Eur. J. Entomol. **110**(1): 47–54, 2013
http://www.eje.cz/pdfs/110/1/47
ISSN 1210-5759 (print), 1802-8829 (online)

Gas exchange patterns in *Platynus assimilis* (Coleoptera: Carabidae): Respiratory failure induced by a pyrethroid

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Key words. Carabidae, *Platynus assimilis*, alpha-cypermethrin, resting metabolic rate, non-target, respiration

Abstract. Discontinuous gas exchange (DGE) is the main (23 individuals) breathing mode in resting adult *Platynus assimilis*. Few of the beetles tested (13 individuals) displayed a pattern of cyclic gas exchange or CGE. The burst of CO_2 release in DGE and CGE was always accompanied by abdominal pumping (active ventilation or V). Seven individuals displayed a pattern of continuous respiration, characterized by regular abdominal pumping. Resting metabolic rate (RMR) in continuously breathing beetles was higher than in those using DGE and CGE. After treatment with sub-lethal doses of alpha-cypermethrin DGE ceased. Treated beetles were characterized by continuous pumping and almost regular periods of activity. RMR increased significantly after treatment with a pyrethroid.

INTRODUCTION

Carabid beetles (Coleoptera: Carabidae) are important polyphagous natural pest control agents in agricultural fields. They are sensitive to anthropogenic changes in habitat quality and affected by intensive agriculture. They can be influenced by tillage as well as by the treatment of crops with pesticides (Kromp, 1990). On conventional farms, pesticides threaten the survival of non-target carabid beetles living in both treated and untreated areas, because carabids move relatively fast. They may contact pesticides directly or feed on chemically-treated seeds and pests (Kromp, 1999). Basedow (1987) considers pesticide application to be the main reason for the reduced numbers of carabids in conventionally-farmed wheat fields. Pesticide use on conventional farms may also cause ecological damage to neighbouring organic farms by killing carabid beetles.

Pyrethroids are currently the most commonly used insecticides in the world (Horton, 2011) and alpha-cypermethrin, the most widely used active ingredient. These pesticides are highly toxic to insects and aquatic organisms (Mueller-Beilschmitdh, 1990; Solomon et al., 2001; Karise, 2007) but of relatively low toxicity to terrestrial vertebrates (Solomon et al., 2001; Yarkov et al., 2003). Pyrethroids are similarly toxic to both pests and non-target organisms with the molecular targets in insects analogous to those in mammals (Marrs & Ballantyne, 2004). Pyrethroids are primarily toxins targeting the functioning of the nervous system (Narahashi et al., 1998). Thus, they owe their insecticidal potency to a rapid functional disruption of an insect's neuromuscular system and the secondary consequences of this, rather than to any direct cytotoxicity (Ray & Fry, 2006). Pyrethroids may become more concentrated in organisms in the upper parts of food webs (Solomon et al., 2001).

Treatments with chemical pesticides never kill all the insects in a population and may induce poorly studied sub-lethal and delayed effects in the survivors. Several methods have been used to assess the effects of insecticides on the physiology of beetles. Sláma & Miller (1987) used a hydraulic transducer to record the neurotoxic effects of a pyrethroid on pupae of *Tenebrio molitor.* Zafeiridou & Theophilidis (2006) used a force displacement transducer attached to the dorsal surface of the second abdominal segment of *T. molitor* and to monitor the respiratory contractions before and after sublethal poisoning with a pyrethroid.

It is very important to determine the normal physiological state of an insect before studying pathological effects. The physiological state of an insect is most often measured in terms of standard metabolic rate (SMR) or resting metabolic rate (RMR). The effects of toxicants on the SMR are well recorded (Keister & Buck, 1974). Gas exchange patterns are also used to characterize the physiological state of insects, but there are few studies on the sublethal and delayed effects of insecticides. In discontinuous gas exchange (DGE) CO_2 is released periodically in bursts. The classic DGE consists of three periods or phases, originally termed CFO based on the spiracular activity (Schneiderman, 1960): constriction (C), fluttering (F) and open (O) phases. During the C-phase, the spiracles are tightly closed and no gas exchange occurs, during the F-phase the spiracular valves open and close rapidly and finally, in the O-phase, CO_2 is released in a burst (Lighton, 1996; Chown & Nicolson, 2004; Gibbs & Johnson, 2004; Marais et al., 2005; Chown et al., 2006). Oxygen uptake occurs in the F phase by the principle of Passive Suction Ventilation (PSV), which reduces water loss (Kestler, 1980, 1982). The DGE is commonly termed the CFO cycle when there is no evidence of active ventilation during the release of CO_2. The term, CFV emphasises muscular ventilation (V) during CO_2 emission (Kestler, 1971, 1985). A specific strategy to reduce loss of water is the replacement of diffusive O-phases by convective V-phases, wherever possible (Kestler, 2003).

