INSECTICIDE RESISTANCE IN *BLATTELLA GERMANICA* (L.) IN THE UNITED KINGDOM

P.A. CHAPMAN, J. LEARMOUNT AND D.B. PINNIGER
Central Science Laboratory (CSL), Ministry of Agriculture Fisheries and Food, London Rd. Slough, Berkshire, SL3 7HJ, UK

Abstract—Resistance to organochlorine, organophosphorous (OP) and carbamate insecticides in the German cockroach *Blattella germanica* has been documented for many years. In the past, significant bendiocarb resistance has been reported in the United Kingdom. More recently, resistance has been reported to the pyrethroids particularly in the United States of America and Denmark. Over the last few years considerable use has been made of pyrethroids for cockroach control, although little accurate information about the current resistance status to pyrethroids in UK German cockroaches has been available until now. We have investigated the resistance status of three strains of *B. germanica* collected from sites in London and one from the USA to a range of OP, carbamate and pyrethroid insecticides. The assessments were made by topical application of a range of doses of technical insecticide in 0.5 µl of organic solvent to 2–3 week old male German cockroaches to produce dose response data. A tarsal contact method based on the WHO cockroach resistance test, was also used to assess resistance to some of the insecticides. High levels of resistance were found to pyrethrins and cypermethrin using both test methods. Results obtained with the topically applied synergists piperonyl butoxide and S,S,S-tributylphosphorotrithioate indicated the presence of a *kdr*-type resistance. Although the strain from the USA showed high resistance to chlorpyrifos, only low OP resistance was found in the London strains. Resistance to carbamates was also detected in the London strains. The resistance data obtained by the two methods is presented and the differences and similarities in the levels of resistance that were obtained using the two methods are discussed in relation to their value as methods for assessing resistance.

INTRODUCTION

The German cockroach, *Blattella germanica*, is a significant pest worldwide, and presents a serious health risk in spreading disease and causing contamination of food, food preparation and other areas in the urban environment, as well as being one of the major causes of household allergies (Cornwell, 1976; Brenner, 1991).

Insecticides have been widely used to control this pest since the development of DDT. However, the development of resistance was soon reported to the organochlorine, organophosphate (OP) and carbamate insecticides both from laboratory selection experiments (Grayson, 1960; Collins, 1975) and following field use (Batth,1977; Cochran 1989; Rust and Reierson, 1991).

Pyrethroids have been extensively used in recent years and initially showed great potential for control of a number of OP-resistant (Koehler and Patterson 1988) and carbamate-resistant (Schall 1988) populations, but as with other insecticide groups there were reports of resistance to pyrethroids from the United States of America (USA) (Cochran,1989; Scott, *et al.*, 1990; Zhai and Robinson,1991), Japan (Umeda *et al.*, 1988), and Denmark (Vagn-Jensen,1988).

In the United Kingdom (UK), dieldrin resistance was reported in 1960 by Gradidge followed by resistance to chlordane and DDT (Green *et al.* ,1961). Low levels of resistance were reported to the OP diazinon (Tyler,1964; Cornwell,1968) and, following reports of control failure in 1975 resistance was confirmed to bendiocarb (Barson and McCheyne,1978). Low level resistance to malathion and permethrin was detected in 1976 but no resistance was reported to propoxur or to other OPs (Barson and Renn,1983).

Currently the most widely used insecticides for cockroach control in the UK are the synthetic pyrethroids permethrin, cypermethrin, deltamethrin and alphacypermethrin. Bendiocarb is still in use and, recently, chlorpyrifos, hydramethylnon and methoprene have been introduced; other OP and carbamate insecticides are used to a lesser extent.

Since 1983 there has been very little published work on the assessment of insecticide resistance in *B. germanica* in the UK. The aim of this work was to investigate the current situation in the light of reported control failures.

