Cytochrome P450 MA Expression in Insecticide-Resistant German Cockroaches (Dictyoptera: Blattellidae)

MICHAEL E. SCHARF,[1] CHOW-YANG LEE,[2] JONATHAN J. NEAL,[3] AND GARY W. BENNETT

Center for Urban and Industrial Pest Management and Department of Entomology, Purdue University, West Lafayette, IN 47907–1158

J. Econ. Entomol. 92(4): 788–793 (1999)

ABSTRACT Cytochrome P450 monooxygenases are detoxification enzymes commonly involved in insecticide resistance by insects. Recently, an overexpressed form of this enzyme, P450 MA, was purified from an insecticide-resistant strain of German cockroach, *Blattella germanica* (L.), and polyclonal antisera (anti-P450 MA) was produced. To test hypotheses that the overexpressed condition of P450 MA has evolved in >1 geographic location and that P450 MA might be involved in insecticide resistance to specific insecticides, investigations were conducted using 4 insecticide-resistant and 1 susceptible German cockroach strains. In western blots that used anti-P450 MA antiserum as a probe, substantial differences in expression of P450 MA were observed. Strains showing the highest P450 MA expression had both the highest tolerance to the organophosphate insecticide chlorpyrifos and cytochrome P450-mediated demethylation activity. Results support the hypothesis that cytochrome P450 MA is potentially overexpressed in insecticide-resistant populations on a global scale.

KEY WORDS *Blattella germanica*, cockroach, cytochrome P450, insecticide resistance

BECAUSE OF ITS close association with humans, the German cockroach, *Blattella germanica* (L.), is intensively treated with insecticides (Schal and Hamilton 1990). A common outcome resulting from this persistent selection pressure is the development of insecticide resistance, which can be caused by a number of mechanisms (Cochran 1995a). One such resistant strain is the Munsyana strain (MA), collected in 1994 from a public housing site in Muncie, IN. In relation to susceptible strains, the Munsyana strain initially possessed LD_{50} resistance ratios of 80-fold to the pyrethroid insecticide cypermethrin and 5-fold to the organophosphate insecticide chlorpyrifos. Elevated content and activity of esterase, cytochrome P450, and glutathione-*S*-transferase detoxication enzymes (Scharf et al. 1997, Wu et al. 1998), and elevated esterase and cytochrome P450-based metabolism of ^{14}C-fenvalerate also were identified in the Munsyana strain (Wu et al. 1998). In addition, decreased knockdown on pyrethoid residues and the point mutation responsible for this *kdr*-type resistance have been identified in Munsyana (Dong et al. 1998).

A polyclonal antibody to purified cytochrome P450 from the Munsyana strain identified a cytochrome P450 isoform of M_r = 49 kDa (P450 MA; Scharf et al. 1998a) with elevated expression in the Munsyana strain. Selection studies using a hybrid of the Munsyana strain crossed to the susceptible Johnson Wax strain identified increased expression of P450 MA only after selection with the organophosphate insecticide chlorpyrifos (Scharf et al. 1998b). To provide information that can be applied toward the development of insecticide resistance management programs, it is first necessary to investigate the geographical occurrence of this suspected resistance-conferring protein. The current study was undertaken to determine if overexpression of the P450 MA protein occurs in association with insecticide resistance and other affiliated factors in German cockroach strains originating from geographically distant locations.

Materials and Methods

Cockroach Strains and Rearing. Cockroaches (see Table 1 for detailed information on strains) were maintained under a photoperiod of 12:12 (L:D) h at 27°C and 65% RH. Unlimited quantities of water and HT-8604 laboratory diet (Harlan-Teklad, Milwaukee, WI) were provided. All cockroaches used in bioassays and biochemical assays were males and they were used 1–2 wk after molting to adults.

Chemicals, Reagents, Buffers, and Solutions. The technical grade insecticides chlorpyrifos (Dow AgroSciences, Indianapolis, IN) and cypermethrin (Zeneca, Richmond, CA) were provided as gifts. All reagents were purchased from Sigma (St. Louis, MO) unless otherwise specified. Water used for all biochemical analyses was deionized by a MilliQ deionizer

[1] Current address: Department of Entomology, University of Nebraska, Lincoln, NE 68583–0816.
[2] Current address: School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia.
[3] To whom reprint requests should be addressed: Department of Entomology, Purdue University, West Lafayette, IN 47907–1158.

