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THE EFFECTS OF CADMIUM ON THE LIFE HISTORY TRAITS OF *LYMANTRIA DISPAR* L.

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Abstract - Gypsy moth (*Lymantria dispar* L.) females and males were chronically exposed to three sublethal cadmium concentrations (10, 30 and 50 mg/g dry food mass) in order to assess the effects of cadmium on larval and pupal duration, pupal mass and longevity. On average, the presence of cadmium in food did not affect larval duration while shortened pupal duration and reduced pupal mass and longevity were recorded. The most significant effects were obtained at the highest cadmium concentration. Females and males did not differ in sensitivity of life history traits to cadmium exposure. It is concluded that (1) cadmium exerts a strong adverse impact on the growth and development of gypsy moths, and (2) the significance of the cadmium effects depends on the dose.

Key words: Cadmium, gypsy moth, larval and pupal duration, pupal mass, longevity

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INTRODUCTION

Cadmium is a non-essential toxic heavy metal. It is highly ranked on the EU list of hazardous substances and there is a lot of concern about increased cadmium accumulation due to anthropogenic releases to the environment. A significant amount of cadmium can be found near mines and zinc smelters, on fertilizer-treated land, municipal and industrial sewage sludges, disposal sites of batteries and metal-contaminated waste from nonferrous-metallurgy (Alloway, 1995). Cadmium exerts a negative impact on living organisms and accumulates in food chains (Roberts et al., 1979).

At the cellular level cadmium is involved in oxidative stress and the production of reactive oxygen species which causes many structural and functional disturbances such as reduced stability of the lysosomal membrane (Breackmann et al., 1999), enhanced lipid peroxidation (Korsloot et al., 2004), depletion of GSH (Augustyniak et al., 2005), and a decrease in the activity of antioxidative and detoxification enzymes (Kafel et al., 2003; Wilczek et al., 2004; Lijun et al., 2005; Augustyniak et al., 2009). At organism level cadmium affects food consumption and digestibility (Migula and Binkowska, 1993; Fountain and Hopkin, 2001; Van Oik et al., 2007), the synthesis and secretion of neurohormones (Ilijin et al., 2010) and functionality and synthesis of hormone receptors (Cervera et al., 2005; Planello et al., 2010).

All these physiological changes may underlie the reduced growth, longevity, fecundity and hatchability recorded in many insects and other arthropods exposed to cadmium in natural and laboratory conditions (Gintenreiter et al., 1993a; Rayms-Keller et al., 1998; Moe et al., 2001; Cervera et al., 2004; Van Oik et al., 2007; Bechard et al., 2008). Results vary depending on exposure time, dose (Cervera et al., 2006; Wu et al., 2006), species (Clubb et al., 1975), life stage (Nascarella et al., 2003) and interaction with other stressors (Vuori 1994; Stone et al., 2001; Augustinijak et al. 2005). The negative effects of cadmium can be transmitted to offspring generations (Mozdzer et al. 2003).

Insects are capable of adaptive responses to cadmium which include cadmium excretion through exfoliation of the midgut epithelium at every molt, sequestration via binding to metallothionein and other cadmium-binding proteins and higher immune response (Posthuma and Van Straalen, 1993; Niu et al., 2000; Sterenborg and Roelofs, 2003; Van Oik et al., 2007; Janssens et al., 2009). The capacity for adaptive responses varies within a population enabling evolution of mechanisms of tolerance after a few generations of exposure to cadmium (Shirley and Sibly, 1999; Sterenborg and Roelofs, 2003; Cervera et al., 2006; Roelofs et al., 2007; Augustyniak et al., 2009). Regarding the energetic costs of tolerance mechanisms, an altered life history is expected to be a part of both physiological acclimatization and genetic adaptation (Posthuma and Van Straalen, 1993; Janssens et al., 2009).

The present study deals with chronic cadmium effects on the life history traits of a polyphagous folivorous insect, the gypsy moth *Lymantria dispar* L. The cadmium effects on folivorous insects depend on its concentration in plant leaves where it enters either from soil through the roots and xilem or directly from the leaf surface through the stomata (Reimann et al., 2001). In natural conditions folivorous insects can be affected by cadmium either directly or through changes in the nutritional value of their food, i.e. plant leaves (Koricheva et al., 1998). Our study was aimed at examining the direct influence of and increased cadmium concentration in an artificial diet on the development and growth of gypsy moth females and males. Since sexual dimorphism is described for many of the fitness (Lazarević and Perić-Mataruga, 2003) and physiological traits (Stockhoff, 1993; Lindroth et al., 1997) of this species, our second aim was to determine whether females and males respond differently to cadmium exposure.

