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Calorimetric investigations of insect metabolism and development under the influence of a toxic plant extract

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Abstract

The cyclic gas exchange in the diapausing pupae (DP) of the cabbage butterfly *Pieris brassicae* was monitored by use of simple and sensitive twin differential calorimeter combined with automatic respirometer. Usually, the periodic CO_2 releases in DP occurred as large, intermittent and micro bursts (i.e. in the form of respiratory cycles).

By treating the DP with low doses of the extracts of the marguerite *Tanacetum roseum* the large cycles of gas exchange were abolished, but the intermittent and micro bursts were preserved. These treatments did not result in any neuromuscular hyperactivity. After the treatment the body mass loss of DP increased from 1.72±0.16 (females (2×) and 2.23±0.31 (males 2×)) mg g^{-1} day^{-1} for the untreated pupae to 2.25±0.38 (females (2×) and 3.05±0.76 (males 2×)) mg g^{-1} day^{-1} for the treated ones.

All the DP who survived the treatment developed into pharate stages, but adult emergence failed. The untreated and control DP kept at room temperatures (20–25°C) did not initiate adult development and died after having lost more than 25% of initial body mass.

It is assumed that pyrethrum, a nonhormonal agent, acts directly on the brain, stimulating the release of the prothoracicotropic hormone (PTTH).

Keywords: Direct calorimetry; Respirometry; Insects; Gas exchange; Standard metabolic rate; Water loss; *Pieris brassicae*; *Tanacetum roseum*

1. Introduction

One of the most exciting fields of environmental biology is that of chemical ecology studying the role of chemical compounds in the interactions between organisms [1]. Most of the results originate from plant/herbivore interrelations and here specially from insects. The products of secondary plant metabolism serve in the defence against herbivorous insects acting upon their nervous system or mimicking their hormones, and thus disturbing the larval development into proper adults [2]. One of these products of secondary metabolism, pyrethrum, is derived from a species of chrysanthemum and contains a bouquet of different

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compounds, among them the pyrethrines as main components. They act as contact poisons on the insect and in proper concentrations lead to quick paralysis and death while they are without harm to homeothermic animals. The aim of the present paper was to investigate the action of pyrethrum on a typical pest insect, the cabbage butterfly or large white butterfly, *Pieris brassicae*, by means of microcalorimetry.

Pyrethrum, the dried flowers and extracts of *Tanacetum (Pyrethrum) cinerariifolium* with pyrethrins as insecticidal ingredients, is first of all known as a neurotoxic nonhormonal agent against insects [1,2]. Often the action of pyrethrum involves contributions from both, the direct toxicity and antifeedant characteristics [1–3]. It is obvious that besides the direct toxic actions the secondary and delayed effects by the sublethal doses of pyrethrum are also of scientific and practical interest. Until now, the physiological changes in diapausing lepidopterous pupae by the action of pyrethrum as a neurotoxic and nonhormonal agent have been scarcely studied (if at all). Nonlethal treatment of the last larval instars of various insects with pyrethrum do not produce any larval-pupal intermediates as they are typical for hormonally active substances and thus prove that its action is neurotoxic but not hormonal [1,3].

The cases when certain chemical nonhormonal substances exert mild toxic effects and at the same time stimulate adult development in diapausing lepidopterous pupae are of particular interest. According to our preliminary studies sublethal doses of extracts of *T. cinerariifolium* and *T. roseum* stimulate adult development in some lepidopterous pupae associated with toxic influences.

Effects of various chemical compounds on the respiration of larval and adult stages of insects are intensively discussed in literature [4]. The respiratory responses of insect pupae on toxicants are much less investigated, and only little is known about the way how the sublethal doses of toxic substances affect cyclic gas exchange in insects pupae [5].

