Bulletin of Entomological Research (1996) **86**, 675–682

Insecticide resistance and synergism in field collected German cockroaches (Dictyoptera: Blattellidae) in Peninsular Malaysia

C.Y. Lee, H.H. Yap,* N.L. Chong

Vector Control Research Unit, School of Biological Sciences,
Universiti Sains Malaysia, Penang, Malaysia

R.S.T. Lee

Pest Dynamics Pte Ltd., Taman Shamelin Perkasa, Kuala Lumpur, Malaysia

Abstract

Twelve strains of German cockroach, *Blattella germanica* (Linnaeus) were collected from various locations in Peninsular Malaysia and tested for their susceptibility to three groups of insecticides applied topically. The levels of resistance were low to high (2.8 to 92×) for carbamates (propoxur and bendiocarb), low (2.0 to 7.6×) for organophosphate (chlorpyrifos) and low to moderate (1.0 to 23×) for pyrethroids (cypermethrin and permethrin) when compared to a susceptible strain. Five strains, resistant to both cypermethrin and permethrin, were also resistant to DDT, phenothrin and deltamethrin. Propoxur resistance in ten strains was suppressed with the synergists, piperonyl butoxide (PBO) and *S,S,S*-tributylphosphorotrithioate (DEF®), suggesting monooxygenase and esterase involvement in the resistance. However, the levels of resistance for cypermethrin and permethrin were not affected when using either PBO or DEF®.

Introduction

Extensive usage and heavy reliance on insecticides have led to the development of insecticide resistance in the German cockroach, *Blattella germanica* (Linnaeus) (Dictyoptera: Blattellidae). Insecticide resistance is a major threat to chemical and pest control industries worldwide. Since resistance to chlordane in the German cockroach was first detected in Corpus Christi, Texas, USA in 1952 (Heal *et al.*, 1953), an increasing number of cases have been documented in the USA (Bennett & Spink, 1968; Cochran, 1989; Rust & Reierson, 1991; Zhai & Robinson, 1991), Canada (Batth, 1977), Europe (Chapman *et al.*, 1993; Jensen, 1993) and Japan (Umeda *et al.*, 1988). Currently, resistance to all the major groups of insecticide (organochlorines, organophosphates, carbamates and pyrethroids) in *B. germanica* has been reported (Cochran, 1995). Increased tolerance and potential resistance to other novel insecticides such as sulfluramid (Schal, 1992) and abamectin (Cochran, 1994) along with behavioural changes in responding to glucose attractant (glucose-aversion) in cockroach bait (Silverman & Ross, 1994) have been reported recently.

Many synergists such as the methylenedioxyphenyls (e.g. piperonyl butoxide and sesamex) and phosphorothioates (*S,S,S*-tributylphosphorotrithioate or DEF®) used at non-toxic doses are known to inhibit monooxygenase and esterase-based resistance mechanisms, respectively (Sun & Johnson, 1960; Casida, 1970; Scott, 1990). Synergists may be used to gain preliminary information on possible metabolic resistance mechanisms involved in resistant strains; however, biochemical, metabolism and electrophysiological studies are still needed to provide direct evidence for the mechanisms involved (Scott, 1990).

Cochran (1987) reduced the resistance ratios drastically in some bendiocarb- and pyrethrin-resistant strains of *B. germanica* with piperonyl butoxide (PBO), thus indicating that an enhanced monooxygenase system is involved in

*Author for correspondence: Professor H.H. Yap, Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang Malaysia.

the resistance. Through the use of either PBO or DEF, Scott *et al*. (1990) managed to partially suppress bendiocarb resistance in the Kenly and Rutgers strains of the German cockroach and suggested that both oxidative and hydrolytic metabolism were involved in the resistance. Permethrin and cypermethrin resistance in a highly pyrethroid-resistant strain (Village Green) was partially suppressed with PBO and DEF®, thus suggesting the involvement of enhanced metabolism as well as target site insensitivity in the mechanism of resistance (Atkinson *et al.*, 1991).

Although insecticide resistance in *B. germanica* has been reported in many parts of the world, it has not been documented in the South East Asia region. Baygon® EC (containing 20% propoxur w/w) has been used as the major insecticide formulation for German cockroach control by pest control operators (PCOs) in Malaysia over the past two decades since control failures by organochlorines were observed. In recent years, numerous complaints were received from local PCOs that Baygon® EC no longer provided effective control against German cockroaches. Propoxur resistance was suspected to occur in the insect due to the high frequency of application and long-term usage of Baygon® EC.

This study characterizes the resistance profile of *B. germanica*, collected from different parts of Peninsular Malaysia, to some commonly used insecticides. In addition, the synergists (PBO and DEF®) were used to suggest the possible underlying resistance mechanisms in those strains under study.

Materials and methods

Cockroach strains

Twelve strains of the *B. germanica* were collected in Peninsular Malaysia (table 1). Collections were made by PCOs through the use of glass jar traps (0.5 l) with the upper inside surface smeared with Vaseline® and baited with a piece of white bread. The collected cockroaches were reared at 26 ± 2°C; 60 ± 5% r.h. with a 12 h photoperiod. Between generations 2–5, the adults were used for insecticide susceptibility and synergism bioassays. A known highly insecticide susceptible strain (ICI) originating from Zeneca Agrochemicals, United Kingdom, was used as a standard. Before insecticidal testing, the cockroaches (aged 3–14 days) were weighed in groups of ten individuals and the mean mass per individual was generated.