MATERIALS AND METHODS

Insect rearing and experimental groups

The gypsy moths analysed in the experiment were hatched from egg masses collected from the oak forest at Lipovička šuma (20 km south of downtown Belgrade). The nearest source of cadmium pollution is a thermal power plant in Obrenovac which uses coal for combustion and is situated approximately 15 km to the west of Lipovička šuma. Also, Lipovička šuma was used as a chemical center of the Yugoslav army and reservoirs of rocket fuel were bombed during the 1999 NATO attacks on Serbia.

Egg masses were collected in December 2003 and refrigerated until April 2004, when they were set for hatching. After removing the hairs the eggs were surface-sterilized in 0.1% sodium hypochlorite. Hatched larvae were reared in Petri dishes (d=9cm) on an HWG artificial diet (O'Dell, 1984) at a temperature of 23°C and photoperiod 12L : 12D.

Cadmium treatments

To assess the cadmium effects on gypsy moth life history traits, the larvae were randomly assigned to four experimental groups. The control group (C) was reared on an artificial diet without cadmium while cadmium nitrate was added to the artificial diets of 3 treated groups. These groups were exposed to 3 different cadmium concentrations from hatching to pupation: 10 (Cd1), 30 (Cd2) and 50 μg/g dry food mass (Cd3). It has been shown previously that concentrations of 10 and 30 μg/g are the no-observed-effect-concentration (NOEC) and lowest-observed-effect-concentration (LOEC) for the growth of 4th instar larvae, respectively (Vlahović et al., 2001).

Determination of life history traits

Hatching, molting, pupation, adult eclosion and adult death were checked daily. Larval duration was defined as the period from larval hatching to pupation, while pupal duration was the period from pupation to adult eclosion. The pupal mass was measured on the 2nd day of pupal development. The longevity assay was performed using mated pairs in separate cups.

Data analysis

All data analyses were performed using the GLM procedure (Type III SS) in SAS (SAS Institute,