In cyclic gas exchange (CGE) there are distinct bursts and inter-burst periods and at least some CO_2 production is detectable even during the inter-burst periods (Marais & Chown, 2003; Nespolo et al., 2007). Thus, during CGE, CO_2 release does not decrease to zero or even close to zero (Gray & Bradley, 2006).

There is no evidence of cyclic or discontinuous release of CO_2 during continuous respiration in which the spiracles open and close continuously and asynchronously (Gibbs & Johnson, 2004; Terblanche & Chown, 2010). Active individuals typically show continuous respiration (Marais et al., 2005).

There are several hypotheses of the evolutionary origin of discontinuous or cyclic release of CO_2 (Chown et al., 2006; Woods, 2011). According to older hypotheses the DGE originated as a water-saving mechanism (Schneiderman, 1960). However, DGE may have other useful functions.

The theory of a non-adaptive or mechanistic origin of DGE (Chown et al., 2006, 2011; Chown, 2011) must also be considered. The model proposed by Förster & Hetz (2010) assumes that both CO_2 and O_2 are involved in the regulation of spiracle behaviour. The CO_2 partial pressure threshold for spiracle opening is sensed by the spiracle muscle and the O_2 partial pressure threshold by the segmental ganglia of the central nervous system (Förster & Hetz, 2010; Chown et al., 2011). Separate CO_2 and O_2 systems as a non-adaptive explanation for DGE behaviour does not contradict several adaptive explanations (Hetz & Bradley, 2005; Förster & Hetz, 2010; Chown et al., 2011). The lengthening of the C-phase and shortening of the O-phase restrict water loss (Schimpf et al., 2012). A very long C-phase, lasting nearly one day, is characteristic of diapausing pupae of the cabbage white butterfly, *Pieris brassicae* (Jõgar et al., 2011).

Kestler (1991) reports sublethal effects of toxicants on CFV cycles (DGE), which are transformed into a pattern of continuous respiration. Some early studies also report that the Colorado potato beetles, *Leptinotarsa decemlineata* (Kuusik et al., 2001a), *Hylobius abietis* (Sibul et al., 2004) and pupae of *P. brassicae* (Harak et al., 1999; Jõgar et al., 2006, 2008) treated with toxic substances do not respire using DGE. Appel et al. (1997) report that insects do not respire using DGE following treatment with contact insecticides, which makes them more susceptible to desiccation and possibly easier to control.

The present study focused on the carabid beetle *Platynus assimilis*, a mainly night-active, soil-surface-dwelling beetle, which is a common predator in agricultural fields and forests. One aim of the present study was to measure the resting metabolic rate (RMR) and to characterize the gas exchange patterns in adult *P. assimilis* at rest. Another aim was to investigate the effect of sublethal doses of alpha-cypermethrin on the levels and patterns of gas exchange in this beetle.

MATERIAL AND METHODS

Insects and treatments

Adult beetles of *P. assimilis* were collected from hibernation sites (tree stumps), in Tartu County, Estonia, in January 2010. They were kept for one day in the laboratory prior to the experiment. Based on preliminary experiments temperature compensation of respiration occurs each day at room temperature. These results were similar to earlier studies on temperature compensation in several insects (Kuusik et al., 1995). For the experiments, 43 beetles (40–42 mg) were selected and placed individually in Petri dishes. Experiments were performed at 22 ± 1°C in a thermostat.

The respiration of the beetles was measured for at least 3 hours before and after treatment. During these measurements the temperature and humidity conditions were recorded by means of HygroClip probes (HygroPalm, Rotronic Company).

