCR decreased only to an insignificant degree, or its level remained unchanged. It is evident that the vanishing of DC cycles is connected with the inhibition of the general musculatory activity of pupae. Affected pupae did not respond to prodding, or else the reaction was very slack. In the case of weak poisoning, DC cyclicity was restored within 2—3 h, although the amplitude of  $\text{CO}_2$  or  $\text{O}_2$  peaks on MRA records did not usually achieve its former extent.

In a number of JHA-treated (injection of JHA-1 10  $\mu\text{g}$ /pupa) cyclic *T. molitor* pupae intermittent gas exchange was preserved, while on MRA recordings the typical DC pattern was mashed due to muscular hyperactivity resulting from neurotoxic effects. When such affected pupae were deprived of mobility by being fixed in foam rubber, the normal DC gas exchange pattern was restored (Fig. 8).

**Ordinary abdominal movements and their changes in affected pupae.** In normal undisturbed *G. mellonella* and *T. molitor* pupae at least four well-distinguished types of active body movements caused by the contractions of certain groups of muscles were observed. These abdominal movements were not evoked by mechanical excitations or by any handling stresses, since they persisted and were characterized by periodicity and regularity.

We regard the rhythmical forward bent of the abdomen on the dorso-ventral plane of the body as weak "bending" (WB). These movements are in most cases not perceivable externally with the naked eye; on MRA recordings they are noted as regular upward peaks ("combtooth pattern") (Fig. 3). We have also registered WB movements with the use of the displacement transducer (Kuusik et al., 1992). In *G. mellonella* pupae WB obviously occurred due to the contractions of ventral muscles between the 4th and 5th abdominal segments, since under the microscope their foldings and unfoldings were perceivable on the dorsal side of the intersegmental

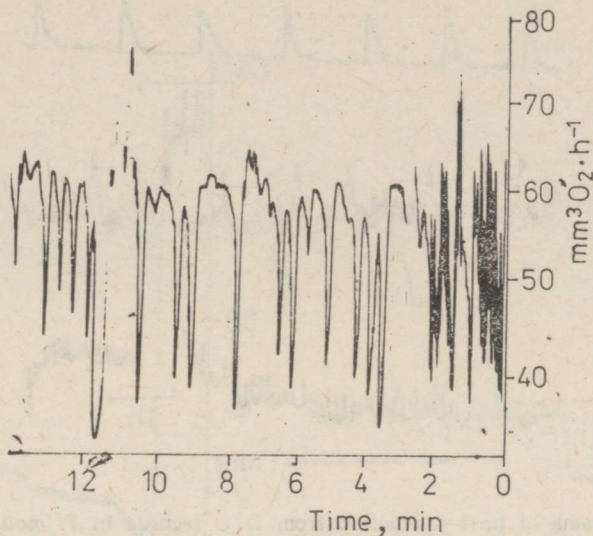


Fig. 8. Right, an MRA record showing muscular hyperactivity in an affected *T. molitor* pupa (dipping in 10% permethrin for 60 s). Left, the same treated pupa after fixing in foam rubber. Muscular contractions are eliminated and therefore DC cycles of gas exchange can be seen.

soft membrane between the mentioned abdominal segments. In *T. molitor* pupae certain dorsoventral muscles participate additionally in WB, while every microcontraction and consequent relaxation cause a decreasing-increasing cycle of the body volume. In *T. molitor* pupae, also the flattening and reflattening of abdominal segments occurred. During WB periods OCR had not risen, and in cyclic pupae the normal DC gas-exchange rhythm was preserved (Fig. 3). This is the reason why we cannot regard the WB motion as real pumping ventilation.

The main feature of weak toxication was the lengthening of WB periods, but in some cases chemical excitation resulted in permanent WB, which lasted for several hours (Figs. 5 and 9).

Strong bending (SB) consisted in motions analogous to WB but performed with larger amplitudes and therefore visible with the naked eye. During SB, the respiratory rate had usually risen by 10–40% and DC gas-exchange cycles disappeared (Fig. 2). Thus, it is obvious that SB acts as a kind of pumping ventilation in these pupae. We suppose that, during SB, the spiracles were opened or half-opened.

A typical symptom of weak or moderate toxicoses (injections of 10 µg JHA-2 or JHA-3; dipping in 1% WSEE of *H. sosnowskyi*, 2% WE of *T. vulgare*, *A. absinthum* 3% WE or *A. vulgaris* 5% WE) was the strengthening of muscular contractions during SB movements and the lengthening of SB periods (Figs. 5 and 9).