Normally, the diapause – a period of dormancy with low energy turnover, interrupting the developmental activity of insects – is broken only by a long exposure to cold (chilling or cold reactivation) in lepidopterous pupae. For *P. brassicae* diapause is terminated by chilling them at 4°C for 12 weeks, and adult emergence occurs after 3–4 weeks at 20–25°C [6–8]. If *P. brassicae* diapausing pupae were kept at room temperature (20–25°C) they could not initiate their adult development at all. They died after having lost more than approximately 25% of their initial body mass.

Only a restricted group of chemical compounds proved to be the inducers of adult development in diapausing pupae. Certain juvenile hormone analogues (JHA) stimulate the development in the diapausing pupae of the cabbage moth *Mamestra brassicae* [9]. The diapause of the pupae of the cabbage butterfly, *Pieris brassicae*, was interrupted by treatments with JHA [10]. The stimulatory effects of organic solvents on initiating the development in the diapausing pupae of flesh fly (*Sarcophaga crassipalpa*) and the tobacco hornworm (*Manduca sexta*) were also described [11,12].

The intermittent gas exchange in the diapausing pupae of *P. brassicae* in general is similar to that described for large lepidopterous pupae showing large cycles and microcycles of CO_2. The cyclic release of CO_2 commonly regarded as an adaptation for reducing the transpiratory water loss has been repeatedly reviewed in literature [13–18].

We studied the respiratory responses of the diapausing pupae of the cabbage butterfly *Pieris brassicae*, caused by the extracts of the Painted Daisy (*Tanacetum roseum*; regarded also as identical to *Chrysanthemum coccineum* and *C. carneum* including pyrethrins as active components). At the same time, we tried to evidence the developmental changes in the pupae associated with mild toxicosis.

Among the various equipments available for studying gas exchange rhythms, we preferred a combination of microcalorimetry [19] and microrespirometry, sufficiently susceptible to record single CO_2 bursts [20]. This method allowed us to monitor the variations in gas exchange during several weeks without adjusting or manipulating the apparatus and thus avoiding any mechanical disturbances of the insects. Preliminary observations showed us that the diapausing pupae of *P. brassicae* are very susceptible to any handling and environmental influences in spite of a highly suppressed metabolic rate (MR).

The combination of calorimeter and respirometer allows to record the rhythms of CO_2 bursts, air suction uptakes into tracheae (passive suction ventilation – PSV) and body movements, even if the latter are not discernible with the naked eye.

2. Materials and methods

2.1. Insects

Cabbage leaves with the egg clutches of the cabbage butterfly, *Pieris brassicae*, were collected from outdoors (in Estonia near Tartu), and after hatching the larvae were reared in the laboratory on cabbage plants leaves in transparent plastic boxes with ventilated lids at 20°C and with 80±3% relative humidity (RH) [7,8] with an 8 h light and 16 h dark photoperiod regime [21,22].

2.2. Calorimetry

Six simple twin differential calorimeters were constructed of vessels made from copper foil (0.1 mm) connected with four copper-constantan thermocouples [20,23]. The volumes of both the insect and reference vessels were 0.45 ml while the sensitivity of the calorimeters amounted to values from 40 to 60 μV mW^{-1} with a detection limit of 4 μW (twice the background noise). The calorimeters were calibrated electrically by the Joule effect [24].

The volume and shape of the calorimetric vessels were manufactured as near as possible to the pupae dimensions of *Pieris brassicae*. One half of the copper vessel was conical to hold the pupae abdomen and the other half cylindrical. In this way the spiracles of pupae were very close to the vessel surface resulting in a high sensitivity to the gas exchange cycles (i.e. CO_2 bursts) and abrupt air uptakes into tracheae.

2.3. Respirometry

The new type of differential electrolytic microrespirometer-actograph (DEMRA) which we used in the present investigations has been described in some previous publications [23,25–27]. In this respirometer no electrodes are used for switching the oxygen generating unit on and off as it is done commonly in electrolytic respirometers. In the DEMRA instrument the oxygen generation occurs continuously while the current level in the electrolysis circuit is varied according to the changes in the insect's demands for oxygen. In this way the level of oxygen consumption is recorded. Every discrete burst of CO_2 results in a corresponding air volume increase in insect chamber and in a corresponding adequate screening of the photosensitive area of the photodiode. Air suction into trachea causes an abrupt decreasing of air pressure in the insect chamber and an immediate upward peak.