Chemicals

Technical grade insecticides used for baseline susceptibility tests and synergism studies were: propoxur (99.5%), chlorpyrifos (97.4%), cypermethrin (94.5%), bendiocarb (95.0%), permethrin (95.1%), deltamethrin (99.8%), phenothrin (94.2%) and DDT (>99.8%). Two synergists were used in this study: piperonyl butoxide (PBO) (99.0%), a monooxygenase inhibitor and *S,S,S*-tributylphosphorotrithioate (DEF) (98.3%), a hydrolase inhibitor. All insecticides and synergists used were diluted in analytical grade acetone.

WHO glass jar test

2.5 ml of 0.1% propoxur (in acetone) was pipetted into a 0.5 l glass jar (surface area=336.5 cm^2) with its upper inside lip smeared with petroleum jelly. The insecticide was deposited evenly in the inner jar surface by rolling the jar on a flat surface until all the acetone had evaporated. Ten adult males were introduced into the jar and knockdown of the cockroaches was recorded at selected time intervals. Ten replicates were carried out for each strain tested (ICI, Kuantan, LipSin, Lumut, TsimShaShui).

Topical bioassay

Ten adult male cockroaches were anaesthetized with carbon dioxide (pressure: 20 kpa) for 20 seconds prior to treatment. When immobilized, each individual was topically treated with 1 μl of a known concentration of insecticide on the first abdominal segment, using a Burkard's micro applicator (Burkard Scientific Ltd., Middlesex, UK) fitted with a 1-ml hypodermic glass syringe and a size-27 gauge needle.

For synergism studies, PBO and DEF at concentrations of 100 μg/μl and 30 μg/μl, respectively, were applied in the form of a band along the abdomen. This was done approximately 2 h prior to treatment with the insecticides propoxur, cypermethrin or permethrin. The concentrations of synergist chosen were approximately one-third of the concentration which caused <3% mortality of the ICI

Table 1. Information on susceptible and field collected *Blattella germanica* used in this study.

Premise	Strain	Collection site	Origin[1]	Date	Treatment history (last year prior to collection)	Treatment frequency
–	ICI (susceptible)	–	Zeneca, UK	Oct 93	–	–
Hotel	GoldenSand	Main kitchen	Penang	Dec 93	Baygon® EC	once/month
	Melia I	Main kitchen	Kuala Lumpur	Mar 94	Baygon® EC	once/month
	Melia II	Main kitchen	Kuala Lumpur	Jun 94	Baygon® EC, ICON® EC	twice/month
	Melia III	Oven	Kuala Lumpur	Aug 94	Baygon® EC, ICON® EC	twice/month
	Subang	Main kitchen	Kuala Lumpur	Mar 94	Baygon® EC	once/month
Food outlets	ChilliPadi I	Main kitchen	Kuala Lumpur	May 94	Baygon® EC	once/month
	ChilliPadi II	Main kitchen	Kuala Lumpur	Aug 94	Baygon® EC, ICON® EC	once/month
	LipSin	Dining area	Penang	Feb 94	unknown	twice/year
	HangTuah	–	Kuala Lumpur	Aug 94	Baygon® EC, ICON® EC	once/month
Theatre/ Karaoke Centre	TsimShaShui	–	Kuala Lumpur	May 94	Baygon® EC, ICON® EC	once/month
Naval ships	Lumut	–	Lumut, Perak	Jan 94	Baygon® EC	twice/year
	Kuantan	–	Kuantan, Pahang	Jan 94	Baygon® EC	twice/year

[1]All collection sites are in Peninsular Malaysia.

susceptible strain, to achieve maximum inhibition of the metabolic enzymes without causing any mortality to the insects (Scott, 1990).

A series of four to six doses spanning the range of 5–95% mortality for each insecticide was tested on each strain. Each test was replicated three to five times. After treatment, the insects were kept in clean polyethylene petri dishes lined with filter paper with ten individuals per container and provided with food and water. Mortality was scored at 48 h post treatment. Cockroaches were considered dead when they were unable to right themselves to a normal posture within 2 minutes when touched on their abdomen with a pair of forceps.

Data analysis

Results from all replicates for each insecticide were pooled and subjected to probit analysis (Finney, 1971) using a computer program developed by Daum (1970). A minimum of 120 insects (30 insects per dose) were used in the analysis to ensure reliability in the estimation of LD_{50} and LD_{90} values (Robertson *et al.*, 1984). All LD_{50} values were converted from μg/insect to μg/g insect to avoid the possible effect of weight differences on insecticide susceptibility. The resistance ratio at LD_{50} (RR_{50}) was calculated by dividing the LD_{50} values of the field strains by the corresponding lethal dose for the ICI susceptible strain.