Table 1. Information pertaining to German cockroach strains used in this study

Strain abbreviation	Strain name	Year collected	Collection location	Status	Reference
JW	Johnson Wax	1930s	Orlando, FL	susceptible	Koehler and Patterson (1986)
M86	Muncie '86	1986	Muncie, IN	resistant	Scharf et al. (1996)
MA	Munsyana	1994	Muncie, IN	resistant	Scharf et al. (1997)
PV	Parkview	1994	Muncie, IN	resistant	NP[a]
ML	Melia	1994	Kuala Lumpur, Malaysia	resistant	Lee et al. (1996)

[a] Information not published.

(Millipore, Marlborough, MA). 4-Chloro-N-methylaniline (4-CNMA) and 4-Chloroaniline were purchased from Aldrich (Milwaukee, WI).

Homogenization Buffer. 0.1 M sodium phosphate, pH 7.5 was used. *Microsome Resuspension Buffer.* Homogenization buffer containing 30% vol:vol glycerol. *Sodium Hydrosulfite Solution.* 220 mM sodium hydrosulfite in water (prepared fresh). *NADPH Generating System.* Resuspension buffer containing 2.5 mM glucose-6-phosphate, 0.05 U glucose-6-phosphate dehydrogenase, 0.5 mM $NADP^+$, and 7.5 mM magnesium chloride. *Demethylation Assay Reaction Mixture.* NADPH generating system containing 10 μM 4-CNMA. *Demethylation Assay Stop Solution.* 134 mM p-dimethylaminobenzaldehyde (PDAB) in 1.0 N H_2SO_4. *SDS Polyacrylamide Gel Electrophoresis (PAGE) Sample Buffer.* 0.5 M tris, 13% vol:vol β-mercaptoethanol, 10% wt:vol SDS, 53% vol:vol glycerol, and 0.05% wt:vol bromophenol blue. *SDS PAGE Running Buffer.* 0.025 M tris, 0.192 M glycine and 0.1% SDS in water. *Transfer Buffer.* 26 mM tris and 192 mM glycine in a solution of 20% vol:vol methanol in water (pH 8.3).

PBST Solution. 10 mM sodium phosphate, 0.9% wt:vol sodium chloride and 0.05% vol:vol Tween-20. *Blocking Solution.* PBST + 1.5% nonfat powdered milk. *Primary Antibody Solution.* Anti-P450 MA serum (Scharf et al. 1998a) diluted 1:5,000 in PBST. *Secondary Antibody-Conjugate Solution.* Sigma A-5278 goat anti-mouse horseradish peroxidase conjugate in PBST (1:1,500).

Insecticide Bioassays. Three, 473-ml glass jars (8 by 15 cm) per strain were treated on their bottom surfaces with acetone-diluted technical grade insecticide stock solutions. Stock solutions were prepared at 15 mg(AI)/ml. Chlorpyrifos was applied at 1 mg per jar (2.6 $\mu g/cm^2$), and cypermethrin at 0.682 mg per jar (1.8 $\mu g/cm^2$). Residues were allowed to dry in a fume hood. The jars then received a mixture of petroleum jelly and mineral oil to confine cockroaches to treated surfaces and to prevent escape. Ten males were introduced per jar, and cumulative mortality was recorded at selected time intervals until all cockroaches were dead. Methods described by Scharf et al. (1995) were used to analyze bioassay data. Six or seven time points were chosen with mortality levels that ranged from 0 to 100%, providing a sample size (n) of 174–240 insects per lethal time probit analysis and acceptable Pearson-chi-square values (SAS Institute 1990). Resistance ratios were calculated at LT_{50} and LT_{95} for each resistant strain in comparison to those of the susceptible JW strain by division.

Preparation of Microsomes. Male cockroaches (30 per preparation, 1–2 wk old) were homogenized in homogenization buffer by using a Vertishear homogenizer (Vertis, Gardiner, NY). Homogenates were centrifuged at 11,000 $\times$ g and 4°C for 10 min and the pellet discarded. Supernatants were filtered through glass wool then ultracentrifuged at 106,000 $\times$ g for 60 min. The resulting microsomal pellets were resuspended in 0.6-ml resuspension buffer by dislodging with a glass rod and repeatedly drawing into and out-of a 10-ml tuberculine syringe by using a 20-gauge needle. Microsomal resuspensions were divided into 50-μl aliquots and stored at −70°C until use.