Fastac 50 EC, which is a commercial formulation of alpha-cypermethrin (a.i. $50g\ l^{-1}$), was used in the experiments on beetles showing only DGE. One ml of the formulation (a 5% emulsion) was diluted in 100 ml distilled water (0.05%, field solution of formulation) and different concentrations (0.01%, 0.001% and 0.005%) were prepared. Preliminary tests showed that concentrations of 0.05% and 0.01% were lethal for carabid beetles, so these concentrations were not used in this study. Thus, beetles were treated with concentrations of either 0.001% or 0.005%. Also, our preliminary studies revealed that topical applications to the thorax or abdomen using acetone as the solvent resulted strong toxic and metabolic delayed effects. For this reason we the dipped the beetles into an aqueous emulsion of the pyrethoid for 10 s. Dipping is used as an alternative contact method for bioassays of substances on several insect species (Van der Stern, 2001; Cetin et al., 2006; Wanyika et al., 2009). The beetles in the control were dipped in distilled water.

Respirometry

To achieve high resolution recordings of active ventilation we used coulometric respirometry. The coulometric respirometry was used to record pumping movements as the volumetric method only records rhythmic changes in body external volume.

Coulometric respirometry

Coulometric respirometers (a volumetric-manometric system) are characterized by a continuously O_2-compensating system (Kuusik, 1977; Kuusik et al., 1996; Vanatoa et al., 2006; Jõgar et al., 2011). The principles of the coulometric respirometry system we used are described by Lighton (2008).

In the present study, we used a modified and compact coulometric respirometer (Fig. 1) suitable for small insects, including *P. assimilis*. In the insect chamber of the coulometric respirometer the beetle could walk in a restricted area and typically remained stationary (resting) occasionally interrupted by short periods of walking (activity).

This respirometer ensures continuous and adequate replacement of consumed O_2 with electrolytically-produced O_2. The insect itself plays an active role in this self-regulating system.

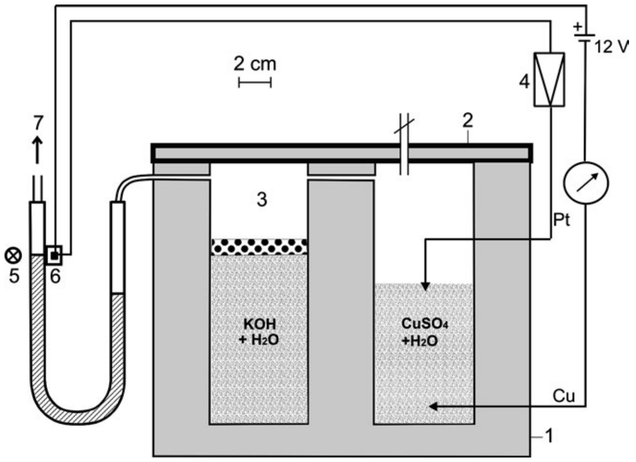

Fig. 1. Design of the continuous recording coulometric respirometer used in the experiments. 1 – plexiglass block; 2 – removable cover; 3 – insect chamber; 4 – current amplifier; 5 – light source; 6 – photo-transistor; 7 – compensating vessel.

Simultaneously, the rates of O_2 production and O_2 consumption by the insect (V_{O_2} ml h^{-1}) are measured. The system also records transient changes in the rate of release of CO_2. In our respirometer, the electrolysis current was directly connected with a photoelement instead of switching the electrodes. High sensitivity of the respirometer to pressure changes in the respiration chamber was achieved by replacing the standard photodiode with the photosensitive element of a transistor (KT302A, Semitronics, Freeport, NY, USA), which has a very small photosensitive area (approximately 0.5 mm^2). In this way, the smallest movement in the meniscus of ethanol inside the U-shaped capillary was reflected as a signal on the recording trace. The electrolysis current depended on the ethanolic meniscus in the glass capillary, which served as a shutter to screen the photosensitive area from light. Every burst of CO_2 produced by the beetle caused a small movement in the ethanolic meniscus, which shuts off the photosensitive area from the light source, resulting in a downward movement in the recording trace (Fig. 2).

