RESISTANCE MECHANISMS IN COCKROACHES – THE KEY TO CONTROL STRATEGIES

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Abstract—Multi-resistant strains of *Blattella germanica* are now common, and strategies for their continued control using conventional insecticides need to be devised rationally if long-term suppression of these nuisance insects is to be achieved. Newer insecticides such as growth regulators may have a role in such strategies, but these cannot be used wholly as substitutes for conventional insecticides, due to their high cost and mode of action, which may result in a residual nymphal population being present over a long period. This may, wrongly, be perceived as control failure by householders.

In order to devise control strategies for multi-resistant *B. germanica* the resistance status of thirty strains from four countries was determined for a range of pyrethroids. Strains were collected on the basis of recently reported pyrethroid resistance. Greater than 2-fold resistance to a range of pyrethroids occurred in fifteen of these strains and, of these fifteen, thirteen strains were resistant to chlorpyrifos and twelve to propoxur. All the field strains tested were heterogeneous with regard to resistance. The mechanisms of resistance were determined in all fifteen multi-resistant strains. The elevated esterase and oxidase-based resistance mechanisms were the most prevalent, with eleven and ten strains respectively having evidence of these mechanisms. Pyrethroid resistance could be synergised by piperonyl butoxide in strains with only the oxidase mechanism. In some strains the elevated esterase mechanisms detected were primarily correlated with organophosphate resistance, while in others they conferred resistance to both organophosphates and pyrethroids. Increased levels of glutathione- *S*-transferase activity were found in four strains and an altered acetylcholinesterase-type, organophosphate and carbamate resistance mechanism was seen in one strain. There was no evidence of resistance to commercially available growth regulators in any of the strains tested.

On the basis of the types of pyrethroid resistance mechanisms detected in field populations of *B. germanica* a two- tiered approach to control of these populations was devised, the optimum strategy being determined by the characteristics of the population to be controlled. Where oxidase-based resistance alone occurs, a synergist/pyrethroid combination can be used, where multiple resistance mechanisms occur a pyrethroid/growth regulator combination is used. The growth regulator is used at a low dosage, which effectively sterilises both male and female cockroaches. The fast-acting pyrethroid controls the majority of the population, resulting in rapid perceived control, while the highly pyrethroid resistant remainder of the population which survive are sterilised by the growth regulator, giving longer term control.

INTRODUCTION

Organochlorines were replaced by organophosphorus, carbamate or pyrethroid insecticides for *Blattella germanica* control after problems with resistance and the environmental acceptability of these compounds occurred. The newer generations of pesticide were more environmentally acceptable, but after a number of years of usage resistance to these compounds also started to occur. The resistance problem is compounded by cross-resistance, where the resistance mechanism selected by one compound confers resistance to other chemically-related or unrelated compounds. This cross-resistance can be understood at a mechanistic level, where common metabolic pathways or similar sites of action are the basis of the resistance mechanism.

Chlorpyrifos is an organophosphorus insecticide commonly used for *Blattella* control but resistance to this insecticide is now widespread (Bennett & Spinks 1968, Milio *et al* 1987, Rust & Reierson 1990). The spread of organophosphate resistance, combined with the increased efficacy of the new pyrethroids, has led to the widespread use of the latter over the last 10 years, either as space or residual sprays. There have been numerous reports of pyrethroid resistance developing in field populations of *B. germanica*, but little is known about the underlying mechanisms (Cochran 1987, Umeda *et al* 1988).

MATERIALS AND METHODS

Insect strains

Resistant strains were chosen for this study on the basis of reports of pyrethroid resistance in the vicinities where the strains were collected. Table 1 gives the countries from which the strains were

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Table 1. Origin of *B. germanica* strains used for this study

Laboratory	USA	Panama	Denmark	Dubai
Goldsboro[a]	El Paso	PQHoc	KoF4	Dubai
	New Jersey	E176-C	BiF3	
	Kansas		Suf2	
	H407			
	E818			
	Seasons			
	Pulaski			
	Purdue			
	Navy 4			

[a]The laboratory susceptible strain has been kept in colony without insecticide exposure for at least 15 yr.

collected. All strains were reared at 28°C and 70% R.H. All biochemical assays and bioassays were done on first instar nymphs, with the exception of the Cytochrome P^{450} assay, which was done on young adult male *Blattella*. Strains were brought into the laboratory between 1987 and 1989 and, because some resistance mechanisms confer a selective disadvantage in the absence of insecticide pressure (Bonning & Hemingway, 1991), nymphs which survived insecticide treatment in any bioassay were returned to the stock cages to ensure that resistance mechanisms were maintained within the population over the three years of this study.

Bioassays were done by exposing the nymphs to insecticide impregnated Whatmans no. 1 filter paper for 1 hr, followed by a 48 hr recovery period in tubes lined with clean filter paper. Throughout the recovery period the nymphs had access to 10% sucrose solutions. Impregnated papers were prepared by making the relevant percentage solutions of organophosphorus insecticides in olive oil, or pyrethroids in Dow Corning silicone fluid. The insecticide solutions were spread on 12 × 15 cm rectangles of filter paper at a rate of 3.6 mg/cm^2, an equal volume of acetone was used to facilitate even spreading of the viscous oil solutions. The testing chamber was a 12 × 4 cm cylinder with the sides and base covered with impregnated paper. The slide at the top of the chamber through which the cockroaches were introduced was coated with fluon to ensure that cockroaches remained on the impregnated paper throughout the test period. At least five insecticide concentrations that caused mortalities between 0 & 100% were tested for each insecticide; 20–25 nymphs were tested per replicate, and each dose was replicated 2–5 times. Data were subjected to log-dosage probit mortality regression analysis. Bioassays with piperonyl butoxide were done by exposing first instar nymphs to 4% synergist impregnated papers for 1 hr, before exposing them to the insecticide as above.

