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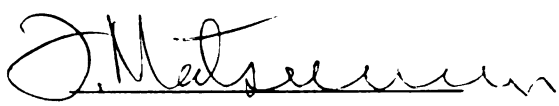
CHARACTERISTICS OF DDT-INDUCED PYRETHROID RESISTANCE IN THE GERMAN COCKROACH, BLATTELLA GERMANICA

presented by

Jeffrey Graham Scott

has been accepted towards fulfillment of the requirements for

M.S. degree in Entomology



Major professor

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CHARACTERISTICS OF DDT-INDUCED PYRETHROID RESISTANCE IN THE GERMAN COCKROACH, BLATTELLA GERMANICA

By

Jeffrey Graham Scott

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ABSTRACT

CHARACTERISTICS OF DDT-INDUCED PYRETHROID RESISTANCE IN THE GERMAN COCKROACH, BLATTELLA GERMANICA

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The mechanism of cross-resistance between DDT and pyrethroids was studied using a DDT-resistant (VPIDLS) strain of Blattella germanica, a strain never exposed to pyrethroid selection. The resistance mechanism is likely a kdr type for the following reasons: (1) decreased sensitivity of the nervous system to DDT and permethrin, (2) cross-resistance to methoxychlor, and (3) lack of difference in the rate of permethrin metabolism between resistant and susceptible cockroaches in vitro. Kdr did not confer substantial cross-resistance to the recently developed synthetic pyrethroids, cypermethrin and decamethrin.

The expression of poisoning by cypermethrin and decamethrin is different from pyrethrin and its synthetic analogue, allethrin, by the following criteria: (1) electrophysiological symptoms, (2) temperature effects on toxicity, and (3) the lack of substantial cross-resistance of kdr roaches to decamethrin and cypermethrin. Such differences suggest that at least a part of the mechanism(s) of action of these newly developed pyrethroids differs from the old type pyrethrin analogs.

To Mom and Dad

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to Dr. Fumio Matsumura for all his advice and direction during the course of this investigation.

To Carol Thomson for her patience, support, faith and encouragement - thank you.

I also wish to express my thanks to Dr. Brian Croft and Dr. Ralph Pax for all they taught me and for their assistance on numerous occasions.

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INTRODUCTION

Due to the increased use of pyrethroids, the question of insect resistance to this new group of insecticides is of increasing importance. Some DDT-resistant insects are cross-resistant to pyrethrins (4,33,39). Several DDT-resistant species also are cross-resistant to the newly developed pyrethroids, to which they have never been exposed (11,26,34). This cross-resistance suggests the possibility of future problems in the use of pyrethroid insecticides (34).

Pyrethroid cross-resistance is related to the kdr (knockdown resistance) factor in houseflies (9,11) which also confers DDT resistance through a decreased nerve sensitivity (9,31,41). The kdr factor appears also to be responsible for pyrethroid-resistance in mosquitoes (25,31), Spodoptera littoralis (13), and the predatory mite, Amblyseius fallacis (35).

Understanding of cross-resistance problems requires basic knowledge on underlying physiological and biochemical mechanisms. The German cockroach, Blattella germanica, is uniquely suited for this type of study for three reasons. First, it has been shown that DDT-resistance in this species involves only reduced nerve insensitivity involving no metabolic or penetration factors (19). Second, it is well suited for electrophysiological studies in an analogous situation with that of the well studied American cockroach. Third, and most important, a DDT-resistant strain (VPIDLS) has an altered Ca-ATPase in its nervous system which is more resistant to DDT inhibition in vitro (14).

The natural pyrethrins were not effective pesticides for field use due to their low environmental stability and high degradability. Elliott and other

workers experimented with a great deal of structure modification to produce many new synthetic pyrethroids which have both greater stability and insecticidal properties. In this paper I will refer to "Type 1 pyrethroids" meaning pyrethrins and allethrin (esters of cyclopropanecarboxylic acids with alkenylmethyl cyclopentenolnes) and "Type 2 pyrethroids" referring to the newer synthetic pyrethroids, especially to cypermethrin and decamethrin (α-cyanophenoxybenzyl esters of dihalovinylchrysanthemic acid).

Many previous studies on Type 1 pyrethroids revealed numerous similarities to DDT. In addition to cross-resistance, pyrethrins, like DDT, show a negative temperature correlation (2,6,16,18) (i.e., greater kill at lower temperature) and both cause repetitive discharges in axons (38).

It is widely believed that all pyrethroids are similar in their mode of action and show cross-resistance due to kdr, even though central vs. peripheral action of pyrethroids is still widely debated (3,22). Recently, differences in insect responses to synthetic pyrethroids have been reported by Clements and May who found that α-cyano pyrethroids did not cause repetitive discharges or enhanced and prolonged muscle contractions as did other types of pyrethroids (8). Gammon (13) previously has shown a type of modified repetitive discharge caused by permethrin and further demonstrated a lack of repetitive discharges with cypermethrin, the α-cyano analogue of permethrin. Clark (7) recently has demonstrated a difference in the inhibitory powers of the Type 1 and Type 2 pyrethroids to two ATPase systems thought to be of importance in the maintenance of electrochemical gradients in neurons. The major goals of this research are to understand the nature of DDT induced cross-resistance to pyrethroids and to clarify the similarities and differences of the mechanism of action among various pyrethroids.

MATERIALS AND METHODS

Materials

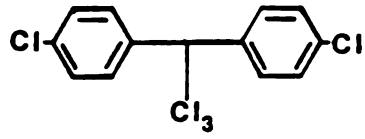
Animals and Chemicals

The sources and purity of all compounds used in this study are given in Table 1. The structures of the pesticides studied are shown in Figure 1.

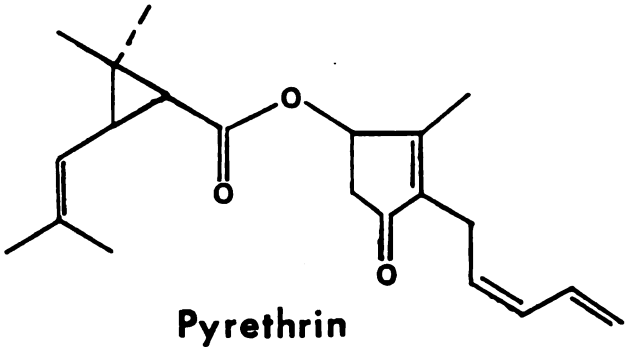
The three strains of Blattella germanica used in this study were: CSMA (Chemical Standard of Manufacturers Association) and Columbus (originally from the University of Western Ontario), both of which are susceptible strains, and VPIDLS, a DDT-resistant (kdr) strain selected from the original VPI (Virginia Polytechnic Institute) strain. All of these strains have been reared by our laboratory for many years (20,37). The CSMA strain was used for all tests except the temperature vs. toxicity tests where Columbus was used. The VPIDLS strain had shown considerable reversion from its previously high level of DDT resistance (14) and was, therefore, subjected to three further selections. Selections were accomplished by placing approximately 40 sixth instar nymphs in a one pint mason jar coated with the desired dose of DDT as described in the Methods section. After 24 hours the roaches were provided with a cube of Purina Dog Chow and a one inch piece of dental wick soaked with water. After about 50 percent mortality had been reached the survivors were placed in a rearing jar and allowed to mate. The offspring were removed and put in a new rearing jar for further testing. The doses and times of the selection process are shown in Table 2. VPIDLS never has been subjected to any pyrethroid insecticide selection.