Carbon Monoxide Difference Spectra. Total cytochrome P450 content was quantified by the dithionite-reduced carbon monoxide (CO) difference spectra, modified from Omura and Sato (1964). One hundred microliters of microsomal suspension at an estimated protein content of 9.4 mg/ml was placed in a glass tube, followed by 1.2 ml of microsome resuspension buffer and 400 μl of fresh sodium hydrosulfite solution. The contents of the tube were then equally divided into 2 matched 1.0-ml quartz spectrophotometer cuvettes and background corrected in a Perkin Elmer Lambda 2 UV:VIS spectrophotometer (Uberlingen, Germany). Carbon monoxide was bubbled into 1 cuvette for 0.5 min, and both cuvettes were then scanned from 500 to 400 nm. The difference in absorbance between 490 and 450 nm was converted to nmol P450/mg microsomal protein by using an extinction coefficient of 91 mM^{-1} cm^{-1}.

4-CNMA Cytochrome P450 Assay. Demethylation of the model substrate 4-CNMA was quantified using 50 μl of microsomal resuspension per assay. All microsomal suspensions were determined to be free of cytochrome P420 by CO-difference spectra before assays were conducted. Metabolism was initiated by adding 400 μl of demethylation assay reaction mixture to microsomes and terminated after incubation for 10 min at 37°C by adding 750 μl of demethylation assay stop solution. Microcentrifuge tubes containing the stopped reaction were centrifuged for 15 min at 11,000 $\times$ g at 4°C. The product, 4-chloroaniline, was quantified by comparing absorbance of supernatants at 445 nm to a simultaneously determined standard curve (0–100 μmol) in a Perkin Elmer Lambda 2 UV:VIS spectrophotometer.

Table 2. Susceptibility of German cockroach strains to cypermethrin and chlorpyrifos in surface contact bioassays

Insecticide	Strain[a]	n[b]	Slope ± SEM	χ^2[c]	LT_{50} (95% CI)[d]	RR_{50}[e]	LT_{95} (95% CI)[d]	RR_{95}[e]
Cypermethrin	Jwax	180	10.6 ± 1.4	0.4	8.5 (8.1–8.9)	—	12.2 (11.2–14.0)	—
	M86	180	14.2 ± 1.9	5.7	11.9 (11.4–12.3)	1.4	15.5 (11.4–12.3)	1.3
	MA	180	6.0 ± 0.8	0.8	38.3 (34.8–41.5)	4.5	71.9 (62.8–89.6)	5.9
	PV	180	3.5 ± 0.7	2.3	169.9 (145.3–220.3)	20.0	498.6 (334.5–1,168)	40.9
	ML	240	7.9 ± 1.1	1.7	40.2 (37.8–42.7)	4.8	64.9 (61.0–68.8)	5.3
Chlorpyrifos	Jwax	180	13.9 ± 1.7	0.3	47.2 (45.4–49.0)	—	61.9 (58.2–68.1)	—
	M86	180	8.6 ± 1.2	1.5	57.4 (53.1–61.1)	1.2	89.1 (53.1–61.1)	1.4
	MA	180	9.2 ± 1.2	2.1	52.0 (48.5–55.1)	1.1	78.5 (72.0–90.1)	1.3
	PV	174	10.5 ± 1.4	0.8	85.8 (81.5–90.1)	1.8	123.1 (113.3–140.7)	2.0
	ML	240	10.1 ± 1.3	1.6	89.6 (85.8–93.0)	1.9	130.7 (125.1–136.2)	2.1

[a] Cockroach strains used in bioassays (see Table 1 for identities and information).
[b] The number of insects on which each probit analysis is based.
[c] Pearson chi-square, goodness-of-fit (SAS Institute 1990).
[d] The LT_{50} and LT_{95} values with 95% confidence intervals (CIs).
[e] Resistance ratios at LT_{50} and LT_{95}, calculated in comparison to LT_{50} and LT_{95} values of the JW strain.