Biochemical assays

First nymphal instars were sieved from the colonies and stored at −70°C until use. Each nymph was subjected to acetylcholinesterase (AChE), esterase, glutathione-*S*-transferase and protein assays. At least 95 nymphs of each strain were tested. Individual nymphs were homogenised in 250 µl of distilled water on ice. A 40 µl sample was removed for the AChE assay, the remainder was centrifuged at 10,000 g for 5 mins at 4°C and the supernatant removed.

The acetylcholinesterase inhibition assay was as described by ffrench-Constant & Bonning (1989). The kinetics of colour formation were measured for each well at 405 nm in a UVmax microtitre plate reader for five minutes. The propoxur concentration used in all tests was initially determined by titration of the AChE from the susceptible WHO strain to a point where a mean activity of 5% of that of the uninhibited control was observed in the propoxur containing inhibited fraction.

The esterase and protein assays were as described by Peiris & Hemingway (1990). The glutathione-*S*-transferase assay was run by adding 80 µl of a mixture of reduced glutathione and 2,4-chlorodinitrobenzene (CDNB) (35 ml 15 mM GSH in 0.1 M phosphate buffer pH 6.5 containing 0.5% Triton X-100 and 280 µl CDNB in methanol) to two 20 µl aliquots of nymph homogenate. The reaction rate was measured at 340 nm for 5 minutes in a UVmax microtitre plate reader.

Cytochrome P450 activity was measured by the method of Omura & Sato (1964). A mass homogenate of adult male cockroaches was made at a ratio of 1.2–1.4 gm wet weight insect to 10 ml

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Tris buffer, (0.25 M Sucrose, 1 mM EDTA, 1% polyvinylpyrrolidone, 0.25 mM phenylmethylsulfonyl fluoride and 1 mM dithiothreitol adjusted to pH 7.6 with 0.5 mM Tris) and used, after ultracentrifugation, as the enzyme source.

Detection of kdr-type pyrethroid resistance was by a modification of the ceral-giant interneurone preparation used for *Periplaneta americana* (Pichon 1974). Recordings were taken extracelluarly between ganglia 5 and 6 of young adult male cockroaches with glass suction electrodes filled with 3M KCl. The preparations were monitored for 20 mins before insecticide application, when pyrethroids diluted to their final concentration were applied in saline with a disposal gravity feed system. The threshold at which the spontaneous rate doubled was considered to be the active concentration. Ten insects were assayed from each strain.

RESULTS

Thirty *B. germanica* populations were analysed for resistance to cypermethrin and lambda cyhalothrin, of these only 15 had >2-fold resistance to one or both of these pyrethroids. These 15 strains were analysed for resistance to chlorpyrifos, propoxur, cyfluthrin and fenvalerate compared to the Goldsboro susceptible strain. Thirteen of the strains were resistant to chlorpyrifos and twelve to propoxur (see Table 2). In the contact assay used, cypermethrin was slightly more toxic than lambda cyhalothrin (1·8x) and cyfluthrin (2.7x) and significantly more toxic than fenvalerate (33x) to the susceptible strain at the LC_{50}.

The shallow slopes of the probit lines of all the resistant strains for all four pyrethroids tested suggest that the strains are all genetically heterogeneous with respect to resistance. Resistance ratios, at the LC_{50} and LC_{90}, were calculated relative to the Goldsboro strain. RR_{LC50} are summarised in Table 2. Combined LC_{50} and LC_{90} resistance ratios to cyfluthrin range from 1.9 to 22.1. Two strains showed no cyfluthrin resistance, showing a low level of negative cross-resistance. The levels of fenvalerate resistance were low (1.7 to 4.6-fold), but the inherent toxicity of this insecticide to the susceptible strain was significantly less than that of the other pyrethroids. All strains had some degree of resistance to cypermethrin, as this was one of the criteria for their selection. Resistance ratios ranged from 2.5–127-fold. All but one strain had resistance to lambda cyhalothrin and the resistance ratios ranged from 2–62-fold. Resistance ratios for chlorpyrifos were from 1.4–462-fold and for propoxur from 1.1–46-fold.

Piperonyl butoxide (PB) treatment, followed by application of each insecticide at the LC_{50} and LC_{90} already estimated for each strain, indicated that chlorpyrifos was less toxic at both the LC_{50} and LC_{90} to the Goldsboro strain after PB treatment. This result was expected because chlorpyrifos

Table 2. Response of first nymphal instar *B. germanica* treated with different insecticides, and their resistance ratios at the LC_{50} compared to the susceptible Goldsboro strain

