

## SELECTED INSECTICIDES AND ACARICIDE AS MODIFIERS OF THE METABOLIC RATE IN THE BEETLE *ANOPLOTRUPES STERCOROSUS* UNDER VARIOUS THERMAL CONDITIONS: THE EFFECT OF PIRIMICARB, DIAZINON AND FENZAQUIN

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**Abstract.** In 2007 and 2008 studies aimed to determine the effect of preparation belonging to carbamate compounds (Pirimor 500 WG), organophosphorus compounds (Diazol 500 EW), and quinazolin compounds (Magus 200 SC), on the oxygen consumption rate by adult beetles *Anoplotrupes stercorosus* were performed. Experiments were carried out under diverse ambient temperatures (14, 19, 24 i 29°C) using two ways of intoxication – contact intoxication or intoxication by ingestion of the biocide. In control insects the ambient temperature affected the oxygen demand only to a small extent. Usually, insecticide preparations which were used, markedly potentiated the oxygen consumption. In those experimental groups significant increases of oxygen consumption as the effect of ambient temperature elevation were noted. The mode of the intoxication influenced oxygen consumption only very slightly. The highest values of oxygen consumption were noted in animals treated by contact intoxication.

**Key words:** *Anoplotrupes stercorosus*, metabolic rate, pesticide

### INTRODUCTION

Many different factors affect numerous metabolic processes of the organism. The most important among them is locomotor activity [Crnokrak and Roff 2002, Reinhold 1999, Suarez 2000] and temperature [Calabi and Porter 1989, Salvucci and Crafts-Brandner 2000]. Investigations have pointed out that intensity of metabolism in insects body could be also determined by their feeding behaviour [Heinrich 1984], the energetic

value of consumed food [Kovac and Stabentheiner 1999, Salvucci and Crafts-Brandner 2000] its chemical composition [Hebling et al. 2000], the presence of scents in the environment [Gouveia et al. 2000], the act of eating alone [Gouveia et al. 2000, Roces and Lighton 1995], digestive processes [Zanotto et al. 1997], or starvation [Needham and Teel 1991]. Many other factors, such as the organism's age [Crnokrak and Roff 2002], body weight [Shelton and Appel 2001], developmental stage [Mbata et al. 2000], caste affiliation and gender [Vogt and Appel 1999, 2000], the concentration of oxygen and carbon dioxide in the environment [Kölsch et al. 2002, Zhou et al. 2001], the socialization of given species [Jaffe and Hebling-Baraldo 1990], colony size [Jaffe and Hebling-Baraldo 1993], the presence of large masses of metabolically inactive tissues [Bradley et al. 2003] or the action of toxins and their synergists [Beleboni et al. 2004, Tęgowska et al. 2004] may also affect the metabolic rate of insect's organism.

Our study was aimed to check out to what extent chosen insecticides, which belong to different chemical groups, may influence oxygen consumption rate by adult *A. stercorosus* beetles under various thermal conditions of the environment.

## MATERIAL AND METHODS

Adult Dung Beetles *Anoplotrupes stercorosus* of both sexes were used for the experiment. Insects were collected in forests near the village Werynia in the Sub-Carpathian Province. Five days before the experiments, the animals were acclimatized to an ambient temperature of 22°C and photoperiod L/D: 12/12. A total of 320 test animals were used.

The following three insecticides were used for the study:

1. Pirimor 500 WG. Producer: Syngenta. Active substance: pirymicarb (a compound belonging to the group of carbamides) – 500 g in 1 kg of the agent. The concentration of the agent in usable liquid: 50 mg/L of water.

2. Diazol 500 EW. Producer: Makhetsim Agan Industries Ltd. Active substance diazinone (a compound belonging to the group of phosphoorganic insecticides) – 500 g in 1 L of the agent. The concentration of the agent in usable liquid: 0.83 ml/1 L of water.

3. Magus 200 SC. Producer: Dow AgroSciences Polska Sp. z o.o. Active substance fezachin (a compound belonging to the group of chinoxalines) – 200 g in 1 L of the agent. The concentration of the agent in usable liquid: 0.33 ml/1 L of water.

Experimental conditions: Laboratory tests of the effect of insecticide and ambient temperature on the oxygen consumption rate by adult *G. stercorosus* were carried out in August 2007 and 2008.

The intoxication was provided using two methods:

- By individual treatment of animals with solutions of given preparations. A drop of the preparation (water in the control), the volume of which 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<sup>2</sup> ±5%) and water. After that, they were transferred to a modified incubator (type: Q-Cell model ERC0750), in previously established ambient temperature of 14, 19, 24 or 29°C and photoperiod (L:D 12:12). Each test was performed on 10 individuals.