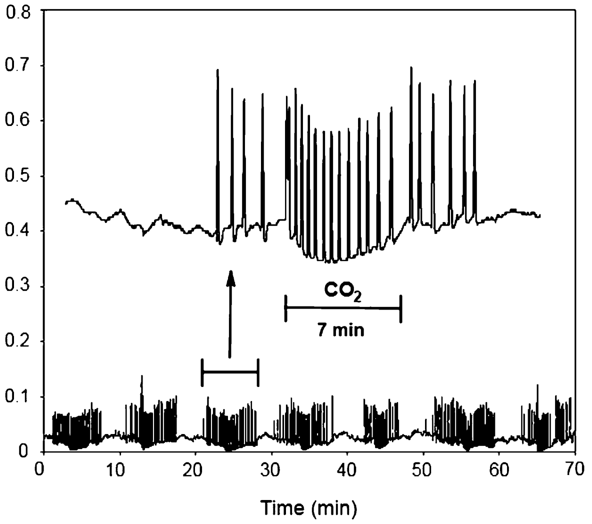

Fig. 2. An example of the gas exchange cycles of adult *Platynus assimilis* recorded by the coulometric respirometer (lower trace). The lower trace indicates in great detail the actographic recording of the pumping movements. Each burst of CO_2 release is actively ventilated by pumping movements of the abdomen. Upper trace is a detailed high resolution record of the pumping movements during a burst of CO_2 release. The downward peak on this recording indicates the bursts of CO_2 release horizontal bar.

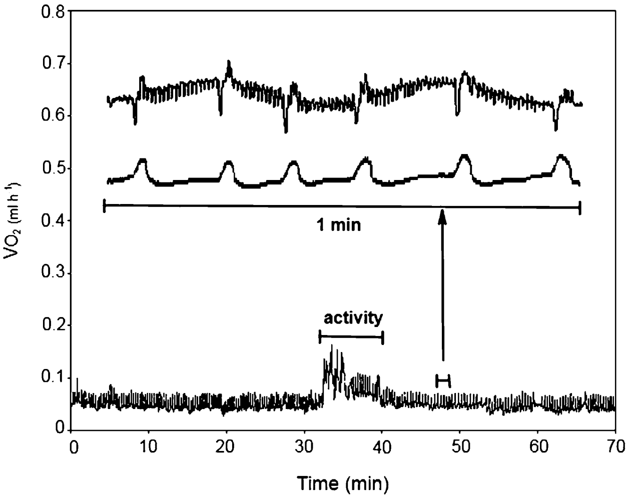

Fig. 3. The pattern in gas exchange of beetles after treatment with pyrethroid (Fastac 50 EC) recorded by the coulometric respirometer (lower trace). Middle trace is a more detailed record of the pumping movements recorded during the fraction of the lower trace indicated by the arrow. The same pumping movements were simultaneously recorded by the infrared actograph (upper trace); the frequent peaks between pumping movements we interpret as heartbeats. Note that the gas exchange is continuous. The horizontal line above the lower trace indicates a period when the beetle was active.

The coulometric respirometer allowed simultaneous recording of O_2 consumption and abdominal pumping movements (Figs 2, 3). Every pumping stroke resulted in a rapid decrease (contraction) and increase (relaxation) in the volume of the body. These body movements caused pressure-volume pulses in the insect chamber, which was connected to the glass U-capillary. The ethanol meniscus in the capillary oscillated in synchrony with the abdominal pumping, which was translated into electrical signals that were recorded as discrete spikes on the recording trace. In this way, the coulometric respirometer served also as an insect activity detector (Fig. 3) and actograph for recording discrete body movements.

Flow-through respirometry and IR-actography

Metabolic rates (V_{CO_2} ml h^{-1}) and gas exchange patterns were measured also by a flow-through system, using a differential gas analyzer (DIRGA) and a pressure compensated URAS 26 (ABB Analytical, Frankfurt, Germany), covering a measuring range of 0 to 500 ppm. The data from the analyzer were sampled at a rate of 10 Hz to PC via the analog output. Ambient air from outside the laboratory was scrubbed of carbon dioxide and water by passing it through columns containing Soda Lime and Drierite. A flow rate of 200 ml min^{-1} was used. The CO_2 channel was calibrated with commercially available span gas (Eesti AGA AS, Estonia; Linde AG, Höllriegelskreuth, Germany).

