GENETICS OF RESISTANCE TO INSECTICIDES IN A PYRETHRIN RESISTANT STRAIN OF HOUSEFLIES

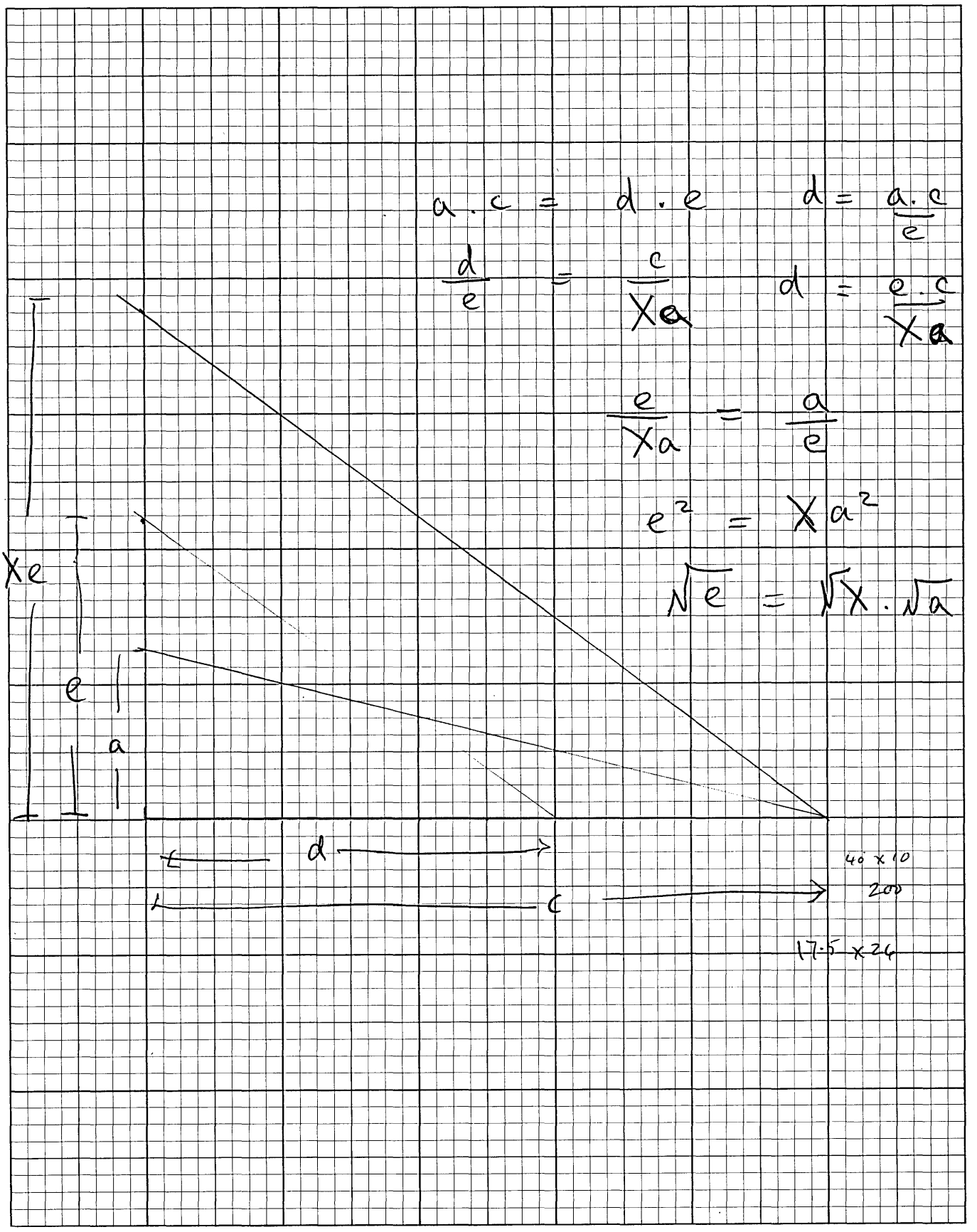
by

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Rothamsted Experimental Station,
Harpenden.


a . c = d . e
d = a . c / e
d / e = c / Xa
d = e . c / Xa
e / Xa = a / e
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Xe
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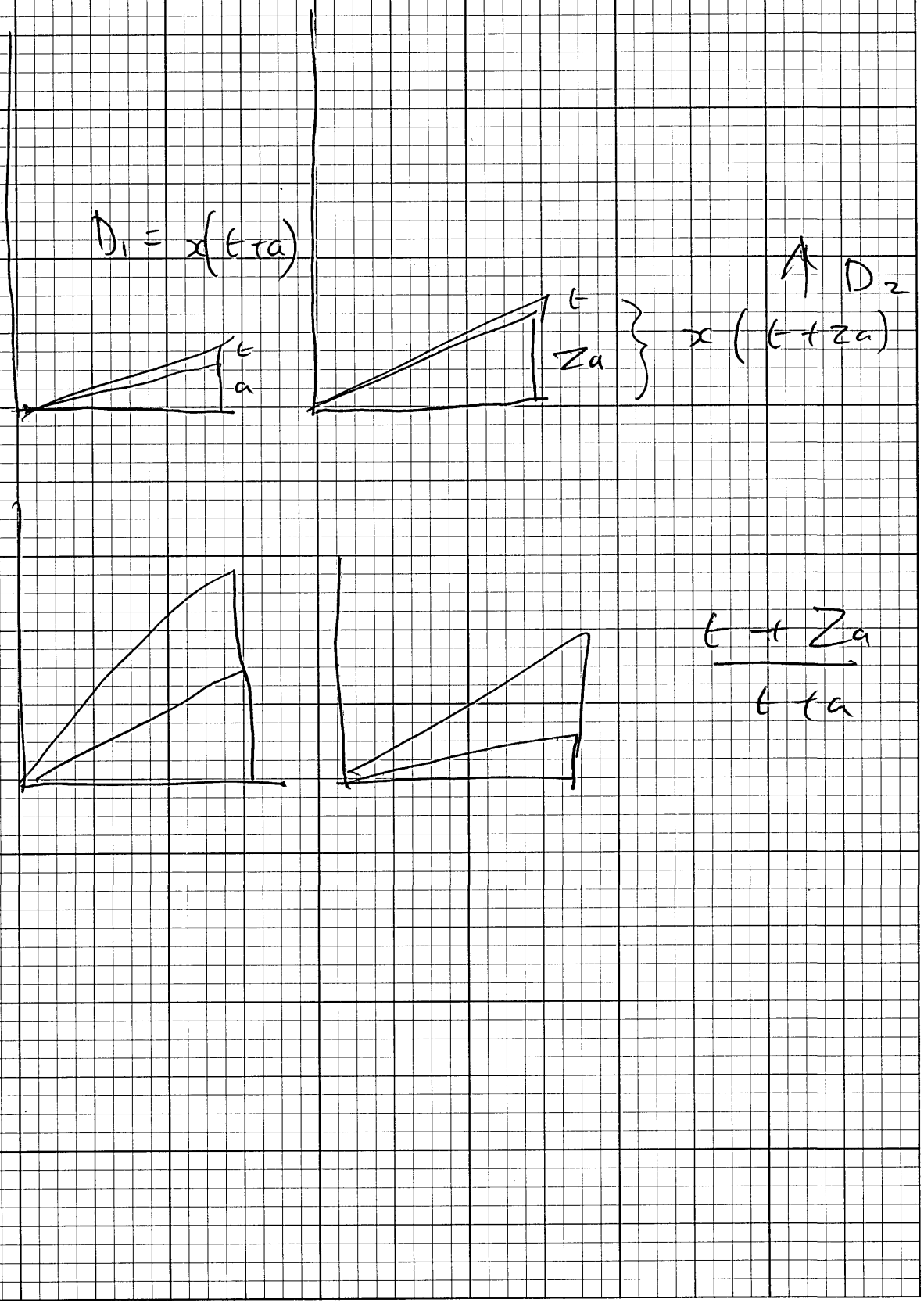

$D_1 = x(t+a)$
t
a
t
$2a$
D_2
$x((t+2a)$
$t+2a$
$t+a$





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ABSTRACT

Genetical analysis of pyrethroid resistance in strain NPR, the natural pyrethrins selected strain of houseflies Musca domestica L. revealed multiplicate resistance. Four major factors contributed to the partial immunity of NPR to the selecting agent (80% survived 50 µg/♀). The factors which were isolated genetically were characterised by their cross-resistance spectra, were

1. kdr-NPR on chromosome III, the most important factor which provided resistance to all pyrethroids and cross-resistance to DDT and also delayed the knock-down effect of these insecticides.

2. Pen also on chromosome III which delayed cuticular penetration, providing very small resistance to all insecticides and also delayed knock-down.

3. py-ses on chromosome V which gave resistance only to pyrethrolone esters which could be suppressed by the synergist sesamex

and 4. R_{381} on chromosome II which gave small resistance to all pyrethroids but stronger resistance to synergised pyrethrolone esters.

No one factor singly gave more than four-fold resistance to natural pyrethrins suggesting strong interaction between them.

Besides this multiplicate resistance, NPR also exhibited multiple resistance to other insecticides. There was triplicate resistance to DDT through factors Pen and kdr-NPR on chromosome III and r-II-DDT on chromosome II; duplicate resistance to zectran on chromosomes II and III and resistance to dieldrin on chromosome IV. Apart from Pen and kdr-NPR these factors were quite distinct from those providing pyrethroid resistance.

INTRODUCTION

1. History and Development of Field Resistance

According to the WHO definition, resistance is the ability of a species, normally susceptible to a given insecticide, to withstand control by that insecticide (Brown and Pal, 1971). It may be thought of as an example of very rapid evolution in response to a change in the environment (Crow, 1960).

Georghiou (1965) stressed the importance of the study of resistance in relation to vector control and eradication programmes of public health agencies. Its history in insects and ticks was reviewed by Brown (1961) who listed thirteen cases of field resistance between 1908 and 1945. The most important were to hydrogen cyanide in three species of scale insect Saissetia oleae, Aonidiella aurantii and Coccus pseudomagnoliarum on citrus in California, and to arsenicals in the codling moth Carpocapsa pomonella, the peach twig borer Anarsia lineabella and two species of cattle tick Boophilus microplus in Australia and B. decoloratus in South Africa. However the problems of resistance really date from the introduction of widespread chemical control of pests with organic compounds of high insecticidal activity.

Resistance to DDT was first noted in the housefly, Musca domestica, in Sweden (Weissmann, 1947) and Italy (Saccà, 1947). Soon DDT resistant houseflies were found in most Danish pigfarms where ɣ-BHC was introduced as a substitute, but failed as little as three months after introduction. By 1951 strains of flies multi-resistant to all chlorinated hydrocarbons were widespread (Keiding, 1953). Use of these compounds for housefly control in Illinois led to similar resistance (Decker and Bruce, 1952) but in Southern California DDT resistant flies were controlled more successfully with BHC (March and Metcalf, 1949).

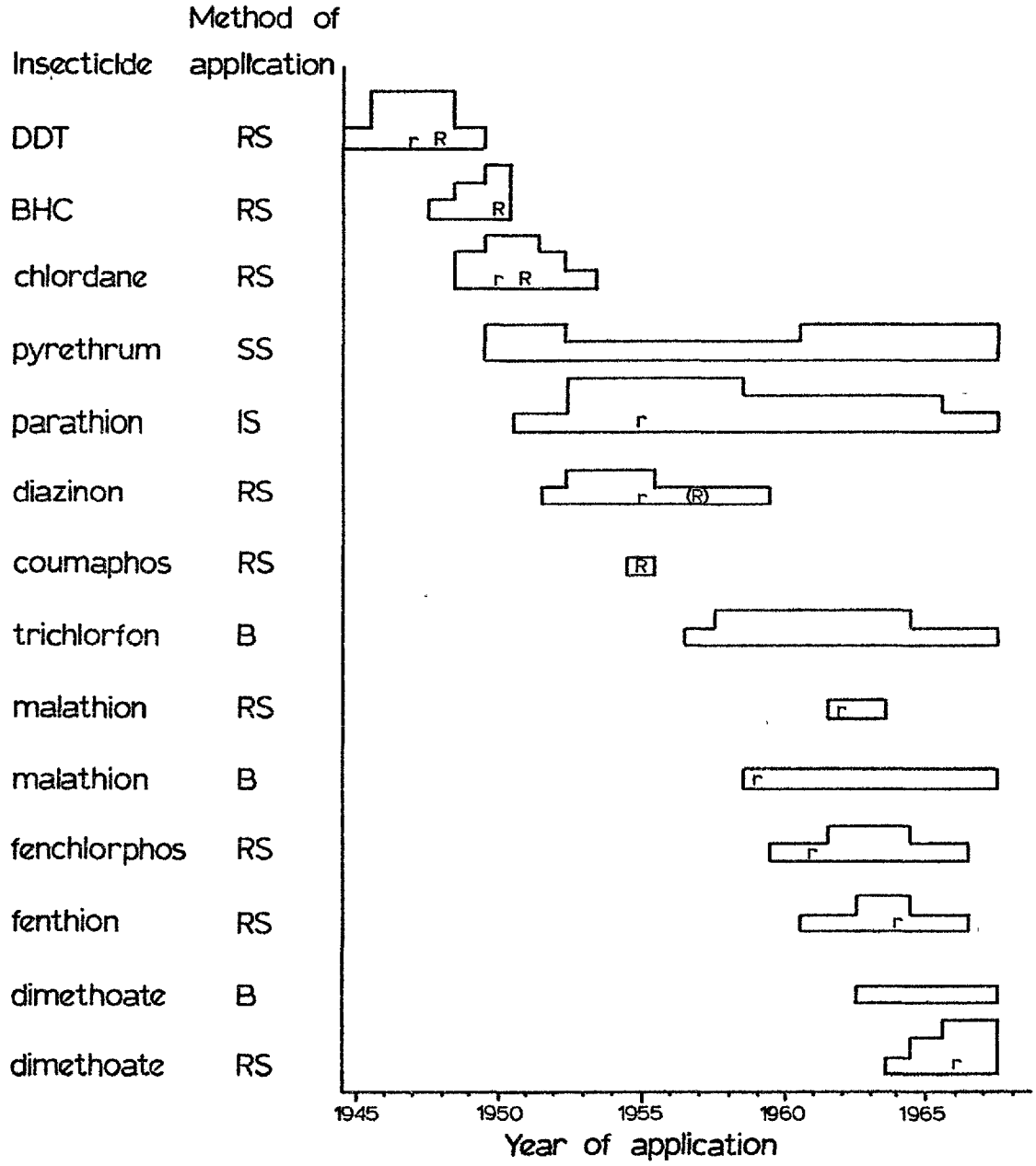
When Bruce and Decker (1950) and Decker and Bruce (1952) studied the rate of development of resistance to organochlorine compounds in houseflies on farms in Illinois, they found that within six years of introduction of DDT all farms had DDT resistant houseflies. Significant resistance took six to twenty generations to appear, but after that the level of resistance increased very rapidly to 100 to 1000 fold over the next six to twenty generations. Keiding (1967) monitored resistance levels over a number of years on farms in Denmark, and found in practice that each new insecticide, initially successful, had an effective period after which satisfactory control failed, necessitating its replacement. Figure I on page 3A shows his data in graphical form.