- Alimentary – By food intoxication. 6 µl of preparation were sprayed onto the surface of apple fruits fragments of approximately 6 cm<sup>2</sup> ±5%. These fragments, together with water, were placed into the plastic containers. Prepared containers, with animals located inside, were placed in a modified incubator (type: Q-Cell model ERC0750), in previously established ambient temperatures of 14, 19, 24 or 29°C and photoperiod (L:D 12:12). Each test was performed on 10 individuals.

Every 12 h, food and water for animals were replaced (so that in case of animals intoxicated by food only first portion of food was treated by xenobiotics).

Expected duration of each measuring cycle was 72 hours after animal's contact with substance. At the end of each experimental day the oxygen consumption rate of each animal was measured. Measurements were performed using the method of closed micro-respirometric test, which had been described previously by Ross [2000]. They were carried out under thermal conditions analogous to those occurring in breeding chambers. The residence time of the animals in the respirometry kit was 2 hours, wherein the data acquisition time included the last 30 minutes of residence time (the paper presents averages of 5 readings performed at intervals of 15 minutes). After performing the analysis, the animals were again placed in the breeding chamber.

$Q_{10}$  values were calculated using mathematical formula:

$$\frac{\left(\frac{W_{T_2}}{W_{T_1}}\right)^{10}}{T_2 - T_1}$$

where:

$W_{T_2}, W_{T_1}$  – values of oxygen consumption were obtained in different thermal conditions (temperatures, ambient temperatures),

$T_2, T_1$  – temperatures.

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

## RESULTS AND DISCUSSION

In most cases, both in the control and in intoxicated groups, an increase of the temperature resulted in an increase in oxygen consumption (Table 1 and 2). Although, in groups tested in 14-24°C also inverse reactions – i.e. decrease in metabolic rate – were noted. These findings are consistent with studies of Neven [2000] and they indicate that, in addition to temperature, the level of acclimation to environmental temperature conditions may also affect the metabolic rate of the studied animals.

Insects intoxicated by insecticides, particularly those, which were kept in warmer environment (24-29°C), usually showed higher oxygen consumption than the control group. This could result from an increase of action potential conductance in excitable cells, caused by acetylcholinesterase inhibition in synapses, by phosphoroorganic compounds and carbamates [Fukuto 1990], although the process of xenobiotics detoxication [Brown and Brogdon 1987, Devonshire and Field 1991, Tęgowska et al. 2004] also could play an important role here.

Table 1. The effect of temperature and mode of application the preparation on in the oxygen consumption of *A. stercorosus* in control group and intoxicated by insecticidesTabela 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, ml O <sub>2</sub> h <sup>-1</sup> · <sup>-1</sup> m.c. Intoksykacja, ml O <sub>2</sub> h <sup>-1</sup> ·g <sup>-1</sup> b.m.							
	contact – kontaktowa				by food – poprzez pokarm			
	14°C	19°C	24°C	29°C	14°C	19°C	24°C	29°C
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
Pirimor 500 WG								
24	0.18 ±0.185	0.60 ±0.206	1.18 ±0.750	1.30 ±0.545	0.20 ±0.136	0.58 ±0.631	0.97 ±0.400	1.18 ±0.281
48	0.20 ±0.173	0.54 ±0.285	1.25 ±0.326	1.24 ±0.395	0.38 ±0.300	0.33 ±0.237	0.75 ±0.358	1.15 ±0.529
72	0.30 ±0.265	0.43 ±0.292	1.10 ±0.547	1.24 ±0.658	0.27 ±0.222	0.24 ±0.185	0.58 ±0.158	1.12 ±0.665
Diazol 500 EW								
24	0.52 ±0.320	0.75 ±0.191	0.82 ±0.604	1.45 ±0.475	0.52 ±0.367	0.64 ±0.250	0.91 ±0.664	1.43 ±0.554
48	0.53 ±0.306	0.75 ±0.368	0.78 ±0.193	0.98 ±0.485	0.67 ±0.395	0.44 ±0.162	0.58 ±0.458	0.94 ±0.425
72	0.50 ±0.287	0.65 ±0.439	0.78 ±0.743	1.25 ±0.527	0.48 ±0.254	0.51 ±0.278	0.67 ±0.411	0.85 ±0.203
Magur 200 S.C.								
24	0.53 ±0.251	0.42 ±0.317	0.69 ±0.339	1.06 ±0.239	0.35 ±0.163	0.39 ±0.240	0.62 ±0.358	0.99 ±0.470
48	0.51 ±0.187	0.44 ±0.330	0.65 ±0.395	1.03 ±0.722	0.31 ±0.207	0.42 ±0.227	0.61 ±0.508	0.99 ±0.617
72	0.53 ±0.193	0.37 ±0.243	0.45 ±0.144	0.95 ±0.651	0.24 ±0.191	0.35 ±0.319	0.48 0.206	0.96 0.849

