Alterations in Esterases are Associated with Malathion Resistance in *Habrobracon hebetor* (Hymenoptera: Braconidae)

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ABSTRACT Biochemical mechanisms of malathion resistance were investigated in a malathion-resistant strain of the parasitoid *Habrobracon hebetor* Say collected from a farm storage in Kansas. General esterase activities were significantly lower in the resistant strain compared with those in a susceptible strain. However, no significant differences were found in activities of malathion specific carboxylesterase (MCE), glutathione S-transferase and cytochrome P450 dependent *O*-demethylase activities, cytochrome P450 contents, and sensitivity of acetylcholinesterase to inhibition by malaoxon between the 2 strains. Because MCE was not elevated in the resistant strain, the weak malathion resistance in *H. hebetor* may result from a different mechanism compared with that hypothesized for some insect species in which reduced general esterase activity is accompanied by an elevated MCE. Decreased esterase activity in the resistant strain suggested that null alleles of some esterases were associated with the resistance. Indeed, E1 and E2, major esterases in the susceptible strain, were not present in the resistant strain on polyacrylamide gels that were stained for esterase activity using the model substrate 1-naphthyl acetate. In contrast, the activity of esterase E3 on the gels was much higher in the resistant strain as compared with that of the susceptible strain. These findings indicate that malathion resistance in *H. hebetor* is associated with both an increased activity of the esterase E3 and null alleles of the esterases E1 and E2.

KEY WORDS parasitoid, resistance, insecticide, detoxification

MALATHION RESISTANCE IS common in many pest insects and in several parasitoids associated with the stored-grain ecosystem. Two parasitoids that have developed significant levels of resistance have different feeding niches within the grain storage system. *Anisopteromalus calandrae* (Howard) (Hymenoptera: Pteromalidae) can penetrate grain masses during the search for hosts such as weevil larvae that are found within grain kernels. A 2nd species, *Habrobracon* [=*Bracon*] *hebetor* Say (Hymenoptera: Braconidae), forages primarily on the grain surface where major hosts are pyralid moth larvae. *A. calandrae* has developed a high level of resistance (>2,500-fold) (Baker 1995) that is hypothesized to result from the presence of protected hosts (i.e., weevil larvae inside grain kernels) when the wasps are searching in grain treated with grain protectants (Baker and Weaver 1993, Baker and Throne 1995). However, malathion resistance in *H. hebetor* is much weaker, ≈7-fold at the LC_{50} based on concentration-response assays, perhaps because it can locate hosts without extensive contact with insecticide residues on the grain mass (Baker et al. 1995).

The high level of resistance in *A. calandrae* is associated with increased activity of a malathion specific carboxylesterase (Baker et al. 1998). There is no evidence that an increase in general esterase activity or increased activity of other detoxification systems, including glutathione S-transferase, phosphotriesterase, or cytochrome P-450 O-demethylase contributed to the resistance in this pteromalid wasp. The mechanism(s) responsible for the much weaker malathion resistance in *H. hebetor* is not understood. In the study reported below, we determined resistance levels of a field strain of *H. hebetor*, increased the resistance frequency of the strain through laboratory selection, and compared activities of several detoxification systems in the selected strain with those of a laboratory strain.

Materials and Methods

Parasitoid Strains. A strain of *H. hebetor* (R strain) was collected during October 1996 in a probe trap placed in wheat that was infested with the Indian meal moth, *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae). The grain storage was located in Dickinson county in north central Kansas. The parasitoid was reared in the laboratory at 27°C and 60% RH on *P. interpunctella* larvae for 4 generations before start of the bioassays. A susceptible (S) strain of *H. hebetor*,

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maintained for >20 yr in the laboratory, was used as a reference strain.

Bioassays. Resistance in adult male and female *H. hebetor* was determined with serial time-response bioassays in glass vials (28 by 60 mm) according to Baker et al. (1995). To compare response of strains, a single concentration of technical malathion (American Cyanamid, Princeton, NJ) (6.1 μg [AI] per vial) was used in these tests. This concentration was the LC_{99} for the SCC strain of *H. hebetor* determined previously with concentration response bioassays (Baker et al. 1995). Adults tested were generally between 3 and 7 d old. In each assay, the parasitoids were briefly anesthetized with CO_2 and placed in the treated vials. Knockdown was determined at 10-, 15-, or 30-min intervals at room temperature. Results are based on 2–3 tests at room temperature on different days, with each test having 3–5 replicate vials (10–15 adults per vial) per sex per strain. Solvent-treated vials were included as controls.

