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An Evaluation of Glutathione S-Transferase Activity in the Tasmanian Lacewing *(Micromus tasmaniae)* as a Biomarker of Organophosphate Contamination

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With pressure from international markets on producers to minimize their use of agrochemicals, the sustainability of agro-ecological systems is of rapidly growing interest and various invertebrate indicators have been proposed as measures of environmental 'health' (Wratten *et al.* 1997, 1998; McGeoch 1998). The Tasmanian lacewing *(Micromus tasmaniae* (Walker); Neuroptera: Hemerobiidae) is a common predator in cereal, vegetable and legume crops in New Zealand (Leathwick 1989). Both adult and juvenile neuropterans are voracious predators of many invertebrate agricultural pests, and the presence of *M. tasmaniae* is justifiably considered beneficial to agricultural systems (New 1975). The species is considered important in integrated approaches to pest management and its use as a biomonitor of stewardship practices in New Zealand farms is being developed (Wratten *et al.* 1997).

As well as using the presence, absence or abundance of key indicator species to denote levels of contamination, sub-lethal enzyme biomarkers have been developed to indicate stresses in the organism's physiological state *(e.g.,* carboxylesterase, cholinesterase, cytochrome P450, glutathione S-transferases). Glutathione S-transferase (GST) is a multicomponent enzyme involved in the detoxification of many xenobiotics and which has an important role in protecting tissues from oxidative stress (Foumier *et al.* 1992). GST is involved in the detoxification pathway for organophosphorous compounds and also has an important role in the development of insecticide resistance (Motoyama & Dauterman 1980; Clark 1982; Yu 1996). In insects, GST has been induced by exposure to a number of chemicals, including pesticides, such as lindane (Lagadic *et al.* 1993), paraquat (Parkes *et al.* 1993) and oxadiazolone (Hunaiti *et al.* 1995), and plant phytotoxins (Lee 1991). The use of GST activity as an indicator of pollutant stress or of potential pesticide resistance has been assessed in a number of insect species (e.g., Starratt & Bond 1990; Gerson *et al.* 1991; Lee 1991; Hunaiti *et al.* 199.5). Nielsen *et al.* (1999) have evaluated GST as a biomarker in wolf spiders and GST has proved useful as a biomarker of pollutant exposure in molluscs (Baturo & Lagadic 1996; Fitzpatrick *et al.* 1997).

In laboratory studies, GST in *M. tasmaniae* has been found to vary between larval instars (Rumpf *et al.* 1997a,b). Also, *M. tasmaniae* larvae are difficult to find in the field and, therefore, the development as a biomarker of the more easily-obtained, and less variable, adults is desirable. The aim of this paper was to examine changes in GST activity in adult *M. tasmaniae* after exposure to organophosphate insecticides (diazinon and chlorpyrifos) and evaluate the potential of this enzyme as a biomarker of

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organophosphate exposure in this species.

MATERIALS AND METHODS

The organophosphates used were chlorpyrifos (Lorsban 40EC; 400 g a.i. per L, DowElanco (NZ) Ltd, New Plymouth, NZ) and diazinon (Basudin 600EW; 600 a.i. g per L, Ciba-Geigy NZ Ltd, Avondale, NZ). These pesticides are commonly used on pasture, cereals and vegetable crops in New Zealand. Rates of pesticide application in these experiments were based on those recommended for use against aphids; 0.5 L for chlorpyrifos and 1.25 L for diazinon in 200 L of water per hectare. For laboratory studies, this equated to dilutions of the formulated product of 1:400 for chlorpyrifos and 1: 160 for diazinon. The doses used in each experiment were based on prior pilot studies and aimed to provide a range of stress levels to adult *M. tasmaniae*, as indicated by mortality or knockdown responses.

The *M. tasmaniae* used were all laboratory-reared specimens, founder populations having been collected from an organic farm in Canterbury, New Zealand. The animals were reared in plastic boxes (30 x 22 x 10 cm) maintained in a constant temperature room (20 ± 1°C 16:8 1ight:dark) and fed on a diet of live pea aphids *(Acyrthosiphon pisum* Harris) and a 1: 1 honey/yeast solution.