	INSECTICIDE											
Strain	Chlorpyrifos		Propoxur		Cyfluthrin		Fenvalerate		Cypermethrin		Lambda cyhalothrin	
	LC_{50}	RR	LC_{50}	RR	LC_{50}	RR	LC_{50}	RR	LC_{50}	RR	LC_{50}	RR
Goldsboro	0.05	1	0.14	1	0.09	1	1.1	1	0.033	1	0.06	1
PQHoc	0.53	10.6	0.32	2.3	0.1	1.1	1.9	1.7	0.1	3	0.14	2.1
El Paso	1.3	26	0.59	4.2	0.32	3.4	0.04	0.03	0.13	3.9	0.03	0.5
E176-C	0.77	15.4	0.45	3.2	0.56	5.9	3.9	3.5	0.81	24.5	0.08	1.3
New Jersey	0.49	9.8	0.38	2.7	0.05	0.5	3.2	2.9	0.08	3.5	0.09	1.4
Kansas	0.33	6.6	0.29	2.1	0.22	2.4	2.3	2.0	0.34	10.3	0.03	0.4
KoF4	0.09	1.8	0.16	1.1	0.11	1.2	0.55	0.5	0.61	18.5	0.13	2.0
H407	2.9	58	0.4	2.9	0.49	5.2	2.8	2.4	0.33	10	0.96	15.1
Navy 4	0.43	8.6	0.43	3.1	0.27	2.9	4.5	4.0	0.24	7.2	1.0	15.6
E818	0.07	1.4	0.05	0.4	0.02	0.2	4.7	4.2	0.12	3.7	0.26	4.1
Seasons	0.4	8	0.02	0.1	0.42	4.5	3.2	2.9	0.1	3.0	0.38	5.9
Pulaski	1.1	22	0.34	2.4	0.11	1.2	2.7	2.4	0.15	4.4	0.14	2.1
BiF3	0.02	0.4	0.21	1.5	0.98	10.4	5.2	4.6	0.08	2.5	0.6	9.4
SuF2	0.61	12.2	0.32	2.3	0.55	5.9	2.85	2.5	0.24	7.2	0.23	3.6
Dubai	0.07	1.4	0.22	1.6	0.09	1.0	4.2	3.7	0.17	5.1	0.18	2.8
Purdue	0.59	11.8	0.24	1.8	0.49	5.4	2.6	2.4	0.41	12.5	0.29	4.8

Table 3. Cytochrome P^{450} levels in the susceptible Goldsboro strain of *Blattella germanica* compared with a range of pyrethroid resistant strains

Strain	nMoles P^{450}/mg soluble protein	Ratio
Goldsboro	0.036	1.0
Suf2	0.836	23.2
E818	0.042	1.2
Bif3	0.229	6.4
Kansas	0.013	0.4
PQHoc	0.014	0.4
New Jersey	0.047	1.3
Seasons	0.102	2.8
H407	0.166	4.6
Navy 4	0.042	1.2
Pulaski	0.021	0.6
Dubai	0.028	0.8
El Paso	0.053	1.5
KoF4	0.199	5.5
E176-C	0.282	7.8
Purdue	0.086	2.4

The ratio given is the amount of P^{450} relative to the susceptible Goldsboro strain. Values are the means of at least three replicates.

requires oxidative activation within the insect, and PB will antagonize rather than synergize organophosphorus insecticides in the susceptible strain. The resistant strains PQHoc, Kansas, KoF4, Navy 4, Seasons, Pulaski, BiF3, Dubai and Purdue also showed an antagonistic effect of PB. In the BiF3 strain, which had negative cross-resistance to chlorpyrifos, the antagonism was particularly marked, suggesting that the negative cross- resistance in this strain may be caused by increased activation of chlorpyrifos. El Paso, E176-C, New Jersey, H407, E818 and SuF2 strains all showed increased mortality after PB and chlorpyrifos treatment, suggesting that oxidative degradation in these strains plays some part in the observed resistance to chlorpyrifos.

Data for synergism of propoxur with PB indicate that strains El Paso, E176-C, New Jersey, KoF4, H407, Pulaski and BiF3 are all synergized by the oxidase inhibitor. The pattern is different from that for chlorpyrifos, where no synergism was obtained in strains KoF4, Pulaski and BiF3 and where strains E818 and SuF2 were synergized with chlorpyrifos but not with propoxur.

Five strains (Suf2, Bif3, KoF4, Seasons and E176-C) showed evidence of synergism with the PB/lambda cyhalothrin combination compared with the insecticide alone. Changes in the LC_{50} values of the other strains were equal to or less than that in the susceptible strain. The rate limiting enzyme on the oxidase system is generally considered to be cytochrome P^{450}; and resistance has been associated with increased titres of this enzyme in other insects.

The cytochrome P^{450} content in all the resistant strains (Table 3) shows that, relative to the susceptible Goldsboro strain, several of the resistant strains had significantly more cytochrome P^{450}; four strains had lower levels of P^{450} than the Goldsboro strain. The highest quantity of cytochrome P^{450} was in the Suf2 strain, which had three times more P^{450} than any other strain. The four strains with the highest levels of P^{450} were those which showed clear synergism of lambda cyhalothrin with PB.

In *Culex* species, broad spectrum organophosphate resistance (including resistance to chlorpyrifos) is strongly correlated with elevated esterase activity (Peiris & Hemingway 1990). In aphids elevated esterases confer organophosphorus and pyrethroid resistance (Devonshire & Moores 1982). We tested esterase activity in *B. germanica* to determine any correlation between the observed chlorpyrifos and pyrethroid resistance and esterase activity in this species. The esterase activity profiles for strains Goldsboro, El Paso, E818, BiF3 and Dubai for 1- and 2-NA are pooled together in Figs. 1a and 2a, as they all had similar levels of activity. The BiF3 strain had the lowest mean level of esterase activity of any of the strains tested with both 1- and 2-NA, and was also the only strain to show negative cross- resistance to chlorpyrifos (see Table 2).