Mortality data from the time-response bioassays were analyzed by the probit procedure developed for correlated data by Throne et al. (1995a). Continuous mortality data (proportion killed) were fit to 6 models. Probability of dying was obtained from back transformations of the transformation giving the best fit to the data as determined by a chi-square goodness-of-fit test (Throne et al. 1995b). Programs for these analyses were written and run in Mathematica. Confidence intervals for median lethal time ratios were calculated according to Robertson and Preisler (1992).

Laboratory Selection. For these biochemical studies, the R strain from Dickinson county was selected with malathion by isolating virgin males and females, exposing them to malathion in the glass vial bioassay, and allowing the survivors to mate. Specifically, virgin males and females were obtained by allowing adult *H. hebetor* from the 4th generation of the R strain to parasitize *P. interpunctella* larvae in plastic petri dishes (15 by 100 mm) dishes. After completion of larval development, individual parasitoid pupae were isolated from the dishes and placed in glass test tubes (13 by 100 mm) with caps. Tubes were observed daily for emergence of adult parasitoids.

Virgin males and females of the R strain (81 ♂ and 59 ♀) were placed separately in glass bioassay vials treated with 6.1 μg (AI) malathion per vial. When ≈50% of the wasps were knocked down in the treated vials, the remaining wasps were briefly anesthetized with CO_2 and transferred to a clean, untreated vial. Adults that were alive after an additional 1 h were transferred to a 3.5-liter jar containing *P. interpunctella* hosts. Progeny from the 1st selection were selected a second time in the same manner. Progeny from these adults constituted the R-selected strain. We did not test this strain against other organophosphate insecticides.

Chemicals. α-Naphthyl acetate (1-NA), β-naphthyl acetate (2-NA), *p*-nitrophenyl acetate (4-NPA), 4-methylumbelliferyl acetate (4-MUA), α-naphthol, o-dianisidine (fast blue salt BN), diethyl p-nitrophenyl phosphate (paraoxon), acetylthiocholine iodide (ATC), ethylene glycol, β-NADP+, isocitrate dehydrogenase, p-nitroanisole, sodium dithionite, and 2,3-^{14}C-malathion were purchased from Sigma (St. Louis, MO). Ethylenediaminetetraacetic acid (EDTA) and sodium dodecyl sulfate were from Fisher (Fair Lawn, NJ). 3,4-Dichloronitrobenzene (DCNB), 1-chloro-2,4-dinitrobenzene (CDNB), and 5,5′-dithio-bis(2-nitro)benzoic acid (DTNB) were purchased from Aldrich (Milwaukee, WI). ReadySafe liquid scintillation cocktail was purchased from Beckman (Fullerton, CA). Malaoxon was a gift from Cheminova Agro (Lemvig, Denmark).

Preparation of Enzymes. Male or female wasps (generally 15–20 adults) were briefly anesthetized with CO_2, placed in cold 50 mM sodium phosphate buffer (pH 7.0), and homogenized with an Ultra-Turrax (Tekmar, Cincinnati, OH) rotary homogenizer. For cytochrome P-450 determination, insects were homogenized in buffer containing 1 mM EDTA by using a glass homogenizer with a Teflon pestle. Homogenates were centrifuged at 5,000 × *g* for 5 min at 4°C and supernatants were collected. These supernatants were subsequently centrifuged at 100,000 × *g* for 1 h at 4°C. The 100,000 × *g* supernatants were used as enzyme source for all assays (except for cytochrome P-450 determination). For cytochrome P-450 determination, the 100,000 × *g* pellets were resuspended in cold 200 mM pH 7.0 sodium phosphate buffer containing 20% (vol:vol) glycerol and frozen at −80°C until assayed. Protein concentrations were determined using the Coomassie Plus Protein Assay Reagent (Pierce, Rockford, IL).