Protein Assay. Protein content was estimated using a commercially available bicinchoninic acid kit (Sigma), according to the instructions of the manufacturer. Protein samples were diluted 1:20 in water, and absorbance of the reaction mixture was converted to protein concentration by analysis against a simultaneously determined standard curve of bovine serum albumin (0–50 mg/ml).

SDS PAGE of Microsomal Proteins. Ten percent polyacrylamide gels (5 cm by 0.75 mm) and 2 cm stacking gels (1 cm wells) were poured into a Bio-Rad mini-gel system (Bio-Rad, Hercules, CA). Before loading, a volume containing 40 μg of resuspended microsomal protein was diluted 1:1 in SDS-PAGE sample buffer and placed in boiling water for 5 min. Proteins were electrophoresed in SDS-PAGE running buffer at 200 V until the dye front completely eluted from the gel (≈50 min).

Western Analysis of Cytochrome P450 MA. Microsomal proteins were transferred from gels to Hybond ECL nitrocellulose membranes (Amersham, Buckinghamshire, England) in 1 h at 100 V and 4°C, by using a Mini *trans*-Blot Electrophoretic Transfer Cell (Bio-Rad) containing transfer buffer. Nitrocellulose membranes were blocked for 1 h at 4°C in blocking solution; washed 2 times for 5 min in PBST; incubated for 1 h in 10 ml primary antibody solution; washed 3 times for 5 min in PBST; incubated for 1 h in 5 ml secondary antibody-conjugate solution; and washed 5 times for 5 min in PBST. Antibody conjugate was detected with an enhanced chemiluminescence (ECL) kit (Amersham) following the manufacturer's instructions, by exposing film to the light emitting-ECL reaction for 60 s. Protein band density of western blots was determined with a Hoefer GS300 transmittance and reflectance scanning dinsitometer (Hoefer Scientific, San Francisco, CA) connected to a Cole-Palmer 8373–10 deluxe laboratory chart recorder (Cole-Palmer, Chicago, IL).

Regression Analyses. Regression analyses were performed using PROC REG (SAS Institute 1990) to plot best-fit lines and obtain coefficients of determination (r^2 values) and significance estimates. Correlation coefficients (r values) were obtained by calculating square-roots of r^2 values. A sample size of 5 points (1 per strain) was used for each pair of variables examined. Variables compared in regression analyses were chlorpyrifos LT_{50}, cypermethrin LT_{50}, total P450 content, demethylation activity, and P450 MA expression.

Results and Discussion

Insecticide Susceptibility. Surface contact bioassays indicated high levels of cypermethrin tolerance, whereas levels of chlorpyrifos tolerance were not as substantial (Table 2). At LT_{50}, cypermethrin, and chlorpyrifos tolerance, respectively, ranged from 1.4- to 20-fold (strain rank: PV > MA = ML > M86), and from 1.1- to 1.9-fold (strain rank: ML = PV > M86 = MA). In comparison to cypermethrin results, the overall lower levels of chlorpyrifos resistance are the result of the stringency of surface-contact exposure (Cochran 1995a) with this insecticide concentration. In the M86 and MA strains, chlorpyrifos resistance ratios of 1.5-fold by the surface-contact approach have equated to ≈10-fold resistance ratios by less stringent topical applications (Scharf et al. 1995, 1997). Chlorpyrifos resistance ratios of 10-fold, generated using a topical application procedure, have been associated with control failures under field conditions (Ballard et al. 1984, Rust and Reierson 1991). Furthermore, the chlorpyrifos resistance ratios at LT_{95} reported here ranged from 1.3- to 2.1-fold, which equate to increased survival times over the susceptible JW strain of 17 through 69 min. Such increases in survival time on this residue (2.6 $\mu g/cm^2$) indicate a substantial selective advantage under field conditions.

