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CROSS-RESISTANCE IN INSECTICIDE-RESISTANT STRAINS OF THE GERMAN COCKROACH, *BLATTELLA GERMANICA* (L.)

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SYNOPSIS

The authors describe a series of experiments designed to determine the extent to which cross-resistance exists in insecticide-resistant strains of the German cockroach. The tests were conducted by exposing females from a DDT-resistant strain, a chlordane-resistant strain, and a non-resistant strain to varying concentrations of several insecticides. From these tests mortality data were obtained and are presented in the form of regression lines.

The insecticides used were DDT, methoxychlor, chlordane, dieldrin, Prolan, malathion, allethrin, and Lethane; these were chosen so that a wide variety of chemical types would be represented.

The results indicate that the DDT-resistant strain is highly resistant to DDT and to its analogue, methoxychlor, but not to any of the other insecticides tested. Similarly, the chlordane-resistant strain is highly resistant to chlordane and to its analogue, dieldrin, but is susceptible to the other toxicants listed.

From these results, as well as those obtained by others, it appears that the picture of cross-resistance in the German cockroach is in essential agreement with that which is emerging for other insects throughout the world.

Control of noxious insects by use of chemical poisons has been rather severely hampered during the last decade by the appearance of insecticide-resistant strains. The problem has been intensified by the additional discovery that insects resistant to one insecticide are frequently either resistant to other insecticides or capable of acquiring such resistance rapidly. This phenomenon is usually referred to as cross-resistance. It was noted by Busvine (1953, 1954) that, in houseflies, resistance to γ-BHC, chlordane, dieldrin, and a number of other cyclodiene insecticides seems to be linked together but is independent of DDT-tolerance. Subsequently, Metcalf (1955) attempted to systematize the phenomenon by establishing seven groups of insecticides, each composed of synthetic organic compounds of similar structure and killing action. These groups are as follows: Group I, DDT and its analogues; Group II, lindane and the cyclodiene insecticides; Group III, nitroparaffins; Group IV, organophosphorus insecticides; Group V, pyrethrins; Group VI, thiocyanates; and Group VII, the carbamate insecticides.

The general picture of cross-resistance is that insects resistant to a member of one group usually exhibit considerable resistance to other members of that group, but little if any resistance to members of other groups. This phenomenon has been rather extensively reviewed by Brown (1958), who pointed out that many exceptions exist. For example, Bruce & Decker (1950) reported a strain of houseflies highly resistant to methoxychlor, but with only negligible resistance to DDT (both Group I). Likewise, Keiding (1953) found a strain highly resistant to lindane but with no resistance to chlordane (both Group II). On the other hand, Pimental et al. (1953) found that a strain of houseflies made resistant to lindane by selection pressure from lindane were also very resistant to DDT but not to dieldrin.

With the German cockroach the work done so far has only partially elucidated the phenomenon of cross-resistance. Fisk & Isert (1953) found that a chlordane-resistant strain was highly resistant to chlordane and dieldrin, but not to a number of other insecticides in other groups. These same authors also tested a strain of DDT-resistant origin which had been reared for nine generations without exposure to insecticide, but found it to have virtually no resistance to any of the compounds tested, including DDT. That resistance to DDT is rapidly lost in this insect after discontinuance of exposure to DDT has been reported by Grayson.[1] In addition, Butts & Davidson (1955) found that a chlordane-resistant strain was resistant to a number of Group II insecticides, including lindane. On the other hand, Grayson (1954) found that a chlordane-resistant strain of cockroaches was quite susceptible to lindane, while Jarvis & Grayson (1957) and Grayson & Jarvis (1958) found that cockroaches from the same strain could be controlled by use of several organic phosphate insecticides.