Organochlorine resistance also developed in other pests, for example to DDT in Culex pipiens in Italy (Mosna, 1948) where chlordane control was effective for about two years before failure resulted in populations which resisted all organochlorine insecticides (Mosna, 1954), and a similar sequence of development of resistance occurred in other mosquitoes (Gjullin and Peters, 1952; Lividas, 1955; Pampana, 1953).

In South Africa, the population of Boophilus decoloratus which resisted arsenicals in 1940 developed resistance to BHC within two years of its introduction whilst retaining its previous resistance to arsenicals, (Whitnall and Bradford, 1949), and six years later to the substituted DDT (Whitehead, 1958). γ-BHC resistance also arose in a sodium arsenite-susceptible population which retained this susceptibility (Bekker, 1953).

Blattella germanica in Texas could not be controlled with chlordane after some years of continuous treatment, and cross-tolerance to γ-BHC and DDT occurred (Head et al., 1953).

After 1951 and following the development of resistance to



1. General use of insecticides for fly control on Danish farms 1945-67 (after Keiding, 1967)

Key
RS residual spray
SS space spray
IS impregnated strip
B bait
r first year resistance of practical importance
R high resistance compelling change to new insecticide
(R) high resistance on farms where insecticide's use resumed

organochlorine compounds in Denmark, organophosphorus compounds such as diazinon and parathion were used for housefly control (Keiding, 1967) but resistance to them arose and imparted cross-resistance to other chemically related and unrelated compounds. For example, flies slightly resistant to parathion on a farm where it had been used for control were very resistant to coumaphos which had never been used in that area (Keiding, 1956).

Other early cases of organophosphorus resistance included that of the European Red Mite Paratetranychus pilosus to parathion, associated with resistance to malathion, but not to EPN (O-ethyl O-p-nitrophenylphosphonothioate) or demeton (Lienk et al., 1952).

Alternatives for organochlorine and organophosphorus compounds have been used, but resistance to them has arisen. For example Boophilus microplus in Queensland resisted organophosphorus control and one population showed high cross-resistance to carbamates when they were tried as substitutes (Shaw et al., 1968). Wharton and Roulston (1970), in a review on tick control, described the development of resistance due to respectively organochlorine, organophosphorus and carbamate compounds in strains from three areas in Australia, Ridgelands, Biarra and Mackay where tick control is proving exceptionally difficult.

However, the introduction of chemical control of a pest is not automatically followed by resistance. Ornithodorus moubata was controlled for twenty years in East Africa with lindane (Msangi, 1968) whilst Brown (1967) cited examples of control of the corn borer, Pyrausta nubilalis and apple maggot Rhagoletis pomonella in North America with DDT, and also of Stomoxys calcitrans which resisted DDT in Scandinavia, Germany and Italy but not in North America.

Once established, resistance may persist when insecticidal

pressure is removed. In one instance malathion resistant houseflies were effectively controlled for five years with dimethoate while resistance to malathion was monitored. The level fell slowly during the first four years but increased during the fifth year (Keiding, 1968).

2. Pyrethroid resistance in the field

Pyrethroids have sometimes been used as substitute pesticides where other control agents have failed, but because of their cost, rapid break-down in daylight and lack of systemic effects, they have not been widely used. This could explain why the number of reports of resistance due to their use is small. Even where they have been used for many years such as for housefly control, development of resistance is rare (Potter et al., 1956; Keiding, 1972).

Up to 1971, the only report of resistance in houseflies to pyrethroids in the field came from a pigfarm in Sweden where, following the failure of DDT, synergised natural pyrethrins were used. Resistance developed to x 15 within three months (Davies et al., 1958). This strain, 213ab, was the parent from which the flies used in this work were derived.

In 1971, a mixture of natural pyrethrins and piperonyl butoxide (1:5) was used as a space spray to control dimethoate resistant houseflies in Denmark. Resistance to the mixture developed on two farms in less than a year, and these flies were found also to resist bioresmethrin (Keiding, 1972).

Following the development of resistance to pyrethroids in the mushroom phorid Megaselia halterata on a farm in Southern England, insects were controlled with DDVP. No other insecticide had been used previously and after natural dilution of the population it could be controlled again with pyrethroids, although they

were not used (Hussey, 1965).

Synergised natural pyrethrins were used to control DDT resistant roaches, Blattella germanica in HMCS Bonaventure and within four months of its use a two-fold tolerance had developed (Wambera et al., 1965).

Other reports of naturally occurring resistance to pyrethroids are in fact instances of cross-resistance in which the pest tolerated pyrethroids, although another insecticide had been used. Fine (1963) cited three such instances: in Blattella germanica in Texas where chlordane, lindane or dieldrin had been the control agent (Keller et al., 1965); in Cimex hemipterus in Kenya (Busvine, 1958) and Boophilus decoloratus in South Africa (Whitehead 1959) where in both cases DDT resistance had developed.

Similarly in houseflies in Russia and Egypt where DDT resistance had developed, sample populations also resisted pyrethrins (Misnik, 1956; Toppozada et al., 1965). Other instances include Pediculus humanus in Marseilles (Nicoli and Sautet, 1955; Sautet et al., 1959).

3. Pyrethroid insecticides

The naturally occurring pyrethroids, known as natural pyrethrins are extracted from the flower head of the pyrethrum plant, Chrysanthemum cinerariaefolium. The plants require temperate conditions including a cold wintry period, and are grown in uplands near the equator such as are found in Ecuador and Kenya, where manual labour for picking and drying the flower heads is cheap.

a. Chemistry and structure of pyrethroids used

The six active ingredients in pyrethrum extract are esters of two acids, pyrethric and (+) trans-chrysanthemic, with three alochols, pyrethrolone, cinerolone and jasmololone, and Table I (p.7 shows the approximate proportion of each ester in the extract

(Elliott, 1967).

Table I % Active ingredients in pyrethrum extract (Elliott 1967)

Alcohols	Acids	
	(+) trans-chrysanthemic	pyrethric
pyrethrolone	35	32
cinerolone	10	14
jasmololone	5	4

Besides the mixture of natural pyrethrins two of the isolated naturally occurring esters, pyrethrin I and pyrethrin II were used, as well as five synthetic pyrethroids. Of these eight insecticides six are esters of two alcohols and three acids, as shown in Table II below.

Table II Names of individual pyrethroid esters

Acid	Alcohol	
	(+) pyrethrolone	5-benzyl-3-furyl-methyl alcohol
(+) trans chrysanthemic acid	pyrethrin I	bioresmethrin
pyrethric acid	pyrethrin II	pyresmethrin
2,2,3,3-tetramethyl-cyclopropane carboxylic acid	(pyrethrolone tetramate)	NRDC 108

The pyrethroid resmethrin is the racemic mixture of all geometric and optically active isomers of 5-benzyl-3-furylmethyl chrysanthemate.

In all the isolated compounds, the ester linkage is attached directly to the cyclopropane ring (for structural diagrams, Fig III see page 31A).

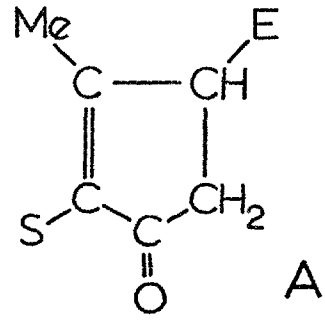
The differences between the acids occur on the substituent attached to the cyclopropane ring at the 3-position which are

$-CH=CMe_2$,H in chrysanthemic acid

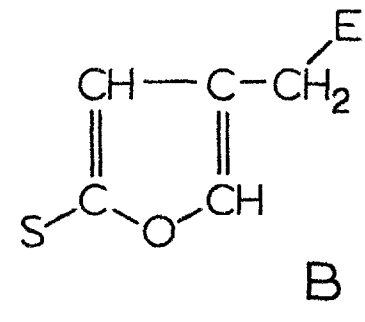
$-CH=C(COOMe)Me$,H in pyrethric acid or

Me, Me
~~H, H~~ in 2,2,3,3-tetramethylcyclopropane carboxylic acid.

On the alcohol part, a side chain is held in the correct stearic relationship to the groups of the acid by a "spacing" unit separating the side-chain from the ester linkage. In pyrethrolone esters this is unit A and in 5-benzyl-3-furylmethyl esters it is unit B.



A



B

In both cases, the bonds through which these units are connected to the ester linkage, E, and the side-chain, S, are at similar angles to each other, so that the synthetic unit, B, successfully imitates the natural unit A. The side chain, S, in pyrethrolone esters is $-CH_2.CH=CH.CH=CH_2$ and in 5-benzyl-3-furylmethy esters is $-CH_2.Ph$. Thus in each alochol a centre of unsaturation is held near the ring by a methylene group.

b. Pyrethroid toxicity

Associated with great toxicity to many insect species (Busvine and Nash, 1953) natural pyrethrins have extremely low mammalian toxicity by dermal application (Malone and Brown, 1968). The extract may have a dermatitic effect on a small proportion of the population (Rickett et al., 1972) but this is due to non-insecticidal constituents which can be removed without affecting insecticidal activity (Lord and Johnson, 1947).

With some insects, particularly Diptera, natural pyrethrins have an extremely rapid initial effect known as knock-down which may be followed by recovery. Ten times the dose applied topically required to give knock-down against houseflies was needed to have a kill effect (Sawicki et al., 1962; Sawicki and Thain, 1962).

c. Synergists

Whilst working on methods for reducing the high cost of natural pyrethrins Pearson (1935) found that pine oil increased the efficiency of cattle sprays. Weed (1938) also found that the addition of ten times by weight of N-isobutyl undecyleneamide increased their effect against houseflies by up to 100-fold although the additive alone had extremely low insecticidal activity and did not potentiate pyrethrins against mammals. Later sesamin (Haller et al., 1942) and piperonyl butoxide (Wachs, 1947) were found to be even more effective additives. These synergists do not potentiate pyrethrins against all insects, for example mosquitoes (Hadaway et al., 1963) and Pieris brassicae (Yule, 1946). Various other additives have been tried such as tropital (Hopkins and Maciver, 1965) and drugs extenders like SKF 525A (Bates et al., 1965) but none has been as commercially successful in terms of potentiation and cost as piperonyl butoxide.

d. Commercial uses

Synergised pyrethrins are mainly used for domestic housefly control but have recently been used for housefly control in farms in Denmark (Keiding, 1972). They have been used for cockroaches and other household pests (Goodwin-Bailey, 1956) but this and the use in widescale control of adult mosquitoes is restricted not only by their high cost but also by their rapid photodegradation (Chen and Casida, 1969). However, moderately successful trials have been made in Tanzania for control of Glossina pallipedes and

G. swynnertoni by low volume aerial spraying (Irving et al., 1969; Tarimo et al., 1971).

As pyrethrins are more stable in the dark, and have low mammalian toxicity they have been used for controlling stored products' pests (Potter, 1935) but this protection is increasingly provided by organophosphorus compounds such as malathion (Papworth, 1961) or by fumigation (Bhambhani, 1964; Thompson, 1966). Other uses include clearing water mains of Asellus aquaticus (Schwarz et al., 1968) and disinfestation of aircraft (Busvine, 1952).