Samples for enzyme analysis were frozen at -80°C. When required, the samples were defrosted on ice and maintained below 4°C (on ice) at all times. Activity of GST in the decapitated bodies was measured by adopting the protocol of Habig *et al.* (1974). Three decapitated bodies were placed in 0.2 *M* potassium phosphate buffer at pH 7.0 (100 µL per body), homogenized and the resulting homogenate centrifuged at 4°C for 5 min at 15000 rpm. In the final assay, 1 m*M* CDNB (1-chloro-2,4-dinitrobenzene) and 6 m*M* glutathione (GSH) were used as substrates. The GST in the sample catalyses the conjugation of CDNB to glutathione producing S-(2,4-dinitrophenyl) glutathione, and enzyme activity is monitored spectrophotometrically at 340 nm. Enzyme activity and total protein concentration of the sample supernatant were determined at 28°C in 96-well microtitre plates using a Reader 340 ATTC and the Biolise software 2000 package v1.6 (SLT Instruments, Salzburg, Austria).

The assay for protein was adapted from that described by Bradford (1976). Bovine serum albumen (BSA, Sigma Chemical Co., St Louis, MO, USA) was used as the protein standard. Activity of GST is expressed as nmoles of GSH conjugated per min per mg protein.

In the topical exposure bioassay, newly-emerged *M. tasmaniae* (O-24 hr old) were placed in a refrigerator (4°C) for 10 min to reduce activity. The lacewings were sorted into batches of 10, and placed in clean plastic containers (60 mm high; 40 mm diameter), housed within a controlled environment room (20 ± 1°C; 16 hr photoperiod). The animals were individually treated with a pesticide solution, by applying a 1µL droplet to their dorsal surface, using a microapplicator (Burkard Manufacturing Co Ltd., Rickmansworth, England). Chlorpyrifos was applied at 1% (4 batches), 2% (4 batches) and 10% (3 batches) of field concentration and diazinon was applied at 0.32% (3 batches), 1.6% (3 batches) and 3.2% (2 batches) of field concentration. Distilled water

was used as a control (3 batches). After treatment, an excess of pea aphids was provided as food.

Although none of the lacewings died during the experiment, many were moribund after 24hr. Therefore, 'knockdown' was estimated using all the lacewings in each treatment and the association between the degree of knockdown and pesticide treatment was estimated using a χ^2 analysis. The lacewings were stored at -80°C for subsequent enzyme analysis. Groups of three lacewings were selected at random from the pooled batches in each treatment and their GST content measured. Differences in GST levels between treatments were then analyzed using a one-way ANOVA, followed by a Dunnett's test to indicate significant differences from the control.

For exposures of lacewings in Petri dishes, dilutions of both organophosphates were made up to represent 100%, 10%, 1%, and 0.1% of field concentration. The pesticides were applied evenly across the surface of a filter paper (Whatman No. 1; 4.25 cm diameter) using a micro-pipette (at 2 µL per cm^2; equivalent to the rate per unit area for field usage). Distilled water applied at the same rate was used as a control. The filter papers were left to dry for 45 min in a fume cupboard and then placed into plastic Petri dishes (4.5 cm diameter). Five *M. tasmaniae* were placed into each dish and the lids put in place. There was a minimum of five replicates for each treatment. No food was provided for the lacewings throughout the exposure period. Mortality was estimated after 24 hr and the survivors frozen for subsequent enzyme analysis. The GST activities of males and females in each treatment were measured separately.

Mortality data from each Petri dish were arcsine transformed and analyzed using a one-way ANOVA to examine differences in mortality between each treatment. The effect of pesticide concentration and sex on GST levels was examined using a General Linear Model (MINITAB 9.2, Minitab Inc., PA, USA).

Dwarf French beans *(Phaseolus vulgaris* L.) were grown in plastic pots in a glasshouse until approximately 15 cm tall. The plants were sprayed to 'run-off' with pesticide using a hand-held plant sprayer. For diazinon, concentrations of 3.2%. 1.6%, 0.8% and 0.32% of field concentration were used. For chlorpyrifos, the concentrations were 4.0%, 2.0%, 1.0% and 0.4% of field concentration. Water was used as a control.