General Esterase. Hydrolytic activities against 4 general esterase substrates, 1-NA, 2-NA, 4-NPA, and 4-MUA, were measured with the procedures of Baker et al. (1998) modified slightly from those of van Asperen (1962) and Abdel-Aal et al. (1992). For 1-NA, 2-NA, and 4-NPA, absorbance values were determined with a BioTek microtiter plate reader. Activity against 4-MUA was determined with a Shimadzu RF5000U spectrofluorophotometer with excitation at 319 nm and emission at 447 nm.

Malathion Carboxylesterase (MCE). Malathion carboxylesterase activity was determined by modifications of the procedures of Halliday (1988), Sakata and Miyata (1994), and Whyard et al. (1994a 1994b) as described by Baker et al. (1998). A stock solution of 2,3-^{14}C-malathion (6.5 mCi/mmol) was prepared in 95% ethanol and used as substrate. Hydrolysis of the malathion was terminated and unhydrolyzed malathion was separated from the malathion monoacids by chloroform extraction. The aqueous phase was diluted into ReadySafe scintillation fluid and counted in a Beckman LS6500 scintillation counter.

Acetylcholinesterase Sensitivity. Acetylcholinesterase (AChE) sensitivity to malaoxon was compared in the R-selected and S strains of *H. hebetor* by measuring the inhibition of hydrolysis of the model substrate acetythiocholine (ATC) by malaoxon with a method modified from Ellman et al. (1961). Reaction mixtures containing 125 μl 12 mM DTNB and 25 μl 75 mM ATC and with or without 25 μl 1.5 mM malaoxon were brought to 2.5 ml with 200 mM sodium phosphate

buffer, pH 7.0. A 100 μl volume of the mixture was added to 50 μl homogenate containing 10 μg enzyme protein in 10 wells of a microtiter plate. Change in absorbance at 405 nm was followed for 5 min at 25°C.

Glutathione S-Transferase (GST). Rates of conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) and 3,4-Dichloronitrobenzene (DCNB) with reduced glutathione were compared in extracts from both R-selected and S strains of *H. hebetor* by slight modifications of the methods of Yu (1982). Reduced glutathione (0.49 ml of 15 mM solution) in 200 mM sodium phosphate buffer, pH 7.0 and 10 μl of 150 mM DCNB or CDNB were pipetted into a plastic semimicro cuvette. After 3 min at room temperature, 250 μl of insect homogenate containing 50 μg of enzyme protein was added to the cuvette and placed in a Beckman DU 7400 spectrophotometer with temperature control. Change in absorbance at 340 nm and 344 nm was recorded for 5 min at 25°C for CDNB and DCNB, respectively.

Cytochrome P-450 Content. Cytochrome P-450 content was quantified by using the method of Omura and Sato (1964) as modified by Jesudason et al. (1988). Microsomal preparations (Hällström et al. 1984) from groups containing both ♂ and ♀ wasps were diluted to 1 mg protein/ml in a final volume of 1.5 ml 200 mM sodium phosphate buffer, pH 7.0. Carbon monoxide was bubbled through the microsomal fraction for 30 s. One half of the microsomal fraction was added to each of two 1-ml quartz cuvettes. Sodium dithionite (2.5 mg) was added to 1 of the cuvettes as a sample. The spectrum difference of the sample was recorded against the reference using an Ultraspec 3000 spectrophotometer and molar concentrations of cytochrome P-450 were determined using the path length of 1 cm and the molar extinction coefficients of 91 cm^{-1} mM^{-1}.

Cytochrome P-450 O-demethylase. O-Demethylase activity of *H. hebetor* was determined by following the O-demethylation of *p*-nitroanisole to *p*-nitrophenol (Kinoshita et al. 1966, Rose and Brindley 1985). The NADPH generating system was prepared in H_2O just before use and included 6.3 mM $MgCl_2 \cdot 6\ H_2O$, 0.732 mM β-$NADP^+$, 11.2 mM DL-isocitric acid, and 1 U of isocitrate dehydrogenase. Integrity of the NADPH-generating system was monitored by observing the absorbance at 340 nm following the addition of isocitrate dehydrogenase. A 200-μl aliquot of the NADPH-generating system was added to 50, 100, or 200 μl of undiluted microsomal fraction in a total volume of 0.6 ml with 0.1 M Tris·HCl pH 8. The microsomal fraction/NADPH-generating system reaction mixture was allowed to incubate with a final concentration of 1 mM *p*-nitroanisole for 30 min at 37°C. Reactions were stopped by addition of 2.5 ml cold acetone. Tubes were capped and after equilibration to room temperature, 0.2 ml of 0.5 M glycine-NaOH pH 9.4 was added to each tube followed by centrifugation at 2,000 × *g* to pellet the precipitate. Absorbance at 410 nm was determined by using a Beckman DU-7400 spectrophotometer. Units of activity were determined using the molar extinction coefficient of 1.7×10^4 $M^{-1}cm^{-1}$ for *p*-nitrophenol.