The purpose of the present communication is to report the findings from experiments designed to determine, in a systematic manner, the extent to which cross-resistance exists in insecticide-resistant strains of the German cockroach, *Blattella germanica.*

Materials and Methods

The cockroaches used in these experiments were adult females between 5 and 15 days in age. They were derived from stock cultures of DDT-resistant, chlordane-resistant and normal strains which are continuously maintained in the authors' laboratory. In order to ensure a constant supply of young adults, several breeding jars were established for each strain. As

[1] In a paper presented at the Fourth International Congress of Crop Protection, Hamburg, Germany 1957 (to be published).

the nymphs from these breeders approached maturity they were transferred to large aquaria cultures. Upon reaching maturity, they became eligible for testing. All rearing was accomplished in a constant temperature and humidity cabinet at 30°C and approximately 60% relative humidity. Other details of rearing procedure have been previously described by Grayson (1951).

The insecticides used were as follows: DDT, methoxychlor, chlordane, dieldrin, Prolan, malathion, allethrin and Lethane. In all cases technical-grade insecticides were used except with DDT, of which water suspensions were prepared from a 75% wettable powder. The technical-grade insecticides were dissolved in acetone to form stock solutions, from which the desired amounts were volumetrically measured in preparing the water suspensions. In the case of chlordane it was necessary to add a suspending agent in equal volume to the toxicant in order to obtain satisfactory suspensions.

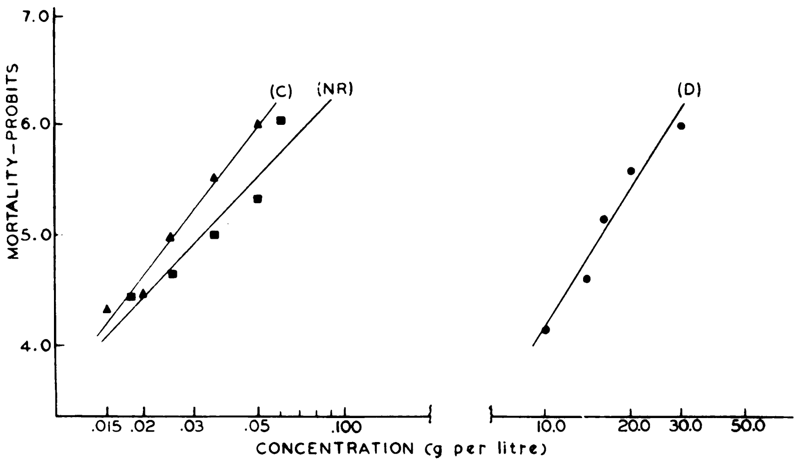
For purposes of insecticide treatment, adult females were placed in small screen cages, usually 25 to 30 per cage, and dipped in the insecticidal preparations at 30°C. They were held in the insecticide suspensions for 20 seconds and then kept in the treated cages for 60 minutes before removal to recovery jars. Food and water were provided during the subsequent period of observation. Mortality counts were made six days following treatment. Dead and moribund cockroaches were combined in the mortality counts. The criterion for regarding a cockroach as moribund was inability to exhibit active locomotion.

The tests were conducted by exposing females from each of the three strains to varying concentrations of the appropriate insecticide. Five concentrations were used to establish the response of each strain to each insecticide. In all cases three replicates of each concentration were obtained. The data were plotted on logarithmic-probability paper, and regression lines were fitted to the points by the method of least squares (Bliss, 1935). The " b " values or slopes of the regression lines were also calculated using this procedure.

