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SELECTED INSECTICIDES AS MODIFIERS OF THE METABOLIC RATE IN *ANOPLOTRUPES STERCOROSUS* UNDER VARIOUS THERMAL CONDITIONS: THE EFFECT OF INDOXACARB AND BETA-CYFLUTHRINE

Bartosz Piechowicz[1], Justyna Gola[1], Przemysław Grodzicki[2]

[1]University of Rzeszów
[2]Nicolaus Copernicus University in Toruń (NCU)

Abstract. In 2007 and 2008 studies aimed to determine the effect of indoxacarb, substance classified as oxadiazines (Steward 30 WG) and beta-cyfluthrine, belonging to synthetic pyrethroids (Bulldock 025 EC), on the oxygen consumption rate by adult *Anoplotrupes stercorosus* were performed. Experiments were carried out under various ambient temperature conditions (14, 19, 24 i 29°C) using two ways of intoxication – contact intoxication, or by an ingestion of the biocide. In insects of control group an ambient temperature affected the oxygen consumption only to a small extent. Conversely – applying insecticides markedly potentiated the oxygen consumption, whereas it was also increasing together with an increase of the temperature. The way of the intoxication affected the oxygen consumption only to quite a small extent. However, its greatest changes were noted in animals treated by contact intoxication.

Key words: beetles, *Anoplotrupes stercorosus*, metabolic rate, pesticide, oxadiazine, pyrethroid

INTRODUCTION

Dung beetles *Scarabeidae* belongs to the group of organisms that play a crucial role in the functioning of the forest environment. Thanks to them there is a rapid conversion of biomass and nutrients restore to the ecosystem (Horgan, 2001; Horgan, 2005), which

Corresponding author – Adres do korespondencji: Dr Bartosz Piechowicz, Department of Ecotoxicology, Institute of Applied Biotechnology and Basic Science, University of Rzeszów, Werynia 502, 36-100 Kolbuszowa, Poland, e-mail: bpiechow@poczta.onet.pl

shortens by about three-times the time of organic matter decomposition (Slade et al., 2007). The acceleration of decomposition processes by *Scarabeidae* beetles limits the process of nitrogen loss from the soil. It contributes to an increase of a primary production, especially for the poor and degraded soils (Bang et al., 2005; Borghesio et al., 1999). It also plays an important role in the elimination of potential epidemiological outbreaks (Boze et al., 2012). *Scarabaeidae* beetles contribute to the renewal of forest stands – either by protection of the seeds against granivorous birds (Andresen, 2003; Shepherd and Chapman, 1998; Hanski and Krikken, 1991) or by entomochoria (Estrada et al., 1999). They also play a considerable role in the process of soil formation, by increasing its aeration and improving water conditions in forest grounds (Bang et al., 2005; Halffter and Matthews, 1966). In spite of their complex influence on the environment those animals belong to the group of the so-called 'environmental engineers', it means, organisms which play a vital role in the ecosystem's functioning (Boze et al., 2012). On the other hand, they are used as indicator organisms because of their distinct sensitivity to anthropogenic degradation of forest environments (Davis et al., 2001; Hill, 1996; Klein, 1989).

Our study was aimed to test the extent to which applied insecticides, with different mechanisms of action, affect the rate of oxygen consumption in adults *A. stercorosus* under different ambient temperature conditions.

MATERIAL AND METHODS

Experiments were performed on adult *Anoplotrupes stercorosus*, both sexes. Insects were collected in forests near the village of Werynia in the Sub-Carpathian Province. For 5 days before the test, animals used in the study were acclimated to a temperature of 22°C and the daily cycle L/D: 12/12. A total of 240 animals were used for the study.

The following insecticides were used for the experiment:

1. Steward 30WG. Producer: Du Pont de Nemours. Active substance: indoxacarb (a compound belonging to the group of oxydiazines) – 300 g in 1000 g of the agent. The concentration of the usable liquid: 2.33 g/dm^3 of water.

2. Bulldock 025 EC. Producer: Bayer AG – Germany. Active substance beta-cyfluthrin (a compound belonging to the group of pyrethroides) – 25 g in 1 dm^3 of the agent. The concentration of the usable liquid: 0.50 cm^3/1 dm^3 of water.