Three strains of *B. germanica* were obtained from sites in London and compared with a strain from the USA. Resistance was assessed to a range of insecticides using topical application methods

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to all strains. In addition, jar tests were carried out using one of the London strains and the American strain. All results were compared with the CSL standard insecticide susceptible strain.

Materials and Methods

Insecticides

The following technical samples of insecticides were used: malathion, diethyl (dimethoxyphosphinothioylthio)succinate, (95% w/w); fenitrothion, *O,O*-dimethyl *O-4*-nitro-*m*-tolyl phosphorothioate (96.7%); chlorpyrifos, *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate (97.8%); cypermethrin, (*RS*)- alpha-cyano-3-phenoxybenzyl (*1RS*)-*cis,trans*-3-(2,2- dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate, with a cis:trans isomeric ratio of 40:60, 91.6% pure; natural pyrethrins, (25%); bendiocarb, 2,2-dimethyl-1,3-benzodioxol-4-yl methylcarbamate (97.3%) and propoxur, 2-isopropoxyphenyl methylcarbamate (99%).

The synergists piperonyl butoxide (PB), 5-[2-(2-butoxyethoxy)ethoxymethyl]-6-propyl-1,3-benzodioxole (90%) and S,S,S-tributylphosphorotrithioate (DEF, 96%) were also used.

Insects

CSL strain: The standard laboratory insecticide susceptible strain of cockroaches against which the field strains were compared has been in laboratory culture for many years without exposure to insecticides.

America strain: Collected from a housing estate in Alabama, USA in May 1991. The history of insecticide use there is unknown.

London 1 strain: A strain collected 1 month after a cypermethrin treatment. Bendiocarb, pyrethrins + PB and propoxur had been used previously.

London 2 strain: A strain collected from a flat 1 week after a hydramethylnon treatment. Bendiocarb and cypermethrin had been used within the last 2 years.

London 3 strain: A strain collected from a flat in a block adjacent to London 2 and connected via heating ducting but where no insecticides were reported to have been used since 1983.

The UK field strains were all collected, from low rise blocks of flats situated in inner London, between June and December 1992 and brought to the Central Science Laboratory (CSL). All cockroaches were reared at 27°C and 45% r.h., with a 10:14h light:dark regime, on a diet of wheatfeed, rolled oats, yeast, fishmeal and ground dog biscuits and peanuts. The ingredients were mixed at a ratio of 14:14:3:6:6:2. Water was available at all times.

Tests using the UK field strains were carried out on 2nd or 3rd generation insects. The America strain had been in laboratory culture for 1 year before testing.

Pre-test handling

Adult male cockroaches were separated from culture tanks when 0-1 weeks old and maintained in holding tanks until 2-3 weeks old, when they were immobilised by chilling and divided into batches for testing. Each batch of insects, held in a 120ml rigid polythene container with a mesh lid, was transferred to the test room maintained at 25°C and 45% r.h. A 10ml plastic beaker containing cotton wool soaked with 10ml water was inverted on the mesh to provide a water source for the insects. The insects were left overnight without food to acclimatise to the conditions.

Tarsal contact jar tests

The tarsal contact jar test was based on a method developed by Keller et al (1956) and adapted as the standard WHO method in 1970. Technical grade insecticides were dissolved in acetone and 2.5ml of the solution was placed in a 350ml glass jam jar. The jar was evenly rolled until the acetone had evaporated, leaving a thin layer of insecticide on the inner surface of the jar at a dose equivalent to a field application rate of active ingredient (table 1). A thin band of petroleum jelly : liquid paraffin (3 : 1) was applied to the top inner surface of the jar to prevent the insects escaping.

A batch of 10 adult male cockroaches, 2-3 weeks old, was immobilised with CO_2 and placed in the treated jar. After the insects had recovered, knockdown (KD) counts were recorded at relevent intervals until 9 out of 10 of the insects were knocked down, or until 48h after the start of the test. No food or water was given during the test period.