The flow-through respirometry was combined with infrared opto-cardiographic measurements (see Metspalu et al., 2001). An IR-emitting diode was placed on the side of the insect chamber near the ventral side of the insect's abdomen, while the IR-sensitive diode (TSA6203) was placed on the opposite side of the chamber. The light from the IR-diode was modulated by

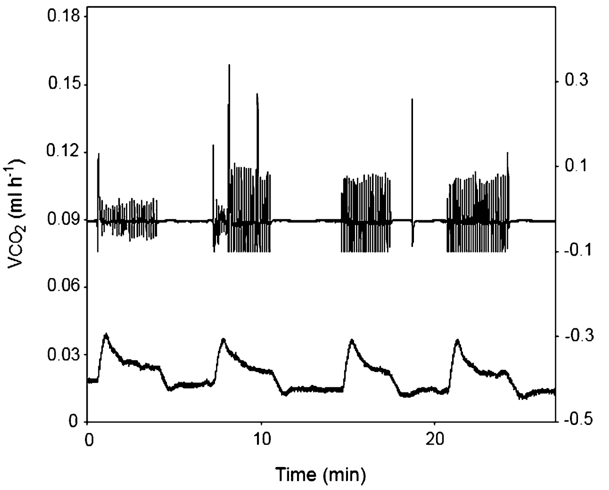

Fig. 4. Carbon dioxide emission of an adult of *P. assimilis* exhibiting cyclic gas exchange recorded using flow-through respirometry (lower trace). Upper trace is a simultaneous recording of the pumping movements during the bursts of CO_2 release recorded using an infrared opto-cardiograph.

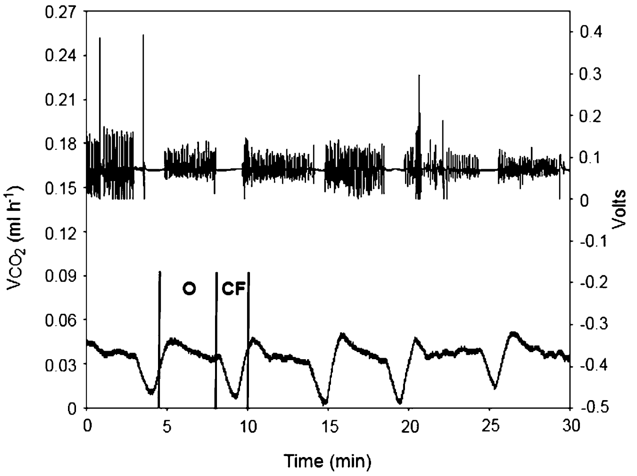

Fig. 5. A flow-through CO_2 respirometer recording of the DGE of an adult *P. assimilis* with a relatively long O-phase and short CF-phase (between vertical lines) (lower trace). Upper trace is a synchronous infrared actograph recording of pumping movements recorded during the bursts of CO_2 release.

abdominal contractions. The level of the output voltage reflected the vigour of the muscular contractions of the insect (Hetz, 1994; Hetz et al., 1999; Mänd et al., 2005; Karise et al., 2010; Kivimägi et al., 2011).

Data acquisition and statistics

Computerised data acquisition and analysis were performed using the DAS 1401 A/D analogue-digital converter as hardware and the TestPoint as software (Keithley, Metrabyte, Cleveland, OH, USA) with a sampling rate of 10 Hz. Mean (± SD) resting metabolic rates were calculated automatically using a statistical program (StatSoft ver. 8, Inc./USA). Statistical comparisons were made using the Wilcoxon Matched Pairs Test and one-way ANOVA (analysis of variance). The significance level was set at $P < 0.05$.

RESULTS

Our results revealed that 23 of the 43 beetles tested used only the DGE mode of respiration, 13 beetles CGE and seven beetles continuous respiration.

The O-phase of the beetles that used CGE made up 40–50% of the whole cycle (Fig. 4). The O-phase was followed by a short C-phase and the F-phase was absent or at least not separated from the C-phase.

The DGE was characterized by a relatively long O-phase (burst of CO_2) making up 80–90% of the whole cycle while the C- and F-phases were not separated from each other (Fig. 5).

In both DGE and CGE the burst of CO_2 release was always accompanied by active (muscular) ventilation (V) or pumping. The mean duration of DGE was shorter than the CGE 444.8 ± 8.1 s and 491.9 ± 5.7 s, respectively ($F_{1,34}$ = 0.17; p = 0.05). No differences were found in resting metabolic rates between DGE and CGE: 0.94 ± 0.01 (V_{CO_2} ml $h^{-1}g^{-1}$) and 0.93 ± 0.01 (V_{CO_2} ml $h^{-1}g^{-1}$), respectively ($F_{1,34} = 0.17$, $p = 0.68$).