Cytochrome P450 Content and Activity. Considerable variability occurred in total cytochrome P450 content among the 5 strains (Fig. 1A). No degradation of cytochrome P450 to cytochrome P420 was observed in any preparations (results not shown). The strains most susceptible to cypermethrin (JW and M86) had the lowest total cytochrome P450 content, whereas total content was significantly higher in the more tolerant strains (MA, PV, and ML). The rank of total cytochrome P450 content by strain (and magnitude of difference over the susceptible JW strain) was PV

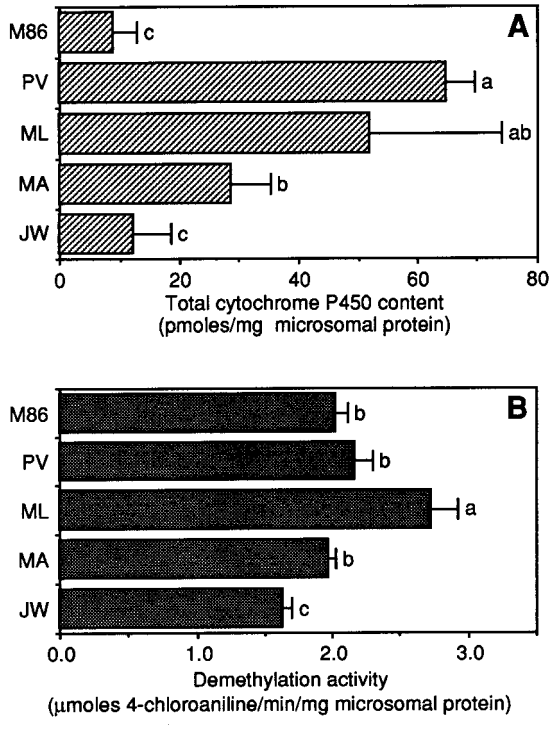

Fig. 1. Mean ± SE (A) total cytochrome P450 content as determined by carbon monoxide difference spectra by using 1 mg of microsomal protein per determination, and (B) cytochrome P450-mediated N-demethylation of 4-CNMA. See Table 1 for strain identities (JW, MA, ML, PV, M86). Values followed by the same letter are not significantly different by the Ryan *Q* test ($P < 0.05$, $n = 3$).

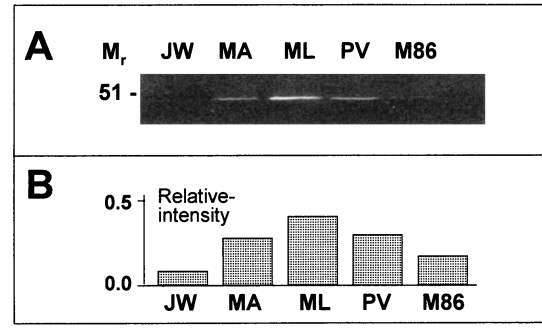

Fig. 2. (A) Negative image of enhanced chemiluminescence-visualized western blot, probed with anti-P450 MA antiserum at a dilution of 1:5,000. (B) Graph of relative intensity data, obtained by densitometrically scanning the autoradiogram shown in Fig. 2A. M_r, molecular mass marker (51 kDa); strain identities (JW, MA, ML, PV, M86), and information are provided in Table 1.

(5.3-fold), ML (4.3-fold), and MA (2.4-fold). Total cytochrome P450 was not statistically different ($P < 0.05$) between JW and M86.

The relative rank of cytochrome P450-mediated 4-CNMA demethylation activity among strains differed from total cytochrome P450 content. The JW strain exhibited lower demethylation activity than the other 4 strains. The Malaysian strain (ML) had the highest demethylation activity, which was 1.6-fold that of the susceptible JW strain. It is of particular interest that the ML strain also had the highest level of chlorpyrifos tolerance. Previously, it was observed that total cytochrome P450 content increased in cockroach populations selected with both chlorpyrifos and cypermethrin (Scharf et al. 1998b), but elevated demethylation activity only occurred in the population selected with chlorpyrifos. It is unknown if this elevated demethylation activity is truly related to insecticide-resistance; however, Valles and Yu (1996) similarly reported a 3.5-fold elevation of demethylation activity toward 4-CNMA (identified as PCMA) in a strain of German cockroach (Marietta) having 7.1-fold chlorpyrifos resistance.

Western Blotting. Microsomal proteins were separated by SDS PAGE, transferred to nitrocellulose membranes, and probed with anti-P450 MA antiserum. The results of the 2 blots that were performed using independent microsomal preparations were nearly identical. One blot is shown in Fig. 2A and indicates that P450 MA protein levels vary among strains (Fig. 2B). The highest quantity of P450 MA expression occurred in the ML strain, followed by PV, MA, and M86. The P450 MA protein was initially purified from the MA strain. The M86 and PV strains also originated in the city of Muncie, and were collected in 1986 and 1994, respectively. Because P450 MA also was identified in the geographically distant ML strain of Malaysia, its overexpression does not appear to be an anomaly unique to populations from the city of Muncie. However, the possibility that P450 MA expression is uniquely underexpressed (i.e., expressed at lower levels) in the susceptible JW strain cannot presently be ruled out.