The experiment

Experiments on the effect of insecticide usage and ambient temperature on the metabolic rate in adult *A. stercorosus* were carried out in August 2007 and 2008. Intoxication of animals was performed using two methods:

1. By individual treatment of the animals solutions of given preparations. A drop of the preparation (water in the control), which volume was 4 µl, was applied using automatic pipette on the ventral part of an insect's thorax, near the paraoesophageal ring. Animals were then placed separately in plastic boxes provided with food (apple pulp fragment of approximately 6 cm^2 ±5%) and water. After that, they were transferred into

a modified incubator (type: Q-Cell model ERC0750) under previously established ambient temperature (14, 19, 24 or 29°C) and photoperiod (L:D 12:12) conditions. Each test was performed on 10 subjects.

2. Alimentary – by food intoxication. The formulations in an amount of 6 cm^3 were sprayed onto the surface of apple pieces of a size approximately 6 cm^2 ±5%. They were placed in a plastic container together with water. The prepared containers with animals located inside were transferred to a modified incubator (type: Q-Cell model ERC0750) under previously established ambient temperature (14, 19, 24 or 29°C) and photoperiod (L:D 12:12) conditions. Each test was performed on 10 subjects.

Every 12 hours the food and water for feeding and watering the animals were replaced (in case of animals intoxicated by food only the first portion was treated by xenobiotics).

The assumed duration of each measuring cycle was 72 hours after the contact of the animal with the preparation. At the end of each day of the experiment the oxygen consumption rate was measured for each animal. Analyses were performed using the method of closed micro-respirometric test described by Ross (2000) under the same thermal conditions as used in the growth chambers. The residence time of the animals in respirometry kit was 4 hours, wherein the data acquisition lasted 30 minutes (this paper presents the average of 3 readings carried out at 15 minutes intervals). After the analysis, the animals were again placed in the breeding chamber.

The calculations

Q_{10} values were calculated using mathematical formula $\frac{\left(\frac{W_{T2}}{W_{T1}}\right)^{10}}{T_2 - T_1}$, where W_{T2} and W_{T1} – values of oxygen consumption obtained in different thermal conditions (temperatures, ambient temperatures), T_2 and T_1 – temperatures.

The obtained test results were analysed statistically using Statistica version 10.0 software. One-way ANOVA with Tukey's post-hoc test was used.

RESULTS AND DISCUSSION

Statistically significant effect of the temperature on metabolic rate of insects was noted in the control group only in few cases, wherein in lower temperatures very frequently the oxygen consumption oscillated in a clearly visible manner. The most intensive gas exchange was noted in insects kept at 29°C. This may indicate that in addition to the temperature, which plays a crucial role as the essential modifier of the intensity of metabolic processes in poikilothermic organisms (Tęgowska et al., 2004; Tęgowska, 2003; Salvucci and Crafts-Brandner, 2000; Vogt and Appel, 2000), also the degree of the organism's acclimatization to environmental conditions affects the intensity of its metabolic processes. Such a phenomenon has been observed in insects by Nielsen et al. (1999). Different results were shown after the intoxication of the insect by insecticides, such as Stewart 30 WG and Bulldock 025 EC, which induced in many cases a statisti-

cally significant increase of the metabolic rate in *A. stercorosus*, together with increasing temperature. In most cases animals of groups intoxicated by biocides showed higher metabolic rate than in the control group (Table 1 and 2). Pyrethroids have a toxic effect, by prolonging the open position of sodium channels in excitable cells (Kakko, 2004; Soderlund et al., 2002; Symington et al., 1999; Aldridge, 1990) and by blocking the GABA receptors, that induces an increase in the probability of an action potential

Table 1. Effect of temperature and mode of application of the preparation on the oxygen consumption of *A. stercorosus* in control group and intoxicated by insecticides

Tabela 1. Konsumpcja tlenu przez osobniki *A. stercorosus* z grupy kontrolnej oraz grup intoksykowanych środkami owadobójczymi w zależności od temperatury i sposobu aplikacji preparatów