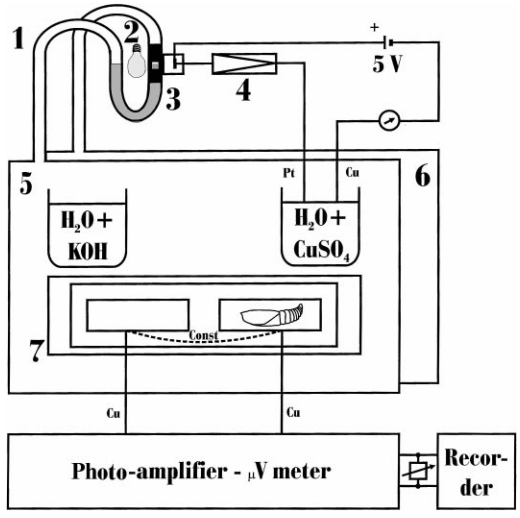

Fig. 1. The principle scheme of the electrolytic differential microrespirometer-actograph combined with the twin differential calorimeter: (1) U-shaped glass tube half-filled with ethanol; (2) light source; (3) photodiode; (4) amplifier; (5) respiration chamber; (6) compensation chamber and (7) calorimeter.

The possible rhythmic body movements are monitored as a series of peaks due to the changes in the external body volume.

The twin differential calorimeter is placed in the respiration chamber (Fig. 1) so that the calorimetric and respirometric signals can be recorded simultaneously.

2.4. Catharometry

A catharometer or the thermal conductivity detector of a chromatograph (Biochrom, 1980, S.U.) was adapted for the entomological research. We replaced the wolfram wire in the catharometer by a platinum spiral (coil diameter 1.2 mm). The general scheme of the flow-through system is designed on the basis of Punt's diapherometric device [28,29]. The air current from the insect chamber is pumped over the glowing platinum spiral of the catharometer, and due to different thermal conductivities of the gases every CO_2 burst of the insect results in a signal of the catharometric recording. This device is sufficiently suscep-

tible to record the microcycles of gas exchanges of the pupae of *P. brassicae*. The signal of the passive air suction intake (PSV) results obviously due to air pressure changes in the thermal conductivity detector.

2.5. Contact thermometry

Contact thermometry by means of heated thermistors can be used to monitor the heart beat rhythms [13,14]. We employ glass thermistors (25 kΩ) in contact with the dorsal midline of the third abdominal segment of the pupa. A current of 1 mA heats the thermistor up to 5–7°C above the ambient temperature together with the body surface in contact with it. Each peristaltic wave propulsing haemolymph in the dorsal vessel (heart) results in a cooling signal recorded by the thermistors.

The systolic amplitude is measured (in mV) by common electrode methods and the heart beat frequency determined by the use of a computing digital impulse counter (Ch-64, 1987, SU).

2.6. Weighing

Pupal body masses were determined to the nearest 0.02 mg at 20°C and 70±2% RH by a microanalytical balance (Meopta A4-20, Meopta, 1978, Czechoslovakia). The weighing of individual pupae was carried out every other day (Table 1).

2.7. Plant extracts and treatments

The flower heads of *Tanacetum roseum* were dried to around 10% of moisture, ground to a fine powder and extracted for 24 h using ethanol (80%) as the solvent. The extract (pyrethrum) was filtered and dried to a paste under vacuum at 80°C. The concentrate was kept at 38°C for 48 h for complete evaporation of the solvent before its use in the experiment. The concentrate was dissolved in water in the double dilutions of: 0.125%; 0.25%; 0.5%; 1%; 2% and 4% calculated as a dry weight of drogue per weight of water (dw/w).