Table 1. Sources and Purity of Compounds Studied

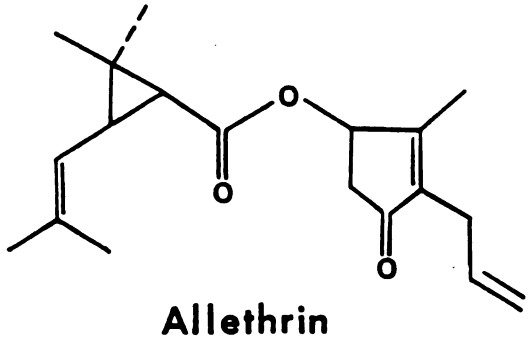
Compound	Source	Purity
DDT	Aldrich Chemical Company	99%
Pyrethrins	Fairchild American Corporation	
S-Bioallethrin	McLaughlin Gormley King Company	96.7%
Permethrin	Penick	91% (cis:trans ratio 35:65)
Fenvalerate	Shell	
Cypermethrin	FMC	
Decamethrin	Roussel UCLAF	99.6%
Piperonyl Butoxide	Pfaltz and Bauer Inc.	



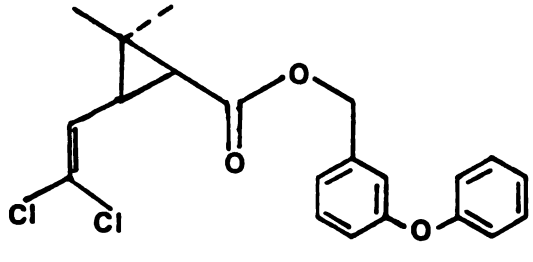
DDT



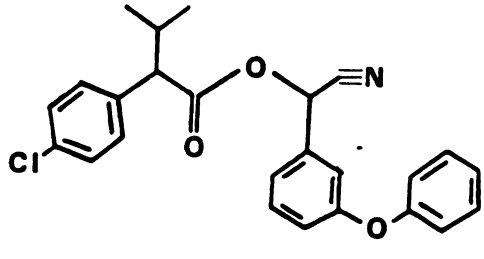
Pyrethrin



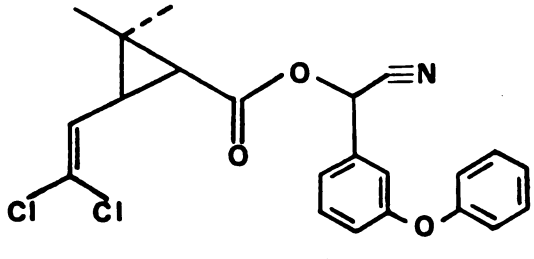
Allethrin



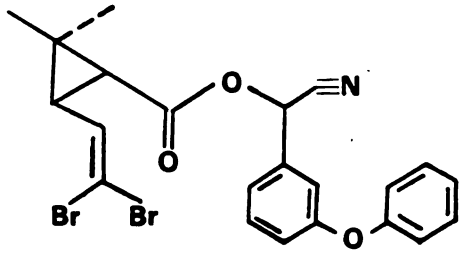
Permethrin



Fenvalerate



Cypermethrin



Decamethrin

Figure 1. Chemical structures of DDT and six pyrethroid insecticides

Table 2. Selection Process for VPIDLS

Selection Number	Resulting Generation	Dose ($\frac{\text{mg DDT}}{\text{1 pt jar}}$)	Duration[a] (Days)
1	VPIDLS G1	4	1
2	VPIDLS G2	10	14
3	VPIDLS G3	10	28

[a]Food and water were supplied after 24 hours.

Periplaneta americana used in some of the electrophysiological studies were from a culture which has been maintained by this laboratory for many years.

Adult male cockroaches were used exclusively for all of the experiments.

Methods

Susceptibility Studies

Surface contact method

The inner surfaces of one pint mason jars were covered with a uniform layer of pesticide delivered in 1.0 ml of acetone. One control per test was run in a jar coated with 1.0 ml of acetone. German cockroaches (25 for the DDT tests, 10 for the dieldrin and pyrethrins tests and 20 for all other tests were placed in each jar and observed at room temperature (24-25^{o}C). Each jar was given a one inch piece of dental wick soaked with water after 24 hours and one cube of Purina Dog Chow after 48 hours. A cockroach was considered knocked down if it was on its back and unable to right itself. Death was defined as being nonresponsive to touch. Three replications were used for all experiments. For synergism studies, 6 µg of piperonyl butoxide (in 1.0 µl of acetone) was applied to the abdominal sternum one hour before exposure to pesticide.

Abdominal topical application

The appropriate amount of pesticide was delivered in 1.0 µl of acetone to the abdominal sternum of the male cockroach. Control cockroaches were treated with 1.0 µl of acetone. They were maintained in the same type of jar with food and water as above. After 4 min., 8 min., 15 min., 30 min., 1 hour, 2 hours, 5 hours, 12 hours, 24 hours, 48 hours and 96 hours, knockdown and mortality were recorded according to the definitions given above. All tests consisted of four doses replicated three times with ten cockroaches at each dose.

Cural topical application

The appropriate amount of pesticide was delivered in 2.0 µl to four legs with each leg receiving approximately 0.5 µl. The placement of the droplet was at the joint between the tibia and tarsus. Controls were treated with 2.0 µl of acetone by the same method. All tests consisted of five roaches per dose with at least four doses and done in duplicate for the cural vs. abdominal study (Figures 5 and 6) or triplicate for the temperature vs. toxicity tests (Figures 7 and 8, Table 6). For the cural vs. abdominal study, the abdominal dose was delivered in 2.0 µl. For the temperature vs. toxicity tests the following post-application conditions were employed: (1) low temperature-11°C, RH 50 to 60%, (2) room temperature-24.5°C, RH 50 to 60% and (3) high temperature-31.0°C, RH 50 to 60%.

Calculations

When there was mortality in the control, the adjusted percent mortality was calculated using Abbott's formula. Data was then calculated by Finney's method (12). The parameters used in this study are: (1) resistance ratio (RR) (resistant strain susceptibility/susceptible strain susceptibility), (2) synergistic ratio (SR) (susceptibility without synergist/susceptibility with synergist), (3) KD_{50} (dose required to knock down 50% of the individuals tested in 24 hours), (4) LD_{50} (dose required to kill 50% of the individuals tested in 24 hours or 96 hours in the case of DDT), (5) KT_{50} (time required to knock down 50% of the individuals tested at the given dose) and (6) LT_{50} (time required to kill 50% of the individuals tested at the given dose).