Densitometric scanning of a representative blot identified relative intensities (Fig. 2B) that ranged from 2.0- to 4.8-fold of the susceptible JW strain. Interestingly, trends identified by densitometric scanning were nearly identical to trends observed for demethylation activity shown in Fig. 1B. These trends are supported by previous results identifying increases in both demethylation activity and P450 MA expression following chlorpyrifos selection (Scharf et al. 1998b). Indirectly, demethylation and densitometric results support the hypotheses that P450 MA is involved in chlorpyrifos resistance and is responsible for the majority of *N*-demethylation activity quantifiable by in vitro assays. However, chlorpyrifos is *O*-ethyl and not *N*-methyl substituted. Therefore, elevated *N*-demethylation activity (as mediated by P450 MA) should not be considered as diagnostic for chlorpyrifos resistance unless it could be determined that P450-mediated *O*-deethylation of [^{14}C] chlorpyrifos or its oxon-metabolite (Siegfried et al. 1990) were inhibited by anti-P450 MA antiserum.

Regression Analyses. Correlation coefficients (*r* values) were calculated using linear regression to determine the correlation relationships of all experimental

Table 3. Regression analyses comparing the relatedness of experimental findings

Independent[a] variable	Dependent[a] variable	dl[b]	*F*-value	Pr > *F*	*P*[c]	*r*[d]
Demeth. activity	P450 MA expression	1, 3	15.39	0.03	***	0.92
Chlorpyrifos LT_{50}	Total P450 content	1, 3	11.79	0.04	***	0.89
Chlorpyrifos LT_{50}	Demeth. activity	1, 3	8.44	0.06	*	0.86
Cypermethrin LT_{50}	Total P450 content	1, 3	7.01	0.08	*	0.84
Chlorpyrifos LT_{50}	P450 MA expression	1, 3	6.83	0.08	*	0.83
Cypermethrin LT_{50}	Chlorpyrifos LT_{50}	1, 3	2.15	0.24	—	0.65
Cypermethrin LT_{50}	P450 MA expression	1, 3	0.71	0.46	—	0.44
Cypermethrin LT_{50}	Demeth. activity	1, 3	0.17	0.71	—	0.22

[a] LT_{50} values were determined by surface-contact bioassays (Table 2), total P450 content determined by carbon monoxide difference spectra (Fig. 1A), demethylation activity determined by quantification of 4-chloroaniline production (Fig. 1B), and P450-MA expression determined by densitometric scanning of western blots (Fig. 2C).

[b] Degrees of freedom associated with the regression model and error terms, respectively.

[c] Significance level for *P*-values: ***, *, and — indicate significance at 95 and 90% confidence, or nonsignificance, respectively.

[d] Coefficient of correlation (values = 1.0 indicate a completely linear relationship between factors being compared).

results (Table 3). Three comparisons suggested little or no relatedness: cypermethrin LT_{50} versus demethylation activity ($r = 0.22$), cypermethrin LT_{50} versus P450 MA expression ($r = 0.44$), and cypermethrin LT_{50} versus chlorpyrifos LT_{50} ($r = 0.65$). Cypermethrin LT_{50} was most highly correlated with total cytochrome P450 content ($r = 0.84$). Other variables (chlorpyrifos LT_{50} and cypermethrin LT_{50} versus total cytochrome P450 content and P450 MA expression) shared moderate relatedness, with *r*-values ranging from 0.83 to 0.89. However, the most highly correlated relationship was demethylation activity versus P450 MA expression ($r = 0.92$).

Recently, the nucleotide sequence conferring *kdr*-like resistance to pyrethroid insecticides was identified in the German cockroach (Miyazaki et al. 1996, Dong 1997). This mutation has been identified in 2 of the strains exhibiting the highest pyrethroid resistance in the current study (PV and MA) but not in the M86 or JW strains, which have much lower tolerance (Dong et al. 1998). In addition, cypermethrin tolerance was not reduced by pretreatment with PBO in the ML strain, suggesting cytochrome P450 has minor or no involvement in pyrethroid resistance in this strain (Lee et al. 1996). These observations support the current observation that cypermethrin tolerance is not well correlated with P450 MA expression, demethylation activity, nor chlorpyrifos tolerance.