Preliminary studies were conducted to determine the sublethal concentrations of the mild toxic effects on the pupae.

We employed a dipping method [30] for the treatments of the pupae to guarantee a homogeneous contact of the extract with all parts of the body. The pupae were dipped for 20 s into a 1% (dw/w) solution. The water gained by wetting was completely evaporated at 25°C during the following 24 h. Exposure resulted in mild poisoning without muscular hyperactivity.

3. Results

3.1. Typical features of gas exchange cycles and heart beat in diapausing pupae

For the present experiments we selected the pupae with standard metabolic rates (SMR) ranging from 0.74 to 1.28 μmol O_2 g^{-1} h^{-1}. This level of SMR is characteristic of the deep pupal diapause of *Pieris brassicae* [21,22]. Only those individuals of three-month-old diapausing pupae were used for treatment and control which exhibited the most distinct and characteristic rhythms of external gas exchange as established earlier for this species [31]. These main typical features revealed in gas exchange and heart beats in the diapausing pupae of *P. brassicae* are briefly described below.

Table 1
Initial body-mass (mg) and rate of mass losses (mg g^{-1} day^{-1}) in control and treated pupae of *Pieris brassicae* (mean±SD)

Sex	*N*		Initial body mass in mg	Body mass losses in mg g^{-1} day^{-1}	
	n_1	n_2		Control	After treatment
Female	43	36	366.5±23.1	1.72±0.16 (a)	2.25±0.38 (b)
Male	38	31	335.4±18.6	2.23±0.31 (a)	3.05±0.76 (b)
All	81	67	348.0±20.3	1.96±0.21 (a)	2.61±0.15 (b)

n_1: The number of control pupae; n_2: the number of treated pupae. Different letters for the same row denote statistically significant differences ($p<0.05$, *t*-test).
SD=Standard deviation.

Table 2
Characteristic data of gas exchange in untreated pupae of *Pieris brassicae*

Characteristic data	Number	Range	Mean	SD
Standard metabolic rate in μmol O_2 g^{-1} h^{-1}	29	0.74–1.28	0.89	0.14
Duration of CO_2 bursts in s				
Large	65	360–910	663.6	168.5
Intermediate	120	121–185	153.9	17.7
Micro	220	2.1–6.3	4.3	1.04
Interval between CO_2 bursts				
Large in h	40	10.3–26.5	14.3	5.8
Intermediate in s	60	485–930	726	126.3
Micro in s	120	65–183	130	37
Endothermic amplitude of CO_2 bursts in mW g^{-1}	50	0.45–3.35	2.66	0.25
Exothermic amplitude of CO_2 bursts in mW g^{-1}	80	0.03–0.12	0.07	0.02

SD=Standard deviation.
The number for standard metabolic rate means individuals. In other cases are given the number of bursts.

Intermittent CO_2 bursts occurred on different levels (large, intermediate and micro bursts) with regard to duration and frequency of CO_2 emission (Table 2). Large CO_2 bursts usually lasted for 6–15 min. The time interval between large CO_2 bursts was 10–26 h. Calorimetric power–time curves showed that the burst resulted in an abrupt exothermic signal at the moment of air suction intake into tracheae. This exothermic signal was rapidly overtaken by an endothermical signal evidently due to evaporative H_2O loss during large CO_2 bursts (Figs. 2 and 3).

Intermediate CO_2 bursts occur between the large CO_2 bursts, lasting 2–3 min and succeeding at the intervals of 8–15 min (Figs. 2 and 4). Usually, these intermediate CO_2 bursts also begin with an abrupt air uptake by suction.