Significance was determined by using a Student's T test for all experiments except the nerve sensitivity studies where the sign test was used. For either test * indicates significance (0.05), ** indicates highly significant (0.01), and *** indicates very highly significant (0.001).

Calculations of the knockdown activities of the pyrethroids were made by the method of Nishimura et al. (25). This model uses the assumption that first order kinetics can be used to describe initial pyrethroid penetration, therefore, when $-\ln(1-KD_{50}^{\infty}/KD_{50}^{t})$ vs. time is plotted, those compounds with the most potent knockdown activity will have the steepest slope.

Metabolism Study

After removal of the head, legs, and wings of 75 adult male cockroaches, their bodies were ground in ice cold insect saline (40) (2 mM phosphate buffer, pH 7.2) with sand for three minutes. The homogenate was first centrifuged at 1200 xg for 90 min. The supernatant fluid thus obtained was re-centrifuged at 100,000 xg for 60 min. The resultant pellet was resuspended in 2.5 ml of saline for use. The incubation mixture contained 10 mM nicotinamide, 150 mM KCl, 8 mM $Na_2HPO_4 \cdot 7\ H_2O$, 2 mM KH_2PO_4 and 0.3 mM NAPDH (pH 7.3). Each treatment contained 1.80 mg of protein and 2.5 µg of permethrin in 2.0 ml of incubation mixture. All incubations were carried out at 30°C. Protein determinations were done by the method of Lowry et al. (21). Quantitation of the permethrin remaining was accomplished by the use of an electron-capture detector (^{63}Ni) coupled with a Varian 3700 gas chromatograph using a six foot class column of 5% QF-1 on Chromosorb W, DMCS at a column temperature of 220°C, and was calculated as a percent of the control (time=0) peak area. Peak areas were calculated as height x width at one-half peak height.

For analysis of cytochromes, the enzyme was prepared as described above and diluted with 0.2 M sodium phosphate buffer (pH 7.2). The CO-binding spectra were measured in the presence of $Na_2S_2O_4$ at room temperature (24°C) with a Varian 635 scanning spectrophotometer. The amount of cytochrome P-450 and cytochrome b_5 was determined by the method of Omura and Sato (28),

using 91 mM^{-1} cm^{-1} at 450 nm (28) and 185 mM^{-1} cm^{-1} at 425 nm (29) as the molar extinction coefficients, respectively.

Electrophysiological Studies

Nerve sensitivity

Tests were conducted on the intact abdominal nerve cord, exposed by opening the dorsal side of the male German cockroach. The dissections were performed essentially as described by Beeman and Matsumura (1). The saline used contained 210 mM NaCl, 3.1 mM KCl, 1.8 mM $Na_2HPO_4 \cdot 7\ H_2O$, 1.8 mM $CaCl_2 \cdot 2\ H_2O$ and 0.2 mM NaH_2PO_4 with a final pH of 7.4 (40). Air was bubbled through the saline for at least one hour prior to use. Pesticide dissolved in 95% ethanol was added to the saline solution immediately before each treatment. The amount of ethanol added never exceeded 0.2%. After dissection, the specimens were treated by flooding the preparation with 7.0 ml of the saline plus pesticide solution (or saline plus ethanol for the controls) for five minutes, prior to monitoring of nerve cord activity. The temperature range of the tests was 23.0 to 24.0°C for permethrin and 23.5 to 25.5°C for DDT. The temperature fluctuation is assumed to be of minimal importance as an equal number of susceptible and resistance cockroaches were run each day.

Recordings of electrical activity were made by placing a pair of fine silver wire electrodes underneath the nerve cord midway between the fifth and sixth abdominal ganglia, and gently pipetting out the saline solution. The entire preparation was then enclosed in a chamber lined with moistened filter paper to prevent rapid desiccation and increase the life of the preparation. Observation of control insects indicated that the nerve preparation gives at least 35 minutes of reliable performance. No effort was made to continue the observations after this time period. A more in-depth discussion of the machinery used is given in Beeman and Matsumura (1).

Susceptibilities of the nerve cords were measured as a function of the time until onset of repetitive discharge. Repetitive discharge was defined as electrical activity of a uniform nature, firing at a frequency of ten milliseconds or less, being of greater amplitude than 10 μ V (minimal detectable voltage) and lasting at least one second for DDT or two seconds for permethrin. Controls were run for the same length of time under identical experimental conditions.

Responses to pesticides

The nerve cord of Periplaneta americana was isolated from just anterior of the first abdominal ganglion to the end of the cercal nerves. The nerve was placed in a trough in a Sylgard 184 dissection dish and the spontaneous activity was monitored by the use of suction electrodes. The saline was the same as previously described, except that air was not bubbled through it before use. The nerve cord was rinsed with 1.0 ml of saline or saline containing pesticide every 10 minutes. Controls of this type of nerve preparation were found to last for many hours.

RESULTS

Susceptibility Studies

It generally has been acknowledged that a kdr factor for DDT resistance confers a cross-resistance to pyrethroid insecticides in the housefly, ticks, Spodoptera littoralis, Amblyseius fallacis and some mosquito species (4,11,13,26,33,34,35,36,39). The data (Figures 2 and 3) confirm this tendency for the Type 1 pyrethroids, but disputes this tendency for the Type 2 pyrethroids as judged by the results of a surface contact test. In these tests resistance was manifested more clearly when assessed by knockdown than by death. The largest resistance ratio was 1000 for the VPIDLS G3 strain to pyrethrins (Figure 3). The susceptibility of the CSMA (susceptible) strain, which was used in the calculation of the resistance ratios in Figures 2 and 3, and the doses used are shown in Table 3.

Two major mechanisms of DDT resistance are known to be kdr (target site insensitivity) and DDTase (metabolic dehydrochlorination) (30). Kdr in houseflies confers cross-resistance to methoxychlor (a DDT analogue) whereas DDTase does not (30,32). The resistance ratios for methoxychlor (Table 4) are similar to those for DDT (Figure 2).

Penetration as a resistance mechanism confers cross-resistance to a large spectrum of pesticides, depending on their polarity. There was no resistance to dieldrin, a pesticide with similar polarity to the synthetic pyrethroids, in any of the strains tested (Table 5), indicating that penetration is not likely a mechanism of resistance in VPIDLS cockroaches.