Once the pesticides had dried (approx. 1 h), transparent acetate cylinders (10 cm diameter, 30 cm tall) were placed over plants and pushed into the soil. The cylinders had mesh sides and tops to aid ventilation. Ten *M. tasmaniae* were placed on each plant. The plants were kept in a 'polytunnel room', with daily temperatures fluctuating between 1l-21 °C. No food was provided for the lacewings throughout the exposure period. After 24 hr, mortality was assessed and any surviving lacewings frozen at -80°C for enzyme analysis. The GST activities of males and females were analyzed separately. Four replicate pots of plants were sprayed for each pesticide at each rate. As each organophosphate was tested with a different batch of lacewings, three separate control replicates were set up for each chemical. Mortality data for each pesticide was arcsine transformed and analyzed using a one-way ANOVA to examine differences in mortality between concentrations. The effect of pesticide concentration and sex on GST levels was examined using a General Linear Model (MINITAB 9.2, Minitab Inc., PA, USA).

RESULTS AND DISCUSSION

In the topical exposure bioassay there were significant differences between treatments in the proportion of individuals suffering knockdown, knockdown being clearly dose related (Fig. 1; $\chi^2 = 110$, $P < 0.001$ for 6 d.f.). GST activity was reduced by exposure to the organophosphates (Fig 1; $F_{6, 13} = 4.9$, $P < 0.01$), although only chlorpyrifos led to a significant reduction compared to the water controls (Dunnett's test, $P < 0.05$).

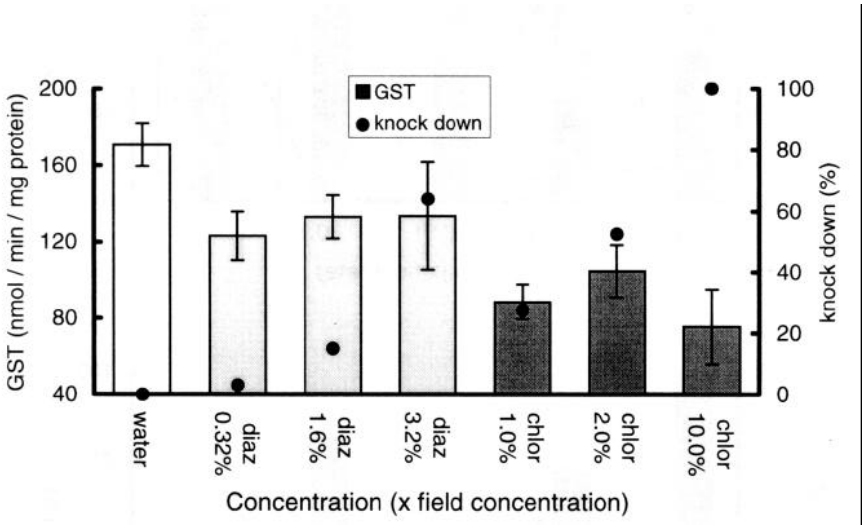

Figure 1. The effect of topical application of diazinon and chlorpyrifos on knockdown and GST activity in *M. tasmaniae* adults (mean ± SE).

Micromus tasmaniae mortality was affected by pesticide residues in the Petri dishes (Fig 2.; $F_{8,49} = 34.6$, $P < 0.001$), although only the field concentrations of both pesticides caused a significant increase in mortality compared to the water control (Dunnett's test, $P < 0.001$). For both pesticides, there was a significant statistical interaction between concentration and sex on GST activity (diazinon $F_{4,11} = 4.7$, $P < 0.02$; chlorpyrifos $F_{3,12} = 9.8$, $P < 0.001$). Diazinon (Fig. 2a) did not affect GST activity in males ($F_{4,2} = 13.4$, $P > 0.05$) or females ($F_{4,9} = 2.1$, $P > 0.15$). Chlorpyrifos (Fig. 2b) did not affect GST activity in females ($F_{3,9} = 0.3$, $P > 0.80$) but caused a significant decrease in males at some concentrations (see Fig. 2; $F_{3,3} = 28.8$, $P < 0.02$). For both pesticides, males on average had higher levels of GST per mg of protein than females (Fig 2; diazinon $F_{1,11} = 62.0$, $P < 0.001$; chlorpyrifos $F_{1,12} = 2.7$, $P < 0.001$).

Micromus tasmaniae mortality was significantly affected by pesticide residues on bean plants (diazinon $F_{4,14} = 33.4$, $P < 0.001$; chlorpyrifos $F_{4,14} = 27.4$, $P < 0.001$), and was positively related to concentration (Fig. 3). Neither pesticide had a significant effect on GST activity in the surviving lacewings (Fig. 3; diazinon $F_{4,10} = 3.5$, $P > 0.05$; chlorpyrifos $F_{4,9} = 1.6$, $P > 0.20$), although GST activity was significantly higher on average in males than in females (diazinon $F_{1,10} = 18.4$, $P < 0.005$; chlorpyrifos $F_{1,9} = 17.3$, $P < 0.005$).