**Rotation of the abdomen** was a typical feature of disturbed and excited *Galleria* pupae, while in *Tenebrio* pupae analogous motion was regarded by us as wriggling. In an undisturbed *Galleria* pupa rotation occurred periodically at 30 °C, while a single period of activity (or bout) consisted mostly of 1–3 rotation strokes (Fig. 1). Every full round of abdominal tip movement corresponded to a decreasing-increasing cycle (rotation stroke) of the body volume similar to pumping movement, though there is no information about the coupling of spiracles during this type of motion. Obviously, rotation plays a more important role in circulation than in ventilation.



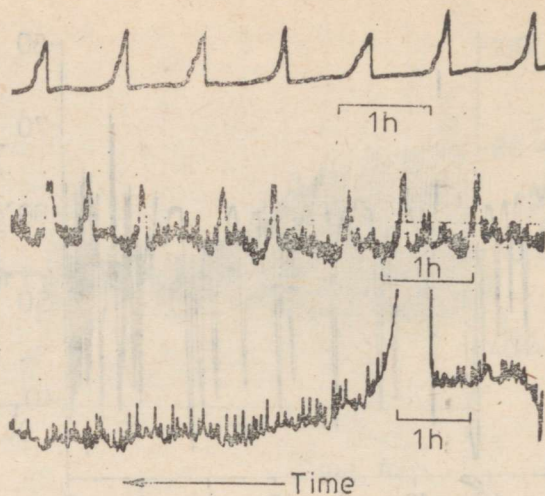


Fig. 9. Thermograms of heat production from DTC records in *T. molitor* pupae. From top to bottom: a normal pattern of heat production due to regular SB movements in a pupa on day-3; a DTC record showing moderate muscular hyperactivity peaks in a treated (injected 10  $\mu$ g JHA-3) pupa on day-1. Nearly regular SB activity periods are still perceivable; muscular hyperactivity with one period of ataxia.

In an undisturbed *Tenebrio* pupa no wriggling normally occurred until adult apolysis; a pharate adult is always characterized by increased muscular activity including wriggling.

Irregular abdominal rotation was a characteristic symptom of moderate neurotoxicosis in *Galleria* pupae. Sublethal doses of toxic substances (dipping in 3% WE of *N. rusticum*, 5% WSEE of *H. sosnowskyi* or in 1% permethrin) caused continuous rotation activity with every stroke being separated from the subsequent ones by a short pause (Fig. 4). These rotation strokes were carried out in a more vigorous manner than those occurring during ordinary rotation in a normal undisturbed pupa; and besides, the lengthening of the acme period of rotation peak due to the prolonged contractions of intersegmental dorsal muscles was typical (Fig. 4).

Irregular wriggle is a common feature of moderate toxicosis in *Tenebrio* pupae, caused by various neurotoxic substances (i. e. 1% WE of *N. rusticum*; 0.5% permethrin). The duration of wriggle activity was largely dose-dependent. OCR did not increase in most cases more than twofold (Fig. 5). In a wriggling pupa, regular WB and SB bouts were fully absent or they left scarcely perceivable traces, as it could be seen in the recordings of MRA and DTC (Figs. 5 and 9).

**Ataxia** was a typical symptom of lethal neurotoxicosis (e. g. dipping in 1% permethrin) in *Tenebrio* pupae, which involved incoordination, high frequency "jitters" and convulsions due to induced muscular hyperactivity. Motions during ataxia differed cardinally from WB or SB movements, while in ataxic pupae various groups of muscles were involved. Ataxic muscular hyperactivity occurred in most cases periodically; high peaks of ataxia were observed on MRA and DTC recordings (Figs. 5 and 9) resulting from an enormous rise of the metabolic rate. Lethal time for ataxic pupae varied individually, but usually they died already before adult apolysis.

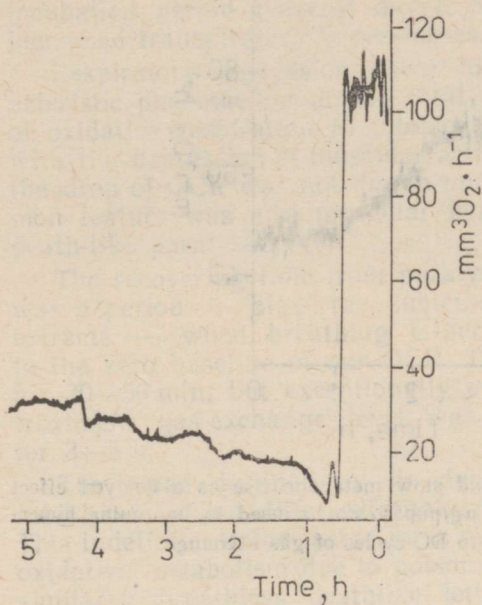


Fig. 10. MRA records of *T. molitor* pupae with irregular DC cycles before and after treatments. A typical pattern of OCR depression (dipping in 16% *H. sosnowskyi* WSEE).