Continuous respiration in *P. assimilis* was characterized by the continuous pumping movements recorded by coulometric respirometry (Fig. 3). Between the pumping movements there were frequent pulsations, which we interpreted as heartbeats (see Fig. 3). Individuals using continuous respiration had higher resting metabolic rates (1.03 ± 0.02 V_{CO_2} ml $h^{-1}g^{-1}$) than those using DGE (0.94 ± 0.01 (V_{CO_2} ml $h^{-1}g^{-1}$) ($F_{1,28} = 11.46$; $p = 0.029$).

After treatment with sublethal doses (0.005%, 0.001%) of the pyrethroid alpha-cypermethrin the beetles ceased DGE and switched to a form of continuous gas exchange (Fig. 6). The treated beetles became active for short periods with a frequency of 1–2 per hour. An increase in CO_2 emission was recorded during each activity period.

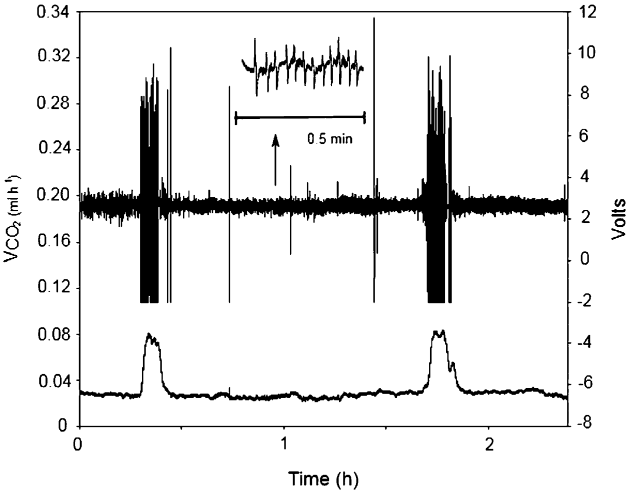

Fig. 6. Typical flow-through respirometry recording of the pattern in gas exchange of *P. assimilis* recorded after treatment with sublethal doses of Fastac 50 EC (lower trace). Note the beetles ceased breathing by DGE and switched to continuous respiration. There are two higher peaks of activity recorded in the upper trace that were synchronously recorded by the infrared actograph; the insert is a more detailed record of the continuous pumping movements. The peaks in CO_2 emission recorded in the lower trace are due to the activity of the beetle and not to cyclic gas exchange.

TABLE 1. The metabolic rates (V_{CO_2} ml $h^{-1}g^{-1}$) of individuals of *P. assimilis* recorded before and after treatment with two concentrations of Fastac 50 EC and distilled water as the control.

Treatment	Indiv.	Metabolic rate (V_{CO_2} ml $h^{-1}g^{-1}$)	
		Before	After
0.005% Fastac 50 EC	1	0.854	1.171
	2	0.976	1.244
	3	0.951	1.121
	4	0.878	1.098
	5	0.976	1.145
	6	0.927	1.122
	7	0.878	1.120
	8	1.122	1.146
	9	1.002	1.122
		t = 0.000 *d.f.* = 8 ***p* = 0.007**	
0.001% Fastac 50 EC	1	0.927	1.098
	2	0.976	1.073
	3	0.878	1.070
	4	1.002	1.171
	5	0.927	1.121
	6	0.976	1.122
	7	0.925	1.049
	8	0.951	1.120
		t = 0.000 *d.f.* = 7 ***p* = 0.012**	
Dist. water	1	0.878	0.902
	2	0.976	0.927
	3	0.927	0.925
	4	0.879	0.976
	5	0.979	1.002
	6	0.925	0.952
		t = 6.00 *d.f.* = 5 *p* = 0.345	

The activity periods may be easily confused with bursts of CO_2 release. These activity periods (struggling) were also confirmed by visual observations under a stereomicroscope. The metabolic rate between active periods was measured. The mean resting metabolic rate of beetles treated with 0.005% and 0.001% Fastac 50 EC were 1.140 ± 0.015 (n = 9) and 1.104 ± 0.016 (n = 8) V_{CO_2} ml $h^{-1}g^{-1}$, respectively. Thus the mean resting metabolic rate significantly increased after treatment in both groups 0.948 ± 0.016 (0.005%) and 1.140 ± 0.016 (0.001%) V_{CO_2} ml $h^{-1}g^{-1}$, respectively (see also Table 1).

DISCUSSION

There was a significant difference in the between-individual variability in gas exchange patterns in adult *P. assimilis*. Variability within and between individuals in physiological characteristics, including gas exchange patterns, is regarded as a normal phenomenon in insects (Chown, 2001; Chown et al., 2002; Marais & Chown, 2003). Essential between-individual variation was found in the patterns of gas exchange of beetles that show DGE, CGE and continuous gas exchange.