Five batches of 10 insects were tested against each insecticide. A further batch of 10 insects was released into a jar treated with acetone only to act as a control.

Topical application tests

Insecticides were dissolved in pentan-3-one and serially diluted to give a range of concentrations. Batches of 15 adult male cockroaches, 2-3 weeks old, were immobilised with CO_2 and individually treated on the ventral thorax, using a hand held, modified Hamilton repeating dispenser and 25 µl gas tight syringe. The dispenser was calibrated to deliver 0.49 ± 0.008 µl of solution. Each batch of treated insects was returned to its holding container. A dog biscuit was added and the water beakers were replenished with 2ml of water.

Preliminary ranging tests were carried out using 2 replicates of 10 insects at each of 4 concentrations. Accurate topical application tests were then carried out using 3 replicates of 15 insects at each of 5 concentrations calculated to give >0% at the lowest and <100% kill at the highest dose. Pentan-3-one alone was applied to 3 replicates of 15 insects as controls.

KD counts were recorded daily for 7 days and 2ml of water was added to the water beakers following each count.

Synergism tests

The synergist PB was topically applied at a rate of 100 µg per insect or DEF at a rate of 30µ g per insect. A synergist was applied to the insects 1h prior to topical treatment with insecticides. The effect of each synergist on the response of the London 1 and CSL strains to natural pyrethrins and cypermethrin was recorded. Controls were treated with synergist alone.

Table 1. Responses of 3 strains of B. germanica to insecticides using WHO jar test method.

Insecticide Strain	KT50 (min)	95% Fiducial limits of KT50	KT95 (min)	RF50	RF95	Slope ± SE
Cypermethrin 50mg/m2						
CSL	9	9, 10	12			14.0 ± 1.7
London 1	184	155, 213	676	20	56	2.9 ± 0.4
America	589	555, 638	924	65	77	8.4 ± 1.1
Chlorpyrifos 200mg/m2						
CSL	30	29, 31	39			14.1 ± 2.0
London 1	46	45, 47	57	2	1	17.2 ± 1.9
America	77	73, 82	119	3	3	8.8 ± 0.9
Malathion 500mg/m2						
CSL	45	42, 47	74			7.6 ± 1.0
London 1	67	44, 95	2094	1	28	1.1 ± 0.2
America	> 2 days #					
Bendiocarb 240mg/m2						
CSL	22	21, 23	31			11.2 ± 1.2
London 1	165	121, 216	2724	8	88	1.4 ± 0.2
America	41	35, 49	130	2	4	3.3 ± 0.5

#No knockdown during the test period.

Statistical analysis

Results from the WHO jar tests were analysed by computer using a maximum likelihood programme for probit analysis compiled at CSL by Mr A.J. Prickett. Data from the field strains were compared with data from the susceptible strain and resistance factors (RF) were calculated. Results from the 7 day counts of topical application tests were analysed and RFs calculated in the same way.

RESULTS

The results from the tarsal contact jar tests expressed as time-probit KD responses are shown in table 1. The dose-response data obtained by topical application is shown for the OP insecticides in table 2, carbamates in table 3, and pyrethroids in table 4. Comparisons of RFs for the four field strains as assessed by topical application at the LD_{50} are shown in fig. 1. The effects of the synergists PB and DEF together with the factors of synergism are shown in table 5.

Resistance to the OP insecticides chlorpyrifos and fenitrothion as shown by the topical test method was low for all three UK strains (tables 2 and fig. 1) and for the London 1 strain for the jar test (table 1). Fenitrothion resistance was also low in the America strain but the resistance to chlorpyrifos as assessed by topical application (table 2) was high although it was similar to the London 1 strain by tarsal contact method (table 1). Resistance to malathion could not be measured in the America strain as there was no KD response in the jar test at 48h, when the test was terminated (table 1), and 0% response to 200 µg applied topically (table 2). The dose-response for the London 1 strain produced a low slope with both application methods giving low RFs at the 50% levels but high RFs at the 95% levels (table 2, fig. 1).