It is likely, however, that additional cytochromes P450 may play a contributing role in cypermethrin resistance in the MA and PV strains, because numerous cytochromes P450 have been identified in some insect species (Ronis et al. 1988, Scott et al. 1994, Pittendrigh et al. 1997). Earlier findings support that additional cytochromes P450 may be implicated in the MA and PV strains: piperonyl butoxide synergizes cypermethrin toxicity (Scharf et al. 1997; unpublished data) and cypermethrin selects for higher total cytochrome P450 content that apparently does not include P450 MA (Scharf et al. 1998b). As identified in the current study, cypermethrin tolerance is also well correlated with total cytochrome P450 content. Finally, NADPH-dependent metabolism of ^{14}C-Fenvalerate was elevated in the MA relative to the JW (S) strain and this conversion also was inhibited by PBO, supporting that additional P450 isoforms are involved in resistance to pyrethroids such as fenvalerate and cypermethrin. Siegfried et al. (1990) identified the involvement of both cytochromes P450 and esterases in chlorpyrifos metabolism in the Dursban-R strain of German cockroach (chlorpyrifos RR = 20-fold). Overexpression of P450 MA was well correlated with chlorpyrifos resistance in our study, agreeing in part with the findings of Siegfried et al. (1990).

Considerations for Resistance Management. Based on results obtained to date, recommendations for resistance management can be made. Our study has identified both elevated P450 MA expression and demethylation activity in association with chlorpyrifos (but not cypermethrin) resistance in cockroach strains of diverse geographic origins. This observation agrees with earlier findings that identified elevated P450 MA expression and demethylation activity following chlorpyrifos selection pressures. The association of cytochromes P450 with organophosphate resistance precludes the use of methylenedioxyphenyl synergists (e.g., piperonyl butoxide) for resistance management, because these synergists also will inhibit bioactivation of the thiophosphate to the more toxic oxon (Siegfried et al. 1990). For resistance management, emphasis should therefore be placed on insecticide rotation (Cochran 1995b, Zhai and Robinson 1996, Scharf et al. 1998b), registered mixtures of insecticides and juvenoids (Scharf et al. 1997, Kaakeh et al. 1997), and integration of nonchemical approaches (Schal and Hamilton 1990, Kaakeh and Bennett 1997).

Acknowledgments

The authors thank the Department of Entomology, Purdue University, for granting a visiting professorship to C.Y.L., and Dan Suiter and Rich Shukle for review of an earlier version of the manuscript. This research was supported in part by Hatch Grant IND 58058 to J.J.N. This is journal article No. 15909 of the agricultural research program of Purdue University, West Lafayette, IN.

References Cited

Ballard, J. B., R. E. Gold and J. D. Rauscher. 1984. Effectiveness of six insecticide treatment strategies in reduction of German cockroach populations in infested apartments. J. Econ. Entomol. 77: 1092–1094.

Cochran, D. G. 1995a. Insecticide-resistance, pp. 171–196. *In* J. M. Owens, M. K. Rust, and D. A. Reierson [eds.], Understanding and controlling the German cockroach. Oxford University Press, Oxford.

Cochran, D. G. 1995b. Looking into cockroach resistance. Pest Manage. Mag. 11: 26–31.

Dong, K. 1997. A single amino acid change in the *para* sodium channel protein is associated with knockdown-resistance (*kdr*) to pyrethroid insecticides in the German cockroach. Insect Biochem. Molec. Biol. 27: 90–100.

Dong, K., S. M. Valles, M. E. Scharf, B. C. Zeichner, and G. W. Bennett. 1998. The knockdown resistance mutation in pyrethroid-resistant German cockroaches. Pestic. Biochem. Physiol. 60: 195–204.

Kaakeh, W., M. E. Scharf, and G. W. Bennett. 1997. Efficacy of conventional insecticide and juvenoid mixtures on an insecticide-resistant field-population of German cockroach. J. Agric. Entomol. 14: 339–348.

Kaakeh, W., and G. W. Bennett. 1997. Evaluation of trapping and vacuuming compared with low-impact insecticide tactics for managing German cockroaches in residences. J. Econ. Entomol. 90: 976–982.