Gel Electrophoresis. Proteins in 5,000 × *g* supernatants of homogenates of individual female *H. hebetor*, or groups of 5 female wasps, were separated by electrophoresis under native conditions in 10% polyacrylamide gels at 125 V for 2.5 h at 4°C with a Novex electrophoresis system. Aliquots containing 5 or 10 μg protein were loaded into each lane. Esterolytic bands of activity were visualized using the substrates 1-NA and 4-MUA from the methods adapted from Ono et al. (1994) and Baker et al. (1998). 1-NA activity bands appear as red bands on a clear background whereas 4-MUA activity bands are visualized using an UV light table.

After electrophoresis, gels were also preincubated with several inhibitors before incubation with 1-NA to determine possible qualitative differences in the esterases between strains. Inhibitors tested were paraoxon, an inhibitor of type B esterases, eserine, an inhibitor of cholinesterases, and triphenyl phosphate, a broad inhibitor of general esterases. Each inhibitor was tested at 4 concentrations from 10^{-4} to 10^{-7} M.

Data Analysis. Significant differences in activity values were determined with PROC *t*-test (SAS Institute 1987).

Results

Susceptibility of Dickinson Strain of *H. hebetor* to Malathion. Males and females of the field strain of *H. hebetor* collected from Dickinson County, KS, were significantly more tolerant of malathion than those of the laboratory strain (Fig. 1A). For comparisons between strains, mortality data were fitted to log-probit transformations. LT_{50}s were 30.9 min (χ^2, 0.65; slope ± SE, 14.0 ± 1.9; 95% CL, 29.2–32.5 min) for ♂ and 37.9 min (χ^2, 4.62; slope ± SE, 10.7 ± 1.1; 95% CL, 35.9–40.1 min) for ♀ from the laboratory strain (designated as S strain) compared with 55.7 min (χ^2, 10.41; slope ± SE, 3.8 ± 0.3; 95% CL, 48.2–64.3 min) for ♂ and 78.3 min (χ^2, 22.23; slope ± SE, 2.6 ± 0.3; 95% CL, 62.9–98.1 min) for ♀ from the Dickinson strain (designated as R strain). Based on LT_{50}s in these tests, lethal time ratios were 1.8-fold (95% CL, 1.5–2.1) for ♂ and 2.1-fold for ♀ (95% CL, 1.6–2.6). In addition, to strain differences, females within each strain were more tolerant to malathion than were males. Within the S strain, females were 1.2-fold (95% CL, 1.1–1.3) more tolerant of malathion than males, and within the R strain, females were 1.4-fold (95% CL, 1.1–1.9) more tolerant.

Selection with Malathion. Results of a typical selection with malathion are shown in Fig. 1B (males) and 1C (females). Males and females were removed from the bioassay vials after 30- and 40-min exposures to malathion, respectively. After removal from the treated vials, the number of adults dying declined compared with the continued death of adults that were not removed from vials. Survivors from these treatments were used to form the R-selected strain.