Results and discussion

The topical bioassay method was chosen for detecting resistance as it was found to be more sensitive than the conventional WHO glass jar residue method (Scott *et al.*, 1986; Milio *et al.*, 1987; Wadleigh *et al.*, 1989; Scharf *et al.*, 1995). A preliminary study comparing the two techniques with propoxur showed that some strains exhibited a lower resistance level by topical bioassay than by the glass jar method. Also, three strains (Kuantan, Lumut and TsimShaShui) showed a different behavioural response to propoxur in the glass jar test. Adult males, when exposed to a propoxur- treated surface remained relatively immobile. This behaviour reduced insecticidal pickup by the tarsal pads and resulted in extended knockdown and kill times (LT_{50} of >120 min for all three strains). The Kuantan and Lumut strains with propoxur resistance ratios of 4.6 and 2.8× by the topical bioassay method, respectively, exhibited RR_{50} of $>10\times$ by the glass jar test method. By contrast, cockroaches of the LipSin strain, which moved around more readily in the jar, exhibited knockdown in a shorter period of time; this behaviour was similar to that of the susceptible strain (LT_{50} for LipSin strain=13.9 min; LT_{50} for ICI susceptible strain=12.1 min).

Earlier, Zhai & Robinson (1992) demonstrated that the amount of walking influenced the rate of knockdown of German cockroaches placed on a cypermethrin-treated surface. They concluded that, in order to obtain a reliable measurement of resistance, both susceptible and field strains should exhibit a similar degree of mobility when placed on a treated surface. In view of this, the topical bioassay method was chosen to avoid behavioural differences interacting with physiological resistance in this study.

Low to high levels of carbamate resistance to the two carbamates tested were recorded in the present study. Propoxur RR_{50} ranged from 2.8 to 92× at LD_{50} values

Table 2. Susceptibility of field collected strains of *Blattella germanica* to propoxur and bendiocarb.

Strain	propoxur					bendiocarb				
	n	48-h LD_{50} (95% FL) μg/g	Slope ± SE	RR_{50}	χ^2 (df)	n	48-h LD_{50} (95% FL) μg/g	Slope ± SE	RR_{50}	χ^2 (df)
ICI (susceptible)	200	9.5 (8.9–10)	3.42 ± 0.23	–	0.27 (3)	120	16 (14–18)	2.93 ± 0.21	–	2.13 (2)
ChilliPadi I	120	350 (306–392)	1.99 ± 0.16	36.8	3.12 (2)	150	770 (700–850)	2.55 ± 0.22	48.1	0.06 (3)
ChilliPadi II	140	320 (280–380)	1.47 ± 0.14	33.7	0.45 (3)	–	–	–	–	–
GoldenSand	160	170 (160–180)	2.60 ± 0.25	17.9	0.12 (2)	150	480 (440–530)	2.42 ± 0.17	30.0	1.26 (3)
HangTuah	150	380 (340–420)	2.28 ± 0.17	40.0	2.86 (3)	150	>990 (23.3%)[1]	–	>62	–
Kuantan	120	44 (39–50)	2.10 ± 0.16	4.6	1.77 (2)	150	82 (73–92)	1.82 ± 0.14	5.1	2.34 (3)
LipSin	150	62 (56–67)	2.57 ± 0.17	6.5	3.32 (3)	150	140 (130–150)	2.25 ± 0.17	8.8	0.47 (3)
Lumut	120	27 (21–31)	1.90 ± 0.27	2.8	0.85 (2)	190	59 (52–66)	1.66 ± 0.12	3.7	0.20 (4)
Melia I	130	630 (520–860)	1.42 ± 0.19	66.3	2.46 (2)	150	>950 (33.3%)[1]	–	>59.4	–
Melia II	150	510 (450–570)	2.11 ± 0.12	53.7	6.92 (3)	120	>960 (26.7%)[1]	–	>60.0	–
Melia III	150	94 (84–105)	1.93 ± 0.13	9.9	2.13 (3)	150	260 (230–300)	1.77 ± 0.12	16.3	0.29 (3)
Subang	120	870 (720–1200)	2.00 ± 0.26	91.6	0.12 (2)	150	>850 (20.0%)[1]	–	>53.1	–
TsimShaShui	150	71 (61–80)	2.22 ± 0.20	7.5	0.91 (3)	150	180 (160–190)	2.48 ± 0.15	11.3	0.53 (3)

[1]Highest dose tested=50 μg/insect. Value in parentheses indicates percentage mortality of cockroaches when tested with this dose.

Table 3. Susceptibility of field collected strains of *Blattella germanica* to chlorpyrifos.

Strain	n	48-h LD_{50} (95% FL) μg/g	Slope ± SE	RR_{50}	χ^2 (df)
ICI (susceptible)	150	5.0 (4.9–5.2)	7.59 ± 0.46	–	3.10 (3)
ChilliPadi I	120	24 (23–25)	6.66 ± 0.35	4.8	0.33 (2)
ChilliPadi II	120	28 (26–30)	3.96 ± 0.43	5.6	0.52 (2)
GoldenSand	120	30 (28–32)	4.56 ± 0.29	6.0	2.54 (2)
HangTuah	180	21 (20–22)	4.45 ± 0.23	4.2	5.81 (4)
Kuantan	120	15 (14–16)	6.00 ± 0.48	3.0	3.04 (2)
LipSin	120	21 (20–22)	5.81 ± 0.39	4.2	0.04 (2)
Lumut	120	15 (14–16)	5.49 ± 0.46	3.0	1.67 (2)
Melia I	120	33 (31–36)	4.43 ± 0.36	6.6	0.81 (2)
Melia II	200	21 (19–23)	3.52 ± 0.27	4.2	0.79 (2)
Melia III	120	12 (11–14)	3.34 ± 0.36	2.4	1.53 (2)
Subang	150	38 (35–42)	2.62 ± 0.21	7.6	4.07 (3)
TsimShaShui	140	9.8 (8.4–11)	3.25 ± 0.31	2.0	0.54 (2)

above. The two strains from the naval ships (Lumut and Kuantan) exhibited low levels of resistance to propoxur (<5×). These cockroach populations were subjected to a low frequency of insecticide application (bi-yearly) while other populations were subjected to monthly or biweekly applications.

