Effects of Mercury on Development of Oxya fuscovittata (Marschall) (Orthoptera: Acrididae)

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Effects of mercury on development of Oxya fuscovittata (Marschall) (Orthoptera: Acrididae)

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Abstract

Grasshoppers are ecologically significant because many animals consume them as a major protein source and thus any change in their population dynamics may have detrimental effects on an ecosystem. This study evaluates effects of mercury (Hg^{2+}) on the developmental periods of different instars of a common short-horned grasshopper, *Oxya fuscovittata* (Marschall). Newly hatched nymphs were fed foods treated with three sublethal concentrations of $HgCl_2$ *i.e.*, dose 1 (d1): 20 mg $HgCl_2$/ kg dry weight in oats, dose 2 (d2): 40 mg $HgCl_2$/ kg dry weight in oats, and dose 3 (d3): 80 mg $HgCl_2$/ kg dry weight in oats, until they reached the adult stage. The experiment was conducted for two consecutive generations (F1 and F2), tested in the same way for the same variables, in order to observe if there is any additional adversity in the latter generation. As $HgCl_2$ concentrations in food increased, the Total Rearing Time (TRT) for each instar significantly increased, whereas survival, adult body weight and adult life span significantly decreased. The results for the F2 generation almost always showed more severe effects than those of the F1 generation.

Key words

developmental periods, ecosystem, heavy metals, short-horned grasshopper, survival

Introduction

Grasshoppers often appear to be the most abundant among ground-dwelling insects, representing up to 20 to 30% of arthropod biomass (Schmidt 1986). They typically consume at least 10% of available plant biomass and often harvest more plant biomass than they consume, influencing the availability and distribution of litter in the environment (Belovsky *et al.* 1996-2000).

Grasshoppers are ecologically significant because they serve as a major food source for other species, especially spiders, reptiles, birds, and small mammals. Without grasshoppers to consume, many vertebrate animals would suffer from lack of a suitable source of animal protein. The decline of grasshoppers may also affect other species, especially those that consume them. According to Belovsky (1993) grasshopper reduction might harm declining or threatened species that depend on these insects as food. It is evident that grasshoppers occupy a critical position in the terrestrial food chain, so that change in the structure of a grasshopper population due to toxic substances may directly affect a whole ecosystem (Schmidt 1986).

Heavy metals are one of the major toxic substances in the environment. The heavy metal mercury can enter the environment through industrial processes, such as chlorine manufacturing (Evers 2005), and through combustion of coal, oil, wood, natural gas and mercury-containing trash. Over the past century, anthropogenic inputs of mercury into the environment have significantly increased (Evers *et al.* 2004). Zheng *et al.* (2008) report that mercury can be accumulated in *Locusta migratoria manilensis* and *Acrida chinensis*. According to them, total mercury concentrations in these species were higher than those in the plants in less or noncontaminated sites. This result demonstrated that mercury can be accumulated in arthropods. This is a concern since mercury could advance through food chains via birds and domestic fowl and ultimately poison human populations (Zheng *et al.* 2008). Mercury in the soil may also cause increased mortality and marked reduction in egg-hatching rates of grasshoppers (Devkota & Schmidt 1999).

Horvat *et al.* (2003) have shown that crop plants such as rice are able to take up mercury from contaminated soil. Mercury and methylmercury concentrations in rice from Guizhou province, China, were 0.569 mg/ kg and 0.145 mg/ kg respectively. Ecological mercury exposure has been reported by many workers. Wang *et al.* (2005) reported significant mercury and methylmercury accumulation in *Rana chensinensis* in an area impacted by gold mining. Tremblay *et al.* (1998) observed that the concentrations of mercury and methyl mercury in emerging insects in recently flooded hydroelecric reservoirs were 0.14-1.50 mg/kg and 0.035-0.80 mg/kg respectively, and may be transferred to fish via the food chain. Lane and Evers (2005, 2006) report that mercury concentration in insect-eating songbirds at several sites in Maine, reached levels that affected their health considerably.