Koehler, P. G., and R. S. Patterson. 1986. A comparison of seven non-resistant strains of the German cockroach. J. Med. Entomol. 23: 298–299.

Lee, C. Y., H. H. Yap, N. L. Chong, and R.S.T. Lee. 1996. Insecticide-resistance and synergism in field collected German cockroaches in Peninsular Malaysia. Bull. Entomol. Res. 86: 675–682.

Miyazaki, M., O. Kazutoshi, D. Y. Dunlap, and F. Matsumura. 1996. Cloning. and sequencing of the *para*-type sodium channel from susceptible and *kdr*-resistant German cockroaches and house fly. Mol. Gen. Genet. 252: 61–68.

Omura, T., and R. Sato. 1964. The carbon monoxide-binding pigment of liver microsomes. J. Biol. Chem. 239: 2370–2378.

Pittendrigh, B., K. Aronstein, E. Zinkovsky, O. Andreev, B. Campbell, J. Daly, S. Trowell, and R. H. ffrench-Constant. 1997. Cytochrome, P450 genes from *Helicoverpa armigera:* expression in a pyrethroid-susceptible and -resistant strain. Insect Biochem. Molec. Biol. 27: 507–512.

Ronis, M. J., E. Hodgson, and W. C. Dauterman. 1988. Characterization of multiple forms of cytochrome, P450 from an insecticide-resistant strain of house fly. Pestic. Biochem. Physiol. 32: 74–90.

Rust, M. K., and D. A. Reierson. 1991. Chlorpyrifos resistance in German cockroaches from restaurants. J. Econ. Entomol. 84: 736–740.

SAS Institute. 1990. SAS/STAT user's guide, vols. 1 and 2. SAS Institute, Cary, NC.

Schal, C., and R. L. Hamilton. 1990. Integrated suppression of synanthropic cockroaches. Annu. Rev. Entomol. 35: 521–551.

Scharf, M. E., G. W. Bennett, B. L. Reid, and C. Qui. 1995. Comparisons of three insecticide-resistance detection methods for the German cockroach. J. Econ. Entomol. 88: 536–542.

Scharf, M. E., J. Hemingway, B. L. Reid, G. J. Small, and G. W. Bennett. 1996. Toxicological and biochemical characterization of insecticide-resistance in a field-collected strain of *Blattella germanica.* J. Econ. Entomol. 89: 322–331.

Scharf, M. E., W. Kaakeh, and G. W. Bennett. 1997. Changes in an insecticide-resistant field population of German cockroach after exposure to an insecticide mixture. J. Econ. Entomol. 90: 38–48.

Scharf, M. E., J. J. Neal, C. B. Marcus, and G. W. Bennett. 1998a. Cytochrome, P450 purification and immunological detection in an insecticide-resistant strain of German cockroach. Insect Biochem. Molec. Biol. 28: 1–9.

Scharf, M. E., J. J. Neal, and G. W. Bennett. 1998b. Changes of insecticide-resistance levels and detoxication enzymes following insecticide selection in the German cockroach. Pestic. Biochem. Physiol. 59: 67–79.

Scott, J. A., F. H. Collins, and R. Feyereisen. 1994. Diversity of cytochrome, P450 genes in the mosquito *Anopheles albimanus.* Biochem. Biophys. Res. Comm. 205: 1452–1459.

Siegfried, B. D., J. G. Scott, R. T. Roush, and B. C. Zeichner. 1990. Biochemistry and genetics of chlorpyrifos resistance in the German cockroach. Pestic. Biochem. Physiol. 38: 110–121.

Valles, S. M., and S. J. Yu. 1996. Detection and biochemical characterization of insecticide-resistance in the German cockroach. J. Econ. Entomol. 89: 21–26.

Wu, D., M. E. Scharf, J. J. Neal, D. R. Suiter, and G. W. Bennett. 1998. Mechanisms of fenvalerate resistance in the German cockroach. Pestic. Biochem. Physiol. 61: 53–62.

Zhai, J., and W. H. Robinson. 1996. Instability of cypermethrin resistance in a field population of German cockroach. J. Econ. Entomol. 89: 332–336.

Received for publication 28 December 1998; accepted 14 April 1999.