Time Czas h	Intoxication – Intoksykacja							
	Contact – Kontaktowa				Alimentary – Poprzez pokarm			
	14°C	19°C	24°C	29°C	14°C	19°C	24°C	29°C
	ml O_2 h^{-1} g^{-1} m.c. – ml O_2 h^{-1} g^{-1} b.m.							
Control – Kontrola								
24	0.27 ±0.044	0.25 ±0.101	0.62 ±0.058	1.00 ± 0.097	0.23 ±0.055	0.29 ±0.070	0.52 ±0.102	0.74 ±0.116
48	0.53 ±0.129	0.41 ±0.046	0.33 ±0.101	0.85 ±0.111	0.43 ±0.142	0.28 ±0.078	0.55 ±0.067	0.72 ±0.099
72	0.35 ±0.134	0.55 ±0.082	0.49 ±0.076	0.60 ±0.229	0.38 ±0.080	0.39 ±0.055	0.58 ±0.118	0.73 ±0.154
Steward 30 WG								
24	0.40 ±0.061	0.55 ±0.068	1.51 ±0.276	1.63 ±0.220	0.44 ±0.065	0.51 ±0.44	1.09 ±0.095	1.19 ±0.156
48	0.30 ±0.075	0.49 ±0.090	1.42 ±0.095	1.40 ±0.133	0.23 ±0.110	0.24 ±0.041	0.57 ±0.109	0.94 ±0.177
72	0.37 ±0.082	0.41 ±0.063	1.27 ±0.186	1.39 ±0.190	0.023 ±0.46	0.21 ±0.044	0.53 ±0.052	0.80 ±0.211
Bulldock 025 EC								
24	0.38 ±0.103	0.63 ±0.094	1.01 ±0.100	1.55 ±0.145	0.19 ±0.081	0.79 ±0.178	0.83 ±0.104	0.99 ±0.185
48	0.32 ±0.053	0.52 ±0.108	1.17 ±0.170	1.16 ±0.178	0.19 ±0.063	0.34 ±0.058	0.61 ±0.079	0.85 ±0.144
72	0.32 ±0.048	0.62 ±0.174	1.00 ±0.082	0.91 ±0.098	0.18 ±0.085	0.38 ±0.085	0.54 ±0.108	0.85 ±0.044

Table 2. List of statistically significant differences in the oxygen consumption rate of *A. stercorosus* treated by water or preparations Steward 30 WG and Bulldock 025 EC
Tabela 2. Istotne statystycznie różnice w tempie konsumpcji tlenu przez osobniki *A. stercorosus* traktowanych wodą oraz preparatami Steward 30 WG i Bulldock 025 EC

Variable – Zmienna				Level of signifance Poziom istotności *P*<	Variable – Zmienna				Level of significance Poziom istotności *P*<
pesticide preparat	temp.	day dzień	intox. intoks.		pesticide preparat	temp.	day dzień	intox. intoks.	
1	2	3	4	5	6	7	8	9	10
k-S	14	1	+	0.05	S	14–29	1	+	0.001
k-S	19	1	*	0.05	S	19–24	1	+	0.01
k-B	19	1	*	0.05	S	19–29	1	+	0.001
k-B	19	1	+	0.05	S	14–29	2	+	0.01
k-S	19	1	+	0.05	S	19–29	2	+	0.01
k-B	24	1	*	0.01	S	14–29	3	+	0.01
k-S	24	1	*	0.01	S	19–29	3	+	0.01
k-B	24	1	+	0.05	B	14–24	1	*	0.01
k-S	24	1	+	0,01	B	14–29	1	*	0.001
k-S	29	1	*	0.05	B	19–29	1	*	0.001
k-B	29	1	*	0.01	B	24–29	1	*	0.01
k-S	29	1	+	0.05	B	14–24	2	*	0.001
k-B	24	2	*	0.001	B	14–29	2	*	0.001
k-S	24	2	*	0.001	B	19–24	2	*	0.05
k-S	29	2	*	0.01	B	19–29	2	*	0.05
k-S	19	3	+	0.05	B	14–24	3	*	0.001
k-B	24	3	*	0.001	B	14–29	3	*	0.01
k-S	24	3	*	0.01	B	14–19	1	+	0.05
k-S	29	3	*	0.05	B	14–24	1	+	0.05
k	14–24	1	*	0.05	B	14–29	1	+	0.01
k	14–29	1	*	0.001	B	14–24	2	+	0.05
k	19–24	1	*	0.05	B	14–29	2	+	0.001
k	19–29	1	*	0.001	B	19–29	2	+	0.01
k	24–29	1	*	0.01	B	14–24	3	+	0.05
k	19–19	2	*	0.05	B	14–29	3	+	0.001
k	24–29	2	*	0.01	B	19–29	3	+	0.01

Table 2 – cont. / Tabela 2 – cd.