Smoke from burning powdered pyrethrum repels mosquitoes and was used in sleeping quarters (Howard, 1911) as the vapour contains a repellent as efficient as dimethyl phthallate (Wright and Burton, 1969). This vapour also has knock-down properties but mosquitoes later recover (Fales et al., 1969). The repellency is not synergised by additives.

e. Synthetic pyrethroids

Because of their desirable properties but high cost, various workers have investigated the possibility of using synthetic pyrethroids, as replacements for natural pyrethrins. The history of these compounds has been reviewed by Elliott (1967). The first commercially successful compound was allethrin (Schechter et al., 1949), which was slightly less toxic than natural pyrethrins to many pests (Busvine and Nash, 1953) although retaining knock-down and some ability to be synergised (Gersdorff et al., 1951). Other compounds were also commercially available such as barthrin and furethrin. Elliott et al. (1967) produced resmethrin which was much more toxic than pyrethrins to a wide range of arthropods (Brooks et al., 1969) and still retained low mammalian toxicity (Miyamoto et al., 1971), but was less effective as a knock-down agent (Brooks et al., 1969) and could not be much

synergised (Elliott et al., 1967).

4. Factors affecting laboratory studies of resistance

Busvine (1971a) stressed the significance of cross-resistance studies in the laboratory in order to find an alternative control agent. He defined cross-resistance as "the existence of one defence mechanism which can give rise to a specific resistance spectrum. The form of the spectrum depends on the mechanism and should be independent of which member of the group was used for selection. Double resistance is due to the simultaneous presence in the same organism of two distinct resistance mechanisms which give protection to different types of poison. By extension one may have triple or even multiple resistance. Duplicate (triplicate or multiplicate) resistance is the situation where two (three or more) mechanisms exist in the same organism to protect it from the poison".

Busvine (1971b) stressed the importance of sound standardised methods for measuring resistance. With the appearance of field resistance in many arthropod pests, WHO and FAO devised standard test methods for detecting and monitoring levels and spread of resistance, which are simple and reliable and can be used in the field (Busvine, 1960). However, laboratory techniques do differ and make the comparison of results for different workers difficult. For example the solvent may greatly affect results with DDT against houseflies (Busvine, 1951 and 1968) whilst both carrier and droplet size affected the absolute LD50 values for diazinon with susceptible or resistant flies (Farnham, 1966). Tests done against mixed sexes of many pests may give different results to those done against the separated sexes as males are

much more susceptible than females (Hoskins and Gordon, 1956). It is equally important to use homogeneous populations whenever possible (Busvine, 1971b).

Application technique can also affect comparisons between author's results. Sawicki et al. (1962) suggested that topical application of pyrethrin I and pyrethrin II to susceptible houseflies may give different results to the spray methods used by Gersdorff (1947) and Incho and Greenberg (1952). Differences in results between Sawicki and Lord (1970) and those of Hoyer and Plapp (1968) using parathion against genetically reconstituted housefly strains apparently bearing the same resistance factors could be caused by the difference between topical application and film techniques.

Many authors quote only 24 hour mortalities, but kill end point, i.e. the point beyond which no change in kill due to the insecticide can be detected, may take longer. For example resistant Sitophilus granarius treated with DDT took up to 8 or 9 weeks to reach kill end point (Lloyd, 1969). With resistant houseflies, dieldrin took up to 96 hours to reach kill end point and a dose that gave no kill after 24 hours finally gave 100% mortality (Sawicki and Farnham, 1968a). With susceptible flies kill end point is much faster which could explain the very high levels of resistance to dieldrin quoted by some authors (Decker and Bruce, 1952; March, 1959). Failure to use kill end point as the criterion of comparison between compounds and/or strains of insect can therefore invalidate results.

5. Development of resistance in the laboratory

a. Rate of development of resistance

The rate of development of resistance and the effect of a selecting agent on the cross-resistance pattern have been studied

in the laboratory by treating strains of insects and breeding from survivors. Often, selection pressure has been designed to kill about 70% of the population. The rate of development depends not only on the initial tolerance of the population, but also on the selecting agent and the pressure applied.

By starting with susceptible houseflies, resistance developed more quickly under organochlorine, e.g. DDT, pressure than when paraoxon or pyrenone (a pyrethrins/piperonyl cyclonene mixture) were used (Bruce, 1950; Dekker and Bruce, 1952).

When DDT resistant flies collected from New York State were laboratory selected, resistance again increased more quickly under DDT than under parathion pressure. However, both rates were faster than when the starting strain was susceptible (Pimental et al., 1953).

Farnham (1971) selected the pyrethrins resistant houseflies of Davies et al. (1958) (strain 213ab) with pyrethrins (NPR) and with resmethrin (104). The level of resistance to pyrethrins in NPR rose slowly until the 40th generation, when it suddenly jumped to a level where most of the strain were immune to 50 µg, but the level of resistance to resmethrin remained approximately the same at a very high level. In strain 104, resistance to resmethrin also remained unaltered through more than 60 generations of selection, but to pyrethrins, it fell slightly.

b. Effect on cross-resistance spectrum

The effect of the selecting agent on the ultimate cross-resistance spectrum is rarely predictable. Houseflies selected with various organochlorine compounds showed that whilst DDT selection could impart resistance to methoxychlor, the contrary was not true. The DDT resistance factor(s) were separate from the factors for cyclodiene resistance (Bruce, 1950; Decker and

Bruce, 1952; Busvine, 1953).

Linquist and Wilson (1948) selected susceptible houseflies with DDT for fourteen generations and they tolerated pyrenone (a mixture of pyrethrins and piperonyl cyclonene) (Wilson and Gahan, 1948). This cross-resistance between DDT and pyrethrins occurred not only in houseflies (Harrison, 1950; Busvine, 1951; Winteringham, 1964) but also in cockroaches (Keller et al., 1956) body lice (Nicoli and Sautet, 1955) bed bugs (Busvine, 1958) grain weevils (Parkin and Lloyd, 1960; Lloyd and Parkin, 1963; Lloyd, 1969) and blue ticks (Whitehead, 1959). Although most populations selected with pyrethrins resisted DDT, not all DDT resistant insects tolerated pyrethrins (Winteringham, 1964).

The level of resistance in pyrethrins selected susceptible Pediculus humanus reached a maximum at the 10th generation and remained unaltered through 46 more generations of selection. Surprisingly cross-resistance to DDT developed more slowly so that by the ninth generation it was five-fold, by the 19th 200-fold, by the 38th 300-fold and at the 50th generation, 10% DDT gave only 12% kill (Cole and Clark, 1961).

Resistance to the knock-down and kill effects of DDT in houseflies were separately controlled (Harrison, 1951) and there was a connection between the resistance to knock-down and to pyrethrins (Busvine, 1953).

Selection with organophosphorus compounds can give various results, but generally selection with one leads to resistance to another, and to DDT, but not to pyrethroids or cyclodienes. In one of the earliest examples of laboratory selection Garman (1950) studied resistance to the red spider, Tetranychus bimaculatus which resisted parathion, but not tetraethylpyrophosphate.

The diazinon selected strain of houseflies, SKA, resisted

diethoxy-organophosphorus more than the equivalent dimethoxy-compounds. It was only slightly resistant to malathion, but very resistant to chlorthion and its ethyl homologue (El Bashir, 1971). This strain strongly resisted DDT (El Bashir, 1967). By detailed examination of the cross-resistance patterns of four organophosphorus resistant strains of housefly from various sources with fourteen insecticides, Busvine (1959) concluded that organophosphorus resistance was no simple phenomenon, but that two separate mechanisms were involved.

Selection with pyrethrins besides imparting DDT resistance as described earlier, usually gives resistance to other pyrethroids For example the pyrethrins resistant housefly strain 213ab also resisted allethrin, furethrin and resmethrin (Davies et al., 1958; Fine, 1961; Farnham, 1971). Resistance to allethrin was increased by selection with natural pyrethrins but not with resmethrin (Farnham, 1971). Pyrethrin resistant grain weevils tolerated allethrin (Lloyd and Parkin, 1963) as did resistant body lice (Cole and Clark, 1961). One exception was a housefly strain derived from the NAIDM susceptible strain by selection with pyrenone (resistance factor 20) which was only two-times resistant to DDT (Decker and Bruce, 1952).

By accumulating these and other data from various sources pesticides can be grouped according to the effect of the selecting agent on cross-resistance spectra, and this is shown in Table III, on page 16.

6. Other Techniques for the Study of Resistance

Apart from the simple studies of broad cross-resistance spectra to insecticides of different groups, various methods have been used to study factors affecting resistance and the mode of

action of poisons.

Table III Groups of insecticides and their effect on cross-resistance

Group	Selecting Agent	Induced cross-resistance
I	DDT	Related compounds, e.g. methoxychlor, TDE
II	γ-BHC and cyclodienes	Other cyclodienes, e.g. dieldrin, aldrin, heptachlor
III	Organophosphates	Other organophosphates and insecticides of group I
IV	Carbamates	Other carbamates and often insecticides of group I
V	Pyrethroids	Other pyrethroids and insecticid of group I
VI	Miscellaneous	e.g. arsenicals, tin compounds

a. Structure toxicity studies

A more refined technique of cross-tolerance observations involves the relationship between the chemical structures of a closely related series of compounds and their toxicities to resistant and/or susceptible insects. For example, an organophosphorus series (Ludvik and Decker, 1951) a carbamate series (Metcalf et al., 1960) and an organotin series (Blum and Pratt, 1960) have been tested against susceptible houseflies to deduce molecula configurations necessary for potency.

Hennessey et al. (1961) made a series of analogues by halogen substitution in the ortho and para positions on one benzene ring of DDT and bioassayed them against DDT-resistant and susceptible houseflies. The derivative orthochloro-DDT reduced the high level of resistance (x 400) to two-fold, because the enzyme system DDT-dehydrochlorinase (Sternberg et al., 1950) could not act on the modified DDT to produce non-toxic metabolites

(Hennessey, 1960).

A more detailed study was made on all possible isomers of allethrin against susceptible and pyrethrins' selected houseflies. The selected flies were most resistant to those isomers least toxic to the susceptible flies. Toxicity involved both optical and geometrical specificity with the asymmetric carbon atoms on the chrysanthemate apparently dominant (Barker and Edmunds, 1963).

Other synthetic pyrethroids have been used in structure/toxicity studies to investigate their mode of action against susceptible houseflies (Viel and Chancogne, 1956; Katsuda and Ogami, 1966; Katsuda et al., 1966; Berteau et al., 1968). But as Barthel (1961) wrote "perhaps the most noticeable and yet discouraging aspects of synthetic pyrethroids have been the lack of correlation between structure and activity".

The history of synthetic pyrethroids was reviewed by Elliott (1967) who later pointed out that the problem of predicting a probable conformation at the site of action may be formidable (Elliott, 1969). It is against this background that the work of Schechter et al. (1949) in synthesising allethrin and Elliott et al. (1967) in producing resmethrin, two highly toxic commercially available synthetic pyrethroids must be viewed.

b. Use of additives

Another approach to resistance and mode of action studies involves the use of non-toxic additives with the insecticide which either enhance (synergist) or reduce (antagonist) the potency. As seen above, synergists may be used for reasons of economy, but in the laboratory they can be used as specific inhibitors of individual resistant detoxification mechanisms. In houseflies, the mechanism DDT dehydrochlorinase (Sternberg et al., 1950) may

be blocked by F-DMC (bis-(p-chlorophenyl)-trifluromethyl carbinol) (Cohen and Tahori, 1957). Thus by toxicological studies alone, this technique can reveal the presence of a second or third mechanism in a polyfactorial resistance system which may be difficult to identify when masked by the presence of the inhibitable factor.

A second DDT resistance factor was characterised by its property of being inhibited by sesamex (Oppenoorth, 1965). Diazinon selected SKA flies strongly resisted DDT (El Bashir, 1967) A fraction of the population was susceptible to a mixture of DDT and FDMC and part was susceptible to a mixture of DDT and sesamex indicating the presence of two resistance factors (Sawicki and Farnham, 1967a). Resistance to DDT in the pyrethrins selected NPR strain was not affected by sesamex or FDMC alone or in combination indicating that neither mechanism present in SKA was a major factor in the high level of resistance in NPR (Farnham, 1971).

However many available data are difficult to compare, again due to variation in treatment techniques. Often the commercial practice of using a constant ratio of additive to insecticide is applied such that at the lowest concentrations of the combination, the effect of the additive is far less than at the higher levels. For this reason in laboratory practise Sawicki and Farnham (1968b) and Farnham (1971) treated houseflies with a constant amount of additive and only varied the dose of applied insecticide.

Resistance to pyrethrins alone in Sitophilus granarius was almost completely lost when piperonyl butoxide was added to the insecticide (Lloyd and Parkin, 1963). With houseflies, the pyrethrins selected NPR strain was greatly affected by pre-treatmen with 2 µg/fly sesamex, but there was still strong residual resistance at about the same level as in the resmethrin selected 104 strain. From these data and from the cross-resistance spectra,

Farnham (1971) concluded that two major factors were responsible for the very high level of resistance in strain NPR. One was due to attack on the alcohol part of the ester which could be affected by sesamex. The other was acid moiety attack, unaffected by sesamex but also present in strain 104. This latter defense mechanism was responsible for resistance to the new synthetic esters in both selected strains.

c. Biochemical approaches

A more direct method for determining the effect of detoxifying mechanisms by an insect is to identify metabolites produced at various times after treatment using either whole insects or homogenised extracts. Although the technique can be successful with susceptible insects, it is much more suited to resistant species as larger quantities of insecticide can be applied without mortality and hence more metabolites produced making identification easier. Thus the nature of the breakdown of DDT to non-toxic DDE in DDT resistant houseflies by the enzyme DDT-dehydrochlorinase was characterised (Sternberg et al., 1950) and isolated (Sternberg et al., 1953).