Insects not only serve as primary consumers and recyclers but also as secondary consumers and as food for animals of higher trophic levels (Jensen *et al.* 2006). Among the four or five trophic levels commonly present in many freshwater and terrestrial ecosystems, insects occupy the critical middle links (Thompson 1984). Despite these important roles, very little is known about how pollutants affect insects, possibly because of insect diversity, ubiquitous distribution and perceived lack of importance in most anthropomorphic activity (Trumble *et al.* 1998). The objective of the present study was to determine the effect of this important heavy metal, on the development, survival and lifespan of *Oxya fuscovittata* (Marschall), by feeding the insects food contaminated with different doses of Hg^{2+}. The experiment was conducted for two generations (F1, F2) in order to observe any additional negative effects in the second generation.

Table 1. Total Rearing Time (TRT) in days ± SD of *O. fuscovittata* under different dosages of $HgCl_2$ contaminating their food for F1 and F2 generations.

	F1				F2			
Molt	Dose				Dose			
	Control	d1	d2	d3	Control	d1	d2	d3
1	5.73±0.37	6.16±0.23	6.66±0.47*	7.33±0.58*	5.83±0.25	6.75±0.20*	7.66±0.88*	8.83±0.25*
2	11.66±0.44	11.33±0.47	11.66±0.47	13.33±0.48*	12.50±0.39	12.97±0.24*	13.29±0.07*	14.91±0.34*
3	19.33±0.89	18.66±0.95	18.33±0.47	24.00±0.87*	19.83±0.65	22.25±0.20*	24.66±2.39*	35.16±1.33*
4	26.66±0.44	25.33±0.47	25.33±0.47	34.66±0.49*	28.00±0.86	32.75±0.20*	38.00±3.84*	47.00±2.77*
5	33.33±0.43	35.33±0.47*	35.00±1.64	45.66±0.49*	33.83±0.63	46.75±1.46*	49.66±2.68*	56.00±1.53*
6	41.66±0.89	43.00±0.82	48.33±0.47*	52.66±0.87*	44.66±2.37	55.25±3.27*	60.83±3.04*	66.00±1.11*

Asterisk indicates a significant difference (P<0.05) in TRT between control and different treatment groups (d1, d2, d3) for a particular molt.

Methods

Rearing of test insects.—Newly hatched nymphs of *O. fuscovittata* were collected from insectariums of the Dept. of Zoology, Visva-Bharati University, Santiniketan, West Bengal, India; here a mass culture of this species has been maintained using methods proposed by Hinks & Erlandson (1994) since 1998. This culture is derived from individuals that originally collected from nearby agricultural and grassland fields of Santiniketan (lat 23 39′ N, long 87 42′ E), Birbhum, West Bengal, India; they are reared under laboratory conditions providing 32 ± 1º C, 70-80% relative humidity and 14:10 LD photoperiod using the method proposed by Haldar *et al.* (1998).

To reduce natural mortality and to avoid cannibalism due to intraspecific competition, 27 to 30 insects were placed in one cage. Three cages per treatment dose were used to carry out the experiment. These cages were specially designed for rearing under laboratory conditions. Each cage of nylon mesh on a wooden frame measured 70 × 40 × 30 cm. The floor of the cage was wooden and in the middle of the floor was a square hole measuring 5×5 cm fitted with a slide for clearing fecal matter and exuviae from the cage. Fine, freshly washed and sterilized sand in standard enamel trays measuring 26 × 21 × 5 cm were placed on the floor of the cages. Sufficient distilled water was sprinkled daily to keep the sand moist.

Food and its contamination.—For the control experiments, the young hoppers (F1 and F2) from untreated parents were fed on pellets of white oats (*Avena sativa*, a whole grain of the family Poaceae). To test for any adverse effects of consuming mercury through feeding in a short time span, oats were contaminated at three dosages: low dose (d1) 20 mg $HgCl_2$/kg in oats; medium dose (d2) 40 mg $HgCl_2$/kg in oats; or high dose (d3) 80 mg $HgCl_2$/ kg in oats — much higher concentrations than those recorded under field conditions. For contamination in white oats, various amounts of $HgCl_2$ were dissolved in 20 ml of double-distilled water, mixed with known amounts of white oats and formed into pellets. After drying, contaminated pellets were given to hoppers. A small amount of green grass (*i.e.*, *Cynodon dactylon*) was provided in each cage as a natural food source. The insects of the F2 generation were fed on the same diets as the F1 generation.

