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Heavy Metal Concentrations of the Endoparasitoid *Glyptapanteles liparidis* Bouche (Hymenoptera) in Contaminated *Lymantria dispar* L. Larvae (Lepidoptera)

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The braconid wasp *Glyptapanteles liparidis* is one of the main parasitoids of the forest pest insect *Lymantria dispar (*gypsy moth) and therefore a regulator of the pest population. The eggs of the endoparasitoid are deposited in early larval stages of the host. The parasitoid larvae develop in the haemolymph of the host and feed exclusively on the nutrients of the haemolymph.

Several previous studies report on the effects of metal contamination on developmental and metabolic parameters of *L. dispar* (e.g., Gintenreiter et al. 1993 a, b).

Führer (1985) suggested that parasitic Hymenoptera are more sensitive to anthropogenic toxicants than their hosts. Contamination with metals may change the development as well as the metabolism of nutrients within the host and finally disturb the development of the parasitoid. Consequently the function of the parasitoid as a regulator of the pest may be endangered.

Effects of metal stress on the relationship of *L. dispar* and *G. liparidis* were investigated by Ortel et al. (1993). Results of that study led to the conclusion that the applied metals at the NOEC (No-observed-effect-concentration) level for *L. dispar* did not affect *G. liparidis* directly. Instead the parasitoid development is probably influenced by alteration of the trophic situation within the host due to its metal stress.

Heavy metal concentration of adult *G. liparidis* that eclosed from contaminated *L. dispar* (Ortel et al.1993) and the heavy metal concentration of the haemolymph of *L. dispar* (Gintenreiter 1994) have been analyzed recently. The present study provides information on the metal concentration of the parasitoid larvae shortly before their eclosion from the host. In order to identify the existence of possible heavy metal regulation mechanisms in the parasitoid the four metals (Cd, Pb, Cu Zn) were applied in two concentrations each.

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MATERIALS AND METHODS

Larvae of *L. dispar* were reared in the laboratory and fed on an artificial diet according to Bell et al. (1981). On the first day of the second larval stage the larvae were parasitized by female *G. liparidis*. Starting with the day of parasitization the lepidopteran larvae were fed on contaminated food. Heavy metals were added as nitrates to the diet yielding in the following concentrations: Cd: 2 ppm ("Cd2"), 6 ppm ("Cd6"), Pb: 4 ppm ("Pb4"), 12 ppm ("Pb12"), Cu: 6 ppm ("Cu6"), 10 ppm (Cu10"), Zn: 60 ppm ("Zn60"), 100 ppm ("Zn100") (concentrations based on nutrient medium fresh weight). A control group ("C") was fed on uncontaminated artificial diet.

At the third day of the 4th larval stage (a few days before the eclosion of the parasitoids) the parasitoids were dissected from their hosts, pooled (10 to 30 individuals/sample; depending on their size) and dried at 70°C to constant weight. The samples were digested with 0.4 ml conc. HNO_3 in polypropylene microtest tubes at 70°C for 3 hours and diluted with 0.4 ml distilled water. Metal contents were assayed by flame (Zn) and flameless (Cd, Pb, Cu) atomic absorption photospectrometry (VarianAA30 + GTA96).

Statistical methods: Distribution of data was examined with Kolmogorov-Smirnov-test. Differences between contamination groups and control group were determined by t-test statistics. Concentration factors were calculated by dividing heavy metal concentrations of parasitoid larvae through the mean metal concentration of the *L. dispar* haemolymph (5th instar) of each contamination group. Mean ± S.E. of the concentration factors were determined.

RESULTS AND DISCUSSION

The results of metal analyses in *G. liparidis* larvae show clear differences between the essential and the non-essential metals. Whereas the Cd and Pb concentration are significantly elevated in the contaminated groups (according to the level of contamination) the concentrations of copper and zinc did not change significantly in the "Cu10", "Zn60" and "Zn100" groups (Fig.1). The concentration of copper is higher in the "Cu6" group than in the highly contaminated group, which suggests the existence of a metal regulation mechanism. Cadmium and copper concentrations of the parasitoid control individuals are very similar to those reported for *G. liparidis* imagoes eclosed from uncontaminated *L. dispar* larvae (Ortel et al. 1993), whereas zinc concentrations are about three times higher in larvae and five times higher in pupae than in the corresponding imagoes. Pupae zinc levels exceeded those of the larvae which is probably due to the weight loss during metamorphosis. Although further weight reduction occurred from pupal to adult stage and hence, Zn levels should be expected to increase, imagoes proved to be contaminated least. This may be attributable to a possible metal elimination via meconium as shown for several other species (e.g., Gintenreiter et al. 1993).