Results

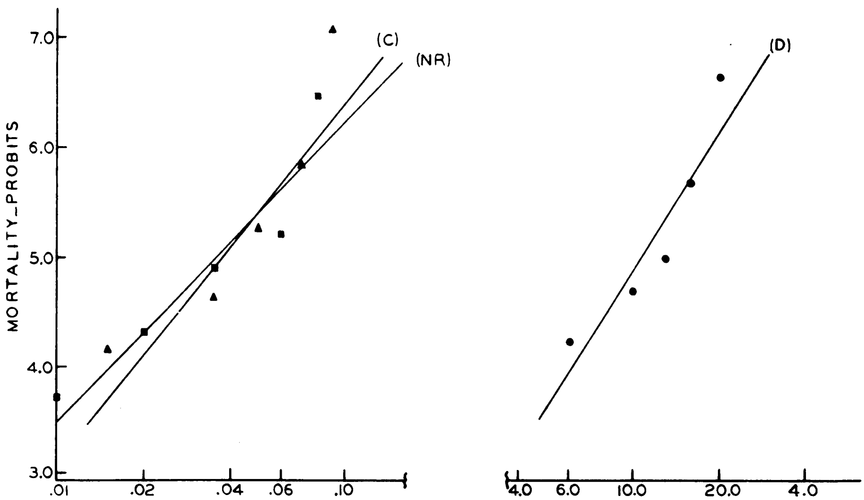
The responses obtained by exposing females from each of the three strains to a variety of insecticides are depicted in Fig. 1–8. The data show that the DDT-resistant strain is resistant to DDT (Fig. 1) and its closely related analogue, methoxychlor (Fig. 2). Based on the resistance at LC_{50} (concentration of the toxicant which kills 50% of the insects), the female cockroaches of this strain are 497 times more resistant to DDT and 310 times to methoxychlor. This same strain exhibited only slight increases in LC_{50} when exposed to the following compounds: 3.4 times to chlordane (Fig. 3), 2.7 times to dieldrin (Fig. 4), 1.5 times to Prolan (Fig. 5), 2.8 times to malathion (Fig. 6), 1.9 times to allethrin (Fig. 7), and 2.3 times to Lethane

FIG. 1. TOXICITY OF DDT TO FEMALES OF THREE STRAINS OF GERMAN COCKROACH



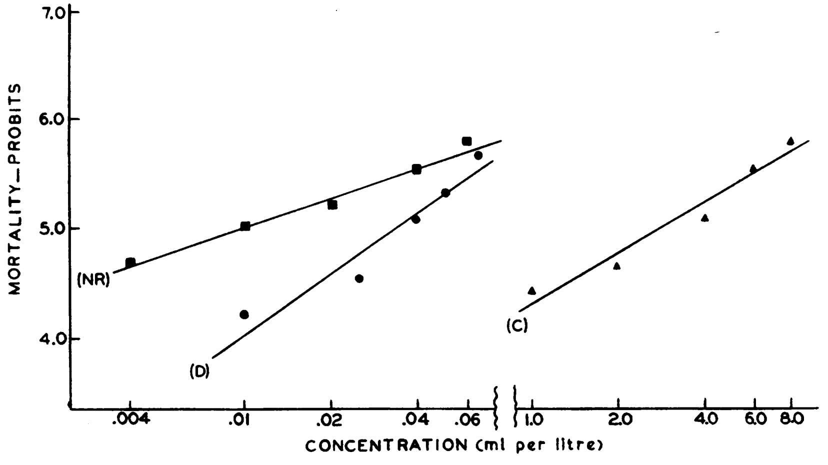
D = DDT-resistant strain C = chlordane-resistant strain NR = non-resistant strain