Baygon® EC was used continually to control the Melia strain, but was replaced with Icon® EC (containing lambda cyhalothrin as active ingredient) shortly after the collection of Melia I. Melia II and Melia III were collected after six and ten applications of lambda cyhalothrin, respectively. The three strains of *B. germanica* collected from hotel Melia were cultured for two generations prior to bioassay. It was observed that propoxur RR_{50} decreased from 66× in Melia I to 54× in Melia II and 9.9× in Melia III. This showed that in the absence of propoxur selection pressure, resistance levels to propoxur can decrease. A similar example of a decline of insecticide resistance in *B. germanica* following removal of a particular insecticide was reported recently by Robinson & Zhai (1994). The authors found that when cypermethrin usage was relaxed for a period of three years and replaced with chlorpyrifos against a cypermethrin-resistant population (Robinson & Zhai, 1990), the population reduction after treatment increased from 20% to 76% when cypermethrin was used again.

Low to high levels of bendiocarb resistance were also observed with the field strains under study (table 2). Resistance ratios ranged from 3.7 to >62 at LD_{50}. The exact LD_{50} and RR_{50} values for four strains – Melia I, II, HangTuah and Subang, were not determined due to blockage of the applicator needle by bendiocarb (>50 μg/μl) due to acetone evaporation.

All field strains exhibited a low level of resistance (<10×) to chlorpyrifos (table 3). The resistance ratio at the LD_{50} ranged from 2.0× in the TsimShaShui strain to 7.6× in the Subang strain. Reierson *et al.* (1988) have suggested that a 10× resistance as measured by topical application is the critical point above which operational control failures are likely to occur. Inconsistent control levels were achieved by Reierson *et al.* (1988) with resistance levels of 5–10×, while good control was obtained at RR_{50} of 5× and below. Thus, the choice of organophosphate insecticides – chlorpyrifos and pirimiphos-methyl, may still provide adequate control of those strains with low resistance levels.

The resistance ratios to cypermethrin ranged from 1.2 to 22× (table 4). Six out of twelve field collected strains (ChilliPadi I, HangTuah, Melia I, Melia II, Melia III and Subang) tested had resistance ratios of 10× or more. The HangTuah strain exhibited the highest resistance level (RR_{50}=22.5). When tested against permethrin, resistance ratios ranged from 1.0 to 15× (table 5). Only nine strains were tested.

The ChilliPadi I, Melia I, II and III and TsimShaShui strains were also resistant to DDT, deltamethrin and phenothrin (table 5). The exact LD_{50} for DDT for all the strains tested was not determined because of needle blockage when concentrated solutions were dispensed.

Cross resistance often occurs between insecticides which are chemically related or which share a common mode of action. Although the carbamate bendiocarb has not been used for German cockroach control in Malaysia, most of the field collected strains were already resistant to it, probably due to the long term usage of Baygon® EC. Some strains of *B. germanica* demonstrated low to moderate levels of resistance to chlorpyrifos and cypermethrin. These chemicals were only recently introduced for public health usage in Malaysia in 1994 and have not been used commercially against all of the field strains collected. Other cases of cross resistance in *B. germanica* have been reported. A carbaryl-resistant strain from the USA showed cross resistance to other carbamates (McDonald & Cochran, 1968). A highly bendiocarb-resistant strain collected from Baltimore, Maryland, USA was also resistant to several other insecticides including chlordane, diazinon, malathion and propoxur (Nelson & Wood, 1982).

Levels of propoxur resistance in field collected strains were reduced when propoxur was used with either PBO or DEF (table 6). All strains with RR_{50} of propoxur of >30× (ChilliPadi I, Melia I and Melia II and HangTuah) were reverted to <10× by PBO and DEF. For example, the RR_{50} of Melia I for propoxur was reduced from 66 to 4.0× with PBO and to 8.0× with DEF. The synergism of propoxur in the Subang strain was not determined, as its resistance levels reverted significantly during this study. Synergism with PBO and DEF indicated the possible involvement of monooxygenases and esterases in propoxur resistance. These findings were similar to those of Siegfried & Scott (1991), where propoxur resistance in *B. germanica* was only partially suppressed by both PBO and DEF. Total non-specific esterases activity in some of the resistant strains (Melia II, ChilliPadi I, TsimShaShui and HangTuah) was also higher than in the susceptible strain (CY Lee & J. Hemingway, unpublished data).

The activity of cypermethrin and permethrin against *B. germanica* was synergized with either PBO or DEF in ChilliPadi I, Melia I, II and III and TsimShaShui strains

Table 4. Susceptibility of field collected strains of *Blattella germanica* to cypermethrin and permethrin.