1	2	3	4	5	6	7	8	9	10
k	14–29	1	+	0.01	k	19	1-3	*	0.05
k	19–29	1	+	0.01	k	24	1-2	*	0.05
k	19–29	2	+	0.05	S	19	1-2	+	0.001
S	14–24	1	*	0.001	S	19	1-3	+	0.001
S	14–29	1	*	0.001	S	24	1-2	+	0.01
S	19–24	1	*	0.01	S	24	1-3	+	0.001
S	19–29	1	*	0.01	B	19	1-2	+	0.05
S	14–24	2	*	0.001	B	29	1-3	*	0.05
S	14–29	2	*	0.001	S	19	2	*-+	0.05
S	19–24	2	*	0.001	S	19	3	*-+	0.05
S	19–29	2	*	0.001	S	24	2	*-+	0.001
S	14–24	3	*	0.001	S	24	3	*-+	0.01
S	14–29	3	*	0.001	B	24	2	*-+	0.01
S	19–24	3	*	0.001	B	24	3	*-+	0.01
S	19–29	3	*	0.001	B	29	1	*-+	0.05
S	14–24	1	+	0.001					

c – control, S – Steward 30 WG, B – Bulldock 025 EC, + – alimentary intoxication, * – contact intoxication.
c – kontrola, S – Steward 30 WG, B – Bulldock 025 EC, + – intoksykacja poprzez pokarm, * – intoksykacja pokarmowa.

generation in nervous and muscle cells (Soderlund et al., 2002). Also the repolarization of the membrane potential by ionic pumps, that are impaired by pyrethroids (Kakko et al., 2003), requires a considerable high energetic effort. Indoxacarb acts paralysing by blocking sodium channels of excitable cells in the closed position (Wing et al., 2000), which, as it seems, should result in the weakening of nerve and muscle conduction. This, in turn, may lead to reduce the load of membrane ATP-ases and in that way it restricts the oxygen demand of cells. In the case of indooxadazines the main inhibitor of sodium channels is DCJW – biologically active metabolite of indoxacarb. It is formed in large quantities only by *Lepidoptera* (Zhao et al., 2003; Lapied et al., 2001; Wing et al., 2000). In case of *Scarabeidae*, this substance, most likely, is not synthesized in sufficient concentrations to induce a strong effect. It means that changes in the metabolic rate may be a result of intensified detoxification processes occurring in the beetle's organisms rather than by DCJW inhibiting nerve conduction.

Also the animal's origin from regions with significant atmospheric stability (Lofroth, 1998; Harmon et al., 1986) could be the reason of considerable increase in its metabolic processes occurring in higher temperatures in the control group, and simultaneously its deceleration in the intoxicated group. These results are consistent with those obtained by

Neven (2000). They indicate that near the upper temperature tolerance limit of the organism a shut-down of their many less important physiological functions occurs. An approach to a thermal tolerance limit in insects kept under 29°C is confirmed by Q_{10} values. It is reduced with the increase of an ambient temperature (in pyrethroid treated group, in temperatures of 24-29°C, during third day of the experiment it was 0.8) (Table 3).

Table 3. Q_{10} values in control and intoxicated groups of *A. stersorosus*, depending on temperature and the mode of the preparation application

Tabela 3. Współczynnik Q_{10} wyznaczony dla osobników *A. stersorosus* z grupy kontrolnej oraz grup intoksykowanych środkami owadobójczymi w zależności od temperatury i sposobu aplikacji preparatów