Neven [2000] indicates that achieving the upper temperature tolerance limit of the organism, that causes shutting-down of many less important physiological functions, frequently occurs. We were able to note the same phenomenon in insects intoxicated by Pirimor 500 WG in temperature ranging from 24-29°C (Table 1 and 3). On the other hand, this phenomenon was not apparent in animals treated neither by Diazol 500 EW

Table 2. The list of statistically significant differences in the oxygen consumption rate of *A. stercorosus* treated by water or preparations Pirimor 500 WG, Diazol 500 EW and Magus 200 SC

Tabela 2. Istotne statystycznie różnice w tempie konsumpcji tlenu przez osobniki *A. stercorosus* traktowane wodą oraz preparatami Pirimor 500 WG, Diazol 500 EW i Magus 200 SC

Substance Preparat	Temperature Temperatura °C	Day of experiment Dzień	Method of intoxication Sposób podania preparatu	Level of significance Poziom istotności $P <$
1	2	3	4	5
k-D	14	1	*	0.05
k-M	14	1	*	0.05
k-D	14	1	+	0.05
k-P	19	1	*	0.05
k-D	19	1	*	0.001
k-D	19	1	+	0.01
k-P	24	1	*	0.05
k-P	24	1	+	0.05
k-D	29	1	*	0.05
k-P	29	1	+	0.01
k-D	29	1	+	0.01
k-P	14	2	*	0.05
k-D	19	2	*	0.05
k-P	24	2	*	0.001
k-D	24	2	*	0.01
k-P	29	2	*	0.05
k-P	29	2	+	0.05
k-P	24	3	*	0.01
k-D	29	3	*	0.05
k	14-24	1	*	0.05
k	14-29	1	*	0.001
k	19-24	1	*	0.05
k	19-29	1	*	0.001
k	24-29	1	*	0.01
k	19-19	2	*	0.05
k	24-29	2	*	0.01
k	14-29	1	+	0.01
k	19-29	1	+	0.01
k	19-29	2	+	0.05

Table 2 cont. – Tabela 2 cd.

1	2	3	4	5
P	14-24	1	*	0.001
P	14-29	1	*	0.001
P	19-24	1	*	0.05
P	19-29	1	*	0.05
P	14-24	2	*	0.001
P	14-29	2	*	0.001
P	19-24	2	*	0.001
P	19-29	2	*	0.001
P	14-24	3	*	0.01
P	14-29	3	*	0.001
P	19-24	3	*	0.05
P	19-29	3	*	0.01
P	14-24	1	+	0.001
P	14-29	1	+	0.001
P	19-29	1	+	0.05
P	14-29	2	+	0.001
P	19-29	2	+	0.001
P	14-29	3	+	0.001
P	19-29	3	+	0.001
D	14-29	1	*	0.001
D	19-29	1	*	0.01
D	24-29	1	*	0.05
D	14-29	2	*	0.05
D	14-29	3	*	0.05
D	14-29	1	+	0.01
D	19-29	1	+	0.01
D	19-29	2	+	0.05
D	14-29	3	+	0.05
M	14-29	1	*	0.01
M	19-29	1	*	0.001
M	24-29	1	*	0.05
M	19-29	2	*	0.05
M	19-29	3	*	0.01
M	24-29	3	*	0.05

Table 2 cont. – Tabela 2 cd.

1	2	3	4	5
M	14-29	1	+	0.001
M	19-29	1	+	0.01
M	14-29	2	+	0.01
M	19-29	2	+	0.05
M	14-29	3	+	0.01
M	19-29	3	+	0.05
P	24	2	*-+	0.01
P	24	3	*-+	0.01
D	19	2	*-+	0.05
D	29	3	*-+	0.05
M	14	2	*-+	0.05
M	14	3	*-+	0.01
k	19	1-3	*	0.05
k	24	1-2	*	0.05
P	24	1-3	+	0.05
D	29	1-2	+	0.05
D	29	1-3	+	0.05

k – control, P – Pirimor 500 WG, D – Diazol 500 EW, M – Magus 200 SC, + intoxication by food, \* – contact intoxication.

k – kontrola, P – Pirimor 500 WG, D – Diazol 500 EW, M – Magus 200 SC, + – intoksykacja poprzez pokarm, \* – intoksykacja kontaktowa.