Contamination of insects.—The experiment was started with nymphs hatched the same day and continued for two consecutive generations (F1 and F2). Insects of each set were fed *ad libitum*. Ten grams of new contaminated food (d1, d2, d3) per cage were offered daily, after removing the leftover.

Collection of F1 generation egg pods and experimental set up for F2 generation.— Grasshoppers lay eggs in moist soil. To recreate such an environment for the experiment, sterilized dried sand was provided as the incubation medium. One hundred gms of sand was taken in each plastic cup (10 cm deep and 6 cm diameter). Some amount of water was sprinkled every day to keep the sand moist. For every concentration of metal chlorides and the control, separate plastic cups were provided in which the insects could lay eggs. When F2 individuals started to hatch, for different dosage treatments including control, 3 replications each were made, keeping 30 same-day hatched insects in a particular set.

Calculation of different parameters.—Newly molted insects were tagged on their pronota with marker pens for future identification. The same mark was used for same-day molted insects. The exuviae of insects indicated duration of a particular molt. By counting the time between two successive exuviae marked with identical tags, duration of a particular molt was obtained. Thus the durations of the Total Rearing Time (TRT) were calculated. To express survivorship, percentages of individuals attaining adulthood were calculated. Adult body weight and adult lifespan were calculated in milligrams (mg) and days respectively.

Analysis of data.—The experiment was set up with three replications. Different variables were analyzed by two-way analysis of variance (ANOVA). Significant differences between the means of treated and control samples were analyzed by Fisher's t test using S-Plus 4.0 software.

Table 2. Adult body weight (mg±SD) of *O. fuscovittata* exposed to different sublethal doses of mercury for two consecutive generations (F1and F2).

	F1		F2	
Doses	Male	Female	Male	Female
Control	174.18±2.85	342.11±2.05	176.09±1.74	342.59±1.36
d1	166.21±2.90*	337.81±2.62*	151.55±1.06*	302.35±2.92*
d2	164.70±1.03*	326.65±3.65*	125.28±2.11*	125.28±3.35*
d3	160.60±2.89*	317.93±1.31*	114.51±1.76*	247.16±2.15*

Asterisk indicates a significant difference (P<0.05) in adult body weight between generations within dose and within generation between doses.

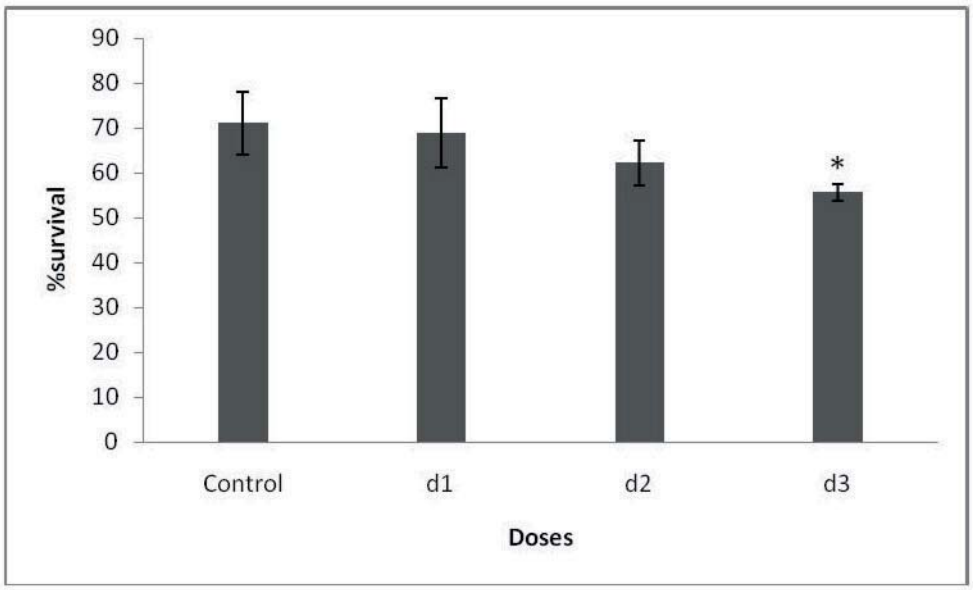

Fig. 1. Effect of mercury on nymphal survival (days) of *O. fuscovittata* up to adult stage in F1 generation. Asterisk indicates a significant difference (P<0.05) in Fischer's test between treatment and control groups.