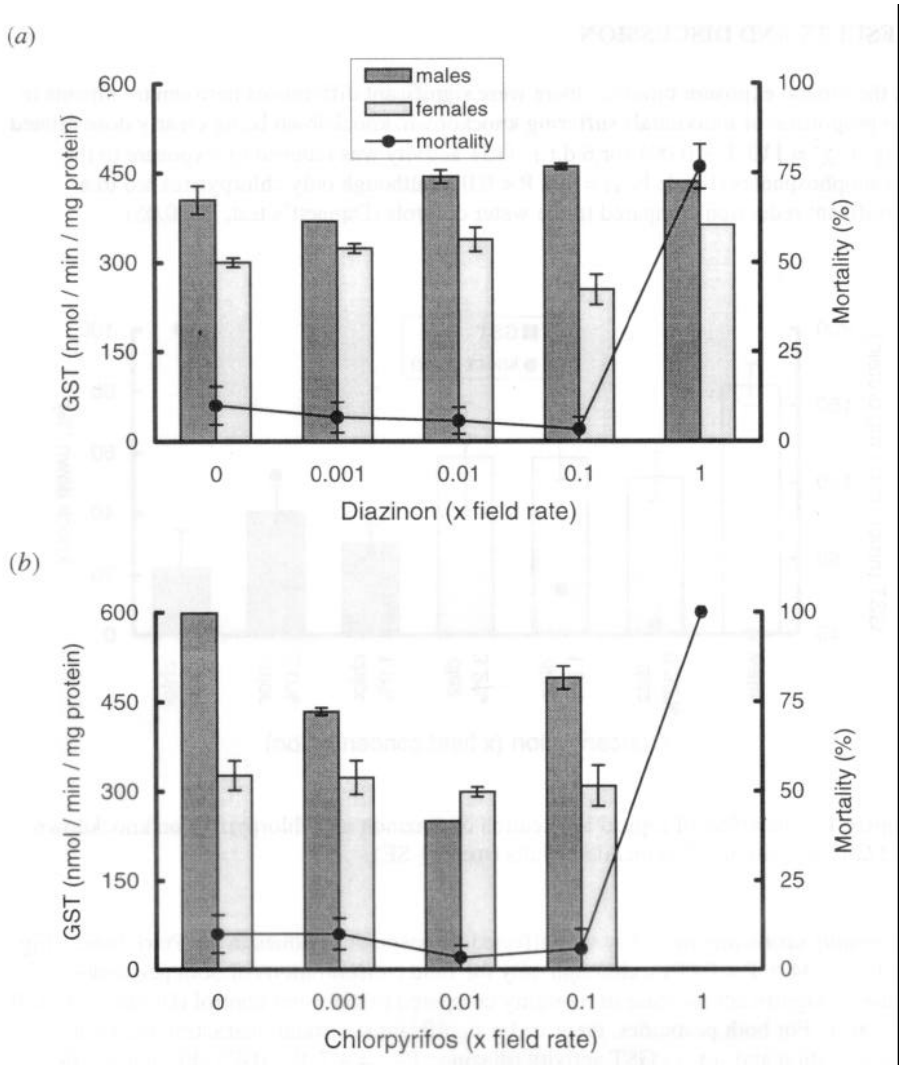

Figure 2. The effect of exposure to *(a)* diazinon and (b) chlorpyrifos residues in Petri dishes on mortality and GST activity in *M. tasmaniae* adults (mean ± SE).

Throughout these experiments, the activity of GST in *M. tasmaniae* adults was within the range reported by Siegfried & Young (1993) for terrestrial and aquatic insects. On average, males had higher GST activity per mg protein than females. The reason for this is not known, although it can be speculated that the overall protein content of females may be higher due to their reproductive anatomy and the storage of eggs. This difference in GST activity between the sexes further highlights the need to account for intrinsic variables between individuals when using physiological biomarkers (see Rumpf 1996).

