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Short Communication

ACUTE EFFECT OF CADMIUM ON LARVAL GROWTH OF *Lymantria dispar L.*

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Dose-dependent effect of cadmium on relative growth rate (RGR) was examined in 4th instar gypsy moth (*Lymantria dispar L.*) larvae. No-observed-effect-concentration (NOEC) was shown to be 10 μgCd/g and lowest - observed - effect - concentration (LOEC) was 30 μgCd/g dry food weight. Linear relationship was found between percentage of RGR reduction and log-transformed values of cadmium concentrations. Concentrations of cadmium that lead to 50, 75, 90 and 100% RGR reduction were calculated as 65, 195, 377 and 584 μgCd/g dry food weight, respectively.

Key words: Lymantria dispar larvae, cadmium, relative growth rate

Many countries are facing the potential risk, associated with heavy metal contaminants. Human activities, including industrial emissions, waste disposal, agricultural practices leave their impact on nature. Detection of toxicants, analysis of their influence on living organisms and prediction of their effects on the dynamics of natural populations are major objectives of

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ecotoxicology. Highly toxic non-nutritient metals, to which cadmium belongs, attract particular attention. In most organisms cadmium has the ability to cause oxidative damage of tissues including lipid peroxidation and oxidation of sulfhydryl groups in structural proteins and enzymes (STOHS, BAGCHI 1995). It is highly persistent and accumulative and thus may show delayed negative effects (MOE *et al.* 2001).

Metal accumulation in organisms is tissue specific (SUZUKI *et al.* 1984; VAN STRAALEN 1994), and species specific (VAN STRAALEN, VAN WENSEM 1986). Insects represent one of the most common and diverse group of animals which readily accumulate heavy metals (HARE 1992). They are frequently used in researches on heavy metal effects at individual (body size, longevity, fertility) or population level (population growth rate, age distribution, biomass turnover).

Due to its outbreaking population dynamics and polyphagous feeding habit, gypsy moth (*Lymantria dispar*) is a serious pest of deciduous and coniferous forests of Europe, Asia and North America. Despite its economic importance, studies on heavy metal effects on gypsy moth have started recently (GINTENREITER *et al.* 1993; ORTEL 1995a, 1995b, 1996).

This work was aimed to investigate dose-dependant effects of cadmium on relative growth rate (RGR) of 4th instar gypsy moth larvae. Short-term bioassay was performed in order to determine no-observed-effect-concentration (NOEC), the lowest-observed-effect concentration (LOEC), and concentrations of cadmium that lead to 50, 75, 90, and 100% reduction of RGR (EC_{50}, EC_{75}, EC_{90}, EC_{100} respectively).

Gypsy moth egg masses were collected in poplar forest at Opovo locality (30 km far from Belgrade). They were kept at 4°C from October to March when they were set for hatching. Larvae were reared on artificial HWG (high wheat germ) diet (O'DELL *et al.* 1984) at 23°C and photoperiod 12L:12D until molting into the 4th instar. During the 4th instar, larvae were reared individually in Petri dishes (9 cm diameter). Depending on cadmium concentration, ten to fifteen larvae were randomly assigned to five experimental groups (0, 10, 30, 100 and 250 μg Cd/g dry food weight).

Relative growth rate was calculated on wet weight basis according to formula

$$RGR=(W_5-W_4)/(W_4*D_4)$$

Larval weight at the begining of 4th instar (W_4) and after the molting into the 5th instar (W_5) as well as duration of the 4th instar (D_4) were

recorded. Larval weights did not differ between the experimental groups at the beginning of toxicity assay. To achieve normality and homogenity of variances data on RGR were log-transformed and than subjected to one-way ANOVA.

Results presented in Fig 1a show that concentration of 10 μgCd/g has no significant effect on RGR of 4th instar gypsy moth larvae (LSD test, $P<0.1166$) while higher concentrations lead to significant reduction of RGR. The lowest-effect-concentration (LOEC) is 30 μgCd/g.

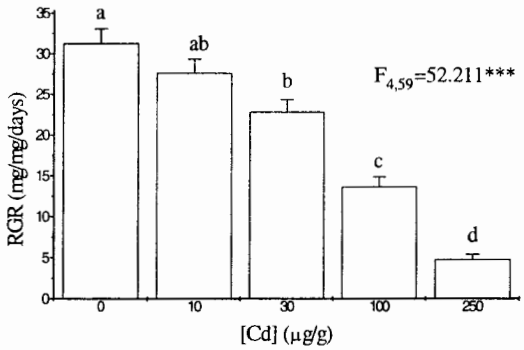

Fig. 1a. Relative growth rate (RGR) of 4th instar gypsy moth larvae exposed to various cadmium concentrations. Values indicated by differnt letters (a, b, c, d) are significantly different (LSD test, $P<0.05$).