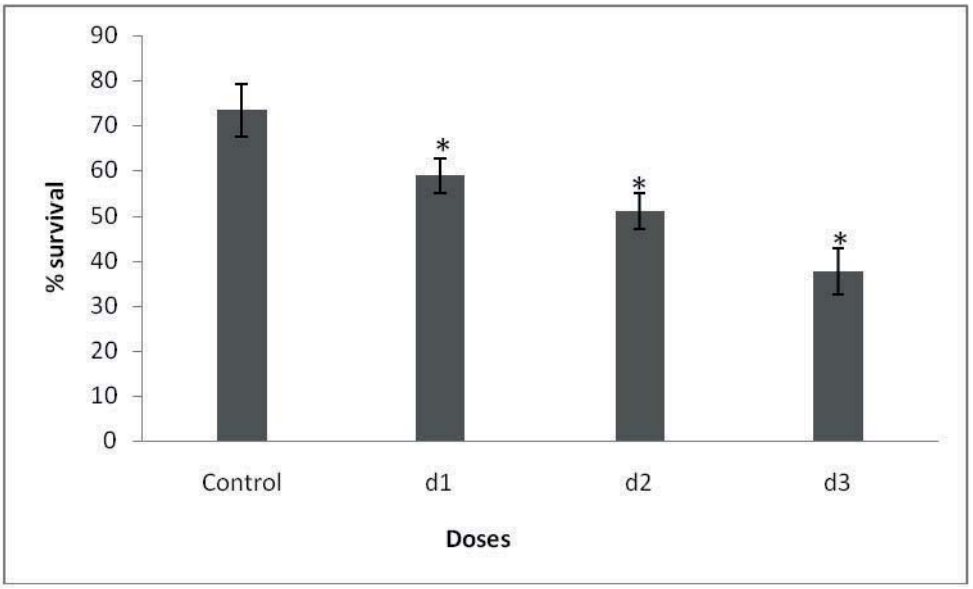

Fig. 2. Effect of mercury on nymphal survival (days) of *O. fuscovittata* up to adult stage in F2 generation. Asterisk indicates a significant difference (P<0.05) in Fischer's test between treatment and control groups.

Results

Effect of mercury on Total Rearing Time (TRT).—Newly hatched nymphs were exposed to $HgCl_2$-treated diet and allowed to feed *ad libitum* until they attained maturity; this continued over two consecutive generations. The TRT for each instar was recorded throughout the experiment. From the results it is evident that TRTs increased significantly in F1 (F=242.94, df=3, 48, P<0.05, ANOVA) and F2 (F=115.85, df=3, 48, P<0.05, ANOVA) generations. Results summarized in Table 1 show that in the F1 generation, only for the fifth molt of d1, and the first and sixth molts of d2, significantly increase (P<0.05), but at the highest dose (d3) , from the first molt onwards a significant increase in TRT was observed (P< 0.05).

Mercury exerted more severe effect in the F2 generation. Significant increases in TRT were recorded (P< 0.05) for all three doses throughout the developmental periods.

Effect of mercury on survival.—Around 40 to 75% of individuals attained adulthood in both generations. Percent survival of insects gradually decreased with increased doses in the F1 generation. Insignificant variations were observed for d1and d2 (df= 4, P= 0.365 and df= 4; P= 0.074 respectively; t test), but for d3, per cent survival significantly decreased (df= 4, P< 0.05, t test) (Fig. 1). Per cent survival in the F2 generation for all three doses (d1, d2, d3) decreased significantly (P<0.05) (Fig. 2). Statistical analysis also revealed that both the mercury dose and generation had significant effects on per cent survival of the selected insects (F=3.516; df = 3, 16; P< 0.05; ANOVA).

Effect of mercury on adult body weight.—Adult body weight of male and female individuals in both generations (F1, F2) showed a significant decrease (males: F=138.83; df =3, 16; P<0.05 and females: F=6.67; df =3, 16; P<0.05; ANOVA) with increasing dose. All the results between generations within dose and within generation between doses, showed significant decreases (df =4; P<0.05 in all cases; t test) (Table 2).