Table 3.  $Q_{10}$  values in control and intoxicated groups of *A. stersorosus*, depending on temperature and the mode of the preparation applicationTabela 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			by food – poprzez pokarm		
	24 h	48 h	72 h	24 h	48 h	72 h
1	2	3	4	5	6	7
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

Table 3 cont. – Tabela 3 cd.

1	2	3	4	5	6	7
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
Pirimor 500 WG						
14-24	6.8	4.9	6.2	4.9	6.2	2.0
19-29	2.2	2.0	2.3	2.0	2.3	3.5
14-19	15.0	11.8	9.7	11.8	9.7	1.6
19-24	3.9	2.8	5.4	2.8	5.4	5.4
24-29	1.2	1.5	1.0	1.5	1.0	2.3
14-29	3.8	3.3	3.4	3.3	3.4	2.1
Diazol 500 EW						
14-24	1.6	1.7	1.5	1.7	1.5	0.9
19-29	1.9	2.2	1.3	2.2	1.3	2.1
14-19	3.6	2.7	3.4	2.7	3.4	1.1
19-24	1.2	2.0	1.1	2.0	1.1	1.7
24-29	3.1	2.5	1.6	2.5	1.6	2.6
14-29	2.0	2.0	1.5	2.0	1.5	1.2
Magus 200 S.C.						
14-24	1.3	1.8	1.3	1.8	1.3	2.0
19-29	2.5	2.5	2.3	2.5	2.3	2.3
14-19	1.4	2.4	1.6	2.4	1.6	3.3
19-24	2.7	2.6	2.2	2.6	2.2	2.1
24-29	2.3	2.5	2.5	2.5	2.5	2.6
14-29	1.6	2.0	1.6	2.0	1.6	2.2

with mode of action similar to carbamates, nor the Magus 200 SC acting inhibitory on mitochondrial complex I [Wood et al. 1996].

In few cases of animals intoxicated by food, the application of all tested preparations induced markedly lower oxygen consumption rates comparing to insects intoxicated by contact (Table 1 and 2). It could be due to food attractiveness (the effect of Diazol 500 EW on the palatability of food has been noted in the case of invasive snails *Arion lusitanicus* [Piechowicz et al. 2012]), and different dynamics of biocide action depending on the mode of application (quinqzolin is characterised by strong toxicity against insects only after contact intoxication).

Kovac and Stabentheiner [1999] have observed a relaxation of the energy economy of wasps as a result of unrestricted access to food. This could have been the reason of



increased oxygen consumption occurring in control and its decrease in animals treated by insecticides that cause paralysis (Table 1 and 2).

The *Scarabeidae* beetles, organisms performing a crucial role in the functioning of forest environments, not only accelerate the circulation of matter in ecosystems [Bang et al. 2005, Horgan 2005, Slade et al. 2007], but also they are a group responsible for the spread of many plant species [Andresen 2003, Estrada et al. 1999]. Its population, however, is often limited because of the application of various chemical preparations in the woods exploited economically by man. Our results indicate, that temperature, combined with plant protection products, can significantly affect the functioning of *A. stercorosus*. This means therefore, that one should take into account its impact when planning protective treatments.

## CONCLUSIONS

Increase of ambient temperature is not tantamount to the increase of oxygen consumption rate in *A. stercorosus* of control group.

In most cases insecticides applied caused the increase of metabolic rate, wherein the metabolic rate increased also with increasing an ambient temperature of intoxicated animals.

Contact intoxication caused greater changes in the metabolic rate of insects than intoxication by ingestion.

Expected time of measurement did not affect the metabolic rate of the animals in a significant way.

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## WYBRANE INSEKTYCYDY JAKO MODYFIKATORY METABOLIZMU U *ANOPLOTRUPES STERCOROSUS* W ODMIENNYCH WARUNKACH TERMICZNYCH OTOCZENIA: PIRYMIKARB, DIAZYNON I FENAZACHINA

**Streszczenie.** W 2007 i 2008 roku przeprowadzono badania wpływu preparatów owadobójczych z grupy karbaminianów (Pirimor 500 WG), insektycydów fosfoorganicznych (Diazol 500 EW) i pochodnych chinazolin (Magus 200 SC) na tempo konsumpcji tlenu dorosłych osobników *Anoplotrupes stercorosus*. Badania przeprowadzono w odmiennych 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. Zastosowane preparaty owadobójcze zwykle nasilały konsumpcję tlenu. W grupie zwierząt intoksykowanych ulegała ona również istotnemu zwiększeniu wraz ze wzrostem temperatury otoczenia. Większe wartości tempa metabolizmu odnotowano u zwierząt traktowanych kontaktowo w stosunku do zwierząt traktowanych pokarmowo.

**Słowa kluczowe:** *Anoplotrupes stercorosus*, tempo metabolizmu, pestycyd

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