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DETOXICATIVE ENZYME ACTIVITIES IN FIVE SPECIES OF FIELD-COLLECTED MELANOPLINE GRASSHOPPERS (ORTHOPTERA: ACRIDIDAE)

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Detoxicative enzyme systems, such as the cytochrome P450 monooxygenases, gluthione *S*-transferases, and general esterases, have been widely studied in holometabolous insects (e.g. Lepidoptera, Diptera, and Coleoptera). These, and other enzyme systems, play important roles in insecticide resistance, but are also important in insect–host plant relationships, because host range can partially depend on the ability of an insect to cope with putatively toxic allelochemicals in an otherwise suitable host plant (e.g. Lindroth 1989). In some cases, differences in the relative activities of these enzymes between closely related insect taxa can have significant biological consequences (Siegfried and Mullin 1989).

These enzyme systems have been far less extensively studied in hemimetabolous insects, but we recently investigated the tissue distribution and development changes in detoxicative enzymes in the migratory grasshopper, *Melanoplus sanguinipes* (Fab.), a major pest of rangelands and cereal crops in western Canada. However, *M. sanguinipes* is only one member of a melanopline grasshopper community in western North America, consisting of about 10 common *Melanoplus* species and additional species in related genera (Vickery and Kevan 1985). Many of these species apparently have a wide host range including grasses and forbs, and in some years several species constitute significant pests of cereal and oilseed crops in the Prairie Provinces. At present, grasshopper control is based on aerial and ground-sprayer applications of pyrethroid, carbamate and organophosphate insecticides (WCCP 1995).

Herein we report analyses of detoxicative enzyme systems in five species of melanopline grasshoppers collected in southern Alberta, to determine if interspecific differences occur. Such information could be useful in predicting relative susceptibilities to certain types of insecticides.

Adult grasshoppers of the following species were collected with sweepnets in mixed wheat and pasture at Turin, Alberta, in July 1994: *M. sanguinipes*, *M. bivittatus* (Say), *M. gladstoni* Scudder, *M. packardii* Scudder, and *Phoetaliotes nebrascensis* (Thomas). The collection site was selected because it contains a mixed grasshopper community from which all of the aforementioned species can be collected simultaneously. More importantly, this site has not been treated with insecticides in the past 4 years. Therefore potential interspecific differences are not confounded by prior pesticide exposure or diet. Grasshoppers were shipped by air to the University of British Columbia where they were maintained in 40- by 28- by 28-cm cages and fed seedling wheat and dry wheat bran for 24 h prior to dissection.

Grasshoppers were dissected in cold 0.15 *M* NaCl solution and their midguts, including the gastric caeca, removed, rinsed, and homogenized as previously described (Feng and Isman 1994). These tissues were previously found to have the highest levels of detoxicating enzymes in *M. sanguinipes*. Homogenates were prepared using midguts taken from eight adults (four male, four female); three preparations were made for each species except for *M. bivittatus* from which two preparations were made. Preparations were assayed for cytochrome P450 level, glutathione *S*-transferase activity, and general esterase activity, using previously described methods and instruments (Feng and Isman 1994).

Results of our enzyme assays are shown in Table 1. With respect to cytochrome P450, the specific level is somewhat lower in *M. gladstoni* than in the other four species, although *P. nebranscensis* is equally low on a per insect basis. The only outstanding differences are between values for the field-collected *M. sanguinipes* and those from a long-standing laboratory colony. Those from the laboratory have more than double the amount of cytochrome P450 and over 4-fold the amount on a per insect basis. However, we have not measured activity of cytochrome P450 from the different species against specific substrates. Regarding general esterase activity, the only outstanding species is *P. nebrascensis*, which has much less activity than the *Melanoplus* species, both in terms of specific activity and especially on a per insect basis. For this enzyme system, specific activity was not much greater for the laboratory-reared *M. sanguinipes* compared with the field insects, but the laboratory insects have about double the activity on a per insect basis. For glutathione *S*-transferase activity, *M. packardii* has the lowest specific activity, but there are no significant interspecific differences on a per insect basis, although *M. bivittatus* has the highest level by both measures. Again, laboratory-reared *M. sanguinipes* had significantly greater activity of this enzyme system compared with their field-collected conspecifics: 6-fold greater in terms of specific activity and 4-fold on a per insect basis.