A wide variety of compounds have been found to induce GST (see Introduction),

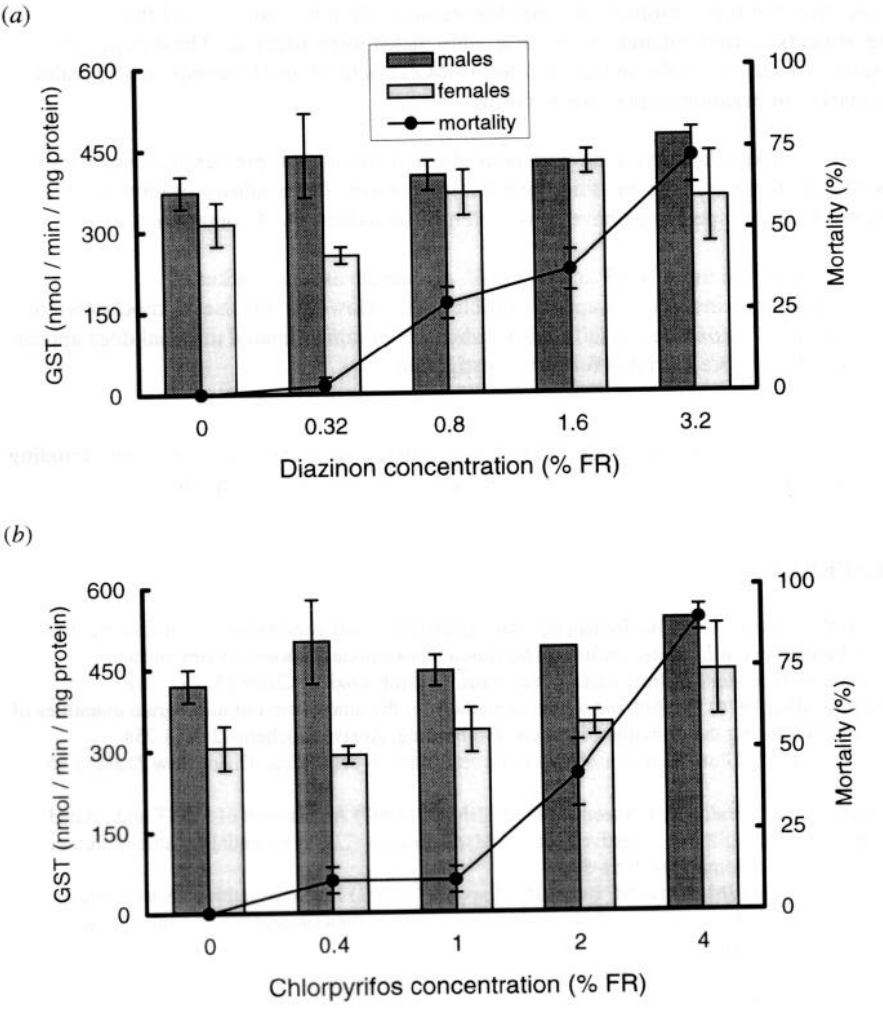

Figure 3. The effect of exposure to *(a)* diazinon and *(b)* chlorpyrifos residues on beans on mortality and GST activity in *M. tasmaniae* adults (mean ± SE).

including organophosphates, although other substances have been found to lower GST activity (Lee 1991; Yu , 1996; Rumpf 1997b). Rumpf *et al.* (1997b) reported that the organophosphates, methyl-parathion and azinphos-methyl, and insect growth regulators, diflubenzuran and tebufenozide, had no effects on GST activity in 3rd instar *M. tasmaniae* larvae, though GST activity was increased by cypermethrin and decreased by fenoxycarb.

In the current experiments, the response in GST activity in *M. tasmaniae* after exposure to the organophosphates varied between experiments. The general indication was that diazinon did not affect GST activity. Exposure to chlorpyrifos caused GST inhibition in

some cases but this inhibition was not dose related, did not occur in all of the experiments carried out and, in one case, only males were affected. These equivocal results cast serious doubt on the usefulness of GST activity in *M. tasmaniae* as a valid biomarker of organophosphorous exposure.

Mortality (or knockdown) of *M.tasmaniae* showed significant, predictable responses in each of the three systems and this suggests that the use of knockdown or mortality responses in this species do have some potential as indicators of contamination.

In conclusion, the use of GST activity in *M. tasmaniae* as a biomarker of organophosphate insecticides appears problematic. However, the use of knockdown or mortality in *M. tasmaniae* as laboratory indicators of contaminated material does appear useful and therefore warrants further investigation.

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