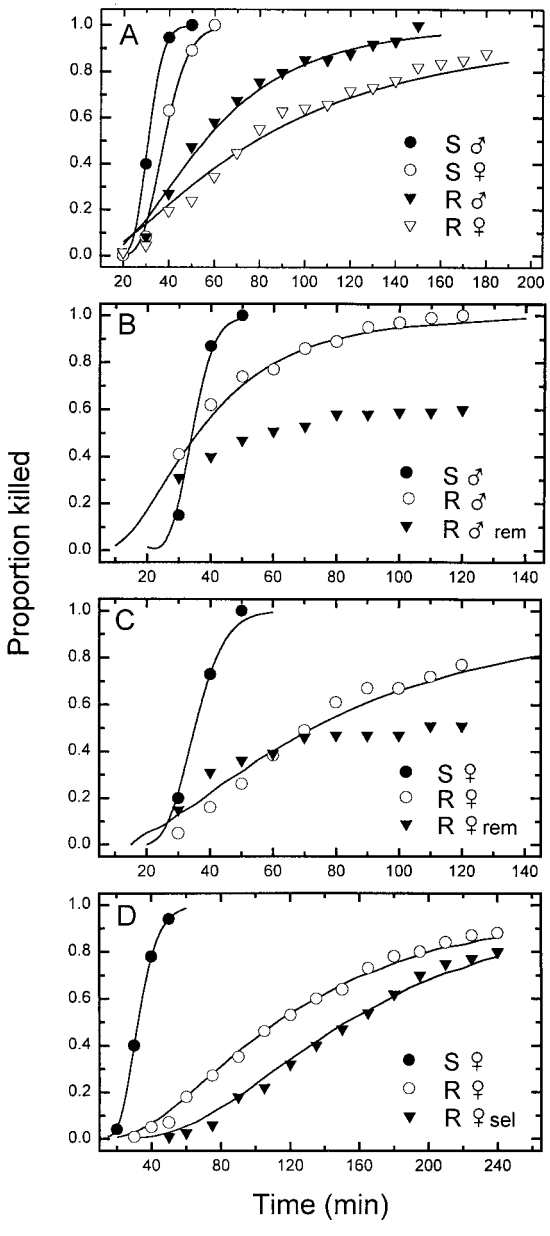

Fig. 1. Time-mortality curves in malathion bioassay of *H. hebetor* after exposure to vials containing 6.1 μg malathion at room temperature. Lines represent back transformations of mortality data based on the log-probit model. (A) Sex and strain differences in response of *H. hebetor* to malathion. (B) Comparisons of time-mortality response between the males that were transferred into untreated vials (R ♂ rem) after a 30-min exposure and the males that were exposed continuously in the malathion treated vials. (C) Comparisons of time-mortality response between the females that were transferred into untreated vials (R ♀ rem) after a 40-min exposure and the females that were continuously exposed in the malathion treated vials. (D) Comparison of time mortality response among females of S, R, and R-selected strains.

R-selected Strain. Based on results of bioassays with female *H. hebetor*, after 2 selections with malathion in the laboratory, the frequency of resistance alleles was increased significantly in the R-selected strain (Fig. 1D). LT_{50}s were 32.2 min (95% CL, 29–35) for the S strain (controls), 112 (95% CL, 98–128) for the R strain, and 153 (95% CL, 136–171) min for adult females of the R-selected strain. The median lethal time ratio based on LT_{50}s, and relative to the S strain (controls), increased from 3.5 (95% CL, 3.0–4.0) in the R strain to 4.7 (95% CL, 4.2–5.3) in the R-selected strain. Relative to the R strain, the R-selected strain was 1.4-fold (95% CL, 1.2–1.6) more tolerant of malathion. The selected strain was used in all biochemical comparisons with the susceptible laboratory strain.

Biochemical Mechanisms of Resistance. Esterase activity against 1-NA, 2-NA, and 4-MUA in females and males from the R-selected strain were significantly lower than those in the S strain (Table 1). Activity levels against these 3 substrates were ≈2.3-, 2.3-, and 2.6-fold lower in the R-selected females compared with corresponding activities in the S females, and 1.6-, 1.7-, and 1.9-fold lower in the R-selected males compared with activities in the S males, respectively. There was no significant difference in activity against 4-NPA among females from the 2 strains. There were no significant differences in MCE activities in both females and males between the R-selected and S strains of *H. hebetor*.

In these assays, there was significantly more acetylcholinesterase activity against ATC in males from the S strain compared with males from the R-selected strain. However, differences between the females were not significant. Activity levels after preincubation with malaoxon were not significantly different between the sexes of the 2 strains. Malaoxon reduced activity by 69 and 73% in females and males from the R-selected strain, and by 74 and 82% in females and males from the S strain, respectively.

Glutathione S-transferase was more active against CDNB than DCNB in both strains, but there were no significant differences in activities between the R-selected and S strain of *H. hebetor* with either substrate.