Strain	cypermethrin					permethrin				
	n	48-h LD_{50} (95% FL) μg/g	Slope ± SE	RR_{50}	χ^2 (df)	n	48-h LD_{50} (95% FL) μg/g	Slope ± SE	RR_{50}	χ^2 (df)
ICI (susceptible)	150	1.2 (1.2–1.3)	6.50–0.43	–	5.97 (3)	150	4.6 (4.3–4.9)	3.40 ± 0.20	–	2.77 (3)
ChilliPadi I	120	10 (9.3–11)	3.29 ± 0.30	8.3	4.88 (3)	130	67 (62–72)	3.19 ± 0.22	14.6	0.91 (2)
ChilliPadi II	200	18 (17–19)	3.45 ± 0.19	15.0	1.82 (2)	–	–	–	–	–
GoldenSand	150	1.6 (1.6–1.7)	6.64 ± 0.43	1.3	4.98 (3)	120	5.5 (5.2–5.8)	4.69 ± 0.32	1.2	0.26 (2)
HangTuah	190	27 (23–34)	2.21 ± 0.22	22.5	2.49 (4)	–	–	–	–	–
Kuantan	130	1.6 (1.5–1.7)	3.53 ± 0.31	1.3	0.21 (2)	160	5.1 (4.9–5.3)	5.68 ± 0.35	1.1	3.69 (2)
LipSin	160	10 (9.7–11)	4.35 ± 0.27	8.3	1.01 (2)	–	–	–	–	–
Lumut	130	1.4 (1.3–1.4)	5.65 ± 0.36	1.2	0.06 (2)	120	4.6 (4.4–4.8)	6.72 ± 0.55	1.0	0.95 (2)
Melia I	160	18 (17–19)	4.23 ± 0.25	15.0	2.25 (2)	120	44 (41–47)	3.39 ± 0.22	9.6	3.81 (2)
Melia II	120	16 (15–17)	4.80 ± 0.34	13.3	3.53 (2)	140	65 (61–69)	4.38 ± 0.42	14.1	2.91 (2)
Melia III	120	13 (12–14)	4.75 ± 0.37	10.8	1.01 (2)	120	52 (48–55)	4.77 ± 0.42	11.3	0.15 (2)
Subang	120	12 (9.9–13)	2.09 ± 0.30	10.0	0.67 (2)	120	53 (49–57)	3.53 ± 0.27	11.5	0.29 (2)
TsimShaShui	160	7.3 (6.7–7.9)	2.84–0.19	5.9	2.29 (3)	120	34 (28–42)	1.21 ± 0.18	7.4	0.62 (2)

Table 5. Susceptibility of some field collected strains of *Blattella germanica* to DDT and selected pyrethroids.

Insecticide	Strain	n	48-h LD_{50} (95% FL) μ/g	Slope ± SE	RR_{50}	χ^2 (df)
DDT	ICI	140	620 (590–660)	4.23 ± 0.28	–	0.61 (2)
	ChilliPadi I	120	> 4000 (16.7%)[1]	–	> 6.5	–
	Melia I	120	> 3800 (30.0%)[1]	–	> 6.1	–
	Melia II	120	> 3860 (0%)[1]	–	> 6.2	–
	Melia III	120	> 3780 (0%)[1]	–	> 6.1	–
	TsimShaShui	120	> 3910 (0%)[1]	–	> 6.3	–
phenothrin	ICI	170	5.2 (3.1–7.1)	3.73 ± 0.75	–	10.48* (3)
	ChilliPadi I	170	270 (260–290)	4.00 ± 0.33	51.9	2.86 (3)
	Melia I	120	69 (61–78)	2.11 ± 0.18	13.3	1.71 (2)
	TsimShaShui	180	80 (68–93)	1.27 ± 0.13	15.4	0.68 (4)
deltamethrin	ICI	120	0.22 (0.21–0.23)	5.86 ± 0.54	–	1.59 (2)
	ChilliPadi I	120	5.2 (4.6–6.5)	3.33 ± 0.41	23.6	1.59 (2)
	Melia I	120	1.4 (1.3–1.5)	4.12 ± 0.33	6.4	0.51 (2)
	Melia II	120	4.4 (4.0–4.7)	4.35 ± 0.43	20.0	0.16 (2)
	TsimShaShui	150	1.3 (1.1–1.4)	1.78 ± 0.14	5.9	1.11 (3)

[1]Percentage mortality upon treatment with 200 μg/insect was shown in parentheses.
*Indicated lack of fit ($P > 0.05$).

Table 6. Synergism of propoxur by piperonyl butoxide (PBO) and *S,S,S*-tributylphosphorotrithioate (DEF) against field collected strains of *Blattella germanica*.