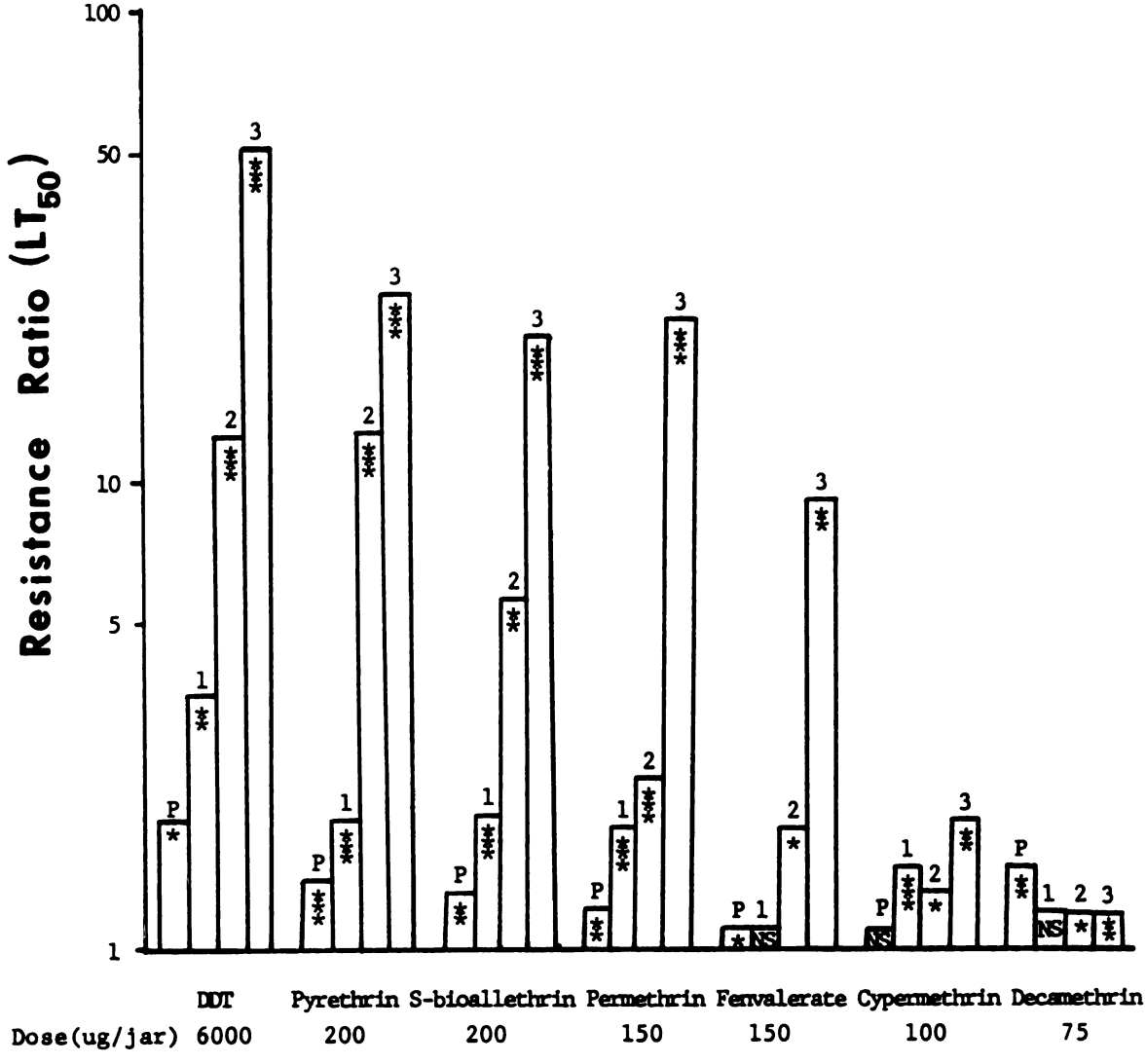


Figure 2. Comparison of mortality resistance ratios in four substrains of B. germanica to DDT and six pyrethroid insecticides by a surface contact method, with P, 1, 2, and 3 referring to VPIDLS parental and successive DDT-selected generations, G1, G2, and G3, respectively.

*Significantly greater than 1.0 (CSMA) at $P < 0.05$.
**Significantly greater than 1.0 (CSMA) at $P \leq 0.01$.
***Significantly greater than 1.0 (CSMA) at $P \leq 0.001$.

NS Not significantly greater than 1.0 (CSMA) at $P \leq 0.05$.

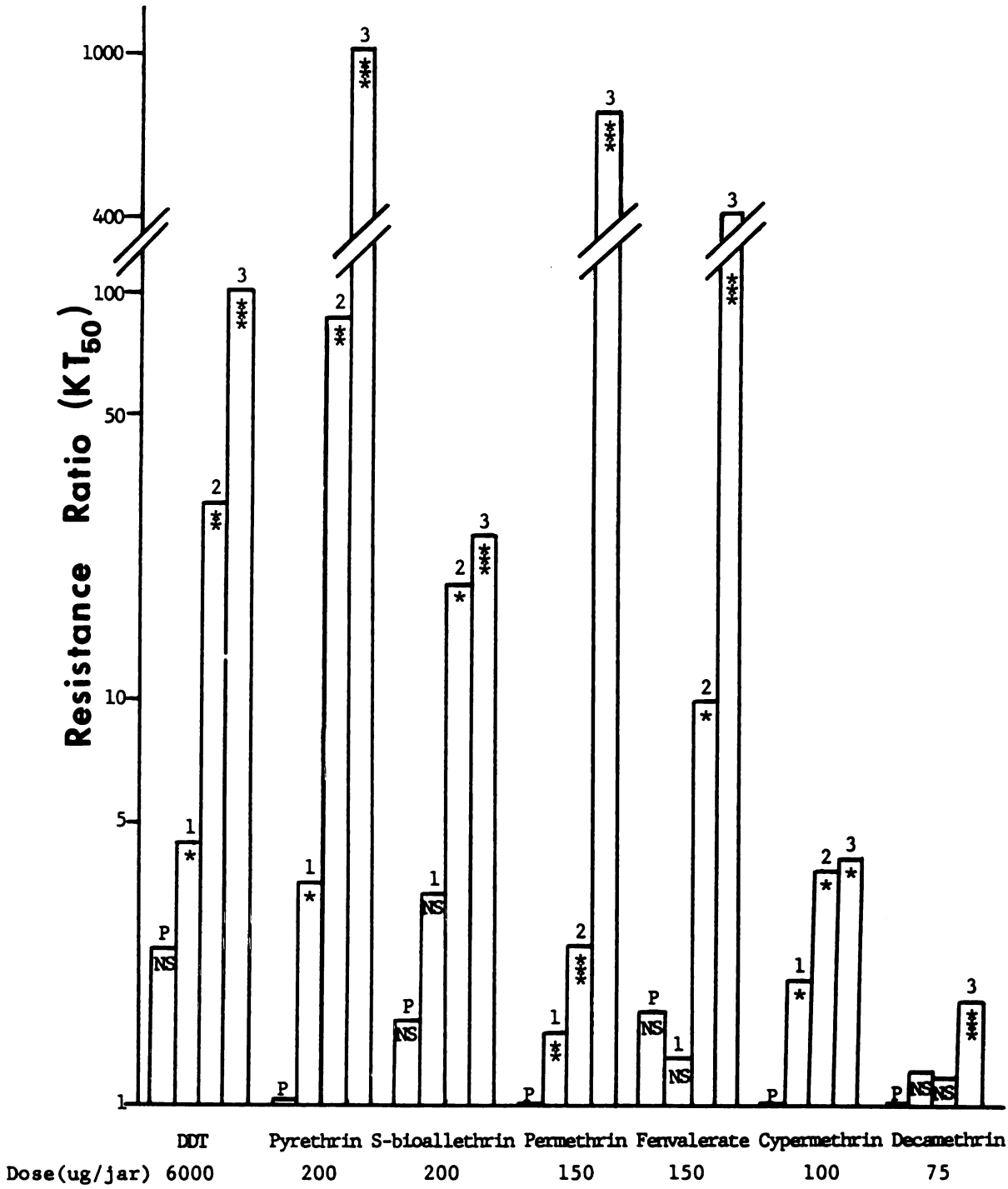


Figure 3. Comparison of knockdown resistance ratios in four substrains of B. germanica to DDT and six pyrethroid insecticides by a surface contact method, with P, 1, 2, and 3 referring to VPIDLS parental and successive DDT-selected generations, G1, G2, and G3, respectively.