Mixed sex groups were used to prepare microsomes from wasps of the R-selected and S strains and there were no significant differences in content of cytochrome P-450 or in O-demethylase activity between preparations from the 2 strains.

PAGE Analysis. There were qualitative and quantitative differences in esterases between the R-selected and S strains of *H. hebetor* when equal amounts of sample protein were analyzed (Fig. 2A). Major bands of activity against 1-NA were found at Rm 0.18 (E1) and 0.24 (E2) in the S strain. The broad band E2 may result from the presence of >1 esterase in this region. E1 and E2 were absent in the R-selected strain, indicating that resistance may be associated with null alleles of these enzymes.

The major band with activity against 1-NA in the R-selected strain was at Rm 0.37 (E3). E3 was also present in the S strain, but the zymograms indicated

Table 1. Comparison of hydrolases, glutathione S-transferase and O-demethylase activities, and cytochrome P-450 content between the R-selected and S strains of the braconid parasitoid *H. hebetor*

Enzyme system	Substrate	Specific activities in *H. hebetor* strains[a]				*t, P* values for comparing strain differences by sex	
		R-selected strain		S strain			
		♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
General esterase	1-NA	77.8 ± 6.7	80.7 ± 7.5	181.4 ± 12.1	133.1 ± 5.4	7.50, 0.000	5.67, 0.002
	2-NA	341.8 ± 24.3	387.8 ± 15.8	796.1 ± 62.8	656.2 ± 45.8	6.74, 0.000	5.54, 0.004
	4-NPA	4.6 ± 0.6	5.5 ± 0.5	4.2 ± 0.4	4.1 ± 0.4	0.52, 0.61	2.07, 0.05
	4-MUA	22.3 ± 2.9	27.6 ± 6.8	58.5 ± 6.1	51.3 ± 4.1	5.35, 0.01	2.98, 0.04
MCE	^{14}C-malathion	96.4 ± 29.6	90.4 ± 12.2	81.2 ± 12.1	158.5 ± 44.1	0.47, 0.65	1.48, 0.17
Acetylcholinesterase	ATC	4.8 ± 1.2	4.4 ± 0.8	6.5 ± 0.3	8.9 ± 0.5	1.37, 0.19	4.77, 0.003
	ATC w/malaoxon	1.5 ± 0.1	1.2 ± 0.3	1.7 ± 0.2	1.6 ± 0.2	0.74, 0.47	1.37, 0.19
Glutathione S-transferase	CDNB	343 ± 34	383 ± 56	324 ± 27	364 ± 43	0.43, 0.69	0.27, 0.80
	DCNB	19.3 ± 1.1	22.7 ± 2.8	19.9 ± 0.9	18.9 ± 1.0	0.42, 0.69	1.24, 0.28
P-450 content[b]	—	1.3 ± 0.6	—	1.0 ± 0.5	—	0.38, 0.72	—
O-Demethylase[b]	p-Nitroanisole	0.24 ± 0.04	—	0.22 ± 0.05	—	0.31, 0.76	—

t, P values from PROC *t*-test.

[a] Specific activities: Means ± SEM based on 3–4 replicates per sex per strain. 1-NA, 2-NA, 4-NPA, glutathione S-transferase and O-demethylase = nmol/min/mg protein; MCE and Acetylcholinesterase = pmol/min/mg protein; 4-MUA = F/min/μg protein; P-450 content = nmol/mg protein.

[b] Microsomes were prepared from samples containing both ♂ and ♀ wasps.

a much higher level in the R strain. Evidence presented below indicates that properties of E3 in the 2 strains are different. The S strain also had an activity band at 0.39 (E4). E4 was weakly visible in the R-selected strain.