FIG 2. TOXICITY OF METHOXYCHLOR TO FEMALES OF THREE STRAINS OF GERMAN COCKROACH



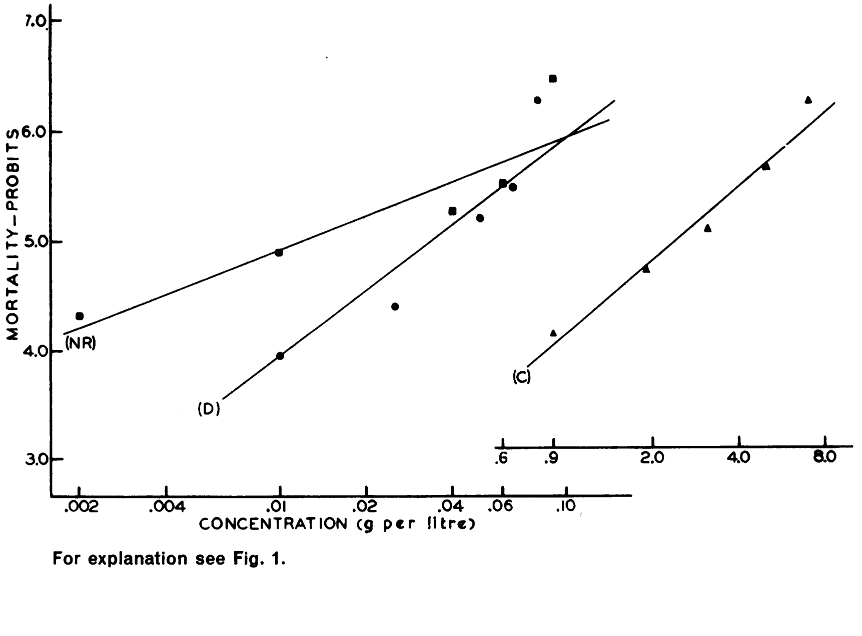
For explanation see Fig. 1.

FIG. 3. TOXICITY OF CHLORDANE TO FEMALES OF THREE STRAINS OF GERMAN COCKROACH



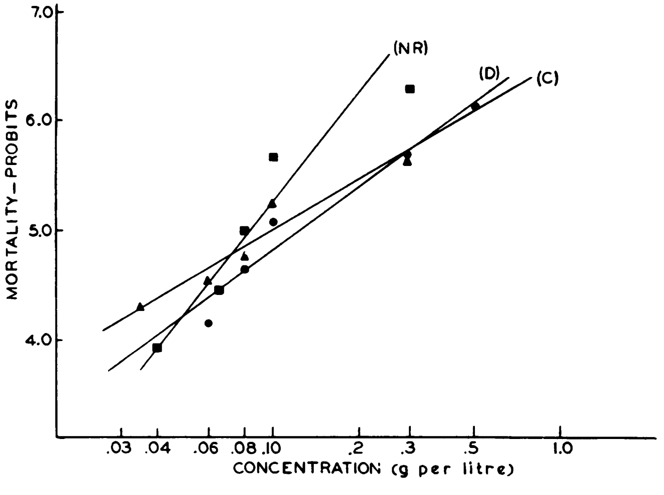
For explanation see Fig. 1.

FIG. 4. TOXICITY OF DIELDRIN TO FEMALES OF THREE STRAINS OF GERMAN COCKROACH



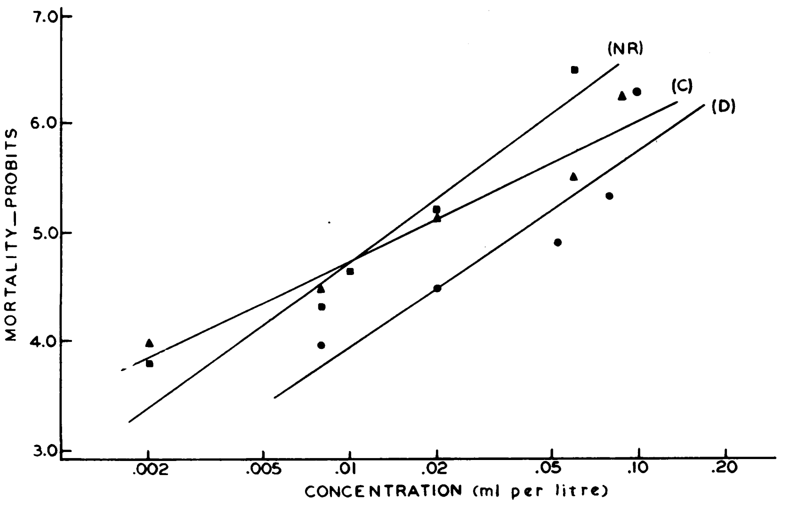
For explanation see Fig. 1.