Temperature range Zakres temperatury °C	Intoxication – Intoksykacja					
	Contact – Kontaktowa			Alimentary – Poprzez pokarm		
	24 h	48 h	72 h	24 h	48 h	72 h
Control – Kontrola						
14–24	2.3	0.6	1.4	2.3	1.3	1.5
19–29	4.0	2.1	1.1	2.5	2.6	1.9
14–19	0.9	0.6	2.5	1.6	0.4	1.1
19–24	6.1	0.6	0.8	3.1	3.9	2.2
24–29	2.6	6.7	1.5	2.0	1.7	1.6
14–29	2.4	1.4	1.4	2.2	1.4	1.6
Steward 30 WG						
14–24	3.8	4.8	3.5	2.5	2.5	2.3
19–29	2.9	2.9	3.4	2.3	3.9	3.9
14–19	1.9	2.7	1.3	1.4	1.1	0.8
19–24	7.4	8.5	9.5	4.5	5.6	6.5
24–29	1.2	1.0	1.2	1.2	2.7	2.3
14–29	2.5	2.8	2.4	1.9	2.6	2.3
Bulldock 025 EC						
14–24	2.7	3.7	3.1	4.3	3.1	3.0
19–29	2.5	2.2	1.5	1.2	2.5	2.2
14–19	2.7	2.7	3.7	17.0	3.1	4.4
19–24	2.6	5.0	2.7	1.1	3.2	2.0
24–29	2.4	1.0	0.8	1.4	2.0	2.4
14–29	2.5	2.4	2.0	3.0	2.7	2.8

In some cases statistically significant effect of pesticides on the insects metabolic rate has been shown. Our results indicate that these preparations operated more efficiently when applied by contact. However, when the biocide was applied with food it appeared to be less attractive to *A. stercorosus* individuals. It is exactly the second hypothesis, that may be confirmed by changes in oxygen consumption rate – usually higher in groups treated by insecticides by contact (Table 1). The phenomenon of Bulldock 025 EC preparation effect on palatability of food has been noted in the case of invasive snails *Arion lusitanicus* (Piechowicz et al., 2012).

A paralysis, the consequence of which appears the inability to refill energy resources, and an increase in metabolic rate, comparing to control animals can quickly cause an exhaustion of energetic resources of the organism in the group intoxicated by pyrethroids. It is confirmed by a statistically significant decrease of the metabolic rate during subsequent days of the experiment (Table 2), noted in many cases. Similar reaction of relaxation of the energy economy of the organism as a result of unrestricted access to food has been observed in wasps (Kovac and Stabentheiner, 1999).

Forests, the most complex terrestrial ecosystems, which also serve as a reservoir of raw materials used in countless industries, require very precise management, including the rational use of protective chemical agents. For this reason, the knowledge of each variable which may affect the protective effects of treatments carried out is particularly important. Our results indicate that the temperature in combination with plant protection products can significantly affect the functioning of such organisms as *A. stercorosus* important for forest environments. This means that one should take into account its impact when planning protective treatments.

CONCLUSIONS

1. The increase of ambient temperature is not tantamount to the increase of oxygen consumption rate in *A. stercorosus* of control group.
2. In the majority of cases applied insecticides caused an increase of metabolic rate, wherein the metabolic rate increased also with increasing an ambient temperature of intoxicated animals.
3. Contact intoxication induced greater changes in the metabolic rate of insects than intoxication by ingestion.
4. Expected time of measurement does not influence, in a significant way, the metabolic rate of the animals.

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WYBRANE INSEKTYCYDY JAKO MODYFIKATORY METABOLIZMU U *ANOPLOTRUPES STERCOROSUS* W RÓŻNYCH WARUNKACH TERMICZNYCH OTOCZENIA: INDOKSAKARB I BETA-CYFLUTRYNA

Streszczenie. W 2007 i 2008 roku przeprowadzono badania wpływu preparatów owadobójczych z grupy oksadiazyn (Steward 30 WG) i pyretroidów (Bulldock 025 EC) na tempo konsumpcji tlenu dorosłych osobników *Anoplotrupes stercorosus*. Badania przeprowadzono w różnych warunkach termicznych otoczenia (14, 19, 24 i 29°C), stosując dwa sposoby intoksykacji – kontaktową oraz poprzez traktowanie biocydami pokarmu. Uzyskane wyniki wskazują, że temperatura otoczenia tylko w niewielkim stopniu wpływała na zapotrzebowanie tlenowe owadów z grupy kontrolnej, natomiast zastosowane preparaty owadobójcze znacznie nasilały konsumpcję tlenu. U zwierząt intoksykowanych ulegała ona również istotnemu zwiększeniu wraz ze wzrostem temperatury otoczenia. Sposób intoksykacji w niewielkim tylko stopniu wpłynął na zapotrzebowanie tlenowe, a większe jego zmiany odnotowano u zwierząt traktowanych kontaktowo.

Słowa kluczowe: chrząszcze, *Anoplotrupes stercorosus,* tempo metabolizmu, pestycydy, oksadiazyny, pyretroidy

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