Data for the H407 and Pulaski strains are combined (see Figs. 1b and 2b). These strains had esterase activity profiles that largely overlapped with the strains in Figs. 1a and 2a, but were significantly different from them on one way analysis of variance ($F = 137$; df = 1,368; P = <0.0001 for 1-NA and $F = 142$; df = 1, 368; P = <0.0001 for 2-NA). The difference in distribution

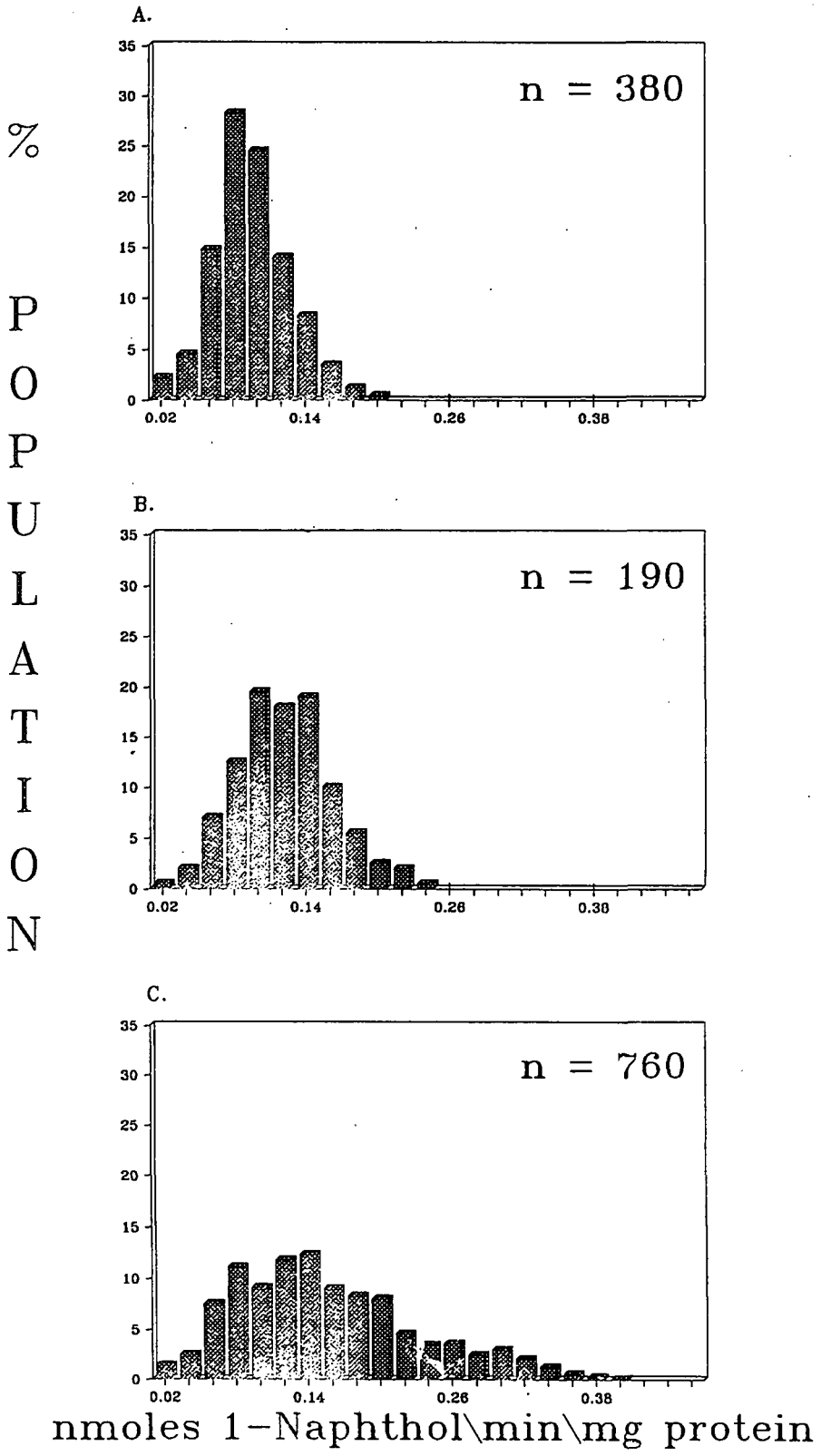

Figure 1. Esterase activity profiles in first nymphal instars of *Blattella germanica* with the substrates 1-naphthyl acetate. (a) strains Goldsboro, El Paso, E818, Bif3 & Dubai. (b) strains H407 and Pulaski (c) strains PQHoc, E176-C, New Jersey, Kansas, KoF4, Navy4, Seasons, SuF2 and Purdue.