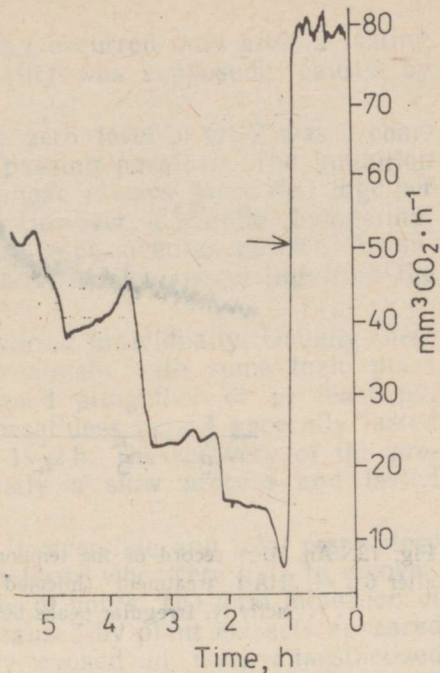


Fig. 11. IRGA records of *T. molitor* pupae before and after treatments (arrow). A pattern of a stepwise recovery after treatment (dipping in 10% *A. vulgaris*).

**Respiratory depression.** A common effect of several plant extracts was the depression of respiration in *T. molitor* and *G. mellonella* pupae. In these experiments we used the pupae who showed neither DC rhythms nor periodical abdominal movements, such as WB, SB, rotation or wriggle before treatment, which could cause a rise in OCR by themselves. However, intoxicated pupae displayed an essentially lower metabolic level than is usual at pretreatment time (Fig. 10). Thus, it is obvious that the fall of OCR was not caused by the inhibition of muscular activity in the affected pupae. The pupae were motionless during respiratory depression (20–30% of normal OCR), and did not respond to prodding. After their recovery from temporal paralysis, normal mobility and OCR were fully or partially restored. The recovery times and its degrees varied individually, but, in general, they were dose-dependent. Sometimes recovery was very slow, lasting for 20–30 h. In the case of a direct lethal effect, the decrease of OCR proceeded slowly during 5–10 h down to the zero level. Often the recovering process occurred step by step, as shown in Fig. 11, where the periods of muscular contractions are indicated by steep rises on IRGA recordings.

**Delayed stimulation of respiration.** Moderate or low doses of some toxic substances or compounds (injection of JHA-1 and JHA-2 20 µg/pupa; dipping in 10% WE of *L. palustre*) caused a delayed increase of OCR. In this case the respiratory rise did not occur immediately after treatment but after 3–4 h. The duration of this incubation period depended on the toxic substance and dose, but it also varied individually to a considerable extent.

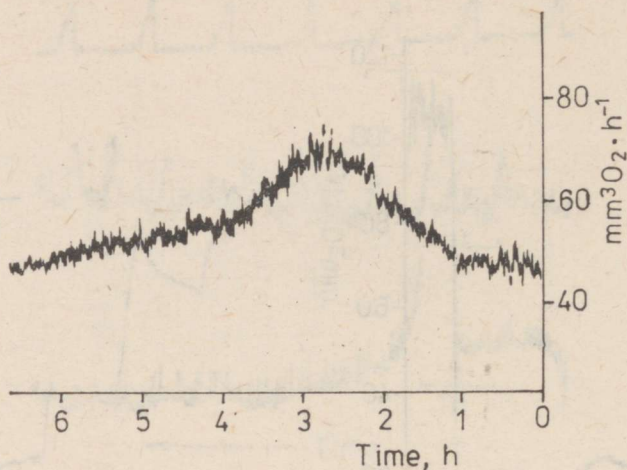


Fig. 12. An MRA record of the temporal and slow metabolic rise as a delayed effect after 6 h of JHA-1 treatment (injected  $15 \mu\text{g/pupa}$ ) not caused by muscular hyperactivity. Irregular peaks belong to DC cycles of gas exchange.