Effect of mercury on adult lifespan.—Mercury had a significant effect

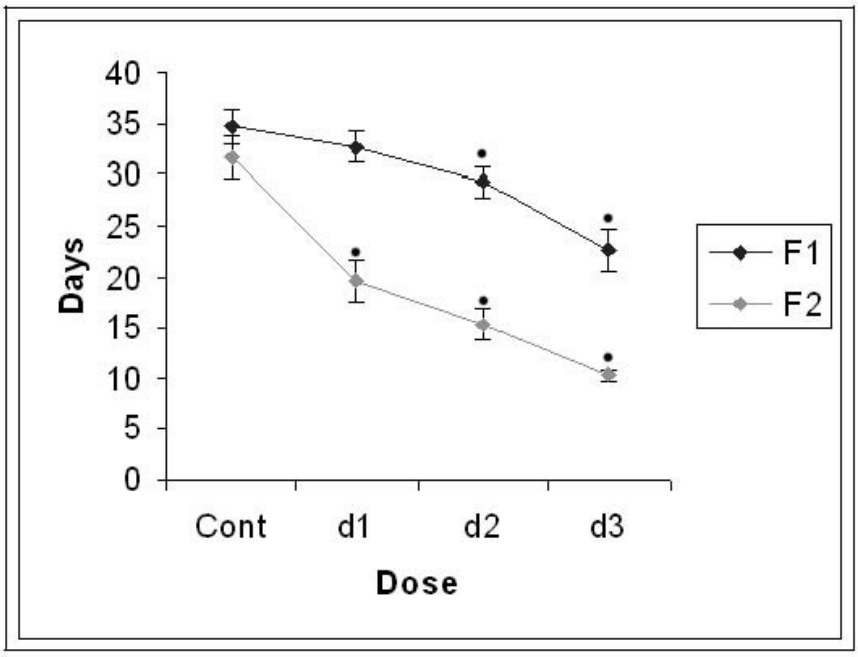

Fig. 3. Effect of mercury on adult male life span (days) of *O. fuscovittata* in F1 and F2 generations. Asterisk indicates a significant difference (P<0.05) in Fischer's test between treatment and control groups within a generation.

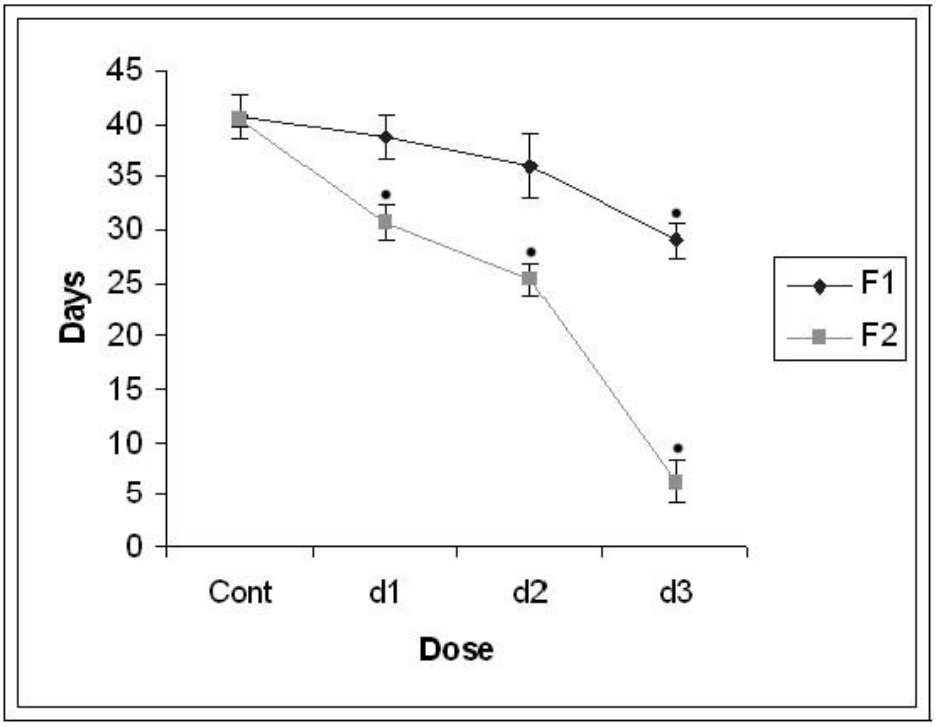

Fig. 4. Effect of mercury on adult female life span (days) of *O. fuscovittata* in F1 and F2 generations. Asterisk indicates a significant difference (P<0.05) in Fischer's test between treatment and control groups within a generation.