*Significantly greater than 1.0 (CSMA) at $P < 0.05$.

**Significantly greater than 1.0 (CSMA) at $P < 0.01$.

***Significantly greater than 1.0 (CSMA) at $P < 0.001$.

NS Not significantly greater than 1.0 (CSMA) at $P \leq 0.05$.

Table 3. Susceptibility of a CSMA Strain of B. germanica to DDT and Six Pyrethroids by a Surface Contact Method

Pesticide	Dose (μg/1 pt mason jar)	KT_{50}[a]	LT_{50}[a]
DDT	6000	9.65 ± 0.40	25.3 ± 0.6
Pyrethrin	200	0.14 ± 0.04	23.1 ± 0.6
Allethrin	200	0.27 ± 0.01	12.5 ± 0.7
Permethrin	150	0.45 ± 0.03	24.0 ± 3.5
Fenvalerate	150	0.43 ± 0.12	46.9 ± 0.2
Cypermethrin	100	0.24 ± 0.02	32.9 ± 1.4
Decamethrin	75	0.19 ± 0.01	48.2 ± 2.3

[a]Values are given as $\bar{X} \pm$ S.E. in hours.

Table 4. Susceptibility of Four Strains of B. germanica to Methoxychlor in the Presence and Absence of the Synergist Piperonyl Butoxide (6 mg/1 pint mason jar) by a Surface Contact Method

Strain	Resistance Ratio	Resistance Ratio with Piperonyl Butoxide	SR
CSMA	1.0 (46)[a]	1.0 (24)[a]	1.9
VPIDLS	1.8	1.7	1.0
VPIDLS G2	2.7	1.3	4.0
VPIDLS G2	> 8	> 14	—

SR Synergistic ratio (Resistance ratio without synergist/Resistance ratio with synergist)

[a] LT_{50} in hours

The results of the comparative toxicity tests (Table 6) indicate that decamethrin was the most insecticidal of all the compounds tested, but that it had the lowest knockdown activity. Indeed the newer type pyrethroids had consistently greater toxicity and lower knockdown activity than the older, less synthetic types. The above findings on toxicity vs. structure are similar to those reported previously for houseflies (10), and American cockroaches (5) but vary somewhat from the order reported for Blattella germanica (5).

Toxicity difference between cural vs. abdominal topical application indicates that both a Type 1 and a Type 2 pyrethroid cause greater knockdown much when applied to the legs versus the abdomen, although there was no significant difference in mortality between the two treatments (Figures 4 and 5).

The results of effects of temperature on toxicity are shown in Figures 6 and 7. A highly significant negative temperature correlation to knockdown was obtained using allethrin, a Type 1 pyrethroid (Figure 6). By contrast there was no significant effect of temperature to knockdown by cypermethrin, a Type 2 pyrethroid (Figure 7). Allethrin also showed a negative temperature correlation to kill, while cypermethrin displayed a positive temperature correlation in terms of mortality (Table 7).

Metabolism of Permethrin in Susceptible and Resistant Cockroaches

There was no significant difference in the rate of in vitro metabolism of permethrin between the CSMA and VPIDLS strain (Table 8).

In accordance with the above observation, there was no significant difference in the amount of either cytochrome P-450 or cytochrome b_5 in the susceptible and resistant strains (Table 9). These results indicate that there is no interstrain difference in the activity of the mixed-function oxidase, the only enzyme system which has been shown to degrade both DDT and permethrin.

Table 5. Susceptibility of Five Strains of B. germanica to Dieldrin (2 mg/1 pint mason jar) by a Surface Contact Method

Strain		LT_{50}[a]	Resistance Ratio
CSMA		39.3 ± 2.2	1.0
VPIDLS		37.6 ± 0.6	1.0^{NS}
VPIDLS	G1	41.1 ± 2.0	1.0^{NS}
VPIDLS	G2	34.9 ± 5.5	0.9^{NS}
VPIDLS	G3	36.9 ± 3.1	0.9^{NS}

[a]Values reported as $\bar{X}$ + S.E. in hours at 2 mg dieldrin per 1 pint mason jar.
[NS]Not significantly different from 1.0 (CSMA) at $P \leq 0.05$.

