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BOOK OF ABSTRACTS

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T1-P-30 Midgut trypsin and lipase activities, hemolymph protein and lipids levels with integrated biomarker response (Ibr) in Gypsy moth (*Lymantria Dispar*) larvae from clean and polluted forest after chronic exposure to benzo[*a*]pyrene

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KEYWORDS: Integrated response of biomarkers; Benzo[*a*]pyrene; Gypsy moth; Forest ecosystems

INTRODUCTION:

Intense anthropogenic influence led to a significant increase in pollution of the biosphere, in which polycyclic aromatic hydrocarbons, especially benzo[*a*]pyrene (B[*a*]P) made a major contribution. Wet and dry deposition gets atmospheric B[*a*]P on the vegetation, an important sink, and a crucial link for B[*a*]P bioaccumulation in animals. The gypsy moth is a phytophagous polyphagous insect that inhabits wide forest areas. Due to its vast appetite, it can pile great amounts of pollutants making it a suitable model system for biomonitoring the adverse effects of B[*a*]P. The larval midgut is the central metabolic place where trypsin and lipases provide efficient digestion of protein and lipids-rich food, showing sensitivity to chemical pollutants. Molecular parameters can be affected by physiological and environmental factors, so different adaptations of insects to the contaminants should be considered during the assessment of biomarker potential.

OBJECTIVES:

The aim was to investigate chronic effects of dietary treatment with B[*a*]P on midgut enzyme activities of trypsin and lipase, as well as the content of total proteins and lipids in hemolymph in gypsy moth larvae from two populations - one from an unpolluted oak forest and the other from a polluted oak forest. Furthermore, we used the method of Integrated Biomarker Response (IBR) to summarize responses of multiple molecular parameters across different tissues to estimate their sensitivity to B[*a*]P exposure in terms of population origin.

METHOD / DESIGN:

Gypsy moth egg masses were gathered in two mixed oak forests – Đerdap National Park forest, free of industrial pollution (unpolluted population of larvae, UP), and Bor forest contaminated by various byproducts of the mining industry (polluted population of larvae, PP). From hatching until the sacrifice (third day of the 5th instar) larvae were fed with a diet containing 0 ng (UP 0 ng and PP 0 ng), 5 ng (UP 5 ng and PP 5 ng), or 50 ng (UP 50 ng and PP 50 ng) of B[*a*]P in 1 g of dry diet. Spectrophotometric assays were used for the determination of specific enzyme activities of trypsin and lipase in the homogenates of the midgut, as well as for the evaluation of total proteins and lipids in the hemolymph of larvae. Two-way ANOVA followed by Tukey's post-hoc test was used for statistical analyses, conducted in GraphPad Prism 8 (GraphPad Software, Inc., USA). Statistical significance was determined at probability (p)<0.05. Excel software (Microsoft, USA) was used to calculate IBR values and to generate star plots9.

RESULTS:

The specific activity of trypsin has significantly inhibited after the treatment with lower B[*a*]P concentration in UP (F=9.412, p=0.0004), while a higher concentration of B[*a*]P significantly induced lipase activity in the same population of larvae (F=8.382, p=0.0007). These enzymes showed no statistically significant changes in the PP. Hemolymph protein content was

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significantly affected by the chronic dietary exposure to the higher concentration of B[*a*]P in both populations of larvae, showing a decrease in the UP, and the elevation in the PP (F=10.16, *p*=0.0002). Lipid concentration was not significantly changed under the B[*a*]P influence regarding the control groups (UP/PP 0 ng) but there was a meaningful difference between B[*a*]P treated larvae among two populations (F=7.16, *p*=0.019). IBR index increased in a concentration-dependent way only in UP after the chronic exposure to B[a]P and the values were higher than the corresponding ones in the PP (IBR index values - UP 0 ng=0; UP 5 ng=1.62; UP 50 ng=4.84; PP 0 ng=2.01; PP 5 ng=1.10; PP 50 ng=3.08).

CONCLUSIONS:
Gypsy moth population from the unpolluted forest showed higher sensitivity to the chronic dietary exposure to B[*a*]P comparing to the population from the polluted forest, especially in terms of trypsin and lipase activity. Hemolymph protein level expressed well correspondence to B[*a*]P concentration in both populations but with inverse trends. The selected set of Gypsy moth larvae molecular parameters possess a good potential for B[*a*]P biomonitoring in the populations from unpolluted forest ecosystems.

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T1-P-31 Sensitivity of midgut phosphatases to thermal stress in Gypsy moth (*Lymantria Dispar*) caterpillars

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KEYWORDS: gypsy moth, alkaline phosphatase, total acid phosphatase, increased temperature, thermotolerance

INTRODUCTION: Environmental temperature directly affects the development of phytophagous insects, and indirectly through their host plants. Alkaline phosphatases (ALP) and total acid phosphatases (tot ACP) are midgut enzymes included in metabolic processes. The previous contact of the insect populations with various stressors and their ability to overcome the effects of the raised temperature (thermotolerance) can modify the response of these enzymes to increased environmental temperature.

OBJECTIVES: We aimed to compare the differences in responses of midgut ALP and tot ACP, with the expression of their isoforms, to increased environmental temperature with and without induced thermotolerance, in gypsy moth 5th instar caterpillars from unpolluted and polluted habitats.

METHOD / DESIGN: Caterpillars were hatched from egg masses collected in unpolluted (UP population) and polluted forest (PP population). They were reared at 23°C (PP23 and UP 23) and 28°C (PP28 and UP28) until the 3rd day of the 5th instar. In both populations, a group of individuals was exposed to 28°C for 24 h (induced thermotolerance) at the beginning of the 4th instar. Afterward, they were returned to 23°C until the sacrification (PP23In and UP23In) or exposed to 28°C for 72h before sacrification on the 3rd day of the 5th instar (PP28In and UP28In). The activity of enzymes was measured spectrophotometrically, using p-nitrophenyl phosphate (pNPP) as substrate, under alkaline conditions for ALP and acid conditions for tot ACP. Isoforms of both enzymes were detected on 12% polyacrylamide gel native PAGE.

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