Strain	synergist	n	48-h LD_{50} (95% FL) μg/g	Slope ± SE	RR_{50}	χ^2 (df)
ICI (susceptible)	PBO	120	12 (11–12)	4.19 ± 0.38	–	2.45 (2)
	DEF	120	3.5 (3.2–3.8)	3.36 ± 0.24	–	0.61 (2)
ChilliPadi I	PBO	150	26 (25–28)	4.23 ± 0.26	2.2	1.48 (3)
	DEF	120	20 (18–21)	3.36 ± 0.26	5.7	4.14 (2)
GoldenSand	PBO	120	64 (60–67)	4.88 ± 0.31	5.3	2.83 (2)
	DEF	120	12 (11–13)	4.59 ± 0.36	3.4	0.35 (2)
HangTuah	PBO	150	79 (75–84)	4.30 ± 0.24	6.6	4.09 (3)
	DEF	150	19 (18–20)	4.03 ± 0.25	5.4	3.28 (3)
Kuantan	PBO	160	18 (17–18)	7.82 ± 0.41	1.5	3.33 (2)
	DEF	200	4.2 (3.9–4.5)	2.55 ± 0.15	1.2	0.31 (3)
LipSin	PBO	120	19 (18–20)	6.75 ± 0.57	1.6	1.37 (2)
	DEF	120	6.5 (6.1–7.0)	3.83 ± 0.29	1.9	1.35 (2)
Lumut	PBO	140	19 (15–24)	3.12 ± 0.42	1.6	6.77* (2)
	DEF	150	3.4 (2.9–3.8)	3.05 ± 0.28	1.0	0.89 (3)
Melia I	PBO	160	48 (45–53)	2.48 ± 0.19	4.0	1.37 (2)
	DEF	130	28 (26–31)	3.03 ± 0.20	8.0	0.52 (2)
Melia II	PBO	120	37 (32–42)	3.28 ± 0.32	3.1	0.27 (2)
	DEF	150	32 (29–36)	2.51 ± 0.18	9.1	1.57 (3)
Melia III	PBO	140	25 (24–27)	3.01 ± 0.23	2.1	2.99 (2)
	DEF	150	20 (19–22)	3.79 ± 0.26	5.7	1.11 (3)
TsimShaShiu	PBO	160	14 (14–15)	5.84 ± 0.35	1.2	2.37 (2)
	DEF	120	14 (14–15)	5.00 ± 0.36	4.0	3.68 (2)

*Indicated lack of fit ($P > 0.05$).

(table 7). However, resistance levels to cypermethrin and permethrin in most strains tested increased with the addition of PBO and DEF. For example, in the Melia I strain, when cypermethrin was used with PBO, the resistance level increased from 15× to 27×. The five strains also showed resistance to DDT, phenothrin and deltamethrin. This suggests possible involvement of a target site insensitivity (*kdr*-type) resistance mechanism to these compounds. Scott & Matsumura (1981, 1983) reported a DDT resistant strain of *B. germanica*, selected from a laboratory culture (VPI strain) that was cross-resistant to all pyrethroids thus providing evidence for the first time of a *kdr*-type mechanism in *B. germanica*.

An increase in RR_{50} after the addition of synergists (PBO and DEF) with pyrethroids does not indicate an antagonistic action of either synergist. As the RR_{50} is generated by dividing the LD_{50} of the resistant strain with the corresponding lethal dose of the susceptible strain, the ratios can be increased if the degree of synergism in the susceptible strain is greater than that of the resistant strain. This is possible when the two strains compared are of different origins (Scott, 1990). This has also been observed in a highly pyrethroid-resistant strain of the house fly, *Musca domestica* Linnaeus (Diptera: Muscidae) where the RR_{50} for permethrin increased from 5900 to 19,000 in the presence of DEF (Scott & Georghiou, 1986). The authors suggested that the permethrin-susceptible strain had greater hydrolytic activity when compared with that of the permethrin-resistant strain.

Bioassays in the laboratory to measure resistance may not necessarily reflect or predict control failure in the field due to differences in the actual nature of insecticide exposure in the field (Hemingway *et al.*, 1993). However, they are

Table 7. Synergism of cypermethrin and permethrin by piperonyl butoxide (PBO) and *S,S,S,*-tributylphosphorotrithoate (DEF®) against some field collected strains of *Blattella germanica*.

Strain	cypermethrin						permethrin				
	synergist	n	48-h LD_{50} (95% FL) μg/g	Slope ± SE	RR_{50}	χ^2 (df)	n	48-h LD_{50} (95% FL) μg/g	Slope ± SE	RR_{50}	χ^2 (df)
ICI (susceptible)	PBO	120	0.23 (0.22–0.24)	6.12 ± 0.41	–	2.89 (2)	160	2.8 (2.7–2.9)	6.82 ± 0.37	–	3.65 (2)
	DEF	120	0.70 (0.67–0.73)	7.96 ± 0.53	–	0.43 (2)	120	2.1 (2.0–2.2)	6.12 ± 0.40	–	0.02 (2)
ChilliPadi I	PBO	120	5.6 (5.2–5.9)	4.05 ± 0.36	24.3	1.81 (2)	160	48 (43–53)	2.30 ± 0.18	17.1	1.45 (2)
	DEF	150	9.2 (8.6–9.7)	3.73 ± 0.24	13.1	1.60 (3)	150	45 (40–50)	1.98 ± 0.17	21.4	0.38 (3)
Melia I	PBO	200	6.3 (6.0–6.5)	5.05 ± 0.25	27.4	0.93 (3)	190	47 (44–50)	2.93 ± 0.19	16.8	1.25 (3)
	DEF	120	10 (9.6–11)	4.71 ± 0.36	14.3	2.31 (2)	200	31 (29–33)	3.21 ± 0.16	14.8	2.47 (3)
Melia II	PBO	180	4.9 (4.7–5.1)	4.41 ± 0.25	21.3	7.91 (4)	130	48 (45–51)	3.90 ± 0.25	17.1	3.47 (2)
	DEF	185	16 (15–17)	4.17 ± 0.23	22.9	4.61 (4)	120	45 (42–48)	3.69 ± 0.26	21.4	0.45 (2)
Melia III	PBO	140	5.6 (5.3–5.9)	5.32 ± 0.34	24.3	4.28 (3)	140	50 (48–53)	4.83 ± 0.30	17.9	0.53 (2)
	DEF	120	9.6 (9.1–10)	5.30 ± 0.45	13.7	2.65 (2)	–	–	–	–	–
TsimShaShui	PBO	150	1.9 (1.7–2.1)	2.06 ± 0.16	8.3	3.56 (3)	120	43 (38–47)	2.43 ± 0.26	15.4	0.84 (2)
	DEF	120	4.9 (4.2–5.7)	1.60 ± 0.17	7.0	1.43 (2)	140	30 (26–34)	1.65 ± 0.15	14.3	3.09 (2)