In most cases, so-called flutter [16,17] takes place between these intermediate bursts. The flutter in diapausing *P. brassicae* pupae consists of the discrete microbursts of CO_2 following one another in time intervals of 1–3 min. It is seen in the respirometric and catharometric recordings that every microburst begins

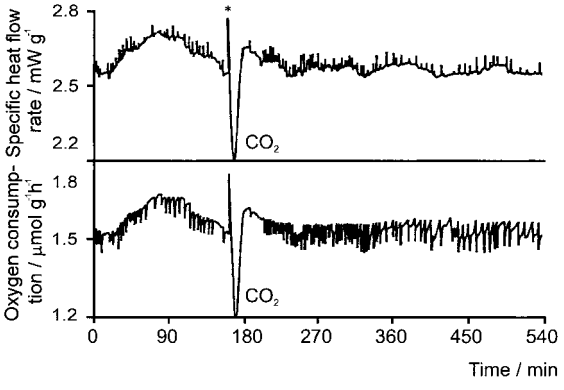

Fig. 2. A calorimetric record of intermediate CO_2 bursts (short upward peaks) and of one large CO_2 burst of an untreated diapausing pupa of *P. brassicae* (above). The asterisk marks the peak due to the abrupt air uptake into tracheae by suction. Simultaneous respirometric recording (below).

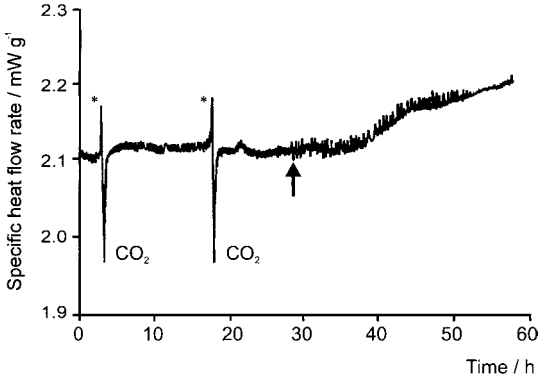

Fig. 3. The calorimetric registration of the abolishing of large CO_2 cycles in a diapausing pupa of *P. brassicae* after treatment (arrow) with the extract of *Tanacetum roseum*. The right part of the record is typical of nondiapausing pupae. Asterisk means the exothermic signal at the abrupt air intake suction into tracheae.

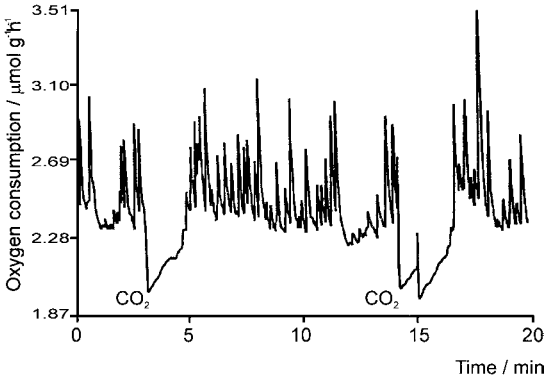

Fig. 4. The gas exchange microcycles of a diapausing pupa of *P. brassicae* (passive suction ventilation+CO_2 micro bursts) alternating with two intermediate CO_2 bursts, recorded by respirometry. This pattern of gas exchange is also characteristic for nondiapausing pupae.

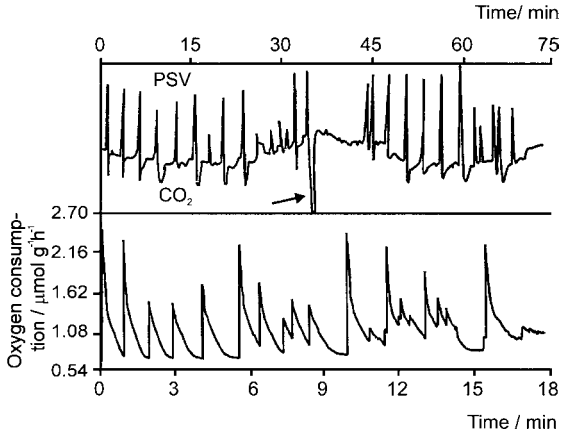

Fig. 5. A catharometric record of a series of microcycles and an intermediate cycle of gas exchange (arrow) of a diapausing pupa of *P. brassicae* (above). PSV (passive suction ventilation) indicates the signals due to abrupt air intake into tracheae by suction overtaken by signals of CO_2 micro bursts. Gas exchanges microcycles in a respirometric recording (below). Abrupt upward peaks are due to passive suction ventilation. The mean metabolic rate amounts to 0.94 µmol g^{-1} h^{-1}.

with an air uptake by suction transforming rapidly into the signal resulting from CO_2 microemission (Figs. 4 and 5).