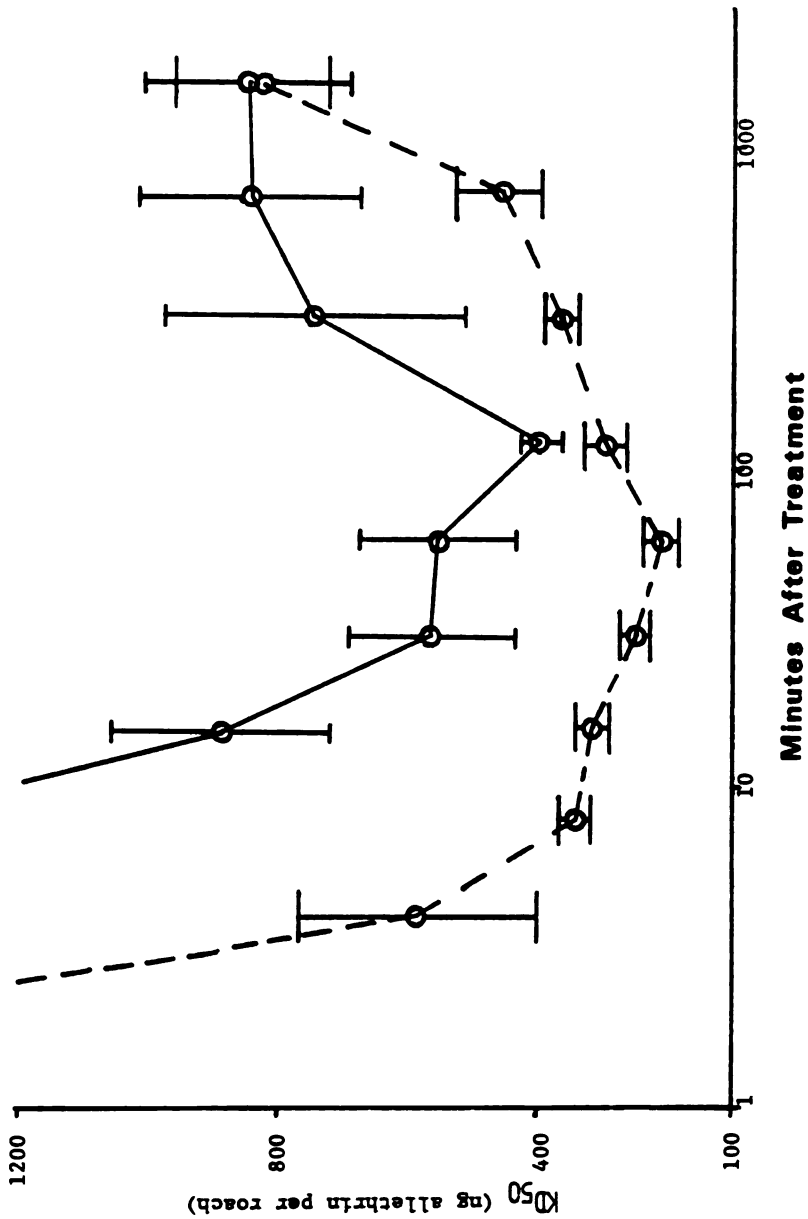


Figure 4. KD_{50}-time curves of allethrin by leg (- - -) and abdominal (——) topical application

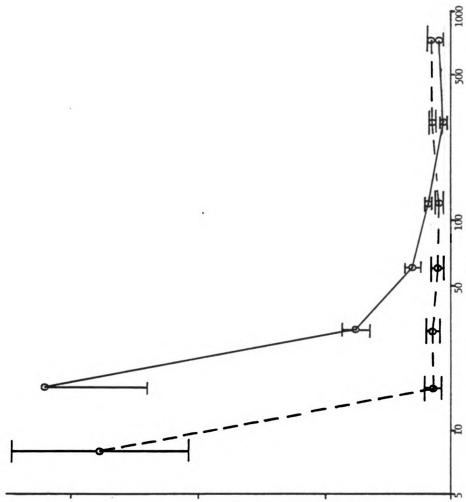


Figure 5. KD_{50}-time curves of cypermethrin by leg (- - -) and abdominal (——) topical application

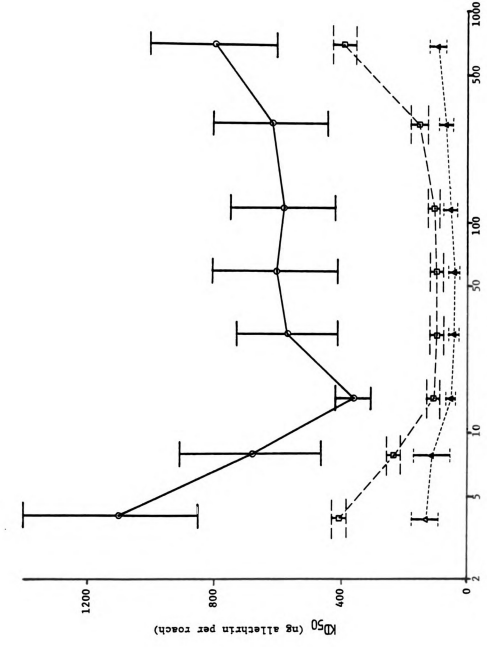


Figure 6. KD_{50}-time curves of allethrin at 31.0°C (●), 24.5°C (■), and 11°C (▲)

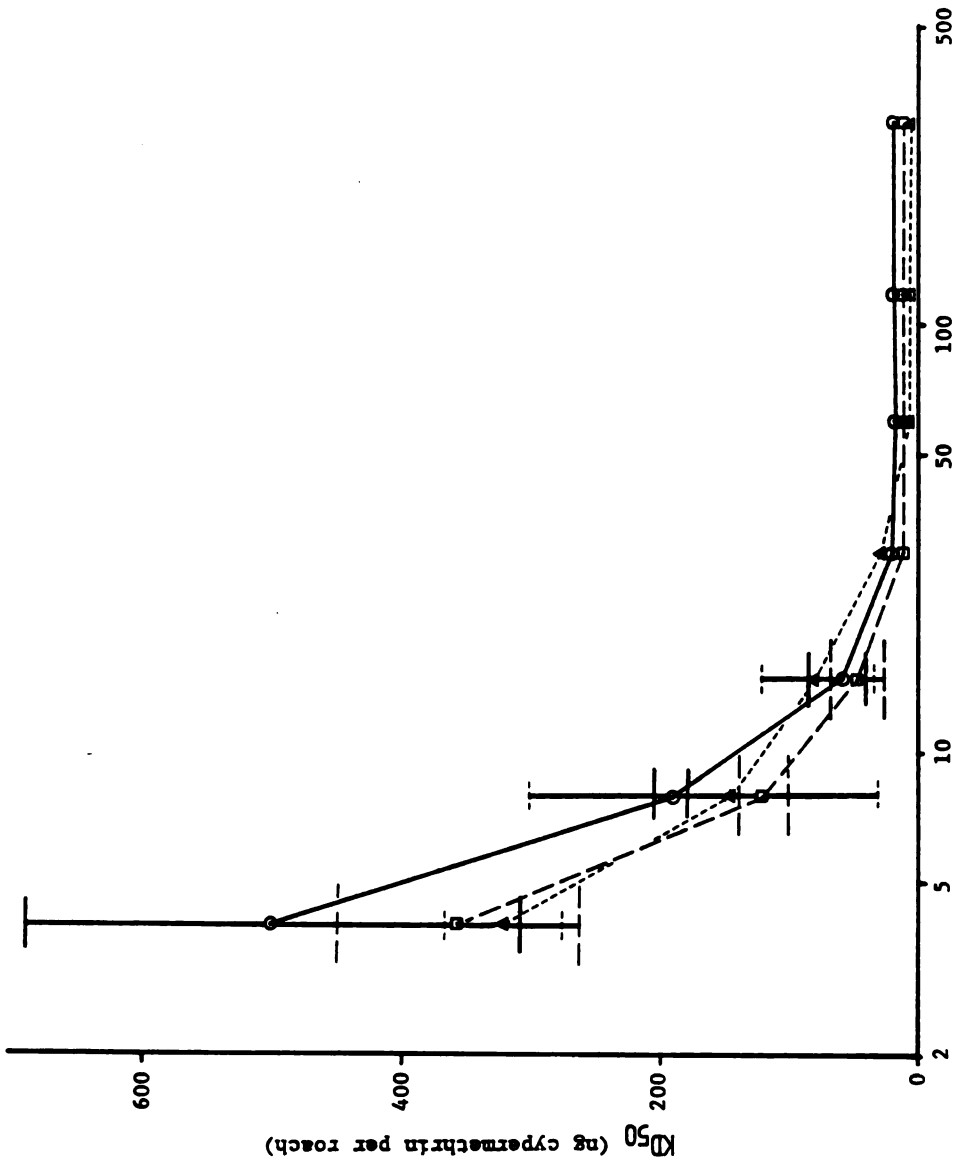


Figure 7. KD_{50}-time curve for cypermethrin at 31.0°C (●), 24.5°C (□), and 11°C (△)

Table 6. Comparative Toxicities of DDT and Six Pyrethroids to a CSMA Strain of German Cockroach by Topical Application to the Abdomen

Pesticide	KD_{50}[a]	LD_{50}[b]	Knockdown Activity[c]	
			Slope x 1000	r^2
DDT	——	11,726 ± 1142	——	——
S-Bioallethrin	390 ± 113	696 ± 116	29.4	.964
Permethrin	177 ± 65	275 ± 14	12.2	.996
Fenvalerate	134 ± 3	239 ± 116	5.9	.998
Cypermethrin	75 ± 16	114 ± 29	5.5	.935
Decamethrin	9.1 ± 0.5	7.5 ± 2.7	2.0	.971

[a]Values are reported as $\bar{X}$ ± S.E. in ng per roach at 2 hours.
[b]Values are reported as $\bar{X}$ ± S.E. in ng per roach at 48 hours.
[c]Calculated as explained in Methods.