500 g of adult pyrethrins-selected Sitophilus granarius, cross resistant to DDT were kept for eight weeks on 3 kg wheat impregnated with 1000 ppm DDT interspersed with a further 7 g DDT. Extraction yielded eight compounds of which dicofol was the major product with quantities of the lesser metabolites TDE and FW152 (1,1 di-p-chlorophenyl-2,2-dichloroethanol) and smaller amounts of DDE and DDA. Further metabolites present in relatively large amounts suggested that detoxification was via dicofol and not DDE as in the resistant houseflies (Rowlands and Lloyd, 1969).

There was no difference in cholinesterase activity between susceptible and organophosphorus resistant houseflies by in vitro

biochemical techniques, but the resistant flies had low ali-esterase activity. This was physiologically connected with resistance to organophosphorus compounds (van Asperen and Oppenoorth, 1959).

In vivo and in vitro tests showed that resistant Culex tarsalis could detoxify malathion by a carboxyesterase system (Matsumura and Brown, 1961).

Penetration of insecticide through the insect cuticle has also been investigated. Diazinon, diazoxon and DDT penetrated more slowly into diazinon selected SKA flies than into susceptible ones / (Farnham et al., 1965; El Bashir, 1967). Abrading the cuticle of SKA flies then treating with diazinon showed that outer cuticula layers included an important protective mechanism (Sawicki and Farnham, 1965).

d. Biochemistry of pyrethroids

Very little work has been done on the biochemistry of pyrethroids in insects, but techniques for separation and identification of metabolites have improved with the use of thin layer and gas liquid chromatography, and by the use of radio tracers for studies with mammalian metabolism of pyrethroids (Casida, et al., 1971; Miyamoto et al., 1971). By using molecules labelled at different sites, both quantitative and qualitative data on metabolites can be deduced.

Biochemical studies of pyrethroids have been done on susceptible insects and early work on houseflies (Chamberlain, 1950) and cockroaches (Zeid et al., 1953) had suggested hydrolysis of the ester linkage. This conclusion was challenged by Chang and Kearns (1964) who interpreted their results on the basis that most metabolism was on the alcohol moiety. Yamamoto and Casida (1966) claimed that the major metabolic products in houseflies were

the result of the oxidative metabolism of the terminal methyl group on the isobutenyl side chain, i.e. on the acid moiety. The main part of this study was done *in vitro* although they asserted that the system also occurred *in vivo*, and suggested how sesamex could affect this system. However this system could not be a major metabolic pathway in NPR the pyrethrins resistant flies as it did not correspond with toxicological data (Farnham, 1971).

Miyamoto and Suzuki (1972) have reported that the synthetic pyrethroid tetramethrin (= neopynamin) can be degraded by susceptible houseflies by cleavage of the ester linkage to give N-hydroxymethyl-3,4,5,6-tetrahydrophthalimide and (±)-*cis*-*trans* chrysanthemi acid. This system is little affected by sesamex but can be inhibited by the organophosphorus synergist NIA 16388. In bioassa with susceptible flies, NIA 16388 was more effective than sesamex with bioneopynamin (= (+) *trans*-tetramethrin) (Synergistic Factors 236, 135 respectively) but not so effective with either S-bioallethrin (SFs 61, 89) or natural pyrethrins (SFs 110, 165) (Farnham unpublished data). This suggests that in susceptible flies ester cleavage of pyrethrins and allethrin is not so important as oxidative metabolism, but that it may occur. This is contrary to earlier theories (Chang and Kearns, 1964, Yamamoto and Casida, 1966).

Reduced penetration of radio-labelled pyrethrin I was also a feature of strain 213ab, a pyrethrins resistant housefly strain, (Fine *et al*., 1967). Reduced penetration from a parathion resistant strain of houseflies (Hoyer and Plapp, 1968) was associated with resistance to organotin compounds (Plapp and Hoyer, 1968) so that the presence of this mechanism can be detected by bioassay rather than by the more laborious washing experiments previously used to determine its presence.

e. Genetical approach

(i) Without the use of marker mutants

The possibility of a multifactorial resistance system was recognised by Busvine (1951) who observed the marked difference in rates of knock-down in two DDT-resistant strains of housefly which had different cross-resistance spectra. These strains were crossed with a susceptible strain and the progenies from sib-matings and back crosses tested. DDT knock-down resistance was due to a single recessive gene allele (Harrison, 1951) and was inherited in a different way from kill-resistance (Harrison, 1953). Busvine (1953) obtained similar results with both houseflies and body lice.

Two resistance mechanisms were separated from a diazinon selected strain of flies by using the cross backcross technique to a susceptible strain. These factors were low aliesterase mentioned earlier as being associated with organophosphorus resistance and a second unnamed factor. Only the latter could be isolated from a malathion selected strain (Oppenoorth, 1959).

By using a similar cross backcross method, Nguy and Busvine (1960) indicated that resistance to parathion and malathion in two strains were each inherited through a single dominant gene pair, and suggested that the two genes were either alleles or closely linked.

(ii) With the use of single markers

The above examples rely on examination and accurate assessment of toxicological data. Tsukamoto (1963) described how these data could be treated to deduce the number of factors involved in a resistance system by examination of log dose-probability (ld-p) lines and without the use of markers. But with the discovery of visible mutants not only in the housefly (Milani, 1954; Sullivan

and Hiroyoshi, 1960; Hiroyoshi, 1960; Tsukamoto *et al.*, 1961) but also in *Culex pipiens* (Laven, 1955) and *Blattella germanica* (Ross and Cochran, 1961), methods of genetical crossing were modified to examine polyfactorial resistance systems (Tsukamoto, 1964) and to draw up linkage maps (Hiroyoshi, 1961).

Linkage group numbering in the housefly, initially confused by conflicting chromosome numbering systems (Milani, 1954; Hiroyoshi 1961) was clarified by Wagoner (1967) who associated various linkage groups with individual chromosomes.

By using single markers Milani (1954) showed that DDT resistance in the housefly was controlled by a single factor *kdr* (on chromosome III) which was later linked to the brown body marker *bwb* (Milani and Travaglino, 1957).

By crosses between three strains of housefly which resisted parathion, malathion, isolan and DDT, and the susceptible single marker strain bearing stubby wings, *stw*, (Chr. II), Hoyer *et al.* (1965) showed that organophosphorus resistance due to low aliesterase was on chromosome II and was linked to a DDT resistance mechanism.

Non-recessive resistance factors were located from the diazinon selected *SKA* strain on chromosomes II and V by using four susceptible strains each bearing one marker for each of four autosomes (Sawicki *et al.*, 1966).

Hoyer and Plapp (1966) crossed the *Grothe* and *Orlando-DDT* strains with *stw* (chr. II) and dot vein, *dov* (chr. III). DDT resistance was found on both chromosomes in the *Orlando-DDT* strain, but only on chromosome II in the *Grothe* strain. This latter common factor was DDT dehydrochlorinase, *Deh*, (previously located by Lichtwardt, 1964) and was susceptible to *o*-chloro-DDT. The other factor on chromosome III gave DDT knock-down resistance

and resistance to o-chloro-DDT. The factor had been isolated and identified by Milani (1960). It was apparently non-allelic with kdr and was called kdr-0.

(iii) With use of multimarker strains

By using multimarker susceptible stocks in which each mutant marks one chromosome more detailed analyses are possible, using the technique described by Sawicki and Farnham (1967b).

DDT resistance was located on chromosome II in two DDT resistant strains of housefly crossed with a triple marked susceptible strain with mutants for chromosomes I, II and III. Extra resistance to DDT present in one of the parent strains was not associated with any one of these three chromosomes (Oppenoorth, 1965).

DDT resistance from fly strains JIR and DMC-R was located on chromosomes II and III by crosses with a susceptible strain with markers on four autosomes. The factor on chromosome II was DDT dehydrochlorinase (Tsukamoto and Suzuki, 1964). The factor on chromosome III controlled resistance to DDT knock-down by reducing nerve sensitivity, but was not identified as either kdr or kdr-0 (Tsukamoto et al., 1965). Since DDT knock-down resistance and resistance to pyrethrins seem intimately linked (Busvine, 1953), it is possible that reduced nerve sensitivity is the common factor involved.

By using single or multimarker susceptible flies crossed with resistant strains, resistance mechanisms have been isolated. These include (i) reduced penetration, Pen (Hoyer and Plapp, 1968); (ii) DDT dehydrochlorinase, Deh (Lichtwardt, 1964); (iii) low aliesterase, gene a (Franco and Oppenoorth 1962); (iv) a factor for DDT and diazinon inhibited by sesamex, ses (Sawicki and Farnham, 1967a); and (v) a dieldrin resistance factor Dld

(Sawicki and Farnham, 1968a). A multimarker susceptible strain was used to isolate these factors from SKA diazinon selected houseflies. Each individual factor was examined by bioassay with many compounds both without and with additives (Sawicki and Farnham, 1968b). The low levels of resistance of the isolated factors did not add up to the very high levels of the parent SKA strain, probably because of interaction between factors (Sawicki and Farnham, 1969). This theory was confirmed by Hoyer and Plapp (1968) who resynthesised a parathion resistant strain bearing isolated factors on chromosomes II and III. This strain was much more resistant to parathion (100 x) than expected from the resistance levels of the separated factors (gene a 30 x, Pen 1 x).

The effect of interaction between Pen and gene a was examined in a strain reconstituted from the individual factors, and bioassay results compared with those for gene a alone. Resistance to all organophosphorus compounds was more than for gene a alone, and was increased to thionates more than to the corresponding phosphates (Sawicki, 1970).

Using a multimarker technique, Tsukamoto and Suzuki (unpublished data quoted in Tsukamoto, 1969) have genetically analysed the pyrethrins resistant strain 213ab. The order of contribution of autosomes to the total kill resistance to pyrethrins in the homozygous state was III $\gg$ II $>$ V $>$ IV. Dominant resistance was only associated with V. In the same analysis for pyrethrins plus piperonyl butoxide a remarkable decrease was noted in the effect of the factor on chromosome V, whilst no significant decrease was noted with the other autosomes.

7. Objects of this work

The programme of study followed similar lines to those described. The object was to

(a) Expand knowledge of the cross-resistance spectrum of the pyrethrins selected NPR strain of houseflies, derived from 213ab (Farnham, 1971).

(b) Explain the toxicological differences previously noted between strains NPR and the resmethrin selected 104 (Farnham, 1971).

(c) Obtain data on the relationship between chemical structure and toxicity of chosen pyrethroids to individual resistance factors and the importance of each on the total resistance.

(d) Examine any other factor that contributed to the cross-resistance spectrum of NPR.

The work was divided into three sections:

(1) Preliminary work to extend the cross-tolerance examination of strains NPR and 104 whose history has been described (Farnham, 1971), and to prepare a susceptible quadruple marker strain bearing one marker on each of four of the five autosomes.

(2) The genetical crossings to locate then isolate the various resistance factors from NPR.

(3) The detailed examination of the cross-tolerance of the isolated mechanisms in an attempt to explain the cross-resistance spectrum of NPR and to suggest how it differs from 104. For this last part, the synergist sesamex was used, as well as various other compounds which NPR resisted.

MATERIALS

1. Housefly strains

All strains of houseflies (Musca domestica L.) used in this work were derived from four standard strains:-

(a) NPR, a wild type resistant strain selected with natural pyrethrins.

(b) 104, a wild type resistant strain selected with 5B3FC

(c) Cooper, a wild type susceptible strain obtained from the Cooper Technical Bureau, Berkhamsted, Herts. and bred free from insecticidal selection.

(d) 2345 SRS, a quadruple marker strain bearing four visible recessive markers:

ali curva, ac, marking chromosome I
aristapaedia, ar, marking chromosome II,
brown body, bwb, marking chromosome III, and
ochre eye colour, ocra, marking chromosome V.

These markers were combined in one strain at Rothamsted Experiment Station from the single marker strains inbred into the WHO SRS strain and obtained from Milani. 2345 SRS was susceptible to organophosphorus insecticides, DDT and dieldrin but part of the population resisted the carbamate Zectran and the knock-down effect of pyrethroids.

a. History and development of strains

(i) Resistant strains

Both NPR and 104 originated from strain 213ab, which had developed 14-fold resistance to a 1:8 mixture of natural pyrethrins and piperonyl butoxide in the field.

NPR was selected for 45 generations with natural pyrethrins alone after which 50 µg/♀ fly killed only 20% of the population.

104 was selected with 5B3FC over the same period during which its resistance to the selecting agent fell from 125 to 52-fold.