The heart pulsation periods of 2–4 min alternate with heart beat pauses, i.e. a complete stillness of heartbeat. The pause periods are 3–5 times longer than the pulsations. The mean heart beat frequency amounts to 0.86±0.03 Hz, ranging from 0.68 to 1.18 Hz (Table 3). The initial and final parts of heart activity periods exhibited lower frequencies than the main part. More details of the heartbeats in *P. brassicae* are observed earlier [32].

3.2. Gas exchange and heartbeat in developing pupae

After cold reactivation the first symptoms of initiating development appear already after 5–6 days at 20°C. The SMR begins to increase step by step from the diapausing level (0.74–1.28 µmol g^{-1} h^{-1}) and exceeding 20 µmol g^{-1} h^{-1} after 15 days. At this time

Table 3
Heart pulsation in pupae of *Pieris brassicae*

Characteristic data	Number of the periods	Range	Mean	SD
Heart pulsation before treatment				
Pulsation period in s	30	123–240	193	33.7
Pause period in s	30	365–1220	870	245
Beat frequency in Hz		0.68–1.18	0.86	0.03
Systol's amplitude in mV		1.35–2.3	1.89	0.28
Heart activity 5–7 days after treatment				
Pulsation period in s	50	1260–2520	1860	336
Pause period in s	50	168–432	336	85
Beat frequency during higher amplitude (2.6–3.5 mV) period in Hz		0.405–0.630	0.518	0.11
Beat frequency during lower amplitude (0.2–0.4 mV) period in Hz		0.710–1.21	0.91	0.18

SD=Standard deviation.

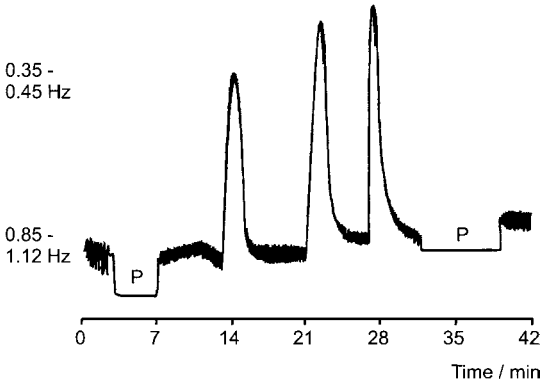

Fig. 6. Heart beats in a diapausing pupa of *P. brassicae* recorded 10 days after treatment by means of a thermistor. The three typical features of heart beats for a developing pupa are seen: the periods of high pulsations alternate with short periods of low frequencies (three high upward peaks) and heart beat pauses (P).

the large bursts of CO_2 have been lost and only intermediate and microcycles of gas exchange are seen (Figs. 3 and 4).

The typical features of heartbeats in a developing pupa are represented in Fig. 6. The period of high frequent pulsations alternates with the periods of low frequencies and heart beat pauses.

3.3. Post-treatment changes in gas exchange, heartbeat and body mass loss

After treatment with plant extracts the large CO_2 bursts were completely abolished. Soon after the treatment (5–10 min) the frequent irregular peaks of 0.12–0.55 Hz appeared on respirometric recordings which were not due to body muscular hyperactivity. No abdominal movements could be detected under a stereomicroscope either. The post-treatment peaks were similar to the chaotic flutter of relatively high amplitude and frequency (Fig. 3). The signals of higher amplitude were due to the irregular intermediate bursts of CO_2 as seen in the calorimetric power–time curves. The usual pre-treatment rhythms of gas exchange (Fig. 2) were never restored after treatment.