Table 7. Effect of Temperature on Toxicity of Allethrin and Cypermethrin by Cural Topical Application

	LD_{50}		
Compound	11°C	24°C	31°C
Allethrin	249 ± 138	636 ± 163	982 ± 182
Cypermethrin	> 640	96.7 ± 31.9	38.8 ± 2.0

Values are reported as $\bar{X} \pm$ S.E. in ng per roach at 48 hours.

Table 8. In vitro Metabolism of Permethrin by Microsomal Preparation from the Whole Body (minus head, legs and wings) Homogenate: the Data are Expressed as the Percentages of Originally-added Permethrin Remaining in the Incubate

	Time Elapsed (minutes)			
	0	15	30	90
CSMA	100 ± 2.0	69.3 ± 0.6	74.3 ± 3.0	60.8 ± 4.9
VPIDLS	100 ± 2.1[NS]	70.1 ± 0.3[NS]	68.0 ± 2.2[NS]	59.6 ± 13.4[NS]

Numbers given represent $\bar{X} \pm$ S.E. calculated as a percentage of the peak area relative to time = 0 as anaylzed by GLC.

[NS]Not significantly different than CSMA at $P \leq 0.05$.

Electrophysiological Studies

Nerve sensitivity

The VPIDLS strain took a significantly longer time to develop repetitive discharges than the CSMA strain for both permethrin and DDT ($P \leq 0.10$ for permethrin and $P \leq 0.01$ for DDT, Table 10). It must be noted, however, that, at these doses, symptoms were induced more slowly with permethrin than DDT. Indeed, after one generation of selection the nervous system had become even less sensitive to both DDT and permethrin as can be seen by the results of the comparison test of VPIDLS and VPIDLS G1 in Table 11.

The electrophsyiological effects of DDT and permethrin are different. DDT causes repetitive firings of single axons resulting in a burst of activity, uniform in amplitude, which ends abruptly (35) as shown in Figure 8a-b. Permethrin's poisoning symptoms were less predictable. The initial symptom noted in many, but not all, permethrin-treated nerve preparations was a repetitive disharge similar to DDT-type symptoms, except that it had less uniform amplitude and slightly decreasing frequency before it terminated (Fig. 9). The next symptom of permthrin poisoning to appear was a repetitive discharge of decreasing amplitude and slightly increasing frequency (Figure 10a-c). The latter symptom was frequently recurring, becoming progressively weaker with each discharge. These permethrin-induced discharges of decreasing amplitude (Figure 10a-c) were long lasting; i.e., they lasted an average of about seven seconds and some lasted as long as thirty seconds. This is in sharp contrast with DDT's repetitive activity which lasted an average of about two seconds with only rare bursts exceeding ten seconds. There was no difference in amplitude of sporadic action potentials observed between DDT and permethrin treatments.

Table 9. Comparison of Cytochrome Levels in a DDT-resistant and Susceptible Strain of Blattella germanica

Strain	Cytochrome P_{450}	Cytochrome b_5
CSMA	.782 ± .045	.253 ± .025
VPIDLS	.859 ± .054[NS]	.287 ± .051[NS]

The above figures are $\bar{X}$ ± S.E. in nmoles per mg protein. Cytochrome P_{450} and Cytochrome b_5 were measured at λ = 450 nm and λ = 426 nm, respectively, as described in Methods.

[NS]Not significantly different from CSMA at $P \leq 0.05$.

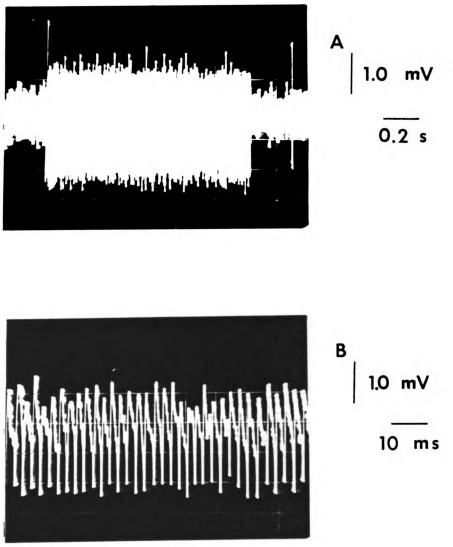


Figure 8. The pattern of a typical DDT-induced repetitive discharge as viewed by a slow scan (A) and a fast scan (b). Note the homogeniety in amplitude and frequency of individual action potentials.

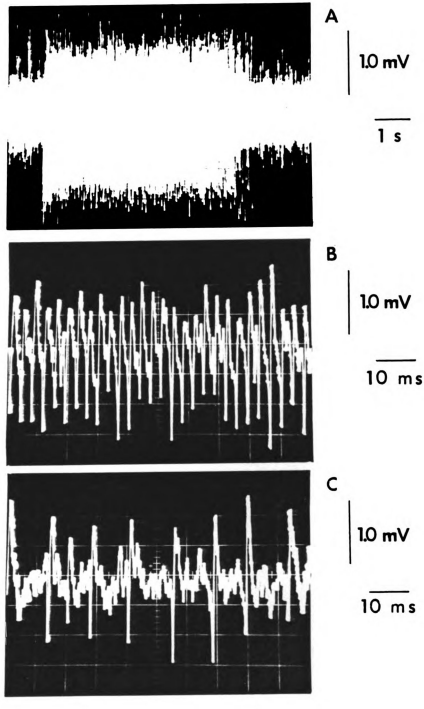


Figure 9. The pattern of a permethrin-induced repetitive discharge. These types of discharges are commonly observed at the beginning of the permethrin poisoning. (A) Entire burst of discharges viewed by a slow scan; (B) A front portion of the burst of discharge by a fast scan; (C) The tail portion of the discharge scanned by the same speed as (B). Note the difference in frequency of (B) and (C).