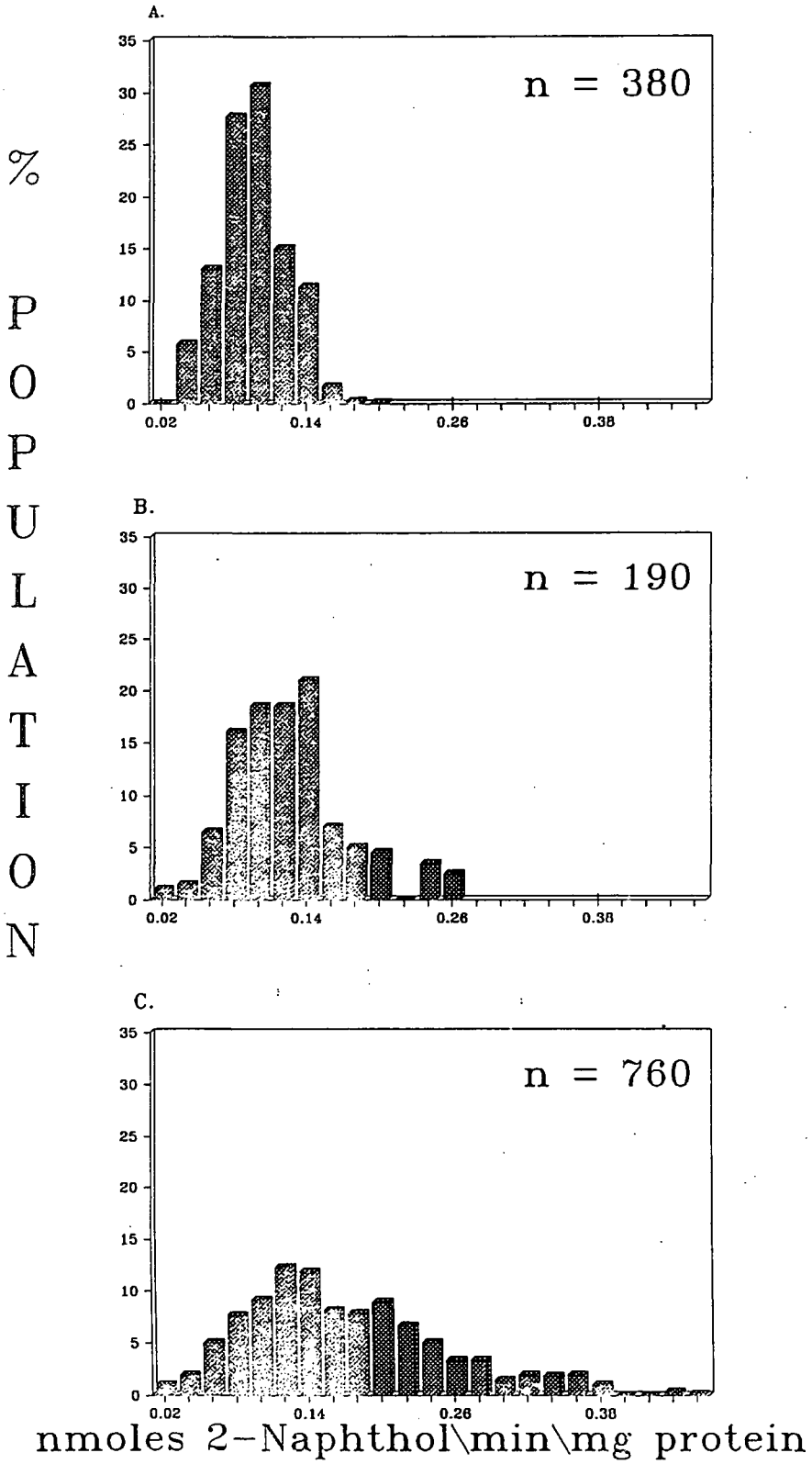

Figure 2. Esterase activity profiles in first nymphal instars of *Blattella germanica* with the substrates 2-naphthyl acetate. (a) strains Goldsboro, El Paso, E818, Bif3 & Dubai. (b) strains H407 and Pulaski (c) strains PQHoc, E176-C, New Jersey, Kansas, KoF4, Navy4, Seasons, SuF2 and Purdue.

