INSECTICIDE RESISTANCE IN *BLATTELLA GERMANICA* (L.) (DICTYOPTERA: BLATTELLIDAE) FROM FOOD PRODUCING ESTABLISHMENTS IN DENMARK

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Abstract—A number of cases of *Blattella germanica* control failure were reported to the Danish Pest Infestation Laboratory from 1987 to 1991. A screening of the insecticide resistance in *B. germancia* in some selected locations was conducted with permethrin using tarsal contact tests to estimate KT_{50} (/WHO/VBC/75.593). Based on these data more detailed measurement of the resistance in the German Cockroaches from chosen locations was then assessed by topical application techniques; 2.5 µl insecticide in acetone on the ventral sclerites of the cockroaches was used. Permethrin, deltamethrin, diazinon, and chlorpyrifos were used in these trials. Probit analysis was used with both methods, according to Finney (1971) and similar results obtained with the DPIL susceptible strain were used to calculate the resistance/ susceptibility ratio (R/S).

The cockroaches were mainly collected from mass food producing kitchens, bakeries, and a zoological garden in Copenhagen and North Zealand, but some were also collected in food producing factories in Aarhus, Jutland, and from residential buildings in Nuuk, Greenland.

In the topical application tests, female cockroaches from ten different locations were tested for their susceptibility to permethrin. The calculated R/S ratio based on the LC_{50}'s varied between 1 and 57. For deltamethrin from eight locations, the R/S ratio varied between 2 and 31, for chlorpyrifos from nine locations, the R/S ratio varied between 1 and 4, and for diazinon from six locations, the R/S ratio varied between 1 and 2.

Based on these findings, the reason for control failure in these particular locations could be ascribed to resistance to the two pyrethroids, permethrin and deltamethrin. Both insecticides were in use for cockroach control in Denmark at that time. The organophosphate, chlorpyrifos, with its low R/S ratio was then used in the locations, and with good results.

INTRODUCTION

In Denmark insecticide resistance in the German cockroach *Blattella germanica* became an established problem shortly after dieldrin was introduced for cockroach control in the late fifties. As early as 1962 dieldrin resistance was detected in a Danish cockroach strains with a resistance ratio of approximately 2000 (Keiding, 1963). In the late seventies dieldrin resistance caused so many problems that dieldrin based products could not be used any more. Chlorpyrifos and diazinon were introduced into the market in the late sixties and in the beginning of the seventies as, e.g. locally produced lacquer-formulations. In contrast to the report of chlorpyrifos resistance by other investigators (Milio et al., 1987, Rust & Reierson, 1991), this type of resistance has not until now caused any major control problems in Denmark although the chlorpyrifos have been and still are often used. In the early eighties, the pyrethroids permethrin and later deltamethrin based spray-formulations became commercially available and seemed to provide new possibilities for cockroach control in Denmark. These products were much easier to apply than the older lacqer-formulations, and they quickly obtained a widespread use by professional pest control operators.

A few years after the above-mentioned pyrethroids had been introduced for cockroach control, control failures were observed, a situation nearly parallel to the control failure with cypermethrin as described by Atkinson *et al.* (1991). In 1987 the Danish Pest Infestation Laboratory was contacted by a municipal pest control organization which had encountered control failure when using permethrin in mass food producing hospital kitchens. Based on some preliminary tarsal contact test, the present study was initiated with the objectives of characterizing the resistance profile of these and other field collected strains to the insecticides available for cockroach control in Denmark.

METHODS AND MATERIALS

The field strains used in this study were collected from mass food producing kitchens, bakeries, food and chocolate producing factories, a zoological garden, and a residential building (Nuuk,

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Greenland). Apart from the zoological garden, only locations with a recognized control problem participated in the survey. The DPIL sus-strain was used as the susceptible reference-strain. The strain has been reared at DPIL since the fifties and is susceptible to a wide variety of insecticides (Jensen, 1987). All strains were reared at 27C at 50% RH with a 12:12 (L:D) photo period on a diet of rat chow (Altromin no.1324) and water.

Tarsal contact test (WHO/VBC/75.593) was used to identify a potential permethrin resistance, and if a resistance problem was identified then new cockroaches were collected and the strain was reared in the laboratory to provide cockroaches enough for a topical application test. In the tarsal contact test, permethrin (Wellcome Res.lab.(UK)) diluted in acetone was used. 2.5 ml of the permethrin solution was applied to the inside of a 250 ml jar to produce a uniform surface residue of 20 µg/cm permethrin on the sides and bottom. No more than ten female cockroaches were released into a jar and the knock-down was registered with a given interval dependent on the strain and insecticide used. With the Pl890211 strain males were only tested to spare the females, and with the two strains Ni881011 and Nu890711 no tarsal contact test was conducted.