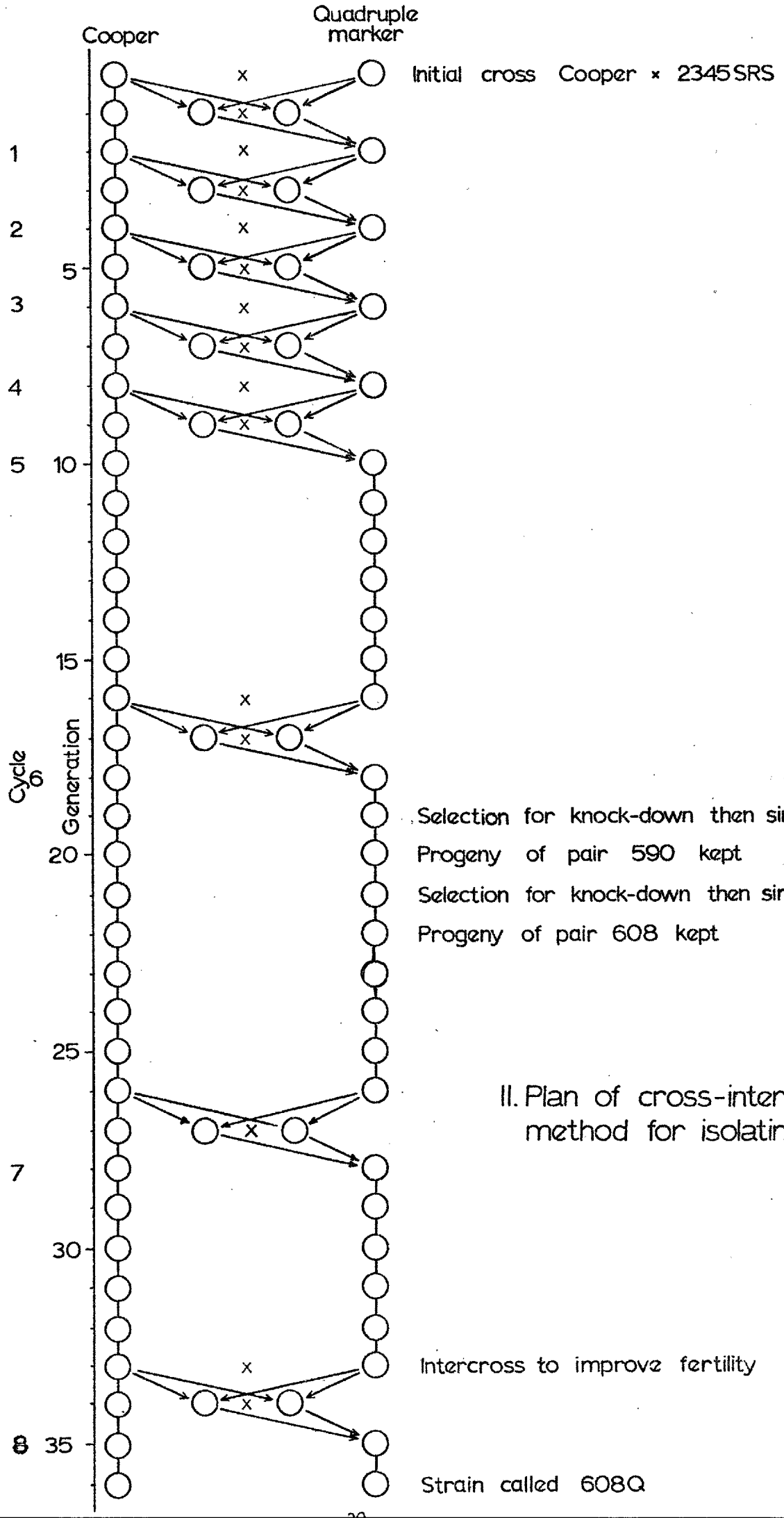
NPR resisted 5B3FC (x 140) but 104 was weakly resistant to natural pyrethrins (x 4). Both strains strongly resisted synergised natural pyrethrins (~ x 100) and DDT (∞), as well as TBTA (~ x 20) (Farnham, 1971).

(ii) Quadruple marker strain

The quadruple marker strain 2345 SRS used in previous work (Sawicki and Farnham, 1968b) was derived from single marker strains outcrossed by Milani to the WHO SRS strain. However this outcrossing did not result in full susceptibility, as the SRS strain slightly resists some carbamates (carbaryl, x 3, isolan, x 2) and natural pyrethrins (x 2.5) (Plapp and Hoyer, 1967). 2345 SRS resisted Zectran and knock-down by natural pyrethrins (Farnham, unpublished data) and was unsuitable for work on the genetics of resistance to pyrethroids.

To eliminate this resistance, the cross-intercross system (Wright and Pal, 1967) was used (see Fig. II on page 29). Quadruple marked flies, initially 2345 SRS, were crossed to the Cooper strain. The F_1 wild type flies were inbred and at F_2, only quadruple marked adults were kept. This was one complete cycle.

After five cycles the quadruple marker flies were bred for six generations, and were then crossed again with Cooper, and one cycle repeated. Approximately 20% of the resulting population slightly resisted Zectran. To eliminate resistance completely, adults were sexed on emergence and selected for increased susceptibility to knock-down by natural pyrethrins when three days old, taking advantage of the negative temperature coefficient for



II. Plan of cross-intercross method for isolating 608Q

knock-down with natural pyrethrins (Lindquist et al., 1945). The procedure was as follows. A small dose of natural pyrethrins (0.05 µg/♀:0.025 µg/♂) was applied topically at 20 ± 1°C. The insects first affected, i.e. the most susceptible, were transferred to the breeding room at 28 ± 1°C where they recovered. Single pairs were set up from these flies (for method, see page 34). From 16 pairs only the progeny of the two most fertile, 584 and 590 were kept. Both strains however still included individuals that resisted Zectran. Strain 584 was discarded after four generations because it was less fertile than 590.

590 was then reselected for rapid knock-down as described above, and another 27 single pairs were set up from the most susceptible individuals. Three pairs yielded fertile progeny: two were discarded, and one, 608 was kept as was the parent 590.

The fertility of 608 F_4 was so poor that the cross intercross with Cooper was repeated, but this failed to improve fertility. Five generations later the cycle was again repeated. The resultant multimarker progeny, called 608Q proved to be both fertile and fully susceptible, so 590 was discarded.

2. Insecticides

The compounds used are listed in Table IV, on page 31.

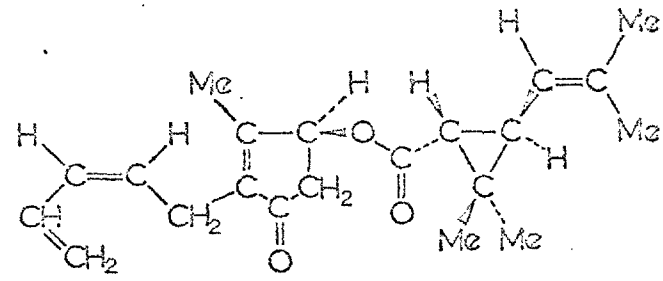
Table IV List of names of compounds used

	British Standard or Manufacturer's Name	Name used in text	Chemical name
1.	Pyrethrum extract	natural pyrethrins	
2.	pyrethrin I	py I	pyrethrolone (+)-*trans*-chrysanthemate
3.	pyrethrin II	py II	pyrethrolone pyrethrate
4.	-	py TMCPC	pyrethrolone 2,2,3,3-tetramethylcyclopropanecarboxylate
5.	resmethrin	5B3FC	5-benzyl-3-furylmethyl (±)-*cis*-*trans*-chrysanthemate
6.	bioresmethrin	5B3F(+) *trans* C	5-benzyl-3-furylmethyl (+)-*trans*-chrysanthemate
7.	pyresmethrin	5B3FP	5-benzyl-3-furylmethyl pyrethrate
8.	NRDC 108	5B3FTMCPC	5-benzyl-3-furylmethyl 2,2,3,3-tetramethylcyclopropanecarboxylate
9.	DDT	DDT	1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane
10.	HEOD	dieldrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-exo-1,4-endo-5,8-dimethanonaphthalene
11.	tributyltin acetate	TBTA	tri-*n*-butyltin acetate
12.	Zectran	Zectran	4-dimethylamino-3,5-dimethylphenyl methylcarbamate
13.	sesamex	sesamex	2-(3,4-methylenedioxyphenoxy)-3,6,9-trioxaundecane

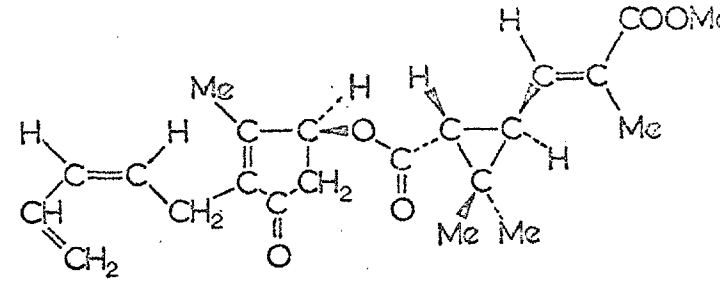
The structures of compounds 2,3,4,6,7,8,9,10,11,12 and 13 are shown in Figs III and IV on pages 31A and 31B.

Pyrethrum extract (25% oleoresin) was given by the Cooper Technical Bureau, Berkhamsted. The other pyrethroids, were

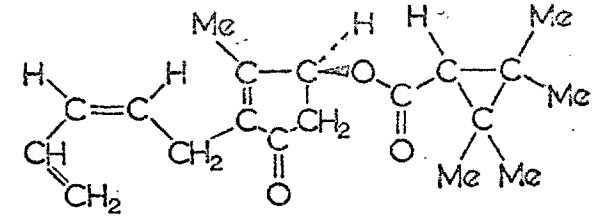
Pyrethrin I



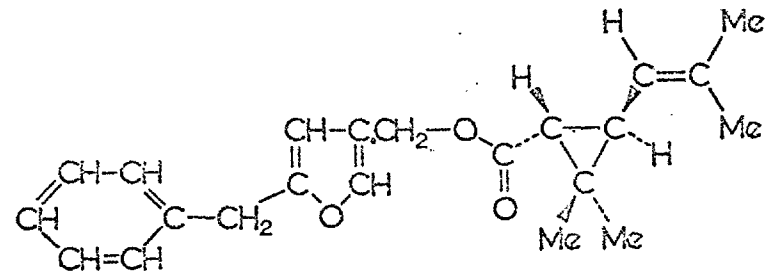
Pyrethrin II



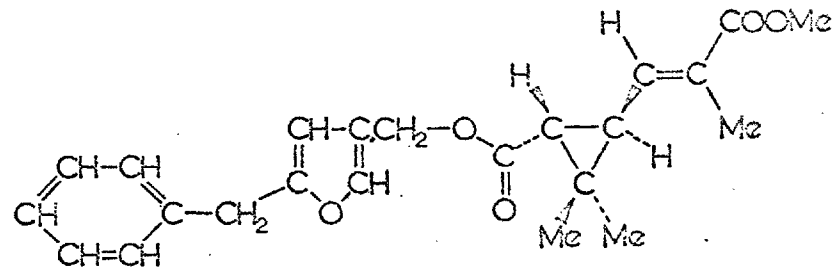
Py TMCPC



5B3F (+) trans C



5B3FP



5B3FTMCPC

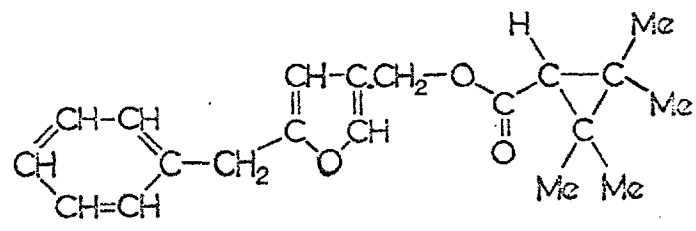
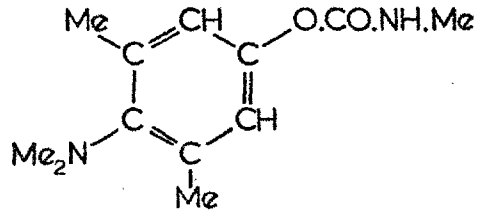
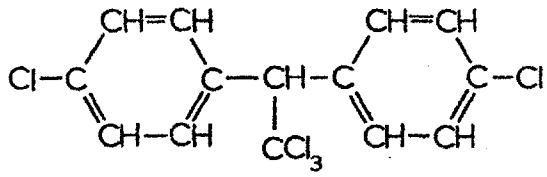


Fig III Structure of pyrethroids

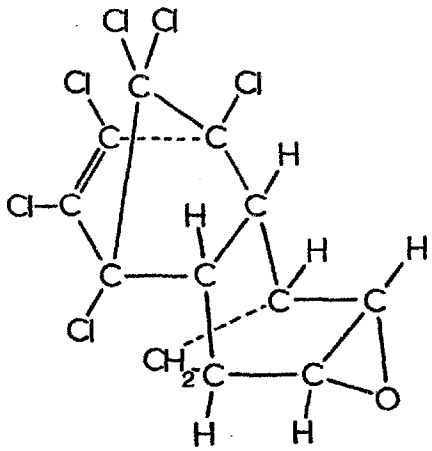
Zectran



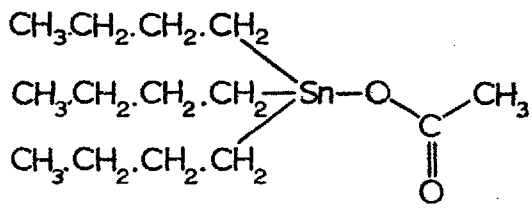
DDT



HEOD



tri-n-butyltin acetate



sesamex

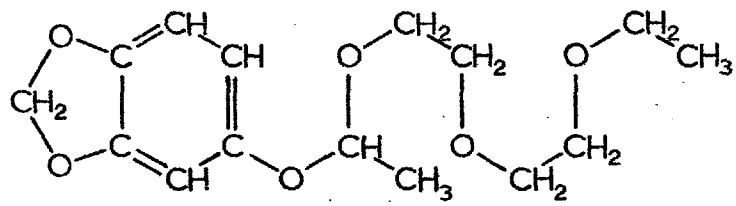


Fig IV Structures of non-pyrethroids

prepared in this department by Drs M Elliott and N F Janes and their colleagues. DDT was re-crystallised from a technical sample given by J R Geigy S A, Basle, Switzerland. Dieldrin was given by Shell Chemicals, U K; Zectran by Dow Chemical International, New York; TBTA by Pure Chemicals Ltd, Liverpool and sesamex by Shulton Inc, New York.