The America strain showed low RFs to bendiocarb (fig. 1), although the slopes of the lines were flatter than those obtained with the CSL strain (tables 1 and 3). The slow rate of KD of the London 1 strain with bendiocarb in the jar tests gave a high RF at the KD_{95} (table 1), but resistance was low as assessed by topical application. The London 3 strain was the most resistant of the UK strains to the two carbamates.

Both the American and London 1 strains showed high levels of resistance to pyrethrins and cypermethrin as assessed by the two methods (tables 1 and 4, and fig. 1). The other two London strains showed higher RFs to pyrethrins but lower RFs to cypermethrin (fig. 1). Both PB and DEF

Table 2. Responses of 5 strains of B. germanica to organophosphate insecticides using topical application test method.

Insecticide Strain	LD50 µg per roach	95% Fiducial limits of LD50	LD95 µg per roach	RF50	RF95	Slope ± SE
Chlorpyrifos						
CSL	0.21	0.20 , 0.22	0.33			8.7 ± 1.0
London 1	0.85	0.78 , 0.95	1.95	4.0	5.9	4.6 ± 0.8
London 2	0.24	0.22 , 0.26	0.46	1.1	1.4	6.0 ± 0.7
London 3	0.59	0.55 , 0.63	1.06	2.8	3.2	6.0 ± 0.9
America	6.60	5.65 , 7.37	13.42	31.4	40.7	5.3 ± 0.9
Fenitrothion						
CSL	0.20	0.19 , 0.20	0.26			13.1 ± 1.4
London 1	0.48	0.45 , 0.51	0.81	2.4	3.1	7.2 ± 1.0
London 2	0.25	0.23 , 0.27	0.36	1.3	1.4	9.4 ± 1.9
London 3	0.74	0.70 , 0.79	1.15	3.7	4.4	8.8 ± 1.0
America	0.59	0.56 , 0.63	0.89	3.0	3.4	9.3 ± 1.1