Results of preincubation with inhibitors before addition of 1-NA indicated there were differences in properties of the esterases between the R-selected and S strains. E1 and E2 in the S strain appeared to be partially inhibited and E3 in the S strain was completely inhibited by preincubation with 10^{-6} M eserine (Fig. 2B). In contrast, E3 was less affected by 10^{-6} M eserine in the R-selected strain. Results of preincubation with TPP (Fig. 2C) indicated that an overall reduction in activity occurred with concentrations increasing from 10^{-7} M to 10^{-4} M. E1 and E2 were inhibited by TPP, whereas E3 in the R-selected strain was apparently less sensitive to TPP. Results with eserine and TPP suggest that esterase E3 in the R-selected strain may have different properties compared with E1 and E2, as well as E3, in the S strain. Preincubation with 10^{-7} M paraoxon (Fig. 2E) reduced the activity of all 1-NA bands relative to controls (Fig. 2D) in both strains. Preincubation with 10^{-5} M paraoxon (Fig. 2F) eliminated almost all activity against 1-NA in both strains. Based on visual observations, no differences were noted between strains in sensitivity of any of the bands to paraoxon.

Esterase zymograms of both wasp strains were prepared with 4-MUA Results (data not shown) were identical to that with the substrate 1-NA except that 2 additional bands with higher mobilities at Rm 0.57 (E5) and 0.62 (E6) were detected in both strains and at equal intensities with this fluorescent substrate.

Discussion

Low levels of resistance to malathion have been documented in strains of *H. hebetor* collected in separate corn and peanut storages in the southeastern United States (Baker et al. 1995). Malathion resistance is common in pyralid moths found in these storage facilities (Zettler 1982) as well as in storage facilities in the north central U.S. (Beeman et al. 1982). It is likely that the presence of resistance in *P. interpunctella* may facilitate resistance development in the associated *H. hebetor* by providing hosts when the parasitoid is under selection pressure (Tabashnik 1986). Malathion has been used extensively in the storage facility in Dickinson County, KS, where the R strain of *H. hebetor* was collected. Low levels of resistance to malathion have also developed in this strain.

Two laboratory selections with malathion significantly increased the frequency of resistance alleles in the R-selected strain compared with that of the original Dickinson County (R) strain and resulted in a resistance level, as measured with the time-response bioassay, that was similar to that found in the SCC strain collected in South Carolina (Baker et al. 1995). During selection, surviving wasps were removed from the treatment vials when about half the group had died or had been knocked down. Among the survivors, the number of adults that died after removal from the vials decreased rather abruptly, indicating a rather quick recovery from their exposure to malathion. The ability to withstand brief exposures to malathion with no apparent loss of vigor may provide additional insight into explaining the lack of extensive resistance development in this species relative to its host.

Several factors have been hypothesized for the low level or resistance development in this parasitoid (Baker et al. 1995). First, *H. hebetor* is a generalist and may attack hosts in ecosystems not treated with insecticides. Second, pyralid moth larvae are generally found on the surface of grain masses. This allows the parasitoid to parasitize hosts without extensive direct contact with insecticide. The finding that *H. hebetor* can withstand a 30–40 min exposure to a malathion concentration based on the LT_{99} in the vial bioassay, and still be an effective parasitoid, provides an equally