All compounds except natural pyrethrins were checked for structure and purity using nuclear magnetic resonance spectroscopy, and were better than 90% pure.

METHODS

1. Rearing technique for mass cultures

All stages of the flies were reared at 28 ± 1°C. The adults (2000-3000) were kept in aluminium cages 0.3 m x 0.3 m x 0.3 m with two open sides covered with polythene netting, and a hole in one side, 0.15 m x 0.15 m, to which a terylene netting sleeve was attached. Smaller cultures (up to 1000 adults) were kept in aluminium cages 0.3 m x 0.15 m x 0.15 m with open ends (0.15 m x 0.15 m), one covered with polythene netting and the other with a terylene sleeve.

Adults of wild type strains not requiring phenotypic checks, sexing on emergence or selection, and mutant strains for test were allowed to emerge in numbers appropriate to the size of the cage. Mutant strains for breeding were obtained from pupae put into a 150 ml polystyrene container in a tray (0.2 m diameter, 80 mm high) covered with polythene netting. Adults emerged and were checked phenotypically within 24 hours before they were put into a breeding cage.

For the first day after the start of emergence, flies were fed on water from saturated cotton wool and lump sugar only. In addition, milk fountains (Fig V page 36) were given from the second day. Strains to be tested had a fresh milk fountain each day. On the third day an egg-laying dish consisting of about 15 40 mm absorbent cotton wool rolls soaked in milk and held verticall in a 30 ml polystyrene container was put into the cages of strains needed for breeding. The egg-dish was rewetted with tap water on the fourth day and a fresh egg-dish was added. These dishes had sufficient eggs on them by the fifth day to 'seed' approximately

0.75 kg of larval medium which was made of dried milk, dried flake yeast, macerated Whatman No 1 filter paper clippings and water in a ratio of 2:2:3:7 by weight. A further 0.25 kg of medium was added two days after seeding. The larvae pupated in the upper layers of the medium a week after inoculation. The pupae were collected by flotation (Basden, 1946), and cleaned by washing in a stream of water over a 10-mesh sieve. After drying, they were transferred to emergence trays or cages, and the adults started to emerge on the ninth day after the inoculation.

2. Rearing techniques for single pairs and their progenies

Small cages for single pair matings were made by punching a 25 mm diameter hole in a 150 ml polystyrene container . Gauze was glued over the hole, and the top closed with a gauze lid (Fig VI on page 36). A three day old virgin female and a male fly were introduced into the container which in turn was put into a similar complete pot. A 50 mm diameter polythene tray with three 40 mm long absorbent cotton wool rolls soaked in milk was put on the bottom of the outer pot. The flies could feed on the milk and the female lay eggs onto the rolls. Each day, the tray was checked for eggs and a fresh tray put in without disturbing the adults. Eggs, rolls and polythene tray were put onto 50 gms of larval medium in a 150 ml polystyrene container and covered with a paper lid to avoid contamination. After two days, the tray was removed and a further 50 gms of larval medium added. The parents were kept until the female had layed four egg batches or until she died. The larvae pupated after a week and the pots were transferred to trays 50 mm deep and 0.2 m diameter covered with polythene netting. After emergence the adults were checked for phenotype and transferred to cages for breeding, if required.

Generally there were few adults from a single pair, so they were bred in cages similar to those for single pairs, but using pots 80 mm high, the system being otherwise identical. At F_2 there were generally enough adults for breeding in a small metal cage, but if not, the larger polystyrene cages were again used.

3. Test and selection techniques

a. Pretreatment procedure

For selection, flies were sexed within 24 hour of emergence, kept in separate cages, and fed on milk, lump sugar and water until they were 3 ± 1 days old.

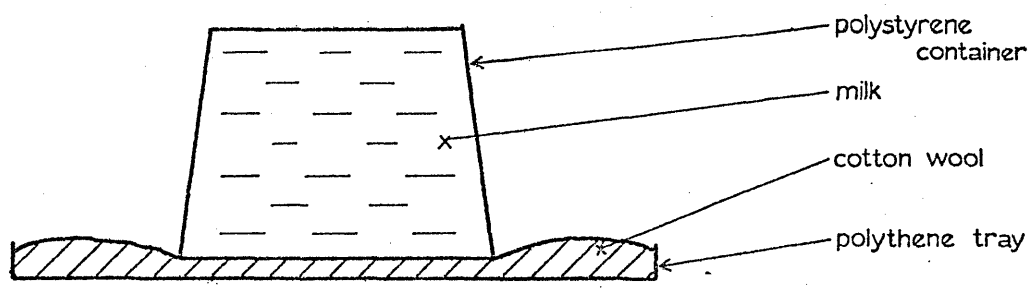
For bioassays, the adults were sexed when 3 ± 1 days old, and tested 24 hours later. Usually only females were bioassayed, but when numbers were small, males were also tested.

Flies for selection or bioassay were lightly anaesthetised with diethyl ether and groups of 15 were put into 150 ml polystyrene containers covered with terylene gauze. The flies took about 15 mins to recover completely from anaesthesia, and from each dish dead or dying flies were removed before treatment. The flies were fed from a cotton wool roll soaked in 2% sugar solution put on top of the gauze lid.

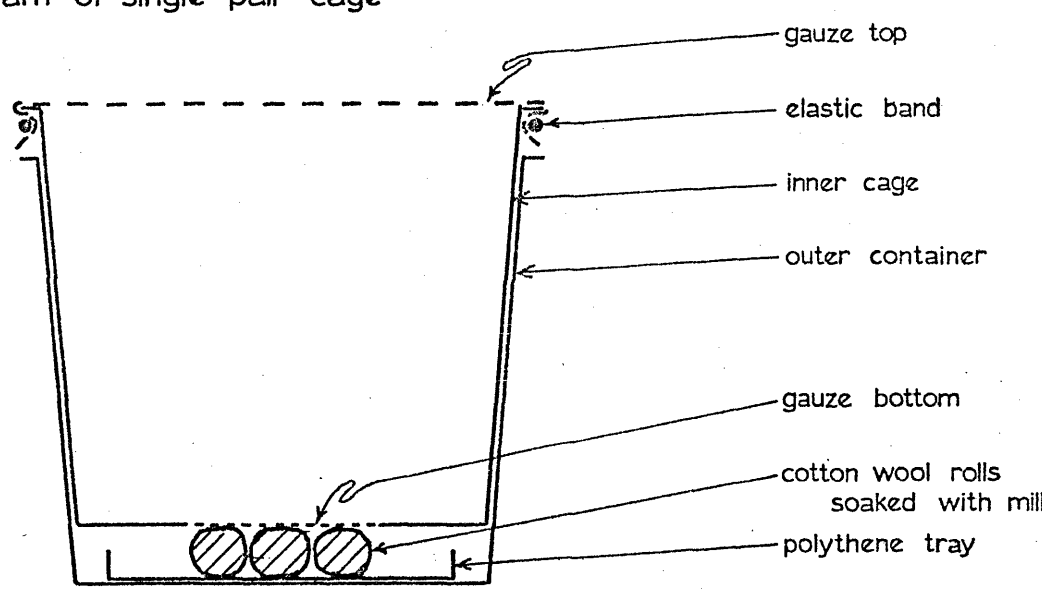
b. Bioassay procedure

Bioassays followed the general design of probit analysis (Finney, 1971) using a topical application technique described previously (Sawicki et al., 1966). All solutions in acetone were prepared on the day of the test from stock solutions kept in a refrigerator. The doses were spread regularly on a logarithmic scale over the kill range, and where possible, six concentrations with two replicates of 15 flies were used at each dose. For heterogeneous populations up to twelve concentrations were

V. Diagram of milk-fountain



VI. Diagram of single pair cage



used to cover the full kill range and detect inflections in the ld-p line (log-dose probit regression line).

Flies lightly anaesthetised with diethyl ether were treated with 1 µl of the insecticide solution on the thorax, using a micro-applicator (Arnold, 1967). In some tests, the flies were pre-treated with 2 µg sesamex on the abdomen 1-3 hours before the application of the insecticide. The tests were done at room temperature and the post-treatment temperature was 20 ± 1°C. The flies were fed with 2% sugar solution and checked for kill at 24 hour intervals after treatment until there was no further change in recorded death. Ld-p lines were drawn by eye and LD50s calculated by the Rothamsted Computer.

c. Selection procedure

Virgin flies of the stock strains were selected by topical application (Farnham, 1971). The survivors were mated either 24 hours after treatment with 5B3FC (strain 104) or 48 hours after treatment with natural pyrethrins (strain NPR). With some strains, each fly was treated whilst held with forceps to ensure that they each received the whole dose. Pyrethrin I and sesamex were applied together when used as a selecting agent.

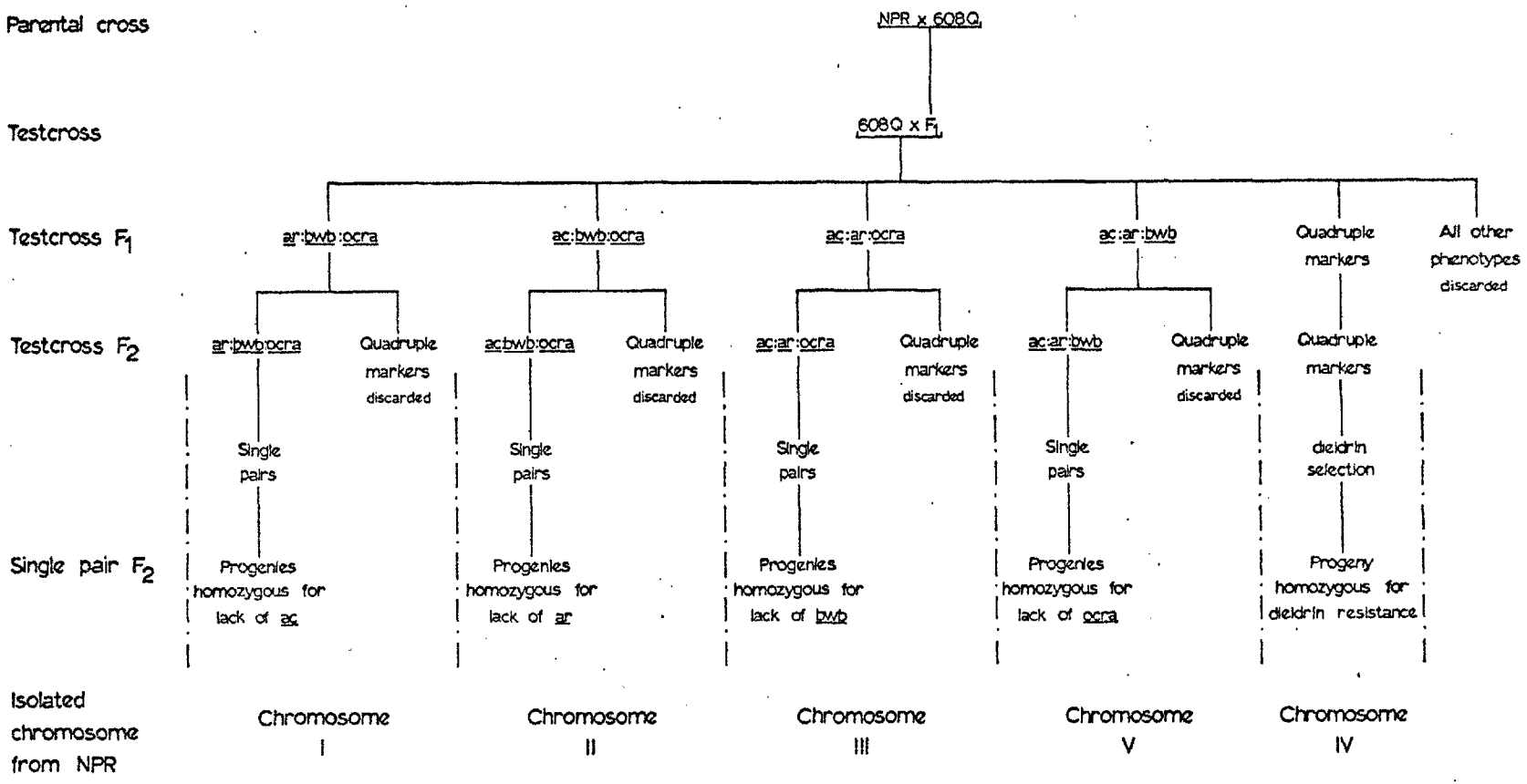
4. Isolation of individual autosomes from strain NPR

Figure VII on page 38 outlines the ideal method for isolating individual chromosomes by substitution from NPR into 608Q. For clarity, the progeny of the testcross (as defined by Bridges, 1934) has been called testcross F_1 (T.C.F_1) rather than F_2.