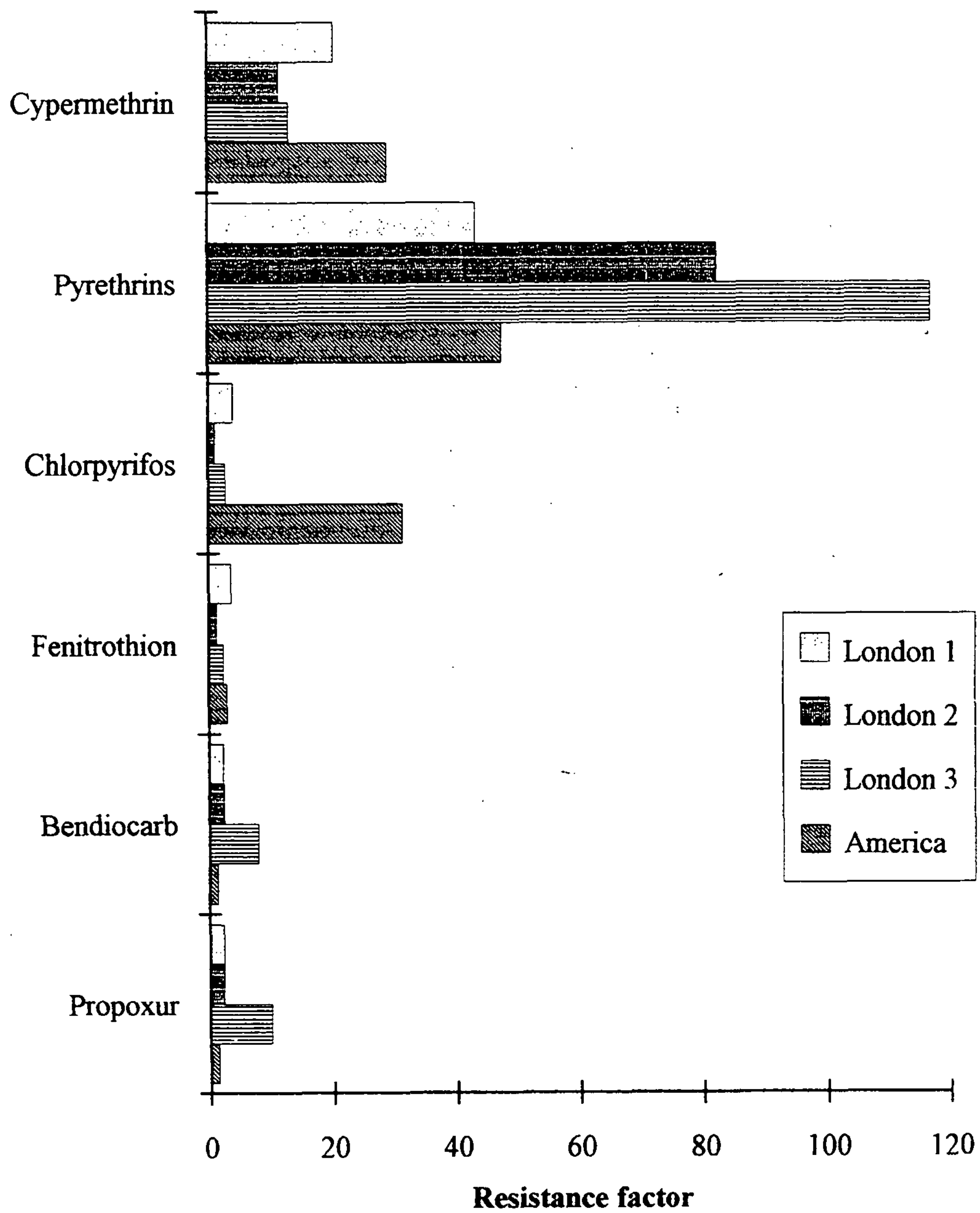

Figure 1. Resistance factors of four strains of B. germanica at the KD50

synergised pyrethrins and cypermethrin in the CSL standard and London 1 strains. The factor of synergism was highest for pyrethrins + PB against the London 1 strain being 1.9 and 2.3 times greater than the CSL strain at the LD_{50} and LD_{95} respectively. The other factors of synergism in the London 1 strain were similar to the CSL strain.

Table 3. Responses of 5 strains of B. germanica to carbamate insecticides using topical application test method.

Insecticide Strain	LD50 µg per roach	95% Fiducial limits of LD50	LD95 µg per roach	RF50	RF95	Slope ± SE
Bendiocarb						
CSL	0.27	0.25 , 0.28	0.41			9.0 ± 1.1
London 1	0.63	0.57 , 0.70	1.49	2.3	3.6	4.4 ± 0.6
London 2	0.68	0.61 , 0.74	1.49	2.5	3.6	4.8 ± 0.7
London 3	2.14	1.82 , 2.54	7.19	7.9	17.5	3.1 ± 0.4
America	0.38	0.29 , 0.48	2.73	1.4	6.7	1.9 ± 0.3
Propoxur						
CSL	0.21	0.20 , 0.23	0.40			6.1 ± 0.8
London 1	0.48	0.44 , 0.53	1.11	2.3	2.8	4.6 ± 0.5
London 2	0.48	0.44 , 0.51	0.88	2.3	2.2	6.2 ± 0.8
London 3	2.11	1.92 , 2.29	4.40	10.0	11.0	5.2 ± 0.7
America	0.29	0.26 , 0.31	0.64	1.4	1.6	4.7 ± 0.5

Table 4. Responses of 5 strains of B. germanica to pyrethroid insecticides using topical application test method.

