PYRETHROID RESISTANCE MECHANISMS IN THE GERMAN COCKROACH (DICTYOPTERA: BLATTELLIDAE)

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Abstract - Resistance to pyrethroid insecticides has become common in field populations of the German cockroach, *Blattella germanica* (L.), in the United States and elsewhere. Some populations are highly resistant to as many as eight or nine pyrethroids. The primary mechanisms of resistance are enzymatic degradation of the insecticide and target-site insensitivity. Tests, using the tarsal-contact method, formed the basis for a further analysis. Resistance to pyrethrins is specific, and can be completely negated by piperonyl butoxide (PBO). Resistance to allethrin is also specific, but PBO has no effect on it. Resistance to several of the more advanced pyrethroids developed later and initially could be negated by PBO. Subsequently and progressively, PBO had little or no effect on resistance to these compounds. Analysis of resistance profiles for a large number of field populations indicates that the PBO-sensitive resistance to permethrin, phenothrin, fenvalerate, and cypermethrin developed independently. Similarly, the PBO-insensitive resistance to these compounds also developed in a stepwise manner. While data of this type do not establish the details of each mechanism, they clearly indicate the existence of numerous pyrethroid-resistance mechanisms in the German cockroach.
Key words - *Blattella germanica*, resistance profiles, tarsal-contact method, synergists

INTRODUCTION

Resistance to pyrethroid insecticides in the German cockroach, *Blattella germanica* (L.), has become widespread in field populations around the world (Atkinson *et al.*, 1991; Cochran, 1989, 1995a; Dong *et al.*, 1998; Lee, 1997; Vagn-Jensen, 1993). The continued use of these insecticides for the control of this ubitiquous household pest is thereby threatened. While no solution for this problem currently exists, the broad outlines of the mechanisms that foster resistance are known (Lee, 1997). In some cases detailed information is available (Dong, 1997; Dong *et al.*, 1998; Miyazaki *et al.*, 1996). This knowledge may aid in the rapid detection of resistance in field populations (Dong *et al.*, 1998), and might foster the development of new control strategies.

It is the purpose of this paper to provide an overview of what is currently known about the mechanisms of resistance to pyrethroids, and to add some additional information concerning the complexity of pyrethroid resistance in this species.

MATERIALS AND METHODS

The insects used were collected from field infestation sites at numerous locations in about 20 states within the continental United States. They were brought into the laboratory and reared continuously (Cochran, 1979). Most populations were allowed to propagate for 2-3 generations prior to testing. The VPI-susceptible strain was the reference strain for bioassays.

Toxicological testing was accomplished as previously described (Cochran, 1989, 1997). Briefly, it consisted of a tarsal-contact method of exposure in which time was the variable. The data from three standard replicates were pooled and analyzed by probit analysis (SAS Institute, 1985). Resistance ratios were calculated as RR = LT_{50} of the test strain ÷ LT_{50} of the VPI-susceptible strain. Testing was done at 21-23^0 C.

The insecticides used, together with their concentrations (AI) and sources, are as follows: from McLaughlin Gormley King Company (MGK), Minneapolis, MN; pyrethrins, 0.3 nl/cm^2; allethrin, 0.3 nl/cm^2; permethrin, 1.5 nl/cm^2; phenothrin, 3.0 nl/cm^2; fenvalerate, 6.0 nl/cm^2; esfenvalerate,

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6.0 nl/cm^2: from Zeneca, Wilmington, DE; cypermethrin, 1.5 nl/cm^2; cyhalothrin, 1.5 nl/cm^2: from Mobay Chemical Corporation (MCC), Kansas City, MO; cyfluthrin, 1.5 nl/cm^2: from AgrEvo, Montvale, NJ; deltamethrin, 1.5 nl/cm^2. The synergists were piperonyl butoxide (PBO) and 2-(2-ethylhexyl)-3a,4,7,7a-tetrahydro-4,7-methano-1H-isoindole-1,3(2H)-dione (MGK 264) from MGK, and S,S,S-tributylphosphorothioate (DEF) from MCC. The insecticide:synergist ratio was 1:5. All chemicals were technical grade except pyrethrins (20%).