Adult NPR and 608Q flies were sexed within 16 hours of emergence and mated in reciprocal crosses when three days old. The F_1

Fig VII

Diagram of scheme for the isolation of chromosomes from NPR



adults were sexed on emergence and the males used for the test-cross to virgin 608Q females in mass culture. Making the test-cross in this direction reduced the chances of crossing over between the marker and resistance factor at the F_1 stage (Hiroyoshi, 1960).

The four triple and quadruple marker flies of the testcross progeny (TCF_1) were kept and separately inbred (i.e. according to phenotype). The triple marked TCF_2 were sexed within 16 hours of emergence, and 40 single pairs of each phenotype set up three days later (for method, see page 34). Pair progenies breeding true for lack of the fourth marker were kept and bioassayed. The phenotypic purity of substrains for the relevant visible markers was usually established by single pair F_3.

Quadruple markers of the testcross progeny were bred by mass culture, called strain 213Q.

Variations on this method were used to obtain homozygous stocks when (i) this method failed to produce a stock homozygous for the relevant marker, (ii) the resulting strain was heterozygous for the separated resistance factor, or (iii) two factors were linked on the same chromosome.

The results of bioassays at the TCF_2 stage on triple marked flies indicated the presence of resistance factors on all MPR's autosomes except I; so, where necessary, flies could be selected with an insecticide appropriate to the chromosome concerned. Wherever possible a non-pyrethroid was used to reduce the chances of selecting for a second pyrethroid factor (e.g. Zectran for chromosome II).

5. Statistical treatment of bioassay results

As all tests, except those for TCF_2 were repeated, each LD50 (see Table X X on page 117) is the weighted mean value

obtained from the formula

$$\text{MEAN LD50} = \text{Antilog} \left[\frac{\Sigma(\log \text{LD50} \times {}^{1}/_{\log \text{SE}^2})}{\Sigma\, {}^{1}/_{\log \text{SE}^2}} \right],$$

and its standard error from the formula

$$\text{Standard error} = \text{Mean LD50} \times \frac{1}{\sqrt{\Sigma {}^{1}/_{\log \text{SE}^2}}} \times 2.303.$$

Resistance factors (RF) were calculated by dividing the Mean LD50 for any one strain by the Mean LD50 for that treatment against 608Q, and any RF between 0.5 and 2 was considered not significantly different from the standard.

Compounds were tested without and with sesamex in the same experiments, and the synergistic factors calculated from the formula

$$\text{Mean SF} = \text{Antilog} \left[\frac{\Sigma(\log a - \log b)({}^{1}/_{\log \text{SE}_{(a)}{}^2} + {}^{1}/_{\log \text{SE}_{(b)}{}^2})}{\Sigma({}^{1}/_{\log \text{SE}_{(a)}{}^2} + {}^{1}/_{\log \text{SE}_{(b)}{}^2})} \right]$$

where a = LD50 for unsynergised treatment

b = LD50 for synergised treatment

$\text{SE}_{(a)}$ = Standard error for a

$\text{SE}_{(b)}$ = Standard error for b

Ld-p lines of heterogeneous populations were plotted on log-probability paper. The lines for 608Q are included. For clarity, points on these graphs are omitted in this thesis.

By observing plateaus on ld-p lines, the proportion of resistant, heterozygous and susceptible flies in a population may be calculated (Tsukamoto, 1963) and where practical, this was done. However, the various triple marker phenotypes of the TCF_2 differed too little in their response to insecticides for accurate analysis.

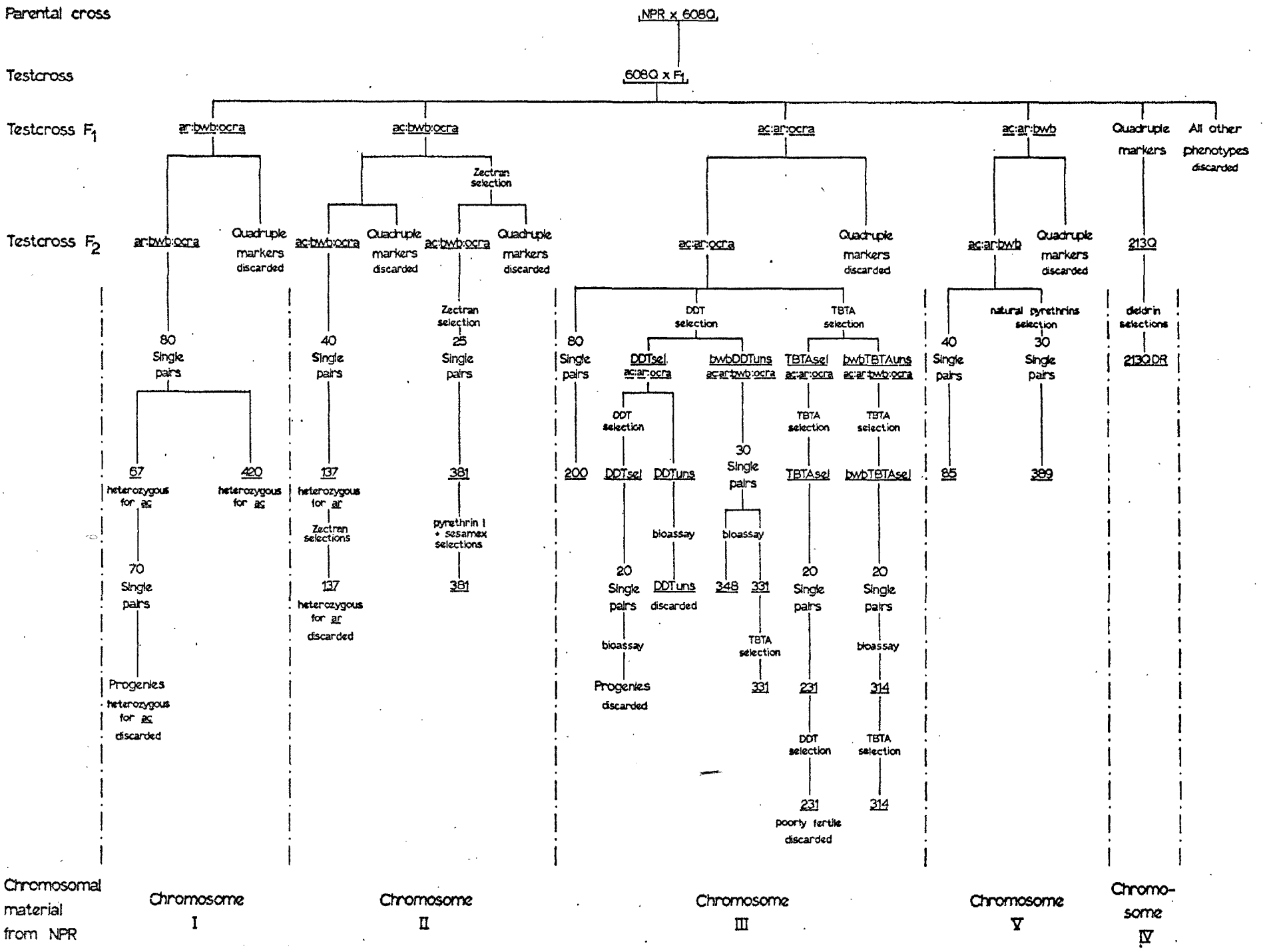
Table V Strains derived from the cross NPR x 608Q

Strain No.	Phenotype	NPR's chromosomal material	Notes
420	ac/+;ar;bwb;ocra	I	No measurable resistance
381	ac;bwb;ocra	II	Heterozygous for resistance to synergised pyrethrolone esters, and to unsynergised and synergised Zectran
200	ac;ar;ocra	III	Homozygous for TBTA resistance. Heterozygous for DDT resistance
314	ac;ar;bwb;ocra	III	Homozygous for TBTA resistance
348	ac;ar;bwb;ocra	III	Homozygous for DDT resistance
331	ac;ar;bwb;ocra	III	Homozygous for DDT and TBTA resistance
213Q	ac;ar;bwb;ocra	IV	Heterozygous for dieldrin resistance
213Q DR	ac;ar;bwb;ocra	IV	Homozygous for dieldrin resistance
85	ac;ar;bwb	V	Homozygous for natural pyrethrins resistance
389	ac;ar;bwb	V	Homozygous for natural pyrethrins resistance

6. Isolation of chromosomes from NPR

Figure VIII, page 41A illustrates the methods used to obtain the individual strains, and Table V, above, lists the derived strains with their phenotypes and the chromosomal material from NPR. The results can be grouped according to the chromosome from NPR under examination.

VIII. Diagram of origin of substrains derived from cross NPR x 608Q



a. Chromosome I

None of the 40 single pairs, 41-80, set up from the TCF_2 adults yielded a strain pure only for ar;bwb;ocra (Table VI, p 43). From the heterozygous strain, 67, which gave flies with ac at F_2 70 single pairs (241-270, 271-310) were set up, but none of the progenies was homozygous for lack of the ac marker, and all were discarded.

The original cross was repeated and 40 more single pairs, 416-455 set up at the TCF_2 stage but again none of the progenies lacked ac. However, strain 420, heterozygous for ac was kept for bioassay because bioassay of ar;bwb;ocra flies at TCF_2 had shown that there was no detectable resistance associated with chromosome I.

b. Chromosome II

None of the first 40 single pairs, 121-160, set up at the TCF_2 stage yielded a strain pure only for ac;bwb;ocra (Table VII, p 44). Strain 137 was kept because it was fertile although it gave quadruple marker flies at F_2. Also it was hetergeneous in its response to synergised pyrethrolone esters and to synergised and unsynergised Zectran, so the strain was selected with Zectran on five successive generations (see Table VIII p 45) using the Zectran resistance as a "marker". This failed both to eliminate the ar marker and to purify Zectran resistance, so 137 was discarded.

At the second attempt ac;bwb;ocra flies were selected at the TCF_1 and TCF_2 stages (see Table VIII, p 45). 25 single pairs were set up from the survivors. Of five of their progenies that lacked ar, the most fertile, 381, was kept. Bioassays later showed that 381 was heterogeneous for the factor which confers resistance to synergised pyrethrolone esters, and for this reason it was selected three times with synergised py I (Table VIII, p 45), but

Table VI Results of single pair matings to isolate chromosome I from MPR

Pair Nos	Pair Parents	Number of single pairs	Number sterile	Layed sterile eggs at F1	Gave too few adults	F1 Quads	Layed sterile eggs at F2	F2 Quads	Quads at later gens	Pure for lack of ac	Result
41-80	$(\underline{ar};\underline{bwb};\underline{ocra})^2$ from TCF_2	40	9	6	2	11	0	9	3	None	all discard
241-270	$(\underline{ar};\underline{bwb};\underline{ocra})^2$ from 67	30	5	0	1	10	1	10	3	None	all discard
271-310	$(\underline{ar};\underline{bwb};\underline{ocra})^2$ from 67	40	8	12	0	6	2	7	5	None	all discard
416-455	$(\underline{ar};\underline{bwb};\underline{ocra})^2$ from TCF_2	40	0	1	2	22	0	15	0	None	420 kept
	Totals	150	22	19	5	49	3	41	11		
	%	100	15	13	3	33	2	30	7		

Table VII Results of single pair matings to isolate chromosome II from NPR

Pair Nos	Pair Parents	Number of single pairs	Number sterile	Layed sterile eggs at F1	Gave too few adults	F1 Quads	Layed sterile eggs at F2	F2 Quads	Quads at later gens	Phenotypical successful pair progenies	Progenies retained
121-160	(ac;bwb;ocra)^2 from TCF_2	40	1	2	1	21	1	13	1	0	None
361-385	(ac;bwb;ocra)^2 from TCF_2 after selection with TCF_2 and TCF_2 with Zectran	25	5	1	1	6	0	7	0	5	All heterozygous for resistance factor - 381 kept
	Totals	65	6	3	2	27	1	20	1	5	
	%	100	9	5	3	42	2	31	2	7	

Table VIII List of selections, dose rates and kills for isolation of NPR's chromosome II

Stage or Strain		Phenotype	Selecting agent	Dose in µg	kill %	Dose in µg	kill %
Testcross	F1	ac;bwb;ocra	Zectran	5	36	1.25	60
	F2	ac;bwb;ocra	Zectran	40	63	20	88
137	F7	ac;bwb;ocra	Zectran	1.0	80	0.5	73
	F8	"	"	10	59	2.5	60
	F9	"	"	40	96	20	95
	F10	"	"	10	66	2.5	93
	F11	"	"	4.0	56	1.0	47
381	F16	"	py I + 2 µg sesamex	0.0063	30	0.0016	78
	F17	"	"	0.025	99	0.0031	96
	F21	"	"	0.04	96	0.01	95

remained heterogeneous. However, it was kept for bioassays.

c. Chromosome III

Bioassay of ac;ar;ocra flies at the TCF_2 stage indicated that there are two factors of resistance on HPR's chromosome III. One gives resistance to pyrethroids and possibly DDT and the other to TBTA. These factors were isolated singly and together and for this reason this chromosome presented most difficulties.