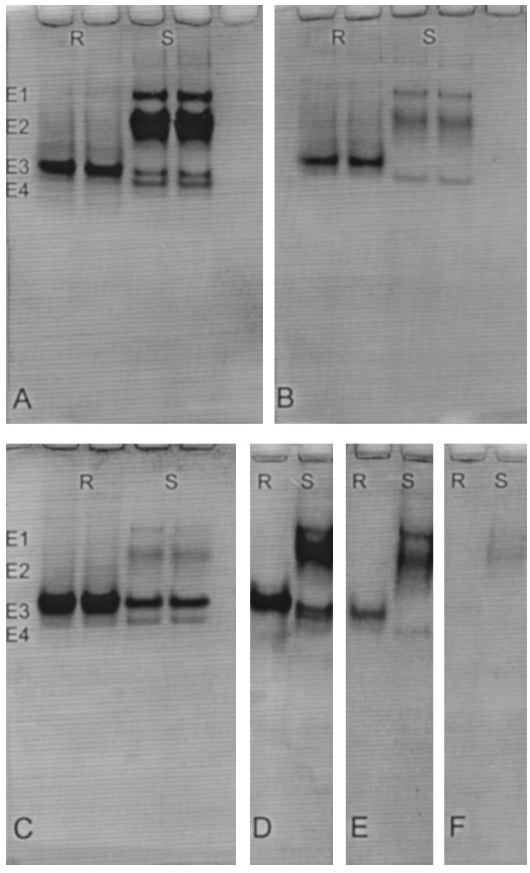

Fig. 2. Esterase zymograms after electrophoresis of 5,000 × *g* supernatants of adult ♀ homogenates from the R-selected and S strains of *H. hebetor* on 10% polyacrylamide gels. (A) Esterase patterns of 10 μg protein/lane from groups of 5 ♀ from the R-selected and S strains against 1-NA. (B) Esterase patterns with 1-NA after preincubation of the gel with 10^{-6} M eserine. (C) Esterase patterns with 1-NA after preincubation of the gel with 10^{-6} M triphenylphosphate. (D, E, and F) Zymograms showing the effect of preincubation of the gel with paraoxon on 1-NA activity: (D) esterase patterns with no paraoxon, (E) esterase patterns with 10^{-7} M paraoxon, and (F) esterase patterns with 10^{-5} M paraoxon.

important means for reducing the effects of selection pressure imposed by chemical protectants applied to grain.

Increased activity of a specific MCE is a primary resistance mechanism in many insects, including *P. interpunctella* (Halliday 1988), a major host of *H. hebetor.* Although the difference in MCE activity between *H. hebetor* strains was not significant in this study, both strains did have a low level of MCE activity and it is possible that the MCE in the R-selected strain has different biochemical properties that might allow it to function more effectively in vivo. Structural modifications that alter the biochemical properties of an ali-esterase with MCE activity have been documented in the Australian sheep blow fly *Lucilia cuprina* Wiedemann (Campbell et al. 1998). In *L. cuprina*, a $Trp^{251} \rightarrow Leu^{251}$ substitution in a MCE had only a slight beneficial effect on the actual kinetics of malathion hydrolysis, but the Leu substitution significantly reduced the sensitivity of the mutant MCE to inhibition by malaoxon. The authors suggested that this decreased sensitivity to malaoxon allows a more rapid reactivation of the enzyme which results in increased metabolism of malathion.

A similar situation may be present in the pteromalid parasitoid *A. calandrae.* Zhu et al. (1999a) found evidence that both a structural mutation and increased expression of a carboxylesterase in *A. calandrae* may be involved in the malathion resistance. In this species a structural mutation $Trp^{220} \rightarrow Gly^{220}$ was found in a carboxylesterase-like enzyme in the resistant strain and the mutation was genetically linked to resistance (Zhu et al. 1999b). The Trp^{220} residue in the carboxylesterase from *A. calandrae* is homologous with the Trp^{251} residue in *L. cuprina*, but it remains to be seen if the $Trp^{220} \rightarrow Gly^{220}$ mutation in *A. calandrae* results in a similar change in biochemical properties of the carboxylesterase-like enzyme. These findings indicate that although the in vitro activity of MCE in the 2 *H. hebetor* strains was similar, different in vivo functional properties of the MCEs may occur and these differences might be associated with the resistance.

In addition to MCE, several other important detoxification systems were present in *H. hebetor*, including glutathione S-transferase, and P-450 dependent O-demethylase. However, the activities of these enzymes were not significantly different between strains. Also, there were no significant differences between strains in sensitivity of acetylcholinesterase to inhibition by malaoxon.

Of the metabolic detoxification systems studied, general esterase activity was most different between *H. hebetor* strains. The activity against 1-NA, 2-NA, and 4-MUA was significantly lower in the R-selected strain compared with that found in the S strain. In addition to quantitative differences in esterase activity between strains E1 and E2, major bands of activity against 1-NA in the S strain as revealed by PAGE were absent in the R-selected strain, whereas the activity of E3, the major activity band in the R-selected strain, was increased relative to that in the S strain. It is not known if structural mutations in E1 and E2 might have resulted in a complete loss of activity against 1-NA in the R-selected strain or if a structural mutation of E3 in the R strain enhances its activity. However, based on sensitivities of the biochemical assays used in this study, there were no significant corresponding increases in MCE activity that might result from mutations in E1 and E2 that would alter the substrate specificity of these enzymes in a manner similar to that proposed by Oppenoorth and Van Asperen (1960). The weak malathion resistance in *H. hebetor* may thus represent a different resistance mechanism in which the loss of general esterase activity is not accompanied by an increased MCE activity. Additional biochemical and molecular studies will be necessary to more completely elucidate and characterize the malathion resistance in this parasitoid.

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