The essential changes in heart beat patterns were seen 5 days after treatment. Heart beat pauses shortened and heart activity periods grew longer. Yet 7–8 days later heart beat pauses occurred only sporadically at irregular intervals and lasted for 3–7 min. The short periods of low frequency (0.40–0.63 Hz) but relatively high amplitude (2.6–3.5 mV) now alternated with the higher frequency pulsations (0.71–1.21 Hz) of lower amplitude (0.2–0.4 mV) (Fig. 6; Table 3). This pattern was also typical of the pupae reactivated from diapause by treatments with JHA [32].

Usually, sufficient amounts of water and energy reserves are stored in diapausing pupae to survive at least 5 months at 20°C and 60% RH. The pupae lost 12–17% (mean 15.4±2%) of their initial body mass during the first 3 months. The critical limit of lethal desiccation was 20–22% of the initial body mass lost during the first 3 months of pupal life. After treatment with the plant extract the body mass loss increased significantly (about 30%) (Table 1).

3.4. The evidence of adult development

The pigmentation of the compound eyes is commonly regarded as the earliest symptom of the initiation of adult development in lepidopterous pupae. But the cuticula covering the eyes stays opaque in the pupae of *P. brassicae*. Therefore, only the adult cuticle visible through the old exuviae could be taken as undoubted morphological sign of the adult development. The imagines failed to emerge from pupal exuviae as a rule.

The earliest physiological evidence of breaking diapause and initiating development is the heart beat rhythm showing transformations typical of pupae after chilling. It is also well known that heart pulsations are very resistant to mild poisoning [33].

4. Discussion

The adverse actions of plants on herbivorous insects have been known for a long time but only in the last decades a more intensive research in this field arose leading to the new branch of chemical ecology [34]. The secondary metabolism of plants produces special substances which are not involved in the basal processes of life but are characteristic of individual plants and vary between them considerably [1]. These compounds may have attracting functions versus potential pollinators, but are usually defensive against herbivorous animals, mainly insects. Different classes of

organic molecules can be found among them, like alkaloids, phenolics, quinones, terpenes, steroids and flavonoids [2].

The original pyrethrum, an extract from the Dalmatian *Chrysanthemum cinerariifolium*, was used as an insecticide already 100 years ago and imported to Europe in larger amounts. Thus it may be considered as the first secondary plant metabolic product with a broad application. Its main components, the pyrethrines as contact-active insecticides, are found in different kind of chrysanthemum, among them the Painted Daisy *Tanacetum roseum*. In the present paper, the influence of a crude extract from *T. roseum* was applied to a typical crop pest, the cabbage butterfly *P. brassicae*. Morphological changes or such in developmental duration were not the special target of this investigation at suboptimal pyrethrum concentrations, but the form of intermittent respiration as a measure for metabolic activities. The method of choice was calorimetry as a general, non-specific and quick means for a long-term monitoring of the diapausing pupae of *P. brassicae* which develop in the calorimetric vessel. The respiratory behaviour is reflected in the calorimetric power-time curves as indicated in the figures of this paper.

At least three levels of CO_2 cycles or bursts: the large, intermediate and microcycles may be distinguished in our observations in diapausing pupae of *P. brassicae*.

The interval between large CO_2 bursts in *P. brassicae* pupae varied individually within wide limits even in specimens with strongly suppressed metabolic rates. Large CO_2 bursts in *P. brassicae* are directly preceded by an abrupt air uptake by suction or by passive suction ventilation (PSV) which fully matches the model of PSV [15,16]. Contrary to our findings Crozier [35] indicated in *P. brassicae* pupae O_2 uptake after the CO_2 cyclic release.