In the topical application tests 2.5 µl of the insecticide solutions in acetone were applied to the ventral sclerites of CO_2 anaesthetized female cockroaches. The insecticides used were: permethrin (Wellcome Res.lab.(UK)), deltamethrin (Roussel-Uclaf), chlorpyrifos (Dow Chemicals), and diazinon (Ciba-Geigy). The doses used were found in a preliminary range finding test. Each concentration was replicated two or three times with upto 10 cockroaches from each strain dependent on the number of cockroaches available for the test. Treated cockroaches were transferred to clean jars and provided with water and food. It has been found that some recovery after treatment with synthetic pyrethroids is common, therefore, mortality was recorded after 48 hours instead of 24 hours as normally done.

Table 1. Response of field and laboratory strains of B. germanica females when exposesd to 20 µg/cm^2 permethrin in glass jars.

Insecticide	N	Strain*	KT_{50}	95% fiducial L.	HR %	HR min.	Locality	I.u.
Permethrin	40	DPIL-sus	13	12 - 14	100	30	Laboratory	-
	20	Bi870911	!	-	55	1440	Hospital	P
	20	Ko871011	!	-	10	4320	Hospital	P
		NA881011	not tested				Factory	P/D
	20	Hø881115	!	-	50	1440	Bakery	P
	18	M Pl890211	186	154 - 223	100	1440	Restaurant	not known
	20	Zo890611	17	15 - 20	100	35	Zool. garden	C
	6	Ni881111	39	32 - 48	100	80	Bakery	P
	20	Su880911	!	-	20	1440	Hospital	P
		NU890711	not tested				Res.buildings	not known
	20	Ar890411	!	-	0	5760	Restaurant	P

* The year in which the strain was collected is given by the two first digits after the letter code.
N= Number of B. germanica tested.
HR= Highest response given as per cent knock-down after a given time in minutes.
M= Males tested.
!= Probit analysis not possible
I.u.= Insecticide used in the nearest past as reported by the pest control operators.
P= Permethrin
D= Deltamethrin
C= Chlorpyrifos

Table 2. Dose-response data (48-h knock-down) for laboratory and field strains of female *Blattella germanica*. Tested against four topically applied insecticides. 2.5 µl of insecticide in acetone was applied to the ventral sclerites of the cockroach.

Insecticide	N	Strain	LC50 %w/v	95% fiducial limits	Slope	± s.e.	chi^2	d.f.	P	R/S_{50}
Permethrin	236	DPIL-sus	0.013	0.012-0.015	4.63	0.49	6.8	4	0.15	-
	158	Bi870911	0.538	0.453-0.640	3.17	0.46	7.7	6	0.27	41
	161	Ko871011	0.522	0.408-0.669	2.06	0.37	1.7	6	0.95	40
	220	Nå881011	0.747	0.655-0.851	4.22	0.47	16.0*	5	0.01	57
	128	Hø881115	0.465	0.386-0.559	3.26	0.61	6.1	5	0.30	36
	115	PL890211	0.272	0.198-0.374	3.01	0.76	0.1	4	0.99	21
	109	Zo890611	0.018	0.016-0.021	5.91	1.01	9.4 *	2	0.01	1
	83	Ni881111	0.527	0.448-0.621	4.65	1.05	19.9 *	3	0.01	41
	140	Su880911	0.577	0.501-0.664	4.46	0.68	4.8	5	0.45	44
	80	Nu890711	0.040	0.032-0.051	3.58	0.70	2.1	2	0.37	3
	150	Ar890411	0.752	0.606-0.932	2.70	0.45	14.6 *	5	0.02	57
Deltamethrin	121	DPIL-sus	0.00081	0.0007-0.009	6.32	1.12	5.4	4	0.27	-
	100	Bi870911	0.017	0.013-0.021	3.09	0.62	1.2	3	0.75	21
	140	Ko871011	0.024	0.018-0.033	1.95	0.34	10.8	5	0.08	30
	140	Nå881011	0.016	0.013-0.019	3.56	0.60	8.7	5	0.15	20
	80	Hø881115	0.018	0.016-0.020	6.50	1.19	16.6 *	2	0.01	22
	120	Pl890211	0.011	0.009-0.015	2.7	0.54	7.4	4	0.11	14
	140	Zo890611	0.001	0.001-0.002	3.23	0.48	1.8	5	0.88	2
	120	Ni881111	0.019	0.016-0.022	4.75	0.75	19.9 *	4	0.01	23
	140	Su880911	0.025	0.020-0.031	2.83	0.42	15.6 *	5	0.01	31
Chlorpyrifos	100	DPIL-sus	0.023	0.019-0.027	4.33	0.78	1.6	3	0.65	-
	119	Bi870911	0.041	0.035-0.048	4.19	0.64	13.6 *	4	0.01	2
	116	Ko871011	0.040	0.034-0.047	4.59	0.72	4.1	4	0.40	2
	132	Nå881011	0.031	0.027-0.035	5.66	0.81	6.6	5	0.24	1
	79	Hø881115	0.038	0.034-0.043	8.55	1.6	0.4	2	0.82	2
	140	Pl890211	0.015	0.011-0.021	2.85	0.57	6.3	5	0.28	1
	122	Zo890611	0.029	0.026-0.033	6.00	0.91	1.4	4	0.87	1
	142	Ni881111	0.070	0.063-0.079	5.59	0.83	5.0	3	0.14	3
	170	Su880911	0.096	0.076-0.120	2.33	0.36	15.0 *	6	0.02	4
	140	Nu890711	0.022	0.018-0.028	3.08	0.52	7.2	5	0.20	1
Diazinon	150	DPIL-sus	0.016	0.014-0.018	4.86	0.56	3.1	4	0.55	-
	90	Bi870911	0.028	0.024-0.034	4.78	0.79	0.89	3	0.85	2
	100	Nå881011	0.027	0.022-0.033	3.62	0.65	5.5	3	0.14	2
	122	Hø881115	0.038	0.032-0.046	3.60	0.61	6.6	4	0.17	2
	180	Pl890211	0.017	0.014-0.020	3.56	0.44	9.6	7	0.22	1
	139	Zo 890611	0.017	0.015-0.020	4.13	0.57	11.5 *	5	0.05	1
	119	Ni881111	0.039	0.031-0.049	2.97	0.56	1.1	4	0.90	2