Insecticide Strain	LD50 µg per roach	95% Fiducial limits of LD50	LD95 µg per roach	RF50	RF95	Slope ± SE
Cypermethrin						
CSL	0.10	0.092, 0.11	0.22			5.0 ± 0.6
London 1	2.02	1.77 , 2.23	4.53	20.4	21.6	4.7 ± 0.8
London 2	1.16	1.08 , 1.24	2.01	11.6	9.1	6.9 ± 0.8
London 3	1.32	1.18 , 1.45	2.74	13.2	12.5	5.2 ± 0.7
America	2.91	2.70 , 3.13	5.54	29.1	25.2	5.9 ± 0.7
Pyrethrins						
CSL	0.74	0.66 , 0.82	1.79			4.3 ± 0.6
London 1	31.23	27.50 , 35.30	93.12	43.4	53.5	3.5 ± 0.5
London 2	60.87	47.86 , 68.88	112.81	82.3	63.0	6.1 ± 1.0
London 3	73.08	55.08 , 90.42	184.35	98.8	103.0	4.1 ± 1.4
America	35.14	32.05 , 38.30	67.21	47.5	37.5	5.8 ± 0.8

DISCUSSION

While the jar test would at first appear to be the more appropriate method of assessing resistance to contact insecticides compared to topical application, it has been shown to seriously underestimate resistance to most OP insecticides particularly where control failure or reduced efficacy of OP insecticides has been observed in the field (Ballard *et al.*, 1984; Milio *et al.*; 1987; Schal,1988). This is further supported by this work where the America strain was shown to have high resistance to chlorpyrifos as assessed by topical application, 31.4 at the LD_{50} (table 2), but only low resistance as assessed by the jar test (table 1). The work of Ballard et al.,(1984) and Rust and Reierson (1991) have indicated that at a RF of 10 at the LD_{50} as measured by topical application, there will be reduced efficacy in control treatments using chlorpyrifos. This level of resistance to chlorpyrifos would not be shown by the WHO jar test, and this method should therefore not be

Table 5. Responses of 2 strains of B. germanica to synergised pyrethroid insecticides using topical appliction test method,

Strain / Insecticide	LD50 µg per roach	95% Fiducial limits of LD50	LD95 µg per roach	RF50	RF95	Synergism factor LD50	Synergism factor LD95	Slope ± SE
CSL								
Pyrethrins + PB	0.20	0.17 , 0.22	0.53			3.7	3.4	3.8 ± 0.5
Cypermethrin + PB	0.023	0.020 , 0.030	0.048			4.3	4.6	5.1 ± 0.8
Pyrethrins + DEF	0.25	0.21 , 0.30	0.54			3.0	3.3	5.0 ± 1.2
Cypermethrin + DEF	0.034	0.030 , 0.040	0.061			2.9	3.6	6.5 ± 1.1
London 1								
Pyrethrins + PB	4.53	3.54 , 5.19	11.74	22.7	22.2	6.9	7.9	4.0 ± 0.8
Cypermethrin + PB	0.55	0.50 , 0.60	1.11	23.9	23.1	3.7	4.1	5.3 ± 0.6
Pyrethrins + DEF	9.99	8.92 ,10.99	22.99	40.0	42.6	3.1	4.1	4.5 ± 0.6
Cypermethrin + DEF	0.96	0.78 , 1.08	1.99	28.2	32.6	2.1	2.3	5.2 ± 1.0

used to assess field resistance to the majority of OP insecticides. The only documented exception to this is malathion where high levels of resistance have been recorded using this technique (Cochran 1989). In this present work the jar test method showed heterozygous resistance in the London 1 strain with high levels of resistance at the KD_{95} (table 1). This suggests that although malathion has not been used for cockroach control in the UK for many years, resistance to it may still be present in some field populations. There appears to be no cross-resistance between malathion and other OP insecticides as shown by the American and London 1 strains (tables 1 and 2) and this is confirmed by other workers in the USA (e.g. Cochran,1989).