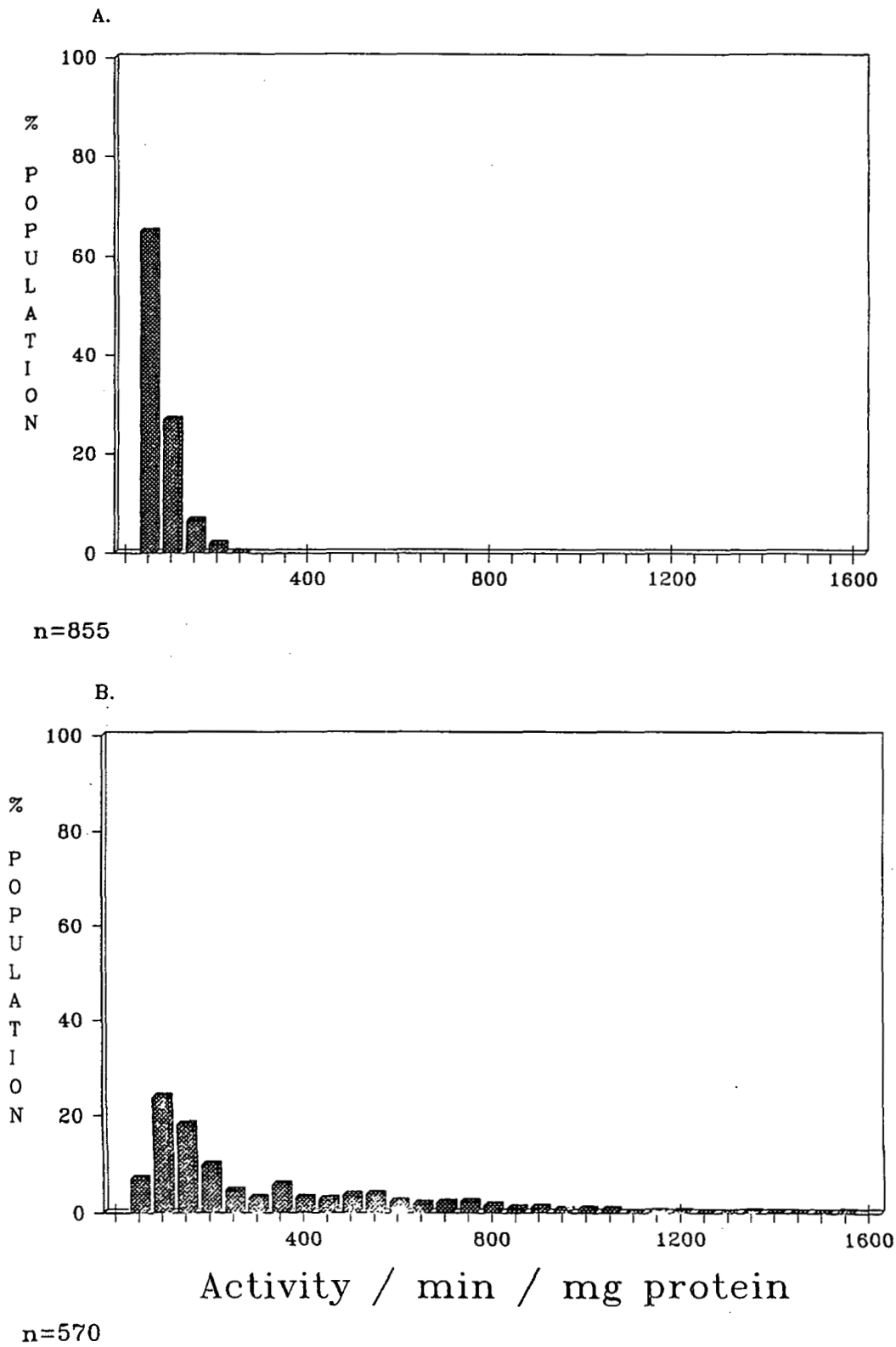

Figure 3. Glutathione-*S*-transferase activity profiles from *Blattella germanica* with chlorodinitrobenzene: (a) strains Goldsboro, Pulaski, Seasons, Pattoc, El Paso, E176-C, E818, Bif3 and Dubai. (b) Strains H407, New Jersey, Kansas, Kof4, Navy4, Suf2 and Purdue.

pattern was greater with 2-NA than with 1-NA. Both these strains had high levels of chlorpyrifos resistance (87 and 152-fold at the LC_{90}). The data for the other nine strains are pooled in Figs. 1c and 2c. Within these strains, the New Jersey strain, with the highest level of chlorpyrifos resistance at the LC_{90}, had a higher proportion of the nymphs with elevated activity with 2-NA than with 1-NA (23 versus 4%). Because the same nymphs were tested with both substrates, a proportion of this population must also have elevated esterases with similar specificity to those of the H407 and Pulaski strains. The PQHoc and Purdue strains also had more individuals with elevated 2-NA than 1-NA. These strains had 66 and 111-fold resistance at the LC_{90}. The remainder of the cockroach strains were segregating for elevated esterase activity equally with both 1-NA and 2-NA.

Glutathione-*S*-transferases are involved in organophosphate resistance in *Musca domestica* (L.) and have been implicated in DDT resistance in this and a number of other insects. The glutathione-*S*-transferase resistance mechanism has been associated with an increased level of activity with the general glutathione-*S*-transferase substrate CDNB (Clark & Shamaan 1984, Herath *et al.* 1988). Figure 3a shows the profile for glutathione-*S*-transferase activity with CDNB in susceptible cockroaches. Data for the Goldsboro susceptible reference strain and strains Pulaski, Seasons, PQHoc, El Paso, E176-C, E818, BiF3 and Dubai are pooled because activity levels did not differ significantly by one way Anova in any of these strains. Data for all the other strains with elevated glutathione-*S*-transferase activity are shown in Fig. 3b.

Both Kansas and New Jersey strains of cockroach had a high frequency of individual nymphs with elevated glutathione-*S*-transferase activity (Fig. 3). The Navy4, Purdue and KoF4 strains had 25% of individuals tested with elevated glutathione-*S*-transferase activity. Both the Suf2 and H407 strains had 1% of individuals with elevated activity.

The Kansas and New Jersey strains had a high frequency of elevated activity and low (= 5-fold) levels of propoxur resistance. The Kansas strain had a low level of chlorpyrifos resistance compared with the New Jersey strain (14-fold compared with 462-fold at the LC_{90}). Both strains had elevated esterase and glutathione-*S*-transferase activity, but the New Jersey strain also had some indication of multifunction mono-oxidase involvement in resistance.

With the altered acetylcholinesterase assay all but one strain had the standard pattern of inhibition expected when their acetylcholinesterase is susceptible to propoxur binding (ffrench-Constant & Bonning, 1989). In contrast, the Dubai strain had an altered acetylcholinesterase based resistance on the basis of its inhibition profile with propoxur. This kind of resistance mechanism should confer a broad spectrum of carbamate and organophosphate resistance to the individuals carrying it, and explains the chlorpyrifos resistance seen in the Dubai strain which is not associated with elevated esterase activity.

Because neurophysiological studies on all the strains of cockroach were not practical, four strains were analysed. Two had resistance levels that suggested a further resistance mechanism not associated with increased esterase or oxidase levels should be present. Two had resistance levels that could be accounted for by the metabolic mechanisms. Before neurophysiological studies were done the resistance levels in the adult males of these strains were determined for permethrin and lambda cyhalothrin. In all cases resistance was present in the adults. Resistance to the two pyrethroids was proportional to that seen in first instars, although LC_{50} differed. Neurophysiological results indicated that two of the four strains tested possessed significant levels of nerve insensitivity (Table 4). These were the strains BiF3 and H407, whose resistance levels could not be fully accounted for by increased levels of metabolic enzymes alone. Our experiments confirmed that nerve insensitivity can play an important part in pyrethroid resistance in *B. germanica*.

Table 4. Electrophysiological threshold response on nerve cords of *Blattella germanica* after infusion with lambda cyhalothrin

Strain	Average[a] insecticide threshold (M)	Rf[b]
Goldsboro	$1 \times 10^{-11} \pm 0.1 \times 10^{-11}$	0
Navy	$2.3 \times 10^{-11} \pm 0.4 \times 10^{-11}$	2.3
Bif3	$7.4 \times 10^{-10} \pm 0.3 \times 10^{-10}$	74.0
H407	$9.2 \times 10^{-10} \pm 0.8 \times 10^{-10}$	92.0

[a] Values are average of 10 insect preparations per strain.
[b] Rf is the resistance factor of nerve preparations compared with the susceptible Goldsboro strain.