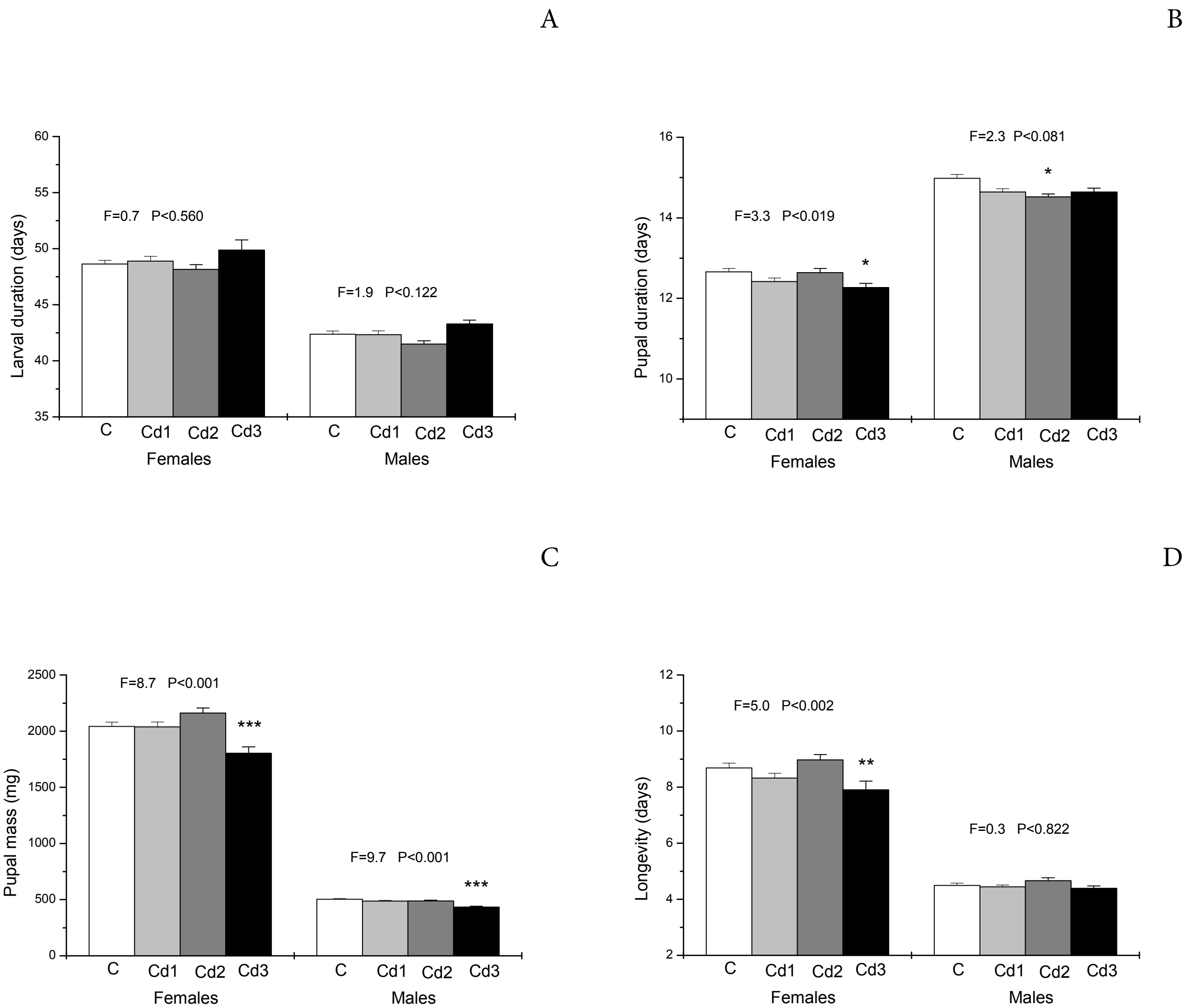

Fig. 1. Means and standard errors for larval duration (A), pupal duration (B), pupal mass (C) and adult longevity (D) in female and male gypsy moths exposed to different cadmium concentrations: C- 0 μg/g, Cd1- 10 μg/g, Cd2- 30 μg/g, Cd3- 50 μg/g of dry food mass. F and P values from one way ANOVA show significance of cadmium effects. Dunnet's test was applied for comparison of cadmium treatments with the control group (* P<0.05; ** P<0.01; *** P<0.001).

2003). Following the examination of the normality and homogeneity of variance assumptions, the appropriate ANOVA models were applied to the log transformed values of life history traits. Two-way analysis of variance was applied to test the significance of the main effects (cadmium and sex) and their interaction. Interaction term tested if there was a significant difference in the response of life history traits to cadmium between the sexes. To evaluate significant differences between the control and treated groups within each sex, a one-way ANOVA with a Dunnett's post-hoc test was used.

RESULTS

As many studies before our experiment have shown, there are highly significant differences in the life history traits of females and males. A longer

Table 1. Mean squares from two-way ANOVA (MS×100) on log-transformed values of life history traits. Sex and cadmium (Cd) are fixed factors. Significant effects are given in bold; df- degrees of freedom.

		Source of variation			
Trait		Sex	Cd	Sex × Cd	Error
Larval	df	1	3	3	746
duration	MS	69.6	0.6	0.1	0.2
	F	297.0	2.4	0.2	
	P	**0.001**	0.069	0.875	
Pupal	df	1	3	3	732
duration	MS	86.8	0.4	0.2	0.11
	F	796.3	4.0	1.8	
	P	**0.001**	**0.008**	0.145	
Pupal	df	1	3	3	746
mass	MS	775.7	1.7	0.1	0.1
	F	7852.4	17.2	1.3	
	P	**0.001**	**0.001**	0.259	
Longevity	df	1	3	3	719
	MS	1317.3	4.8	1.9	1.3
	F	1026.4	3.7	1.4	
	P	**0.001**	**0.011**	0.230	

larval duration, shorter pupal duration, higher pupal mass and adult longevity are characteristic for females (Fig. 1, Table 1). However, two-way ANOVA did not reveal sex differences in the sensitivity of gypsy moth life history traits to cadmium (non significant Sex × Cd interaction term, Table 1).

The presence of cadmium in food did not affect larval duration either in females or males, although individuals exposed to the highest cadmium concentration (Cd3 group) tend to have a prolonged larval development (Fig. 1A, Table 1). Significant cadmium effects were recorded in all other examined traits (Table 1). Pupal duration was shorter in some of the groups treated with cadmium than in the control group (Fig. 1B). Reduction in pupal mass was determined only in the Cd3 group, both in females and males (Fig. 1C), while only females showed a shortened longevity in response to the cadmium concentration of 50 μg/g (Fig. 1D).