Bendiocarb resistance is readily assessed by the jar test (Barson and McCheyne,1978 and Cochran,1989) and in this work the jar test gave high estimates of resistance in the London 1 strain with a flat slope suggesting a heterozygous high resistance in the population which was not shown by topical application. In the London 3 strain, however, high carbamate resistance was shown by topical application. It may be that the jar test is a more sensitive method for assessing carbamate resistance than topical application and further tests need to be carried out to clarify this point.

As in the case of bendiocarb, the jar test has been shown to give high estimates of resistance to the type I pyrethroids such as natural pyrethrins or allethrin (Cochran,1987) and to permethrin and fenvalerate (Scott *et al.*, 1986). However, against the type II pyrethroids particularly those with alpha-cyano groups, such as cypermethrin and deltamethrin, low estimates of resistance have been reported compared to those obtained by topical application (Scott *et al.* 1986, Zhai and Robinson 1991,1992). The strain of cockroaches used by Scott *et al.* was resistant to DDT but had not been exposed to pyrethroid insecticides and was shown to possess only a kdr resistance mechanism. They postulated that it was the behaviour of the insecticides acting on different sites in the nervous system that led to the differences in the levels of resistance as assessed by the two methods. Zhai and Robinson (1991) assessed resistance to cypermethrin in a field strain of insects that had been exposed to OP insecticides, principally chlorpyrifos, and bendiocarb but there was no record of pyrethroid use until cypermethrin was used twice yearly for five years with decreasing efficacy prior to collection of the strain. The level of resistance by topical application was 180 at the LD_{50} but only 2.9 by tarsal contact (Zhai and Robinson, 1991). Further work suggested that the field strain was more active and picked up more insecticide than their laboratory suceptible strain. When the activity of the strains was restricted both picked up a similar amount of insecticide and the resistance to cypermethrin assessed by tarsal contact increased from 2.9 to 22 compared to 123 for topical application (Zhai and Robinson, 1992). Integrated pest management and resistance management programmes require detection of moderate levels of insecticide resistance and Zhai and Robinson (1992) concluded that only topical application was sensitive enough for these purposes. In contrast to the above reports it was found in this current work that both the surface contact and topical application methods gave similar high levels of resistance in both the America and London 1 strains (Tables 1 and 4).

Application of PB and DEF to the CSL strain caused similar levels of synergism with both pyrethrins and cypermethrin (Table 5) suggesting the presence of naturally occuring detoxification mechanisms. There was slightly increased PB synergism to pyrethrins in the London 1 strain but no increased synergism with DEF or to cypermethrin with either chemical. The lack of synergism with cypermethrin was also reported by Scott *et al.* (1990) in their Ectiban-R strain which indicated that at least part of the resistance was due to a *kdr*-type mechanism. Work by Bull and Patterson (1993) on a pyrethroid resistant field strain using synergists PB and DEF with radio-labelled permethrin again suggested a basic kdr mechanism, with some reduced cuticular penetration as a minor modifying factor and, while there was clearly metabolic detoxification in both susceptible and resistant strains there was no appreciable resistance-related enhancement in the resistant strain. It is unfortunate that apparently no tarsal contact tests were carried out on this strain.

It is clear from this work that serious pyrethroid resistance is present in London with *kdr* implicated in the resistance mechanism. The question of why the similar levels of resistance to the type II pyrethroids as shown by both the test methods against both the American and London 1 strains while other workers using different strains with *kdr*-type resistance failed to show significant levels of resistance by tarsal contact needs to be resolved. Resistance to OP insecticides was low in the three UK strains tested although some high levels of malathion resistance may be lingering in some populations it is unlikely to lead to cross-resistance to other OP insecticides. Carbamate resistance is clearly present in strains in London but the significance of this resistance needs to be established. Resistance to all of these compounds needs to be determined for other strains of *B. germanica* to accurately assess the spread and degree of resistance in London and throughout the UK.

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