FIG. 5. TOXICITY OF PROLAN TO FEMALES OF THREE STRAINS OF GERMAN COCKROACH



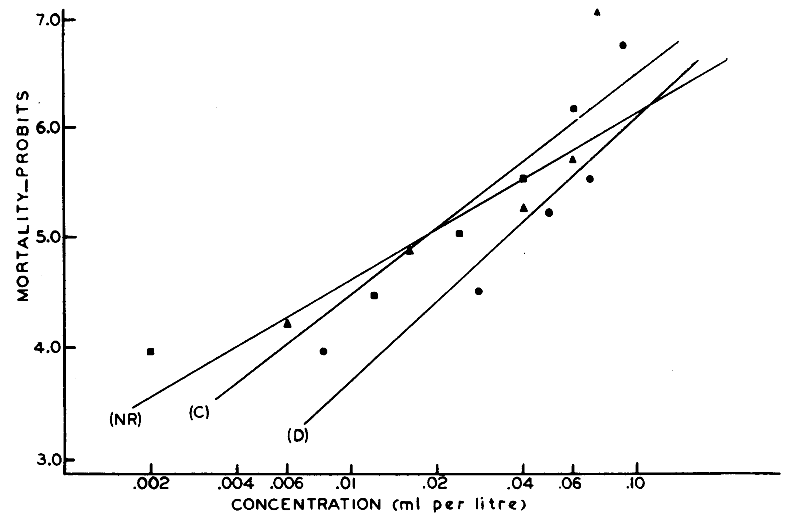
For explanation see Fig. 1.

FIG. 6. TOXICITY OF MALATHION TO FEMALES OF THREE STRAINS OF GERMAN COCKROACH



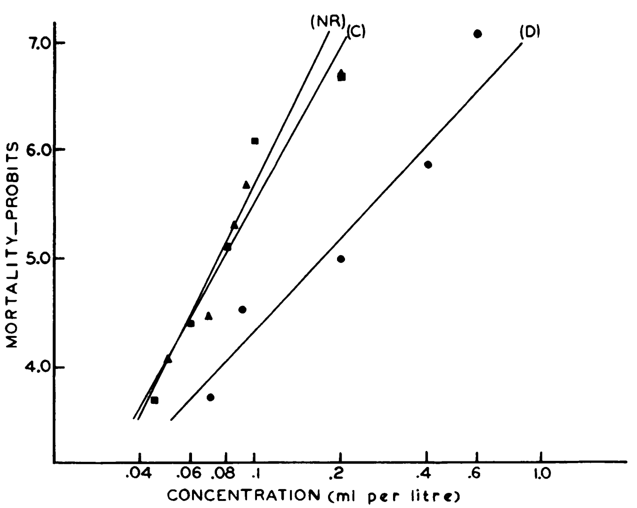
For explanation see Fig. 1.

FIG. 7. TOXICITY OF ALLETHRIN TO FEMALES OF THREE STRAINS OF GERMAN COCKROACH



For explanation see Fig. 1.

FIG. 8. TOXICITY OF LETHANE TO FEMALES OF THREE STRAINS OF GERMAN COCKROACH



For explanation see Fig. 1.

(Fig. 8). In all cases these values were calculated by setting the LC_{50} value of the non-resistant strain equal to one.

Similarly, the chlordane-resistant strain exhibited resistance to chlordane (Fig. 3) and its closely related analogue, dieldrin (Fig. 4). At LC_{50} the strain was 280 times more resistant to chlordane and 212 times to dieldrin. The chlordane-resistant strain responded in virtually the same manner as the non-resistant strain to methoxychlor (Fig. 2), malathion (Fig. 6), allethrin (Fig. 7), and Lethane (Fig. 8). On the other hand, this strain was apparently slightly more susceptible to DDT and slightly less susceptible to Prolan than the non-resistant strain.

The LC_{50} values and the " b " values, or slopes of the various regression lines, are recorded in the table. It can be seen that the slopes vary considerably from strain to strain and from insecticide to insecticide. The only consistent feature of the slopes is that for each strain the values obtained are virtually the same with DDT and methoxychlor. It is also interesting to note that, contrary to the hypothesis of Hoskins & Gordon (1956), there is no general flattening of the regression lines (decrease in slope) from the resistant strains in comparison with the non-resistant strain in cases where significant resistance was found. It must be recalled, however, that these strains are already highly resistant to the insecticides employed in their selection.