Table 5. Summary of possible resistance mechanisms segregating in 15 strains of *Blattella germanica*

Strain	Elevated GST	Elevated esterase	Elevated oxidase	Synergism (PB)		kdr
				Lambda	OP/Carb	
WHO	(a)	(a)	-	-	-	-
Goldsboro	(a)	(+)	a	a	a	a
Suf2	(+)	(+ +)	+ + +	+	(+)	-
E818	(a)	(+)	+	a	(+)	-
Bif3	(a)	(a)	+ +	+	(+)	-
Kansas	(+ +)	(+ +)	a	a	(a)	-
PQHoc	(a)	(+ +)	a	a	(a)	-
New Jersey	(+ +)	(+ +)	±	a	(+)	-
Seasons	(a)	(+ +)	+	+	(a)	-
H407	(+)	(+)	+	a	(+)	+
Navy	(+)	(+ +)	±	a	(a)	a
Pulaski	(a)	(+)	a	a	-	-
Dubai	(a)	(a)	+	a	(+)	-
El Paso	(a)	(a)	+	a	(+)	-
KoF4	(a)	(+ +)	+ +	+	(+)	-
EC176-C	(a)	(+ +)	+ +	+	(+)	-
Purdue	(+ +)	(+ +)	+	a	(a)	-

a not present
± - + + + indicates present at increasing levels of elevation
( ) indicates cumulative data from this paper and Hemingway et al. (in press)
Lambda is PB synergism with lambda cyhalothrin
OP/carb PB synergism with chloropyrifos and/or propoxur
- not tested

Table 5 summarizes the combinations of resistance mechanisms found in the 15 strains assayed. Six strains had only one resistance mechanism, the other nine had two or more resistance mechanisms.

DISCUSSION

This study was prompted in part by widespread reports of pyrethroid resistance in *B. germanica*, particularly in the USA. Strains were obtained from over thirty locations where resistance had been reported. However, on the basis of residual contact insecticide bioassays with two pyrethroids, little or no (< 2-fold) resistance could be detected in late first nymphs in at least half the strains.

In the 15 strains selected for further study, resistance to cyfluthrin, fenvalerate, cypermethrin and lambda cyhalothrin was starting to develop. Resistance levels in this study were determined by mortality of first nymphal instars 48 h after insecticide exposure by tarsal contact. Recovery at 48 h was used because nymphs occasionally recovered between 24h and 48h. The same strains were segregating for intermediate and high level chlorpyrifos and propoxur resistance. Our results indicate that operational resistance to chlorpyrifos and propoxur is widespread in feral cockroach populations. Chemical based control programs are increasingly likely to fail unless strategies for insecticide use which do not rely wholly on these type of compounds are followed. Our analysis of the mechanisms involved in resistance suggested involvement of altered acetylcholinesterase, esterase and oxidase enzyme systems. Elevated glutathione-*S*-transferase activity was segregating in a number of strains but did not confer significant levels of resistance.

Nerve insensitivity to pyrethroids was found in the two strains where it was predicted (on the basis of bioassays and biochemical assays) and not in two strains where it was not predicted. If this is extrapolated to the data on the other 11 resistant strains, then elevated esterase and cytochrome P^{450} mechanisms are more prevalent than *kdr*-type resistance. Preliminary polyacrylamide gel electrophoresis data on the cockroach strains with elevated esterase activity showed that esterases with high staining intensity varied between strains. Most of these pyrethroid resistant strains are also resistant to chlorpyrifos and propoxur. Some of the elevated enzyme levels observed are probably primarily related to organophosphorus resistance, and carbamate resistance (or both) rather than pyrethroid resistance.

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A number of control strategies for delaying the onset or spread of resistance have been modelled (Curtis, 1985, Tabashnik, 1989, MacDonald *et al*, 1983, Mani, 1985). Resistance, once at a level in the population that can be readily detected, will spread rapidly if the same selection pressure is maintained. The rate of increase in resistance is dependent principally on the dominance of the resistance gene(s), the length of generation time and the degree of insecticide exposure in the population. Insects such as *B. germanica* with a long generation time should develop resistance more slowly than insects such as mosquitoes with a rapid generation time. However, the rate at which resistance has been observed to develop in *B. germanica* populations suggests that the majority of individuals in the population are subjected to pesticide selection. This is not surprising, as the populations treated tend to be discrete ones delimited by the building which they occupy.

The spread of resistance may be delayed if two insecticides are used as mixtures or in spatial or temporal mosaics. For these insecticides to be effective in delaying resistance they must not share common metabolic routes or target sites, as cross-resistance will make the strategy ineffectual. This limits the number of insecticides which can be used together.

Rotations of insecticides to delay the onset or spread of resistance have been used effectively in the Onchocerciasis control programme (OCP) in West Africa to prolong the effective lifespan of the organophosphorus insecticide, temephos (Kurtak *et al*, 1987). Rotations rely on the premise that, in the absence of positive selection pressure, resistance to an insecticide will decline due to reduced fitness associated with it. In the OCP programme insecticides are used non-residually, unlike the situation with cockroach control. Where residual insecticides are rotated it is deleterious if insecticide A is allowed to decay during the period of treatment with insecticide B to a point where there is prolonged positive selection for heterozygotes resistant to A, which would have been killed by a fresh deposit of insecticide A. To avoid this possibility, insecticide mixtures, rather than rotations, may be preferable for cockroach control.

Theoretical studies on the use of mixtures suggest that, for them to be effective, various conditions must be met. As well as the lack of cross-resistance these include: resistance to each pesticide being functionally, partially or fully recessive; resistance genes not being linked together on the same chromosome; a low initial frequency of one or both resistances so that doubly resistant individuals are rare; a small population or the presence of refugia so that a proportion of the population remains untreated, and an equal persistence of both insecticides.