Our results indicate that, in *P. assimilis*, the metabolic rate of beetles that show DGE and CGE did not differ, which contrasts with the literature in which the metabolic rate of DGE beetles is commonly recorded as lower than that in CGE beetles. However, *P. assimilis* exhibited an uncommon pattern of DGE: the O-phase (burst) was extraordinarily long compared with the whole cycle. This pattern of gas exchange is similar to that described by Duncan & Dickman (2001) for the carabids *Cerotalis* sp. and *Carenum* sp. and Kivimägi et al. (2011) for *Pterostichus niger*. The O-phase (burst) in all beetles coincided with muscular ventilation.

There were few resting *P. assimilis* that exhibited continuous respiration in which ventilation by muscular abdominal pumping occurred continually. Abdominal pulsations may be easily confused with heartbeats (Sláma, 2000). In *P. assimilis* we recorded continuous heartbeats not coordinated with either bursts of CO_2 release or periods of abdominal pulsation, but Wasserthal (1996) reports that in *Thermophilum hexmaculatum* the heartbeat period is synchronised with ventilatory movements of the abdominal tergites. Breathing by muscular abdominal pumping is well known in insects (Miller, 1974, 1981). The continuous respiration in adult *Tenebrio molitor* is also characterized by abdominal pumping or respiratory contractions occurring continually or periodically (Zafeiridou & Theophilidis, 2004, 2006). Sláma (2008, 2010) also describes weak abdominal movements, such as extracardiac haemocoelic pulsations.

Resting metabolic rate in beetles with continuous active ventilation was significantly higher than in individuals using DGE. That metabolic rate varies with the gas exchange pattern and is lowest in individuals that use DGE and highest in individuals using continuous gas exchange is also reported by Gibbs & Johnson (2004) and Contreras & Bradley (2009, 2011).

Treatments of adult *P. assimilis* with 0.005% and 0.001% emulsions of alpha-cypermethrin resulted in the beetles switching from DGE to continuous respiration via pumping. However, the pumping in treated beetles occurred at higher frequencies and amplitudes than in untreated beetles characterized by continuous gas exchange. Mild desiccation, sublethal doses of toxicant and handling stress also result in a similar pattern of release of CO_2 (Kestler, 1991).

Cessation of DGE may be regarded as the earliest symptom of poisoning in insects. It may be suggested that disturbances in DGE are due to the paralysis of the opening-closing mechanisms of spiracles. However, it is also possible that the pesticide increases metabolic rate due to uncontrolled muscle activity and it is this that causes them to switch from DGE to a continuous mode of gas exchange. However, there are also other factors that might result in the cessation of normal DGE (Kuusik et al., 2001b). The mechanism in insects may be either autointoxication and/or the release of neurohormones, or changes in O_2/CO_2 thresholds, or in the CO_2 capacitance due to changes in the acid-base status (Kestler, 1991).

Resting metabolic rate of treated individuals of *P. assimilis* was significantly higher than that of untreated individuals. The increase in the metabolic rate of treated beetles may be a consequence of the metabolic cost of vigorous pumping. According to Chown & Holter (2000) and Sibul et al. (2008) an increase in metabolic rate may be due to a small increase in the metabolic cost of convective ventilation by muscular contractions.

DGE is advantageous for insect species of different lifestyles living in a wide range of environments. Thus, ceasing to breath by means of DGE is harmful for insects. According to Schimpf et al. (2012) insects breathing by means of DGE survive for longer when deprived of food and water, which indicates that DGE confers a fitness benefit by reducing water loss.

Carabid beetles are important agents of biological control in organic farming programs (Kromp, 1990). Our data indicate that the respiratory system of *P. assimilis* is vulnerable to pyrethroids, which induce a respiratory failure in this beetle. When pyrethroids are applied to agricultural land their physiological effect on predaceous beetles should not be ignored.

ACKNOWLEDGEMENTS. The authors are grateful to editores and the anonymous referees for their critical and valuable comments on this manuscript. The research was supported by the Estonian Science Foundation (grants 9449 and 8895), State Forest Management forest protection project (2012–2015) and Estonian target financing project number SF170057s09.

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Received May 28, 2012; revised and accepted August 17, 2012