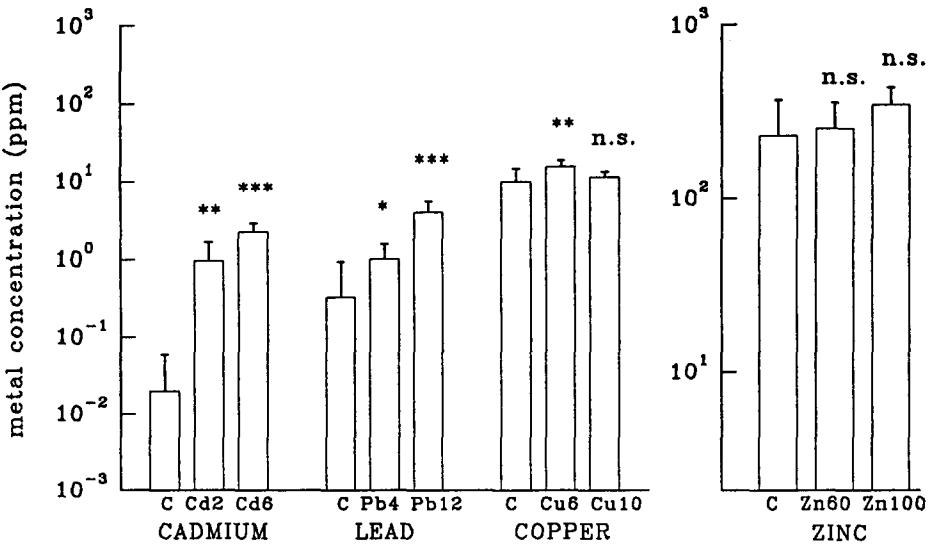

Figure 1. Metal concentrations (mean ± S.D.; n = 12 for each group) of *G. liparidis* larvae shortly before eclosion from the host *L. dispar*. Above the bars the significant differences to the control group. Note the logarithmic scale at the ordinate. (The nomenclature of the groups is explained in Materials and Methods.)

Ortel et al. (1993) calculated concentration factors from the total body burden of the two species (*G.liparidis/L. dispar*), which were mostly below 1. But concentration factors of parasitoid larvae and host haemolymph would be of greater physiological and ecological relevance because of the well documented fact that metals accumulate tissue specifically (Dallinger and Wieser 1984, Hopkin 1989). In accordance, Gintenreiter (1994) analyzed the distribution of metals within *L. dispar* larvae (5th larval stage) and revealed extremely low metal concentrations in the haemolymph. On the condition that metals are not accumulated in the haemolymph and the assumption that their concentrations do not change drastically from stage to stage, the results of Gintenreiter (1994) were used to derive concentration factors for parasitoid larvae and host haemolymph.

Generally, the metal concentrations of the parasitoid control group are very high compared to the metal levels in their source of food - the haemolymph of the host (concentration factors: Table 1). However, the measured heavy metal concentration of the haemolymph only describes a temporary status. As the parasitoids live in and feed on the haemolymph for several weeks, it is possible for them to accumulate such high amounts of heavy metals.

Table 1. Concentration factors "CF" (mean ± S.E.; n = 12 for each group) of metals in *G. liparidis*-larvae (whole body) / *L. dispar* (haemolymph).

	CADMIUM			LEAD		
	C	**Cd2**	**Cd6**	**C**	**Pb4**	**Pb12**
CF	10.0 (4.8)	69.2 (7.2)	67.3 (4.3)	---	9.2 (2.2)	9.0 (1,8)

	COPPER			ZINC		
	C	**Cu6**	**Cu10**	**C**	**Zn60**	**Zn100**
CF	24.7 (3.4)	38.9 (2.7)	20.1 (1.8)	86.2 (8.8)	75.1 (5.5)	69.6 (4.2)

In comparison with data from field studies, metal concentrations of *G. liparidis* are relatively low, even in the high contaminated groups. Knutti et al. (1988) reported Cd concentrations between 0.1 and 1 ppm Cd in insects of a woodland area without discernable Cd pollution, whereas Cd concentrations can increase in herbivorous insects to more than 20 ppm at a contamination site (Hunter et al. 1987). Normal copper concentrations vary between 10 and 40 ppm (Lindqvist 1992: hymenopteran and lepidopteran species; Levy and Cranroy 1973: diverse insect species). Along an air pollutant gradient the Cu levels of four hymenopteran species exceed 100 ppm close to the emission source and range between 10 and 50 ppm at more than 2 km distance from it (Heliövaara 1989). Hence, values of 10 to 15 ppm Cu in *G. liparidis* larvae can be considered moderate. Pb levels are very low in beetles (1-2 ppm: Vogel 1986) and rise to more than 600 ppm in Isopoda (Williamson and Evans 1972), whereas Zn levels may lie between 200 and 250 ppm in caterpillars (Andrzejewska et al. 1990) and between 300 and 600 ppm in Coleoptera (Vogel 1986) for example.

Ortel et al. (1993) found no correlation between the extent of metal contamination and parasitization success, but noted a faster development in the control group than in most of the contaminated groups. Additionally they observed, that developmental rates of *L. dispar* and *G. liparidis* correlated positively. Moreover, metal contamination can alter respiratory metabolism (Migula 1989) as well as the haemolymph composition (Radhakrishnaiah and Busappa 1986) and reduce the content of important nutrients for the parasitoid (Bischof, subm.).

The data in the present study indicate that in spite of the very high concentration factors, parasitoid larvae/host haemolymph metal burdens of the parasitoids are relatively low compared to data derived from field studies, even for uncontaminated sites. Consequently, the hypothesis of Ortel et al. (1993) that the development of *G. liparidis* is not directly affected by the metals themselves, but by alteration of the trophic situation within the host, can be confirmed.

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