Lethal doses of JHA-3 and JHA-2 (injections of  $50 \mu\text{g}$  per pupa) caused the premortem OCR increase at 10–16 h after injection, while the period of the metabolic rise lasted 4–6 h. (Fig. 12). The time of the appearance of MRA increase was largely dose-dependent. As is seen in OCR recordings (Fig. 12), the stimulation of respiration did not result from muscular hyperactivity (frequent rapid peaks of muscular contractions were lacking).

### Discussion

Several types of muscular abdominal pumping are mostly characteristic of adult and larval stages, while passive suction ventilating has been best studied in pupal stage (cf. Miller, 1981; Kestler, 1985). It is also evident that all regular and periodical abdominal movements of a pupa have their distinct functions in respiration and/or circulation. There exist only a limited number of stereotyped motions in *G. mellonella* and *T. molitor* pupae, and these are expressed differently in MRA recordings. Several muscular groups may be involved in these different motions. The process of toxicosis is reflected in the muscular activity pattern, and the corresponding specific features are easily recognized in respirograms.

It can be concluded from the presented results that various toxic substances can abolish normal DC gas-exchange cycles in both species (without causing abnormal muscular contractions), which can be regarded as nonspecific effects. The vanishing of gas-exchange cyclicity in *G. mellonella* and *T. molitor* pupae can be explained as a loss of muscle contractibility involved in DC gas exchange. Consequently, in an indirect way, we have demonstrated the role of muscular contractions in the DC cycles of gas exchange.

Another nonspecific subtoxic effect was the lengthening and strengthening of WB and SB motions in pupae. The period of recovering normal muscular and respiratory activity varies individually to a great extent (from some hours to several days).

The prolonged periods of vigorous rotations in *G. mellonella* and wriggling in *T. molitor* were evoked only by highly neurotoxic substances in large doses (permethrin and extracts of *H. sosnowskyi* and *N. rusticum*),

but death of the treated pupae (80—85%) occurred only after a lasting incubation period (several days). Mortality was supposedly caused by increased transpiratory water losses.

Respiratory depression down to the zero level of OCR was a characteristic phenomenon during total but passing paralysis. The inhibition of oxidative respiration in intoxicated pupae always proceeded together with the depression of muscular activity. However, it is quite obvious that the drop of OCR was not due to muscular impassiveness as such. A common feature was also muscular hyperactivity after recovering from the death-like paralysis.

The recovering time from paralysis varied individually. Usually there was a period — after the sublethal treatment with some toxic plant extracts — when breathing either ceased altogether or at least fell to the zero baseline of our OCR. The breathless period generally lasted for 30—50 min, but exceptionally even 1—2 h. The recovery of the pre-treatment gas-exchange level was mostly a slow process and lasted for 3—5 h.

The pupae destined to die due to irreversible and total respiratory inhibition did not differ externally from those who were able to recover. This indefinite period lasted for a number of hours. The total inhibition of oxidative metabolism due to poisoning caused by plant extracts appeared similar to breathless deathlike lethargy evoked in water-anaesthetized pupae. However, there existed a fundamental difference in the “awakening” or restoring process of breathing and muscular activity. After asphyxia as a result of submerging, both breathing and muscular contractibility restored abruptly (during 3—10 s), independent of the duration of post-anoxic breathless lethargy. The recovery from reversible paralysis in intoxicated pupae was always a slow and gradual process, as it was established on the basis of MRA and IRGA records.

The appearance of death has often been considered as the time when no movements can be elicited by prodding; such a simplified estimation of dying is unsuitable when the toxic properties of plant extracts are to be estimated and lethal time (LT) values determined.

Several screened plant extracts acted, first of all, as effective metabolic inhibitors when treating day-0 pupae. When administered to less susceptible (day-1 and day-2) pupae, the period of inhibited respiration usually lasted 1—2 h, and was followed by one or two OCR peaks due to moderate muscular hyperactivity.

None of the tested plant extracts evoked typical symptoms of ataxia. Among the compounds and substances tested, maximum muscular hyperactivity and respiratory response (over 3-fold prerise level) regarded as ataxia was evoked by pyrethroid (permethrin). Pyrethroids have usually been characterized as axonic poisons which cause supernumerary muscular contractions at moderate doses (Narahashi, 1971; O'Brien, 1978).