To calculate EC values that cause 50, 75, 90 and 100% RGR reduction, growth reduction was plotted against the log-transformed values of cadmium concentration. Results presented in Fig. 1b show linear relationship and EC values are EC_{50}=65 μg/g, EC_{75}=195 μg/g, EC_{90}=377 μg/g and EC_{100}=584 μg/g.

Growth rate is a fundamental measure of physiological fitness/performance and provides one of the most sensitive measures of stress in organisms. Increase in cadmium concentrations lead to reduction of insect growth rate (FOUNTAIN, HOPKIN 2001) and in some insects it is correlated with reduced reproduction (SMIT 1997). ORTEL *et al.* (1992) found that NOEC for gypsy moth reproduction is 10 μg/g. The same concentration was determined as NOEC for gypsy moth RGR in our experiment (Fig. 1a). Maintaining homeostasis at growth level is enabled by plastic responses at physiological level. Comparing to growth responses, physiological responses are more sensitive to low concentrations of pollutants. It has been discov-

ered that chronic exposure of 4th instar gypsy moth to cadmium concentration of 10 μg/g lead to significant increase of total free amino acids and decrease of total proteins, trehalose, glucose and lipids in haemolymph while body protein and carbohydrate content is higher compared with control group (ORTEL 1995a, 1995b, 1996).

Our experiment showed that growth rate responds in a dose-depend manner (Fig. 1b). Dose-dependent and fast response of gypsy moth larval growth to dietary cadmium make it suitable for short-term toxicity bioassays. Low doses may have stimulatory effect (hormesis). It can be calculated from equation presented in Fig. 1b that gypsy moth growth is accelerated for (Cd)<7.335 μg/g. At higher concentrations (>30 μg/g) cadmium significantly reduced growth rate.

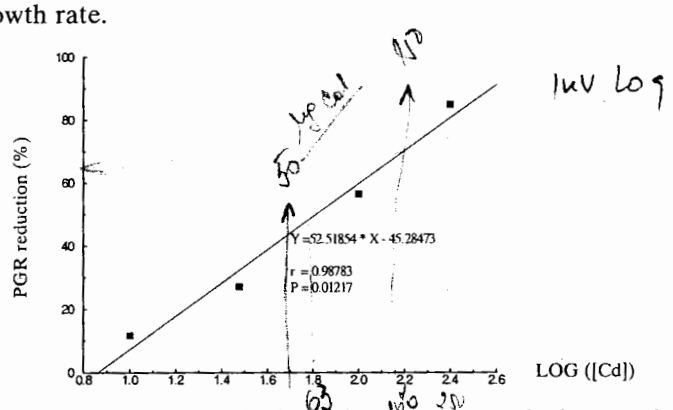

Fig. 1b. Linear regression of RGR reduction against log-transformed values of cadmium concentration.

Pathological and adaptive responses to cadmium have been described in insect cells. Cadmium destroys microtubular structure, impedes cell proliferation (BRAECKMAN *et al.* 1997) and leads to dilatation of perinuclear cisternae, rough endoplasmatic reticulum, and lysosomes (BRAECKMAN *et al.* 1999). It also reduces stability of lysosomal membrane (WERNER *et al.* 2000), inhibits aerobic metabolism, reduces total adenilate charge, ATPase activity, the amount of ATP (MIGULA *et al.* 1997) and induces the synthesis of defense proteins such as metallothionein (HENSBERGEN *et al.* 2000) and heat shock proteins (BOURNIAS-VARDIABASIS *et al.* 1990).

Cadmium accumulates in mitochondria and membrane enclosed granules of insect midgut epithelium. Excretion of cadmium through exfoliation of the midgut epithelium at every molt and sequestration via binding to metallothionein are main mechanisms of metal tolerance in insects

(POSTHUMA, VAN STRAALEN 1993). Insects are capable to some extent to cope with exposure to heavy metal stress without changes in life history traits. Above certain species-specific threshold level energy allocation to defense mechanisms may exceed investment in growth and reproduction. However, as there is no strong evidence that fitness reduction is a consequence of energy allocation to defense mechanisms (VAN STRAALEN, HOFFMANN 2000) reduction in gypsy moth larval growth can be explained by effects of starvation and/or poisoning.

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AKUTNI EFEKAT KADMIJUMA NA RAST LARVI *Lymantria dispar L.*

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I z v o d

Efekat različitih koncentracija kadmijuma, na relativnu brzinu rasta (RGR) ispitivan je kod larvi gubara (*Lymantria dispar L.*) četvrtog stupnja. Pokazano je da je koncentracija koja nema vidljivi efekat (NOEC) 10 μgCd/g, dok je najmanja koncentracija koja ima vidljivi efekat (LOEC) 30 μgCd/g suve težine hrane. Utvrdjena je linearna zavisnost izmedju procenta redukcije rasta i log-transformisanih vrednosti koncentracija kadmijuma. Izračunate su vrednosti kadmijuma koje dovode do redukcije rasta larvi za 50, 75, 90 i 100%, i iznose 65, 195, 377 and 584 μgCd/g suve težine hrane.

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