Where it is proposed to control a population with a mixture which has already developed partial resistance to extend the life of the compound already in use, there should ideally be no resistance to the second insecticide. This is particularly pertinent in small defined populations such as *Blattella*, as selection of such populations with two insecticides, to which a proportion of the population is already doubly resistant, will rapidly result in the build up of linkage disequilibrium (i.e. non-random association of two alleles at two loci). In this case the mixture will quickly become more effective than either insecticide alone, but will be more expensive. The rate at which disequilibrium is established is dependent both on the proportion of the population exposed to the insecticide and on the dominance of the resistance alleles, where the build up of linkage disequilibrium is fastest when both resistances are fully or co-dominant (Curtis, 1985, Mani, 1985).

It has been suggested that resistant *B. germanica* populations could be controlled by a strategy of alternating pyrethroid and organophosphorus insecticides or using them as a mixture (Cochran, 1990). This strategy is unlikely to delay resistance in *B. germanica* from many parts of the world, on the basis of data presented here. as, where resistance gene frequencies are already relatively high to both insecticides, selection with a mixture or rotation would rapidly increase the resistance level to both compounds, as doubly resistant individuals will already be present at significant frequencies.

In some of the strains of *B. germanica* tested changes in the multi-function oxidase system form the basis of the only pyrethroid resistance mechanism segregating. In these strains it would be possible to use a synergist/insecticide combination to overcome resistance and thus allow continued effective pyrethroid control. Synergists such as piperonyl butoxide are readily available, and could be used in formulation with a number of pyrethroids. This strategy would not, however, work if other resistance mechanisms were also segregating in the population, as has been demonstrated in many of these strains. There is also the obvious danger that prolonged pressure with a synergist/ insecticide mixture will select mechanisms, such as kdr-type resistance, which are unaffected by the synergist.

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Insect growth regulators with juvenile hormone like activity, such as fenoxycarb, hydroprene and pyriproxyfen, have been introduced to the public health insect control market over the last few years, and both fenoxycarb and hydroprene are registered for *B. germanica* control. These growth regulators interfere specifically in *B. germanica* with embryonic development, maturation of the last nymphal stage and, when present over the sensitive developmental window in the last instar, induce morphogenetic abnormalities and disrupt the reproductive physiology of the adult female (Das & Gupta, 1974, King & Bennett 1989). These compounds at high dose rates are toxic to the insects, but the dose of fenoxycarb required to sterilise *B. germanica* is 10 to 20 times less than that required to kill young nymphs.

There are two major objections to the use of a growth regulator alone as a chemosterilant. Firstly, the average lifespan of treated adults may be extended from 6–28 weeks (King & Bennett, 1989) and the public is unlikely to perceive this as effective control. Secondly, resistance may also be selected over time, as has occurred with other groups of insects. For example, resistance to the insect growth regulator diflubenzuron has already been reported in the codling moth, *Cydia pomonella*, in the USA.

An alternative strategy would be to use the insect growth regulator mixed with a pyrethroid, which should benefit the long term prospects of both compounds. The pyrethroid would cause an immediate decline in the cockroach numbers by removing susceptible individuals. The remaining pyrethroid resistant individuals would be controlled by the growth regulator, which would prevent an increase in the pyrethroid resistance gene frequency in the population and extend the period of effective use of the pyrethroids.

In putting forward this type of strategy we need to determine whether the theoretical criteria for successful use of a mixture are met. Although the two groups of compounds have completely different target sites, removing the possibility of cross-resistance from kdr-like pyrethroid resistance mechanisms, it is possible that they share common metabolic detoxication enzymes. Oxidation has already been shown to be one of the major pyrethroid resistance pathways in *B. germanica*, and the structure of many of the juvenoids suggests that they may also be susceptible to oxidation. However, information on resistance in other species suggests that oxidative pathways differ between groups of insecticides and it is likely that these two groups are dissimilar enough to require different oxidative enzymes. This should be determined before any large scale use of mixtures of pyrethroids and juvenoids are used against *B. germanica* populations which have oxidase-based pyrethroid resistance. The principle of using a mixture is dependent on doubly resistant individuals being much rarer than the insects in refugia which escape exposure and, therefore, are not selected by either insecticide. In the case of *B. germanica* control which combines small population numbers with high insecticide coverage and very rare (undetected) insect growth regulator resistance, doubly resistant individuals may not exist in many populations and temporary eradication could be achieved in some buildings. The insect growth regulator is also likely to decay more slowly than the pyrethroid which favours this management strategy, as only the extremely rare growth regulator resistant homozygotes are able to survive the treatment at all times.

In summary, pyrethroid susceptible *B. germanica* populations or those with low (<2-fold) levels of resistance can still be controlled by residual pyrethroid treatment, although their efficacy should be carefully monitored to ensure that control is maintained. If resistance at a low frequency is suspected, treatment should be undertaken with a pyrethroid, such as cypermethrin or lambda cyhalothrin, in combination with a juvenoid such as fenoxycarb or pyriproxyfen. The dose of pyrethroid should be sufficient to kill resistant heterozygotes and homozygote susceptibles, and the dose of juvenoid should be sufficient to sterilise any remaining cockroaches. Where there is a moderate level of resistance to pyrethroids this strategy would prevent a further increase in pyrethroid resistance, but unacceptable numbers of cockroaches may remain for some weeks after treatment, and although these would be sterile, the treatment may be perceived to have failed. To avoid this, the pyrethroid could be used in combination with piperonyl butoxide as well as the juvenoid. This would render any oxidase-based pyrethroid mechanism ineffective and would increase the initial knock-down rate of the population which should increase the perceived efficacy of the treatment.

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