DISCUSSION

Our results confirmed that cadmium exerts a strong adverse impact on gypsy moth growth and development. Such a decrease in performance in response to a pollutant is characteristic for chewing insects (Koricheva et al., 1998). However, the cadmium concentrations which we applied were much higher than the concentrations found in plant leaves in natural conditions. Even in urban areas the cadmium content of plant leaves is below 1μg/g (Chronopoulos et al., 1997). The highest concentrations found in plants near lead/zinc smelters and toxic-spill contaminated areas are in the order of 10 μg/g (Rabitsch, 1995; Del Rio et al., 2002). In the Serbian forest ecosystems high (>0.5μg/g) to moderate levels (0.03-0.05μg/g) of cadmium have been determined at most examined sites (Kadović and Knežević, 2002).

Our results did not show a significant change in larval duration, which is in accordance with the results of other authors where a significant prolongation of gypsy moth larval development was obtained after acute or chronic exposure to a cadmium concentration of 100 μg/g and higher (Ortel et al., 1993; Gintenreiter et al., 1993a; Ilijin et al., 2010). Generally, a high cadmium concentration increases the larval duration of holometabolous (Sildanchandra and Crane, 2000; Nascarella et al., 2003; Wu et al., 2006) and hemimetabolous insects (Cervera et al., 2004) while a shortened pupal duration is characteristic for gypsy moths exposed to chemical stress. A chronic exposure of gypsy moth larvae to cadmium (present results) or unsuitable host plants (Barbosa et al., 1983) led to a decrease in pupal duration. Taking into account that there is a strong correlation between pupal mass and fecundity in the gypsy moth (Lazarević et al., 1998), the reduced pupal mass in response to the highest cadmium concentration has implications for female reproductive success. It is also known that larger male insects have a higher mating ability (Santos et al., 1988) and, thus, the reproductive performance of the male gypsy moth could also be affected as a consequence of lower pupal mass. Since the gypsy moth is a capital breeder its longevity depends on resources accumulated during larval development. Accordingly, a reduced body size led to reduced longevity. Cadmium provokes growth retardation in grasshoppers (Malakar et al., 2009), aquatic chironomids (Silachandra and Crane, 2000), milkweed bug (Cervera et al., 2004), ground beetle (Mozdzer et al., 2003), and housefly (Niu et al., 2002).

Similar to results of Silanchandra and Crane (2000) on *Chironomu riparius,* we did not record sex differences in sensitivity to cadmium of any life history trait, while Cervera et al. (2004) found that adult survival in female *Oncopeltus fasciatus* is much more affected by cadmium exposure than that of males. Despite the absence of sex differences in sensitivity to cadmium of growth and development, it is possible that the physiological traits underlying gypsy moth life history traits differ between the sexes. For example, a sex-dependent detoxification strategy against pollutants has been found in spiders (Wilczek et al., 2004).

Cadmium accumulation in gypsy moth larval, pupal and adult body increases with an increase in cadmium concentration in food, and it reaches much higher values compared to the levels in food (Gintenreiter et al., 1993b). Cadmium is partly eliminated via feces, exuviae and head capsules, but cadmium remaining in the body causes damage to metabolic processes and leads to many structural and functional disturbances (Janssens et al., 2009). Its accumulative nature results in a gradual increase in adverse effects on the fitness traits in the gypsy moth (Vlahović et al., 2009) and other insects (Laskowski, 2001). The toxicity of cadmium is apparent above a species-specific threshold level which depends on the capacity of various defense mechanisms to overcome its harmful effects. In the present study, significant effects on life history traits were recorded mainly at the highest cadmium concentration (50μg/g).

Adaptive responses to cadmium include changes in physiology (mechanisms of detoxification) and/or behavior (decrease in consumption rate). Detoxification mechanisms are energetically costly. Under stressful conditions reallocation of resources towards the defense mechanism which increase survival probability leads to a reduction of insect growth and reproduction (Van Straalen and Hoffman, 2000). It has been found that acute exposure to cadmium does not affect the consumption rate in gypsy moth larvae (Jobstl, 1993), Ortel (1995a) suggested that it can be affected under chronic exposure. Although a lower consumption rate means an adaptive decrease in toxicant uptake, it also decreases the uptake of nutrients. A lower content of metabolites in hemolymph and body of the gypsy moth (Ortel, 1995a; 1995b; 1996) and other insects (Wu et al., 2006) exposed to cadmium point to disturbances in energy metabolism. Defense costs, decreased nutrient uptake and damage to metabolic processes could explain the adverse effects of cadmium on gypsy moth life history traits as examined in our work.

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