Delayed respiratory responses to JHA, with a considerable rise in oxygen consumption, which often occurred in treated pupae that were destined to die, are noteworthy. Such increased premortem uptakes of oxygen were not related to muscular activity, as is evident from MRA records (Fig. 12). Therefore we could qualify such respiratory stimulation not as an indirect effect but rather as the direct increase in basal metabolism.

*L. palustre* revealed weak toxic properties, while there still occurred delayed slight stimulation of OCR not due to muscular hyperactivity. The mentioned plant extracts possess distinct properties of insect growth regulators (IGR) evoking morphogenetic lethal effects in *T. molitor* (unpublished data); yet relations between these properties and the respiratory influence have remained unexplored. The mechanisms of delayed respiratory stimulation in pupae caused by JHA are also unclear.

We can conclude that most muscular and metabolic effects in present tests depended less on plant species and more upon the doses (concentrations) of substance used. Remarkable and at the same time discouraging was the great variation in individual susceptibility to tested substances in all the doses irrespective of the age of pupae in a sample. This is why the dose-dependent relation in our tests remains somewhat unclear. Our preliminary data indicate an essential range of variation in an individual daily weight loss in normal (untreated) pupae of *T. molitor* and *G. mellonella* (unpublished). Whether there exists any relation between the individual rate of water evaporation (before treatment) and sensitivity to insecticidal compounds must yet be shown experimentally.

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**LIHASTE AKTIIVSUSES JA HINGAMISES AVALDUVAD VASTUSREAKTSIOONID MÕNEDE TAIMEEKSTRAKTIDE, JUVENOIDIDE JA PERMETRIINI PÕHJUSTATUD KEEMILISELE STRESSILE SUUREL JAHUMARDIKAL (*TENEBRIO MOLITOR*) JA VAHALEEDIKUL (*GALLERIA MELLONELLA*).**

Kasutades mikrorespiromeetrit-aktograafi (MRA), registreeriti ja kirjeldati nelja põhitüüpi tagakehaliigutust suure jahumardika (*Tenebrio molitor*) ja suure vahaleediku (*Galleria mellonella*) häirimatutel nakkudel. Taimeekstraktide, juvenoidide ja püretroidi (permetriini) toimel tagakehaliigutuste kokkutommete rütm muutub ja see kajastub ka MRA üleskirjutusel. Toksilised ained muudavad ka difuusse-konvektsionaalse gaasivahetuse tsüklilisust.

Enamik katsetatud toksilistest taimeekstraktidest mõjus hingamisainevahetuse inhibiitorina.

Aare КУУЗИК, Луле МЕТСПАЛУ, Кюлли ХИЙЕСААР,  
Аво КОГЕРМАН, Урмас ТАРТЕС

**МУСКУЛЯТОРНЫЕ И РЕСПИРАТОРНЫЕ РЕАКЦИИ НА ХИМИЧЕСКИЙ СТРЕСС, ВЫЗВАННЫЙ РАСТИТЕЛЬНЫМИ ЭКСТРАКТАМИ, ЮВЕНОИДАМИ И ПЕРМЕТРИНОМ У КУКОЛОК *GALLERIA MELLONELLA* И *TENEBRIO MOLITOR***

Описаны 4 основных типа периодических и регулярных движений брюшка у куколок пчелиной огневки (ПО) *Galleria mellonella* и мучного хрущака (МХ) *Tenebrio molitor*. Для этого проведены визуальные наблюдения и записи на микрореспирометрактрографе (МРА), регистрирующем даже малейшие движения брюшка одновременно с циклами и уровнем газообмена. При помощи инфракрасного анализатора и дифференциального калориметра изучены мускуляторные и респираторные эффекты, наблюдаемые после обработки растительными экстрактами 6 растений, аналогами ювенильного гормона (АЮГ) и перметрином.

Результатом влияния субтоксических доз явилось превращение перерывистого дыхания в постоянное у обоих видов, поскольку исчезли диффузно-конвекционные циклы поглощения воздуха в трахеях и выделение  $\text{CO}_2$  без понижения скорости потребления кислорода (СПК). Большинство испытанных растительных экстрактов оказалось депрессантами дыхания и ингибиторами мышечных сокращений. Однако падение СПК само по себе еще не послужило причиной мышечной инактивности; подавлен был основной обмен. Длительность полного прекращения дыхания, которое рассматривалось как временный паралич (П), зависела от применяемых доз и продолжительности экспозиции. После прохождения П отмечались один или два пика подъема СПК, связанных с возобновлением мышечной активности. Максимальные пики СПК отмечались во время атакции, вызванной действием токсических доз перметрина.