In summary, the most notable differences found in our study are those between field-collected and laboratory-reared *M. sanguinipes*, the latter having much higher levels of the three detoxicative enzymes systems assayed, particularly in the case of glutathione *S*-transferase. Our laboratory colony represents a satellite of a non-diapause strain originally selected at the Agriculture Canada Research Station in Saskatoon, Saskatchewan (Pickford and

TABLE 1. Detoxicative enzyme activities in the midguts from different grasshopper species

Species		Cytochrome P450		General esterase		Glutathione *S*-transferase	
		nmol mg protein^{-1}	nmol insect^{-1}	nmol min^{-1} mg protein^{-1}	nmol min^{-1} insect^{-1}	nmol min^{-1} mg protein^{-1}	nmol min^{-1} insect^{-1}
Melanoplus bivittatus (Say)		0.41 (0.12)	0.04 (0.01)	441.8 (56.5)	472.8 (30.7)	8.62 (1.08)	8.72 (1.42)
M. gladstoni Scudder		0.25 (0.07)	0.02 (0.00)	282.4 (86.4)	207.0 (59.1)	5.91 (1.15)	4.28 (0.10)
M. packardii Scudder		0.32 (0.18)	0.06 (0.05)	432.8 (75.4)	745.9 (53.0)	3.72 (0.52)	5.95 (0.50)
M. sanguinipes (Fab.)	field	0.45 (0.03)	0.03 (0.01)	368.7 (79.4)	284.6 (34.7)	5.05 (0.67)	5.15 (1.25)
	lab	1.01 (0.15)	0.14 (0.04)	476.0 (49.4)	619.6 (78.2)	30.44 (4.16)	20.49 (2.35)
Phoetaliotes nebrascensis (Thomas)		0.41 (0.06)	0.02 (0.01)	157.1 (6.7)	81.6 (16.6)	5.13 (1.13)	3.32 (0.91)

Values are means (SE) from three different preparations, each with duplicate readings, except for *M. bivittatus* (two preparations). Each preparation was made using the midguts from eight individual insects. "*M. sanguinipes* lab" data are from Feng and Isman (1994).

Randell 1969). This strain has been maintained for over 200 generations in the laboratory, without exposure to pesticides. However, both our colony and the parent colony have been treated periodically with sulfa-based antibiotics to manage the parasitic protozoan *Malamoeba locustae*. It is possible that the elevated level of constitutive glutathione *S*-transferase activity in the lab-reared grasshoppers may reflect past selection with these antibiotics.

In general, detoxicative enzyme and activity levels are comparable to some other insects, notably lepidopterans. For example, Yu and Hsu (1993) reported a cytochrome P450 content for the midgut of last-instar fall armyworm, *Spodoptera frugiperda* (J.E. Smith), as 0.202 nmol/mg protein; contents of the grasshopper species examined herein ranged from 0.25 to 0.45 nmol/mg protein. The same authors also reported glutathione *S*-transferase activities from the midguts of seven species of lepidopterans ranging from 3.4 to 74.8 nmol/min/mg protein. In the present study, specific activities of this enzyme in the grasshopper species ranged from 3.7 to 8.6, on the low end of the scale relative to lepidopterans examined. As to general esterase activity, Wheeler et al. (1993) reported a value of 383.7 nmol/min/mg protein for the midgut of the fall armyworm, which would place that insect within the range of values obtained for the grasshopper species (157.1–441.8) in the present study.

Among the species examined, *M. gladstoni*, *M. packardii*, and *P. nebrascensis* are predominantly grassland species, although their host ranges include grassland forbs. On the other hand, *M. bivittatus* and *M. sanguinipes* are common in both grasslands and forests, and feed on a wide range of forbs in addition to grasses; *M. bivittatus* feeds on a number of "toxic" rangeland plants (W. Majak and D. Johnson, unpublished data). This latter species had the highest specific activities for each of the three enzyme systems assayed in our study, but differences between it and the other species are not likely biologically, let alone statistically, significant.

Interspecific differences in susceptibility of these grasshopper species to insecticides have not been fully examined, but McDonald (1967) determined LD_{50} values for 23 insecticides in both *M. sanguinipes* and *M. bivittatus*. Though there are a few exceptions, in general the two species appear equally susceptible to most of the compounds tested. Based on the present, limited findings, we suggest that differences in susceptibility to insecticides among the five species we examined should be minor.

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