useful in providing an indication of resistance development in field populations of the German cockroach studied. In this study, a topical application method was chosen rather than a residual contact method (although the latter may be more realistic in reflecting field conditions) due to the problem of overestimating resistance levels by the latter method when some cockroaches showed differences in behavioural response to insecticides. The method was also relatively easy to carry out and provided accurate data for comparing resistance levels between strains.

This is the first documented report of broad spectrum resistance in *B. germanica* in South East Asia. Further studies on the biochemical/physiological basis of the observed resistance are being undertaken to further substantiate current findings.

Acknowledgements

The authors are grateful to J. Hemingway (University of Wales, Cardiff), K.H. Tan (Universiti Sains Malaysia) and M.E. Scharf (Purdue University) for their constructive criticisms on this manuscript; S.Y. Lim and E. Bakar for their assistance with the rearing of German cockroaches, and the chemical companies (AgrEvo, Singapore; Bayer AG, Germany; CCM Bioscience, Malaysia; DowElanco, Malaysia; Miles Agriculture Division, Kansas City, Missouri, USA and Sumitomo Chemicals, Japan) for the supply of technical grade insecticides and synergists. This paper constitutes a portion of the PhD thesis of the senior author submitted to the Graduate School, Universiti Sains Malaysia.

References

Atkinson, T.H., Wadleigh, R.W., Koehler, P.G. & Patterson, R.S. (1991) Pyrethroid resistance and synergism in a field strain of the German cockroach (Dictyoptera: Blattellidae). *Journal of Economic Entomology* **84**, 1247–1250.

Batth, S.S. (1977) A survey of Canadian populations of the German cockroach for resistance to insecticides. *Canadian Entomologist* **109**, 49–52.

Bennett, G.W. & Spink, W.T. (1968) Insecticide resistance of German cockroaches from various areas of Louisiana. *Journal of Economic Entomology* **61**, 426–431.

Casida, J.E. (1970) Mixed-function oxidase involvement in the biochemistry of insecticide synergists. *Journal of Agricultural and Food Chemistry* **18**, 753–772.

Chapman, P.A., Learmount, J. & Pinniger, D.B. (1993) Insecticide resistance in *Blattella germanica* (L.) in the United Kingdom. pp. 125–133 *in* Widley, K.B. & Robinson, W.H. (*Eds) Proceedings of the First International Conference on Insect Pests in the Urban Environment.* Exeter, BPCC Wheatons Ltd.

Cochran, D.G. (1987) Effects of synergist on bendiocarb and pyrethrins resistance in the German cockroach (Dictyoptera: Blattellidae). *Journal of Economic Entomology* **80**, 728–732.

Cochran, D.G. (1989) Monitoring for insecticide resistance in field-collected strains of the German cockroach (Dictyoptera: Blattellidae). *Journal of Economic Entomology* **82**, 336–341.

Cochran, D.G. (1994) Abamectin resistance potential in the German cockroach (Dictyoptera: Blattellidae). *Journal of Economic Entomology* **87**, 899–903.

Cochran, D.G. (1995) Insecticide resistance. pp. 171–192 *in* Rust, M.K., Owens, J.M. & Reierson, D.A. (*Eds*) *Understanding* *and controlling the German cockroach.* New York, Oxford University Press.

Daum, R.J. (1970) A revision of two computer programs for probit analysis. *Bulletin of the Entomological Society of America* **12**, 365–369.

Finney, D.J. (1971) *Probit analysis*. Third edition. London, Cambridge University Press. 333 pp.

Heal, R.E., Nash, K.B. & Williams, M. (1953) An insecticide-resistant strain of the German cockroach from Corpus Christi, Texas. *Journal of Economic Entomology* **46**, 385–386.

Hemingway, J., Dunbar, S.J., Monro, A.G. & Small, G.J. (1993) Pyrethroid resistance in German cockroaches (Dictyoptera: Blattellidae): Resistance levels and underlying mechanisms. *Journal of Economic Entomology* **86**, 1631–1638.