Gene frequency estimates were done as described by Cochran (1994a, 1994b). They were based on the Hardy-Weinburg equilibrium expression (Falconer, 1981). The calculations were possible because it is known that all homozygous susceptible and heterozygous individuals are killed by permethrin, phenothrin, fenvalerate, or cypermethrin in the test protocol used (Ebbett and Cochran, 1997). Thus, only homozygous resistant individuals survive, and gene frequency equals the square root of the fraction representing the survivors in a test sample from a population.

Resistance ratios and gene frequency estimates represent the status of each strain at the time of analysis. Since no direct comparison of strains is included, no statistical analysis was needed. In addition, the tests with synergists were employed only to provide yes/no answers and were not analyzed statistically.

RESULTS AND DISCUSSION

There are two primary mechanisms capable of conveying resistance to pyrethroid insecticides in insects, including the German cockroach. They involve either the enzymatic detoxication of the insecticide or insensitivity of a target site to the insecticide. In both cases, it is recognized that multiple forms of these mechanisms may exist (Anspaugh *et al.*, 1994; Dong *et al.*, 1998; Head *et al.*, 1998; Lee, 1997; Park *et al.*, 1997; Pittendrigh *et al.*, 1997; Schuler *et al.*, 1998). Reduced penetration of the insecticide through the cuticle is a third mechanism (Fine *et al.*, 1963), but it is usually considered to be of only minor importance (Soderlund and Bloomquist, 1990).

Of the enzymatic or metabolic mechanisms that are known (Lee, 1997), it is the monooxygenases and esterases that appear to be the most important as possible causes of resistance to pyrethroids. Elevated levels of cytochrome P_{450} and esterases have been reported from various strains of the German cockroach (Anspaugh *et al.*, 1994; Hemmingway *et al.*, 1993; Lee, 1997; Scharf *et al.*, 1996, 1997; Valles and Yu, 1996). While some exceptions may exist (Dong *et al.*, 1998; Scott, 1990), the synergist PBO is usually successful in negating monooxygensae-mediated resistance, as is DEF with resistance due to enhanced esterase activity (Atkinson *et al.*, 1991; Hemmingway *et al.*, 1993; Lee, 1997; Valles and Yu, 1996).

Table 1. The status of pyrethroid resistance in field-collected strains of the German cockroach.

Category	Number of strains
Total tested	150
Strains susceptible to all pyrethroids	44
Strains resistant to pyrethrins only	4
Strains resistant to pyrethrins and allethrin only	17
Strains resistant to pyrethrins and phenothrin, but not allethrin	2
Strains resistant to three or more pyrethroids	53

The target-site insensitivity mechanism that conveys pyrethroid resistance in the German cockroach, as in other insects, is of the *kdr*-type (Scott and Matsumura, 1983; Umeda *et al.*, 1988). When it is present in a population, the commonly-used synergists usually fail to negate resistance (Lee, 1997).

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It is inherited as a simple, autosomal, incompletely recessive trait (Scott and Dong, 1994). While other possibilities have been suggested to explain the *kdr*-mechanism (Pauron *et al.*, 1989; Rossignol 1988), it is now known that the *kdr*-type trait in the German cockroach is attributable to a point mutation in the highly conserved membrane-spanning segment 6 of domain II (IIS6) of the *para* sodium channel protein that results in a single amino acid substitution from leucine^{993} in a susceptible strain to phenylalanine^{993} in the Ectiban-R strain (Dong, 1997). This mutation protects sodium channels from the insecticide. Subsequently, the same mutation has been found in 20 cockroach strains, but the super-*kdr* mutation was not detected (Dong *et al.*, 1998). The latter is known in houseflies, and has been characterized as involving an additional mutation that greatly enhances pyrethroid resistance (Lee *et al.*, 1998; Smith *et al.*, 1997).