LC_{50} VALUES AND SLOPES OR " b " VALUES OF THE REGRESSION LINES OBTAINED FROM EXPOSING THREE STRAINS OF COCKROACH TO VARIOUS INSECTICIDES

Toxicant	DDT-resistant strain *		Chlordane-resistant strain *		Non-resistant strain *	
	LC_{50}	slope	LC_{50}	slope	LC_{50}	slope
DDT	15.900	4.2	0.026	3.5	0.032	2.8
Methoxychlor	10.800	4.2	0.037	3.3	0.035	2.7
Chlordane	0.034	1.8	2.800	1.5	0.010	0.9
Dieldrin	0.033	2.4	2.550	2.2	0.012	1.0
Prolan	0.125	1.9	0.100	1.5	0.082	3.3
Malathion	0.039	1.8	0.016	1.3	0.014	1.9
Allethrin	0.035	2.4	0.018	2.0	0.018	1.5
Lethane	0.175	2.8	0.078	4.7	0.074	5.3

* LC_{50} values are expressed as grams or millilitres of toxicant per litre of suspension.

Discussion

The data presented in this study indicate that limited cross-resistance exists in DDT-resistant and chlordane-resistant strains of the German cockroach. The results appear to agree with the generalizations established by Metcalf (1955) in that the DDT-resistant strain of the German cockroach is resistant to DDT and methoxychlor (Group I insecticides), and the chlordane-resistant strain is resistant to chlordane and dieldrin (Group II insecticides). This agreement is further substantiated by the negligible resistance which the DDT-resistant strain shows to the compounds tested in Groups II-VI and the virtual lack of resistance which the chlordane-resistant strain shows to compounds tested in Group I and in Groups III-VI.

Certain comparisons may also be drawn from the results of this experimentation and those obtained by other investigators. For example, the present results agree with the data of Fisk & Isert (1953) in showing that the chlordane-resistant strain of the German cockroach is highly resistant to chlordane and dieldrin. However, Fisk & Isert used a DDT-resistant strain with a low degree of resistance to DDT. Thus, it is not surprising to find that their results indicate very little resistance of any type in this strain, whereas the strain used here shows high resistance not only to DDT but also to methoxychlor. The present results also agree with Jarvis & Grayson (1957) and Grayson & Jarvis (1958) in showing that chlordane-resistant German cockroaches are not resistant to the organic phosphate insecticide, malathion, but, as indicated, are resistant to chlordane and dieldrin.

Thus it can be seen that the information on insecticide resistance in the German cockroach represents an example of a relatively simple and straightforward situation with regard to cross-resistance. The only exception to this is Grayson's (1954) report of a chlordane-resistant strain being susceptible to lindane. This point is currently being reinvestigated.

It should be pointed out that this agreement according to groups of insecticides is probably what would be expected in view of the history of these strains. With the exception of the early generations of the chlordane-resistant strain, all of the work on this insect has been carried out under closely controlled laboratory conditions in which the DDT-resistant strain was exposed only to DDT and the chlordane-resistant strain only to chlordane. On the other hand, some of the apparent exceptions to Metcalf's hypothesis have been reported from field studies (Hansens, 1953) or laboratory studies (March & Metcalf, 1949) in which the insects were exposed to insecticides from one group followed by exposure to insecticides from another group. Such an occurrence could easily explain these cases of multiple resistance. It is quite probable that the cockroaches resistant to Group I and Group II toxicants described above would develop resistance to other groups of insecticides if they were put under selection pressure with such insecticides. It should also be remembered that, because of the widespread use of insecticides in agricultural and public health programmes, it is extremely difficult to be sure that a wild population has never been exposed to insecticides. The more real exceptions to Metcalf's hypothesis are not so easily explained. One is forced to the conclusion that in certain isolated cases aberrant resistance mechanisms seem to be operative (see, for instance, Pimental et al., 1953).