Jensen, K.M.V. (1993) Insecticide resistance in *Blattella germanica* (L.) (Dictyoptera: Blattellidae) from food producing establishments in Denmark. pp. 135–139 *in* Widley, K.B. & Robinson, W.H. (*Eds*) *Proceedings of the First International Conference on Insect Pests in the Urban Environment.* Exeter, BPCC Wheatons Ltd.

McDonald, I.C. & Cochran, D.G. (1968) Carbamate cross resistance in a carbaryl-resistant strain of the German cockroach. *Journal of Economic Entomology* **61**, 670–673.

Milio, J.F., Koehler, P.G. & Patterson, R.S. (1987) Evaluation of three methods for detecting chlorpyrifos resistance in German cockroach (Orthoptera: Blattellidae) populations. *Journal of Economic Entomology* **80**, 44–46.

Nelson, J.O. & Wood, F.E. (1982) Multiple and cross-resistance in a field collected strain of the German cockroach (Orthoptera: Blattellidae). *Journal of Economic Entomology* **75**, 1052–1054.

Reierson, D.A., Rust, M.K., Slater, A.J. & Slater, T.A.M. (1988) Insecticide resistance affects cockroach control. *California Agriculture* **42**, 18–20.

Robertson, J.L., Smith, K.C., Savin, N.E. & Lavigne, R.J. (1984) Effects of dose selection and sample size on the precision of lethal dose estimates in dose-mortality regression. *Journal of Economic Entomology* **77**, 833–837.

Robinson, W.H. & Zhai, J. (1990) Pyrethroid resistance in German cockroaches. *Pest Control Technology* **18**, 26–28.

Robinson, W.H. & Zhai, J. (1994) Insecticide resistance in German cockroaches. Good news from the field. *Pest Control Technology* **22**, 64, 66 & 98.

Rust, M.K. & Reierson, D.A. (1991) Chlorpyrifos resistance in German cockroaches (Dictyoptera: Blattellidae) from restaurants. *Journal of Economic Entomology* **84**, 736–740.

Schal, C. (1992) Sulfluramid resistance and vapor toxicity in field collected German cockroaches (Dictyoptera: Blattellidae). *Journal of Medical Entomology* **29**, 207–215.

Scharf, M.E., Bennett, G.W., Reid, B.L. & Qui, C.F. (1995) Comparison of three insecticide resistance detection methods for the German cockroach (Dictyoptera: Blattellidae). *Journal of Economic Entomology* **88**, 536–542.

Scott, J.G. (1990) Investigating mechanisms of insecticide resistance: Methods, strategies and pitfalls. pp. 39–57 *in* Roush, R.T. & Tabashnik, B.E. (*Eds*) *Pesticide resistance in arthropods*. New York, Chapman and Hall.

Scott, J.G. & Georghiou, G.P. (1986) Mechanisms responsible for high levels of permethrin resistance in the house fly. *Pesticide Science* **17**, 195–206.

Scott, J.G. & Matsumura, F. (1981) Characteristics of a DDT-induced case of cross-resistance to permethrin in *Blattella germanica. Pesticide Biochemistry and Physiology* **16**, 21–27.

Scott, J.G. & Matsumura, F. (1983) Evidence for two types of toxic actions of pyrethroids on susceptible and DDT-resistant German cockroaches. *Pesticide Biochemistry and Physiology* **19**, 141–150.

Scott, J.G., Ramaswamy, S.B., Matsumura, F. & Tanaka, K. (1986) Effect of method of application on resistance to pyrethroid insecticides in *Blattella germanica* (Orthoptera: Blattellidae). *Journal of Economic Entomology* **79**, 571–575.

Scott, J.G., Cochran, D.G. & Siegfried, B.D. (1990) Insecticide toxicity, synergism, and resistance in the German cockroach (Dictyoptera: Blattellidae). *Journal of Economic Entomology* **83**, 1698–1703.

Siegfried, B.D. & Scott, J.G. (1991) Mechanism responsible for propoxur resistance in the German cockroach, *Blattella germanica* (L.). *Pesticide Science* **33**, 133–136.

Silverman, J. & Ross, M.H. (1994) Behavioral resistance of field-collected German cockroaches (Blattodea: Blattellidae) to baits containing glucose. *Environmental Entomology* **23**, 425–430.

Sun, Y.P. & Johnson, E.R. (1960) Synergistic and antagonistic actions of insecticide-synergist combinations and their mode of action. *Journal of Agricultural and Food Chemistry* **8**, 261–266.

Umeda, K., Yano, T. & Hirano, M. (1988) Pyrethroid-resistance mechanism in German cockroach, *Blattella germanica* (Orthoptera: Blattellidae). *Applied Entomology and Zoology* **23**, 373–380.

Wadleigh, R.W., Koehler, P.G. & Patterson, R.S. (1989) Comparative susceptibility of North American *Blattella* (Orthoptera: Blattellidae) species to insecticides. *Journal of Economic Entomology* **82**, 1130–1133.

Zhai, J. & Robinson, W.H. (1991) Pyrethroid resistance in a field population of German cockroach, *Blattella germanica* (L.). *Japanese Journal of Sanitary Zoology* **42**, 241–244.

Zhai, J. & Robinson, W.H. (1992) Measuring cypermethrin resistance in the German cockroach (Orthoptera: Blattellidae). *Journal of Economic Entomology* **85**, 348–351.

(Accepted 8 May 1996)