Immediately after treatments with the toxic plant extract the large CO_2 bursts disappear completely. The peaks seen in the calorimetric and respirometric signals are due to chaotic intermediate CO_2 bursts. This pattern of gas exchange is characteristic for the developing pupae of *P. brassicae*.

Nevertheless, we do not suppose that the diapause was abruptly interrupted as it may be concluded from the rapid abolishing of large bursts of CO_2. The latter event was undoubtely the toxic effect resulting in a partial perturbation of cyclic gas exchange. However, the cyclic gas exchange in *P. brassicae* pupae was not totally abolished as far as intermediate CO_2 bursts and gas exchange microcycles were preserved.

It was suggested that the main physiological cause of the death of poisoned insects may-be due to respiratory failures such as the loss of cyclic CO_2 release [36]. The diffusive-convective retention of water is thought to be abolished by the toxic action. Such a cyclic CO_2 release has been considered as an effective water-conserving mechanism in a number of insect species [5,17,37]. But this hypothesis is experimentally verified only in a few publications. To our knowledge, Kestler [5] was the first to demonstrate the perturbation of cyclic CO_2 release in an intoxicated insect (*Periplaneta americana*). The clear gas exchange cycles in the pupae of *Galleria mellonella* and *Tenebrio molitor* were also abolished by some toxic plant extracts [20,38].

To initiate adult development in pupae, the prothoracic glands have to be stimulated to secrete ecdyson [39]. The prothoracic glands can be activated directly by JHA or via the prothoracicotropic (PTTH) hormone of the brain [9]. The second way is more probable, because the brain responds directly when the adult development of diapausing pupae is stimulated by organic solvent [12]. Therefore, we suggest that pyrethrum breaks the diapause of *P. brassicae* by its action on the brain and stimulates the release of PTTH.

Of course, it is important to know if the adult development can be stimulated by pyrethrins in debrained pupae. Unfortunately, a part of operated, but non-treated diapausing pupae showed some signs of imaginal development as well, obviously due to a wound shock (unpublished data) so that we cannot take these results into consideration.

In natural environments, the chilling (i.e. cold reactivation) of *P. brassicae* pupae activates the brain neurosecretory cells to secrete the prothoracicotropic hormone. The latter in its turn activates the prothoracic glands to secrete the moulting hormone (ecdysone) which evokes the adult development in pupae. The hormonal regulation of pupal diapause of the cabbage butterfly was reviewed by Feltwell [7] and Beck [6].

The voltage-sensitive sodium ion channels in the membrane are probably the major initial sites of the toxic action of pyrethrum, but although neurosecre-

tory neurones are very sensitive to these toxicants resulting in an abnormal release of neurohormones. The release of a diuretic hormone from neurosecretory cells into the haemolymph may result in a dramatic loss of body fluid in adult insects [3]. In the same way, some organochlorine and organophosphorus insecticides may also induce the release of neurohormones in adult insects [40]. The disruption of the balance of neurohormones in the insect could be a major factor in the toxicity of pyrethrins [3]. The data mentioned above may support the suggestion that pyrethrins stimulate the brain directly to release PTTH in the diapausing pupae of *P. brassicae*.

In the case of mild poisoning by pyrethrins there seem to proceed two processes which are contrary and independent of each other: a toxic action observed as a respiratory failure and the initiating of adult development. As a rule, the imago of *P. brassicae* failed to emerge from pupal exuviae. After treatments with organic solvents, however, adults emerged normally [12].

No literature data could be found indicating the morphogenetic action of pyrethrins and synthetic pyrethroids. Treatments with the pyrethrum of the last instar caterpillars of *P. brassicae* never produce the larval-pupal intermediates (unpublished results) as in the case of treatments with juvenoids [10].

To our knowledge, the mechanisms how non-hormonal neurotoxic agents induce the adult development in diapausing pupae have yet been scarcely investigated. More details of action of pyrethrum on the hormonal centre of the diapausing pupae of *P. brassicae* are yet to be elucidated.

Acknowledgements

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