* indicates heterogeneity.
N= number of tested cockroaches.
d.f.= degree of freedom.
P= goodness of fit.
R/S_{50}= resistance factor

Data was analysed using probit analysis according to Finney (1971). The LC_{50}'s were estimated for each strain tested against each insecticide. Goodness of fit was estimated by extrapolating between tabulated values. Test for statistical significance between LC_{50}'s was the failure of their 95% fiducial limits to overlap. The resistance ratios (R/S) was calculated as LC_{50} resistant strain divided by LC_{50} susceptible strain. R/S was considered significantly different from one when based on LC_{50}'s that was significantly different.

RESULTS

The results of the tarsal contact test are given in Table 1. Apart from the DPIL-sus strain, only strain Zo890611 turned out to be susceptible to permethrin, and all in all only three of the eight field

strains tested reached a percentage mortality high enough to make a probit analysis possible. The remaining five strains gave response between 55 and 0 per cent mortality.

Differences in susceptibility were measured when the strains were topically treated and the LC_{50}'s and the fiducial limits were calculated. The R/S is based on the base line information on the DPIL-susceptible strain given in Table 2, along with the corresponding data for each of the field strains.

Some of the field strains treated with permethrin or deltamethrin displayed different degrees of heterogeneity. Some of them, such as Zo890611 with permethrin and HØ881115 with deltamethrin have their origin in technical problems, due to the fact that the slope was extremely steep and therefore very sensitive to small variations in the response, while others reflect more genuine heterogeneity in the population. The latter calls for some caution when the results are discussed, but as it does not overestimate the LC_{50}'s it still gives some minimum estimation of the R/S.

An R/S of 1-2 means very little or any resistance even though the R/S can be statistically significant in this group. R/S above 2 indicates increasing level of resistance and if the R/S is above 10 it indicates high resistance (Cochran, 1989).

Only two of the field strains display low or some resistance to permethrin, one of them Zo890611 was a strain from a zoological garden where only very limited amounts of insecticides are used, and then preferably chlorpyrifos. The other one was Nu890711, a strain collected in Nuuk, Greenland. The results from the tests with deltamethrin showed high resistance in the same strains as with permethrin, but in all tests the resistance to deltamethrin was considerably lower than the resistance to permethrin.

In the test with the two organophosphates: chlorpyrifos and diazinon, only two strains had an R/S >2, and the highest R/S was 4 in strain Su880911 originating from a hospital kitchen.

DISCUSSION

The present study reveals a number of problems concerning insecticide resistance in Danish cockroaches, even though it is based on a limited number of strains. As the strains were sampled based on reports of control failure they must be assumed to represent the "worst situation" and is by no means representative for the cockroach resistance status in Denmark as such. The study of the "worst situation" is on the other hand the best method to anticipate what will happen if pyrethroid resistance becomes more common.

Permethrin and deltamethrin are today widely used in Denmark for cockroach control by professional control operators, not because of resistance problems with the older compounds, but because these new products are not nearly as labour-consuming as the old ones, and they display a rapid activity and less odour.