The physiological bases for the development of insect resistance to most insecticides are still largely unknown. The one possible exception is with DDT, but even here the mechanism of resistance is not perfectly understood. However, in the present study it seems quite obvious that some sort of biochemical or physiological protective mechanism is functioning in view of the high degree of resistance reported. Furthermore, the data provide presumptive evidence that the mechanism giving protection against DDT is the same as or very similar to that giving protection against methoxychlor. Similarly, the mechanism giving protection against chlordane appears to be the same as or very similar to that giving protection against dieldrin. However, these two types of resistance mechanism appear to be completely independent. Conversely, the slight shifts in LC_{50}, which the DDT-resistant and chlordane-resistant strains show when tested with compounds outside the groups to which they were selected, do not appear to be due to specific biochemical resistance, but rather to some other factor or factors such as increased vigour, elimination of susceptibles, etc. From a genetical point of view, these slight shifts in LC_{50} are probably a reflection of the fact that the three strains are not isogenic populations, and it would be quite absurd to expect them all to respond in the same manner. However, these shifts in LC_{50} and the slopes of the various lines do seem to indicate a trend which should be mentioned. In general, it appears that factors for " vigour tolerance " are either absent or are of minor significance in the chlordane-resistant strain, while in the DDT-resistant strain they are prominent in every case.

In discussing small variations in regression lines, as evidenced by changes in slope and LC_{50} values, a cautionary note needs to be inserted. As indicated by Hoskins & Gordon (1956), by Hewlett (1958), and by Wilson-Jones & Davidson (1958), there are many parameters which may influence the slope and position of a regression line. Among those that have been mentioned are: (1) genetic variability, (2) toxic properties of the insecticide, (3) differences in rate of absorption at high and low dosages, (4) phenotypic variation of the population and (5) chance variation due to sampling errors. In many cases, it is difficult to determine which, if any, of these parameters is exerting a dominant influence. Therefore, small differences in slope and position of regression lines must be interpreted with extreme care.

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RÉSUMÉ

Les auteurs examinent dans cet article le phénomène de résistance croisée, tel que le présentent les souches de blattes, *Blattella germanica*, résistantes aux insecticides. Les expériences ont porté sur des femelles adultes de souches résistantes au DDT, au chlordane, et non résistantes.

Les études de résistance croisée faites ces dernières années ont montré qu'il existe une résistance croisée vis-à-vis de substances de constitution chimique analogue, mais non pas entre substances chimiquement étrangères. Sur cette base, certains auteurs ont tenté de grouper les nouveaux insecticides synthétiques d'après leur structure et leur action létale en 7 groupes. Cette classification n'est cependant pas universelle, et le cas de la blatte, *B. germanica* en particulier, n'avait pas été tiré au clair.

Dans la présente étude, les recherches ont porté sur 6 des 7 groupes, soit: DDT et méthoxychlor (I), chlordane et dieldrine (II), Prolan (III), malathion (IV), allethrin (V) et Lethane (VI). Des blattes ont été trempées dans des suspensions aqueuses de ces insecticides — à 5 concentrations différentes — pendant un temps donné. Les résultats ont été portés sur un papier gausso-logarithmique et les lignes de régression tracées. Chaque point représente le résultat de trois épreuves au moins.

Les résultats indiquent que la souche résistante au DDT l'est aussi au méthoxychlor, mais à aucun autre des composés essayés. De même, la souche résistante au chlordane l'est aussi à la dieldrine, mais à aucun autre des insecticides considérés. Il semble donc que ce sont des mécanismes différents qui provoquent la résistance aux groupes I et II. La blatte en question présente une résistance croisée au sein des groupes I et II, mais vis-à-vis d'aucun autre groupe.

Il apparaît d'après ces résultats que *B. germanica* présente une résistance croisée du même type que celle qui a été observée chez d'autres insectes, dans le monde entier.

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