The work reported here is based on an analysis of 150 field-collected strains (Table 1). Of those strains, 44 were susceptible to all pyrethroids tested and another 34 were resistant only to pyrethrins. That resistance is controlled by a simple, autosomal, dominant gene (Cochran, 1994b) which conveys metabolic resistance that can be completely negated by PBO. An additional 15 strains were also resistant to allethrin. That trait is controlled by a simple autosomal incompletely recessive gene (Cochran, 1995b). Strains with this trait fail to respond to PBO or DEF. The presumption here is that this gene conveys a nerve insensitivity (*kdr*-type) mechanism which is the same as or related to the one discussed above. When present, this trait also protects pyrethrins from the action of PBO.

As mentioned above, there is evidence suggesting a multiplicity of resistance mechanisms in the German cockroach. That concept finds support from the two additional strains resistant only to pyrethrins and allethrin (Table 1) and three other strains (Table 2) in which PBO and MGK 264 completely negated resistance to allethrin. In these strains it appears that either the metabolic resistance to pyrethrins also extends to allethrin or an additional allethrin-resistance trait is present.

Table 2. Strains of German cockroaches in which resistance to allethrin is eliminated by synergists.

Strain	RR[a]	RR + PBO	RR + MGK264
Crawford[b]	>140	1.4	1.3
Hawthorne[b]	>140	1.6	1.6
Frishman # 6[c]	>140	1.4	1.3
Boces[c]	>140	1.8	2.5
King L-11[d]	>140	1.8	1.6

[a] RR = resistance ratio with allethrin alone, or allethrin plus PBO or MGK 264.
Resistance ratio is defined as LT_{50} test strain ÷ LT_{50} VPI susceptible strain.
[b] Strains resistant to pyrethrins and allethrin.
[c] Strains resistant pyrethrins, allethrin, and phenothrin.
[d] Strain resistant to pyrethrins, allethrin, and permethrin.

Many strains showed other combinations of pyrethroid resistance. Two strains were resistant to pyrethrins and phenothrin, but not allethrin (Table 1). The resistance profile for one of them, the Fryers strain, is shown in Table 3. Fifty three other strains were resistant to various combinations of three or more pyrethroids (Table 1). As illustrated in Table 4, the third pyrethroid was variable. Phenothrin and fenvalerate were the most commonly found. Resistance to one of these four pyrethroids did not convey cross resistance to the others. Gene frequency estimates support this contention. For example, in the Boces strain gene frequency is elevated sufficiently to result in high-level resistance (>0.70) only against phenothrin. The low gene frequencies for the other three pyrethroids, as well as the low RRs, indicate that resistance to them was not developing in this strain.

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Table 3. The pyrethroid resistance profile for the Fryers strain.

Insecticide	RR[a]	Insecticide	RR
Pyrethrins	>140	Esfenvalerate	0.8
Allethrin	2.4	Cyfluthrin	1.2
Permethrin	2.0	Cypermethrin	1.4
Phenothrin	>100	Cyhalothrin	1.5
Fenvalerate	2.6	Deltamethrin	0.9

[a] RR = resistance ratio which is defined as LT_{50} test strain ÷ LT_{50} VPI susceptible strain.

Table 4. Strains of German cockroaches with resistance to pyrethrins, allethrin, and one other pyrethroid.

Strain	Permethrin		Phenothrin		Fenvalerate		Cypermethrin	
	GF[a]	RR[b]	GF	RR	GF	RR	GF	RR
Boces	<.20	1.1	0.72	>100	0.28	1.1	<.20	1.3
Morris	<.20	1.6	0.82	>50	0.29	1.3	0.31	0.9
Royal Garden	<.20	1.2	0.72	>80	0.36	2.3	0.44	1.0
Navy # 7	<.20	1.5	0.69	24.4	0.38	1.4	<.20	1.5
Williams	0.22	1.1	0.31	1.3	0.76	>50	0.22	1.1
Army 360	0.53	3.3	0.38	3.0	0.82	>50	<.20	1.3
Navy # 4	0.31	1.8	0.38	2.4	0.90	>50	0.22	2.6
Pruda	0.73	>60	<.20	1.1	<.20	1.2	0.38	2.3

[a] GF = estimated gene frequency of the resistance allele in the test population.