on adult lifespan in both sexes and generations (F1, F2). In males, treatments d2 and d3 for the F1 generation and all the doses for the F2 generation, differed significantly (F=112.37, P<0.05, ANOVA), whereas in females, though the F2 generation showed similar trends to those of males, only the d3 treatment in F1 differed significantly (F=157.67, P<0.05, ANOVA) from the controls (Figs 3 and 4).

Discussion

In the present work, we have investigated some of the effects of elevated Hg concentration on development, survival, adult lifespan (ALS), and adult body weight (ABW) of the terrestrial phytophagous grasshopper *O.fuscovittata*.

Newly hatched nymphs of *O.fuscovittata* were exposed to various sublethal concentrations of $HgCl_2$, throughout a complete life cycle and for two consecutive generations. Total Rearing Time (TRT) increased significantly both in F1 and F2 generations with increas-

ing dose. This is consistent with the study of Schmidt *et al.* (1992) who found in another acridid, *A. thalassinus*, prolonged nymphal stadia from mercury poisoning. Haney and Lipsey (1973) found a prolonged developmental period in the aphid *Macrosiphum gei*, fed on methylmercuric chloride-treated tomato plants. The effect of heavy metal-treated diet on the development of insects was also studied by Mathew and Al-Doori (1976) in *Drosophila melanogaster*. It was found there that development was affected when the larvae were reared on artificial media, having 1.434 mg of the Hg^{2+}-containing fungicide Ceresan M. It took at least 16 d for adult flies to emerge from the treated media but less than 12 d in the control.

Lapointe *et al.* (2004), reported that percent survival may not be associated with the doses of every heavy metal because, according to his study, per cent survival of *Diaprepes abbreviates* did not decrease gradually with the increased Cu^{2+} concentrations. However, in the present study with Hg^{2+}, a gradual decrease in per cent survival along with higher doses was observed.

A few studies are concerned with body-weight loss produced by heavy metals (Edens *et al.* 1976, Cheng 1980, Schmidt *et al.* 1992, Lapointe *et al.* 2004, Cervera *et al.* 2004). This effect is consistent with the present study; we did not measure food intake, however we observed that treated foods were less consumed than by the controls, especially at the higher Hg concentrations. Thus, at least a partial effect of feeding inhibition on the observed reduction in growth may be expected.

Adult lifespan of grasshoppers showed a tendency to decrease with increased dose. Cervera *et al.* (2004) reported a similar result where survival of adult females of *Oncopeltus fasciatus* decreased at concentrations higher than 10 mg Cd/L, while males were only affected at 30 mg Cd/L or higher doses.

Weakness in the legs, which sometimes led to difficulties in walking, abnormal movements of the antennae, in addition to tremors, were evident. It was often observed that the wings were deformed in F2-generation grasshoppers fed on treated diets; development of wings was improper, hence the insects could not fold their wings in a normal manner. As a result, wings became outstretched and bent, especially in the case of the highest dose (d3). Schmidt and Ibrahim (1994) reported a similar result while working on *A. thalassinus*. And Ramel and Magnusson (1969) also reported a similar result in *D. melanogaster* flies which arose from larvae being fed on Hg^{2+} compounds: adult flies had abnormal wings that were outstretched and bent downwards.

An important finding of the present study is that TRT increased significantly both in F1 and F2 generations. Again, in the case of per cent survival, adult body weight and total life span, a pronounced adverse effect was observed. Some morphological distortions in adults were also seen. Such adverse effects upon the physiology and the developmental period of the insects could reduce populations to extinction, provided growth retardation led to death of the insects before attaining maturity.

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