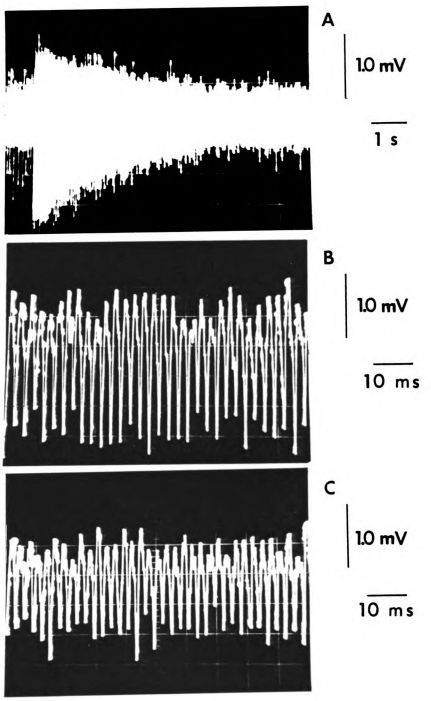


Figure 10. The pattern of a second type of permethrin-induced repetitive discharge which was often observed at the later part of nerve poisoning. (A) A slow scan indicating the gradual decrease in peak amplitudes; (B) A fast scan of the front; (C) A fast scan of the tail part of the burst.

Table 10. Time to Onset of Nerve Poisoning Symptomsin the Abdominal Nerve Cord of Susceptible and Resistant German Cockroaches

	Minutes to Repetitive Discharges	
	CSMA	VPIDLS
DDT (10^{-4}M)		
	10	28
	14	15
	10	17
	13	25
	15	22
	15	> 35
$\bar{X} \pm$ S.E.	12.4 $\pm$ 1.0	> 23.7 $\pm$ 3.0**
Permethrin (2 x 10^{-5}M)		
	20.5	23.8
	23.8	> 35
3	27.3	27.8
4	24.8	22.3
5	21.0	> 35
6	13.5	18.5
$\bar{X} \pm$ S.E.	21.8 $\pm$ 2.0	> 27.8 $\pm$ 2.7[a]

[a]Significantly greater than CSMA at $P \leq 0.10$.
**VPIDLS significantly greater than CSMA at $P \leq 0.01$.

Table 11. Time to Onset of Nerve Poisoning Symptoms in the Abdominal Nerve Cord of German Cockroaches Before and After One Generation of Selection by DDT

	Minutes to Repetitive Discharges	
	VPIDLS	VPIDLS G1
DDT (10^{-4}M)		
	14.6	32.3
	> 35	14.7
	27.8	> 35
	16.3	> 35
	13.7	16.7
	20.2	> 35
$\bar{X} \pm$ S.E.	> 21.3 $\pm$ 3.5	> 28.1 $\pm$ 3.9[a]
Permethrin (3×10^{-5}M)		
	23.9	34.4
	15.8	22.2
	> 35	16.4
	19.5	> 35
	14.6	> 35
	19.5	21.4
$\bar{X} \pm$ S.E.	> 21.4 $\pm$ 3.0	> 27.4 $\pm$ 3.4[b]

[a] VPIDLS G1 significantly greater than CSMA at $P \leq 0.20$.
[b] VPIDLS G1 significantly greater than CSMA at $P \leq 0.10$.

Responses to pesticides

The spontaneous activity of isolated American cockroach abdominal nerve cords in the presence of allethrin and cypermethrin is shown in Table 11. Allethrin caused repetitive discharges to occur in each nerve cord observed. These repetitive discharges could not be differentiated from those caused by DDT (Figure 8) in frequency of repetitive discharges, frequency of spikes during repetitive discharges or duration of repetitive discharges. Cypermethrin had no observable effect on the spontaneous activity of the isolated central nervous system at concentrations of 10^{-10}M to 10^{-3}M, except in one nerve cord where repetitive discharges were observed (Table 12).

DISCUSSION

The three physiological mechanisms by which an insect may develop resistance to insecticides are: (1) decreased penetration, (2) target site insensitivity (i.e., kdr) and (3) metabolic differences.

It has been established in this study that the mechanism of DDT-resistance in the VPIDLS strain of Blattella germanica is a kdr (target site insensitivity) type. Penetration was shown to be an unlikely mechanism of DDT-resistance because the VPIDLS strain showed no cross-resistance to dieldrin, a pesticide with similar polarity, but a different mode of action than DDT. It has been demonstrated that DDT-resistance in VPIDLS is correlated with a decreased nerve sensitivity toward DDT and permethrin which can be measured directly at the level of the central nervous system. It has been shown that VPIDLS is cross-resistant to methoxychlor, allethrin, pyrethrins, and permethrin, all compounds to which kdr houseflies are known to be cross-resistant (9,30,32,33). Lastly, there was no detectable difference in the mixed function oxidase system of susceptible and resistant (VPIDLS) roaches, either in the rate of metabolism of permethrin or in the levels of cytochrome P-450 or cytochrome b_5, indicating that metabolism is not likely a mechanism of resistance in the VPIDLS strain of B. germanica.

The mode of action of DDT has eluded investigators for decades. The mode of action of pyrethroid insecticides, despite intensive studies over recent years, also has evaded researchers. Insects which are resistant by means of a target site insensitivity (i.e., a change at the site of action) provide a useful tool

Table 12. Effects of the Synthetic Pyrethroids Allethrin and Cypermethrin on the Spontaneous Activity of the Isolated American Cockroach Abdominal Nerve Cord

Pesticide	Number of Nerve Cords Examined	Type of Disorder	Number Showing Response	Concentration Range	Time Until Observed Order
Allethrin	7	Repetitive discharges	7	10^{-8}-2X10^{-8}M	17-62 minutes
Cypermethrin	8	None observed	7	10^{-10}-10^{-3}M	1-4 hours
		Repetitive discharges	1	10^{-10}M	40 minutes

for the investigation of the modes of action of pesticides. Comparisons of the VPIDLS strain of the German cockroach to the susceptible counterparts could, therefore, yield helpful insights as to the mode of action of DDT and pyrethroid insecticides.

This study has revealed that there are differences between cypermethrin and decamethrin pyrethroid insecticides (Type 2 pyrethroids) and those closely related to the pyrethrins (Type 1 pyrethroids). Type 1 pyrethroids have a negative temperature correlation for both knockdown and mortality factors, while Type 2 pyrethroids have no obvious temperature correlation for knockdown and a positive temperature correlation for mortality. Electrophysiologically, Type 1 compounds were shown to cause repetitive discharges while Type 2 compounds did not. Lastly, there was only very limited resistance of the VPIDLS strain of DDT-resistant (kdr) B. germanica to Type 2 pyrethroids while resistance to Type 1 pyrethroids was large. It should be noted that permethrin and fenvalerate are intermediate pyrethroids, not clearly fitting into Type 1 or Type 2 classifications. While VPIDLS shows cross-resistance to both fenvalerate and permethrin (a Type 1 action), fenvalerate is more structurally similar to the Type 2 pyrethroids and permethrin causes a modified repetitive discharge (neither a Type 1 nor Type 2 action).

It is my conclusion, from the data presented here, that the newly developed synthetic pyrethroids have, at least in part, a different mode of action from that of the old type pyrethrin analogues, and there is a possibility of overcoming DDT-induced cross-resistance to pyrethroids with the use of these new pesticides.

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