The level of resistance to permethrin and deltamethrin in most strains is high, with R/S_{50} factors between 21 and 57 for permethrin and 14 and 31 for deltamethrin. Higher resistance factors have been found with other pyrethroids when tested on male German Cockroaches (Atkinson *et al.*, 1991). The immediate effect of the pyrethroid resistance found in the field strains is that the two pyrethroids cannot be used in these locations anymore.

The mechanism behind pyrethroid resistance is not known in detail the two pyrethroids used in this test represent two though not completely separate modes of action (Scott & Matsumura, 1983), (Scott *et al.*, 1986). One of the interesting aspects in this context is the development of resistance to deltamethrin, as deltamethrin had never been used in a number of the locations (Table 1), according to the pest control personnel. Cockroaches with a resistance to deltamethrin could have been introduced into the locations, but considering the number of locations in which the phenomenon is observed, it does not seem likely. Therefore, there must be some kind of link between either the permethrin resistance already developed or with some resistance mechanism originating from exposure to earlier used insecticides. The first possibility is supported by the observations by Cochran (1987, 1989, 1991), that selection with one pyrethroid can facilitate resistance to additional pyrethroids. The second possibility, exposure to earlier used insecticides needs some further investigation on the history of the insecticides used. It is very likely that all these strains at least have been resistant to dieldrin in the past, but how cross-resistance is involved is not clear. Hopefully, later studies of these strains can answer some of the questions.

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From a control point of view it is a problem that cockroach control in Denmark relies on only organophosphates, mainly chlorpyrifos, and on the synthetic pyrethroids permethrin and deltamethrin. If the pyrethroids fails in a locality the alternative in Denmark will be to use chlorpyrifos. This was done in all the locations which participated in this trial, and with good results. Even if chlorpyrifos resistance has not developed, although it has been used for more than 25 years that does not mean that it will not develop. The strain Su880911 in this investigation showed some resistance to chlorpyrifos which was confirmed by biochemical assays conducted by Dr J. Hemingway, London School of Hygiene and Tropical Medicine; and as already mentioned, chlorpyrifos resistance in field strains has been reported by (Milio et al., 1987) and (Rust & Reierson, 1991). Serious control problems could develope in Denmark even if only a small number of locations became resistant to both synthetic pyrethroids and chlorpyrifos.

ACKNOWLEDGEMENT

The author wishes to thank Dr M..A. Spinney, Insecticide Research & Development, ICI, UK and Dr J. Hemingway, London School of Hygiene and Tropical Medicine, UK for permission to use data from a biochemical bioassay on strains of cockroaches also used in this study.

REFERENCES

Atkinson, T.H., Wadleigh, R.W., Koehler, P.G. and Patterson, R.S., (1991). Pyrethroid Resistance and Synergism in a Field Strain of the German Cockroach (Dictyoptera:Blattellidae). *J. Econ. Entomol,*. 84(4): 1247-1250.

Cochran, D.G., (1987). Selection for Pyrethroid Resistance in the German Ccockroach (Dictyoptera: Blattellida). *J. Econ. Entomol.*, 80(6): 1117-1121.

Cochran, D.G., (1989). Monitoring for Insecticide Resistance in Field-Collected Strains of the German Cockroach (Dictyoptera: Blattellidae). *J. Econ. Entomol.*, 82(2):336-341.

Cochran, D.G.,(1991). Extended Selections for Pyrethroid Resistance in the German Cockroach (Dictyoptera: Blattellidae). *J. Econ. Entomol.*, 84(5):1412-1416.

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

Jensen, K.-M.,V., (1987). DPIL susceptible B. germancia strains tested by tarsal contact on 9 insecticides. *Danish Pest Inf.Lab.Ann.Re.*, 68-71.

Keiding, J., (1963). Resistance to dieldrin in cockroaches. *Danish Pest Inf.Lab. Ann.Rep.*, 44-45.

Milio, J.F., Koehler, P.G and Patterson, R.S., (1987). Evaluation of Three Methods for Detecting Chlorpyrifos Resistance in German Cockroach (Orthoptera: Blattellidae) Populations. *J. Econ. Entomol.*, 80:44-46.

Rust, M.K., Reierson, D.A., (1991). Chrlorpyrifos Resistance in German Cockroaches (Dictyoptera: Blattellidae) from Restaurants. *J. Econ. Entomol.*, 84(3):736-740.

Scott, J.F.,and 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., and Tanaka K., (1986). Effect of Method of Application on Resistance to Pyrethroid Insecticides in Blattella germanica (Orthoptera: Blattellidae). *J. Econ. Entomol.*, 79: 571-575.

WHO/VBC/75.593. Tentative instruction for determining the susceptibility or resistance of cockroaches to insecticides.