[b] RR = resistance ratio which is defined as LT_{50} test strain ÷ LT_{50} VPI susceptible strain.

As pyrethroid resistance evolved in the field, strains with more complex patterns of resistance appeared (Table 5). Resistance to two or three additional pyrethroids was common, but again each strain exhibited its own pattern. These data also support the position that the traits are independent. However, it is clear from the gene frequency data that resistance to the fourth pyrethroid was emerging in some strains (e.g. Runnaways). Where high-level resistance was present, PBO blocked it completely.

In a separate study, it was shown that high-level resistance to each of the pyrethroids considered in Tables 4 and 5 is inherited as a simple autosomal incompletely recessive trait (Ebbett and Cochran, 1997). From linkage data it was not possible to determine if one or more than one gene is involved, but in each case the resistance was completely negated by PBO. However, the data in Tables 4 and 5 strongly suggest that each trait is inherited independently.

The further evolution of pyrethroid resistance is illustrated in Table 6. Additional strains are shown in which PBO completely blocked high-level resistance to each of these four insecticides, but in other strains the pattern was changing. First, resistance to permethrin (Boston Sub) or phenothrin (Smithtown) could not be blocked by PBO. Subsequently, strains were found in which PBO failed to block resistance to both of these pyrethroids, or to them and fenvalerate. Finally, one strain (Tien Hung) was tested in which PBO was ineffective against all four of these insecticides.

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Taken together, I believe the data presented here, while not definitive, strongly support the contention that resistance to pyrethroids in the German cockroach is extremely complex. The evidence indicates that several independent metabolic resistance mechanisms were present in the strains studied. In all of these cases, PBO, MGK 264, and/or DEF totally reversed resistance. In addition, the original presumed nerve-insensitivity mechanism was specific for allethrin. It was only after high-level metabolic resistance to other pyrethroids was in place that PBO failed to negate resistance to one or more of them. The implication is that other mechanisms, probably also nerve-insensitivity type, gradually evolved until the synergists no longer had an effect on resistance. If there is a *super-kdr* gene in the German cockroach, it would probably exist in a strain such as the Tien Hung (Table 6). The genetic plasticity implicit in these findings is truly remarkable.

Table 5. Strains of German cockroaches with resistance to pyrethrins, allethrin, and two or three other pyrethroids.

Strain	Permethrin		Phenothrin		Fenvalerate		Cypermethrin	
	GF[a]	RR[b]	GF	RR	GF	RR	GF	RR
Hartford	0.37	1.5	0.87	>80	0.79	>40	<.20	2.5
Coretta	0.90	>100	0.97	>140	0.38	2.1	<.20	1.7
Las Palms	0.49	3.2	0.85	>120	0.82	>60	0.90	>80
Runnaways	0.87	>70	0.98	>100	0.58	4.6	0.98	>75
Jacksonville	0.85	>120	0.87	>140	0.69	7.3	0.31	3.0

[a] GF = estimated gene frequency of the resistance allele in the test population.

[B] RR = resistance ratio which is defined as LT_{50} test strain ÷ LT_{50} VPI susceptible strain.

Table 6. The ability of piperonyl butoxide to negate resistance in strains of German cockroaches highly resistant to these four pyrethroids.

Strain	Permethrin	Phenothrin	Fenvalerate	Cypermethrin
Jones	Yes	Yes	Yes	Yes
From Bret	Yes	Yes	Yes	Yes
Salisbury	Yes	Yes	Yes	Yes
Boston Sub	No	Yes	Yes	Yes
Smithtown	Yes	No	Yes	Yes
Barksdale	No	No	Yes	Yes
Jackson	No	No	Yes	Yes
Nassau	No	No	Yes	Yes
Puerto Rico	No	No	No	Yes
Pizza Internat	No	No	No	Yes
Toughkengnon	No	No	No	Yes
Tien Hung	No	No	No	No

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