Table IX, p 47, lists the single pairs set up to isolate the three strains. All the first 40 single pair progenies 1-40 set up at the testcross F_2 stage had bwb and were discarded. The method was repeated (161-200) yielding two pair progenies lacking bwb. Of these, one, 180, was lost through infertility, and the other, 200 proved heterogeneous to DDT and pyrethroids.

After these two failures, the original cross was repeated and ac;ar;ocra flies at the TCF_2 stage were divided into two parts. One was selected with DDT to try to obtain a population that resisted pyrethroids and DDT, and the other with TBTA (see Table X, p 48). Survivors were bred separately in mass culture, giving two strains DDT sel and TBTA sel. The number of adults of each phenotype at the first generation for each strain is shown in Table XI, p 49. The closer a resistance gene is to the marker locus on the chromosome, the less it will be associated with it in this generation. Thus the low elimination of bwb in the progeny of selected flies indicates weak linkage. Hence, Table XI shows that there was more crossing-over between bwb and the DDT resistance factor than between bwb and the TBTA resistance factor. bwb Flies emerged earlier from DDT sel than from TBTA sel but why this occurred is not known.

Table IX Results of single pair matings to isolate factors on NPR's chromosome III

Pair Nos.	Pair Parents	Number of single pairs	Number sterile	Layed sterile eggs at F1	Gave too few adults	F1 Quads	Layed sterile eggs at F2	F2 Quads	Quads at later gens.	Phenotypically successful pair progenies	Progenies retained
1-40	(ac;ar;ocra)^2 from TCF_2	40	9	6	2	11	0	9	3	0	None
161-200	(ac;ar;ocra)^2 from TCF_2	40	7	13	1	9	1	7	0	2	200
201-220	(ac;ar;ocra)^2 from DDT sel	20	2	4	0	2	0	2	0	10	None
221-240	(ac;ar;ocra) from TBTA sel	20	1	5	0	2	0	0	0	9	None
311-330	(ac;ar;bwb;ocra)^2 from TBTA sel	20	7	2	0	-	2	-	-	9	314
331-360	(ac;ar;bwb;ocra)^2 from bwb DDT uns	30	5	3	0	-	2	-	-	20	331, 348

Table X List of selections, dose rates and kills used during isolation of factors on NP3's chromosome III

Stage or strain		Phenotype	Selecting agent	♀ Dose in µg	♀ % kill	♂ Dose in µg	♂ % kill
Testcross	F2	ac;ar;ocra	DDT	1.0	78	1.0	86
	F3	"	"	2.0	65	2.0	87
Testcross	F2	ac;ar;ocra	TBTA	2.0	89	1.0	94
	F3	"	"	4.0	65	2.0	84
	F4	ac;ar;bwb;ocra	"	1.0	55	0.5	23
314	F6	ac;ar;bwb;ocra	TBTA	3.0	80	1.5	74
	F9	"	"	3.0	73	1.5	71
	F21	"	"	3.0	70	1.5	65
331	F6	ac;ar;bwb;ocra	TBTA	3.0	80	1.5	93

Table XI Phenotypic segregation of DDT sel F_1 and TBTA sel F_1

Strain	Females		Males		Total	
	ac;ar;ocra	ac;ar;bwb;ocra	ac;ar;ocra	ac;ar;bwb;ocra	ac;ar;ocra	ac;ar;bwb;ocra
DDT sel	1624	117	1628	113	3252	230
TBTA sel	558	8	546	10	1104	18

To further purify these strains and to compare the two methods of selection, the triple markers from each strain were subdivided further into two of which one was selected as before (Table X, p 48). Mass cultures of these four stocks gave strains that, for the author's convenience, were called DDT sel and TBTA se selected twice with their respective agents and DDT uns and TBTA un selected once only. The numbers of the phenotypes of the progenie of these stocks are shown in Table XII (p 51) and indicate that there was a small proportion of bwb resistant flies after the second selectio.

After bioassay, 20 single pairs 201-220 (Table IX , p 47) were set up from DDT sel to obtain a strain homozygous for both DDT and TBTA resistance. All pair progenies that lacked bwb later proved heterogeneous for DDT and/or TBTA resistance and were discarded.

20 more single pairs, 221-240, were set up from the TBTA sel (Table IX, p 47) to obtain a strain homozygous for TBTA resistance only, but again all pair progenies that lacked bwb were heterogeneous for one or other of the factors, but the progeny of pair 231 was kept because it was homogeneous for TBTA resistance but heterogeneous for DDT. It was later selected with DDT but discarded because survivors bred so poorly that in 30 generations there were never enough for bioassay.

Because the methods used had failed to isolate strains homozygous for either resistance factor alone or in combination, the method was repeated, but crossing over was allowed between bwb and the resistance factors.

Quadruple marker flies bred from survivors of the selections with DDT or TBTA of the ac;ar;ocra flies at the TCF_2 stage were kept and bred in mass cultures, giving two substrains called

Table XII Phenotypic segregation of DDT sel F_2, TBTA sel F_2, DDT uns F_1, and TBTA uns F_1

	Females		Males		Total	
Strain	ac;ar;ocra	ac;ar;bwb;ocra	ac;ar;ocra	ac;ar;bwb;ocra	ac;ar;ocra	ac;ar;bwb;ocra
DDT sel	817	17	924	9	1741	26
TBTA sel	935	18	1217	12	2152	30
DDT uns	1476	51	1551	64	3027	115
TBTA uns	1353	24	1300	42	2653	66

bwb DDT uns and bwb TBTA uns. At F_3, bwb TBTA uns was selected with TBTA (Table X, p 48) and from the strain renamed bwb TBTA sel, 20 single pairs 311-330 (Table IX, p 47) were set up at F_7. Nine pair progenies were fertile and after bioassay one, 314, was kept because it resisted TBTA but not DDT, and even later selection with TBTA at F_{21} (Table X, p 48) failed to change its resistance level.

30 more single pairs, 331-360 were set up (Table IX, p 47) from bwb DDT uns. The fertile progenies were bioassayed and two strains kept: 348 which resisted DDT but not TBTA and 331 which also resisted DDT but was heterogeneous in its response to TBTA. 331 was later selected with TBTA (Table X, p 48) to give a strain homogeneous for resistance to both. Thus these three strains with material from NPR's chromosome III were quadruple markers: 314 had TBTA resistance, 348 had DDT resistance and 331 had both.

d. Chromosome V

40 single pairs (81-120) were set up at the TCF_2 stage and of five progenies lacking ocra one, 85, was kept after bioassay with pyrethrum extract (Table XIII, p 53). Strain 85 was slightly less resistant to natural pyrethrins than anticipated from the bioassay on ac;ar;bwb flies at the TCF_2 (see page 74), so a second attempt was made to isolate this chromosome after selecting the ac;ar;bwb flies at the TCF_2 with natural pyrethrins (Table XIV p 54). From 30 single pairs, 386-415 four progenies lacked ocra and the most fertile, 389, was kept.

e. Chromosome IV

Bioassay of the TCF_2 (see page 75) flies showed that dieldrin resistance was probably associated with this unmarked chromosome. Quadruple marker flies of the TCF_2 were kept and

Table XIII Results of single pair matings to isolate factor(s) on NP3's chromosome V

Pair No.	Pair parents	Number of pairs	Number sterile	Layed sterile eggs at F1	Gave too few adults	F1 Quads	Layed sterile eggs at F2	F2 Quads	Quads at later gens.	Phenotypically successful pairs	Progenie retained
81-120	(ac;ar;bwb)^2 from TCF$_2$	40	4	7	2	11	0	11	0	5	85
386-415	(ac;ar;bwb)^2 from TCF$_2$ selected with natural pyrethrins	30	5	10	0	5	1	5	0	4	389

Table XIV Selection, dose rate and kill used during isolation of strain 382

Stage	Phenotype	Selecting agent	♀ Dose in µg	♀ % kill	♂ Dose in µg	♂ % kill
Testcross F2	ac;ar;bwb	natural pyrethrins	1.0	80	0.5	83

the strain called 213Q. It was heterogeneous in its response to dieldrin, and was selected with dieldrin (see Table XV, p 56) and on four other occasions until homogeneous. The strain was renamed 213Q DR.

Table XV List of selections, dose rates and kills used for isolation of NPR's chromosome IV

Strain	Phenotype	Selecting agent	♀ Dose in µg	♀ % kill	♂ Dose in µg	♂ % kill
2130 F3	ac;ar;bwb;ocra	dieldrin	0.1	26	0.1	42
2130DR F4	"	"	1.0	66	0.5	74
F8	"	"	1.0	42	0.5	40
F10	"	"	4.0	76	0.5	28
F11	"	"	5.0	59	2.5	79

BIOASSAY RESULTS

1. Response of the four standard strains to insecticides

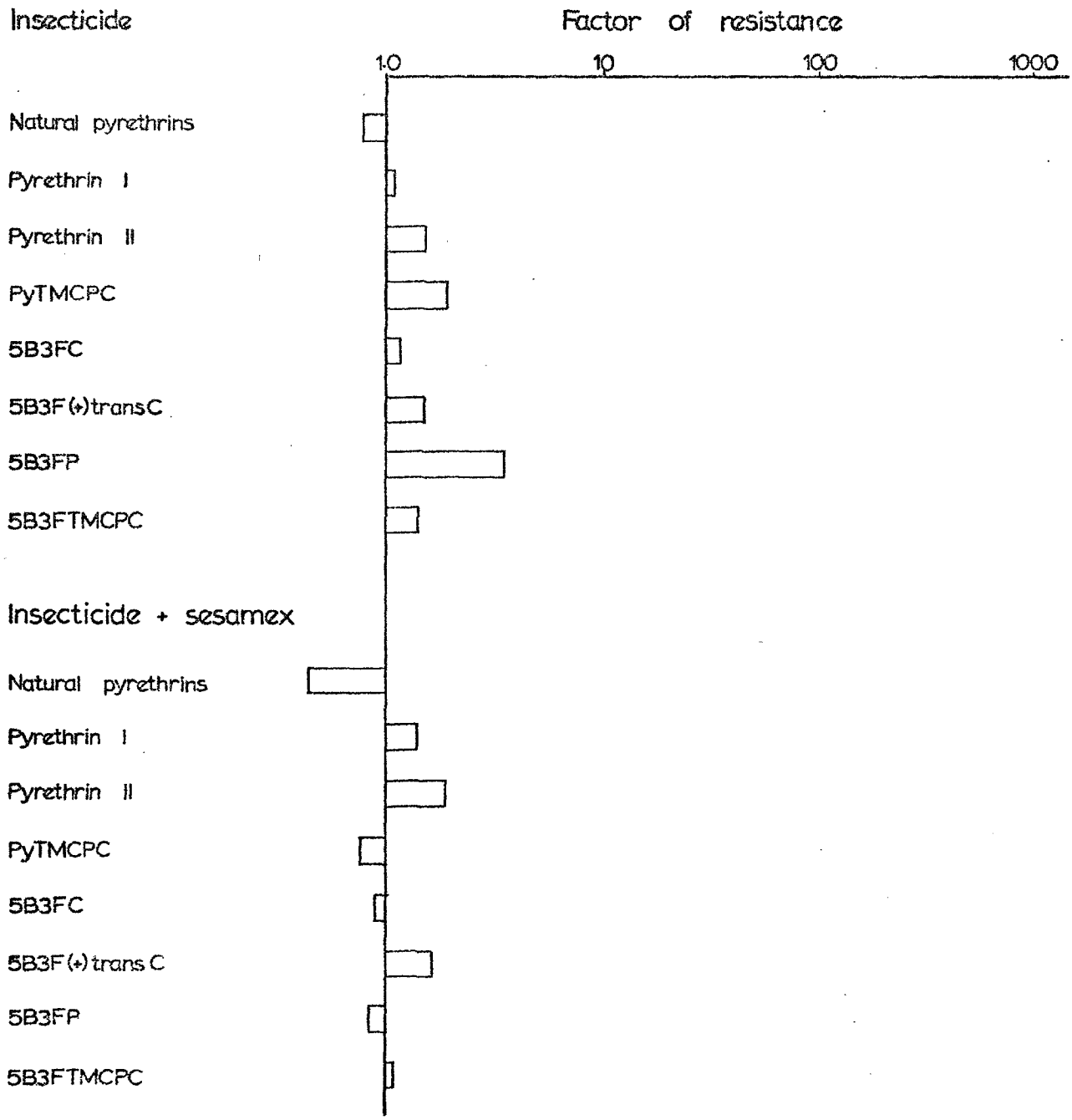
All standard strains, i.e. NPR, 104, Cooper and 608Q were tested with the listed pyrethroids and Zectran without and with pretreatment with sesamex and also with DDT, dieldrin and TBTA. Results obtained with strain 608Q, the susceptible strain used for genetical crosses, were taken as standard throughout the work, and are shown in Table XX on page 116. Histograms of pyrethroid results for other strains (Histograms I-III, pages 58, 59, 60) show the factors of resistance in the absence or presence of the synergist and the factors of synergism for each insecticide. All bioassay results are shown on Tables XX on pages 117, 118 and in the Appendix.

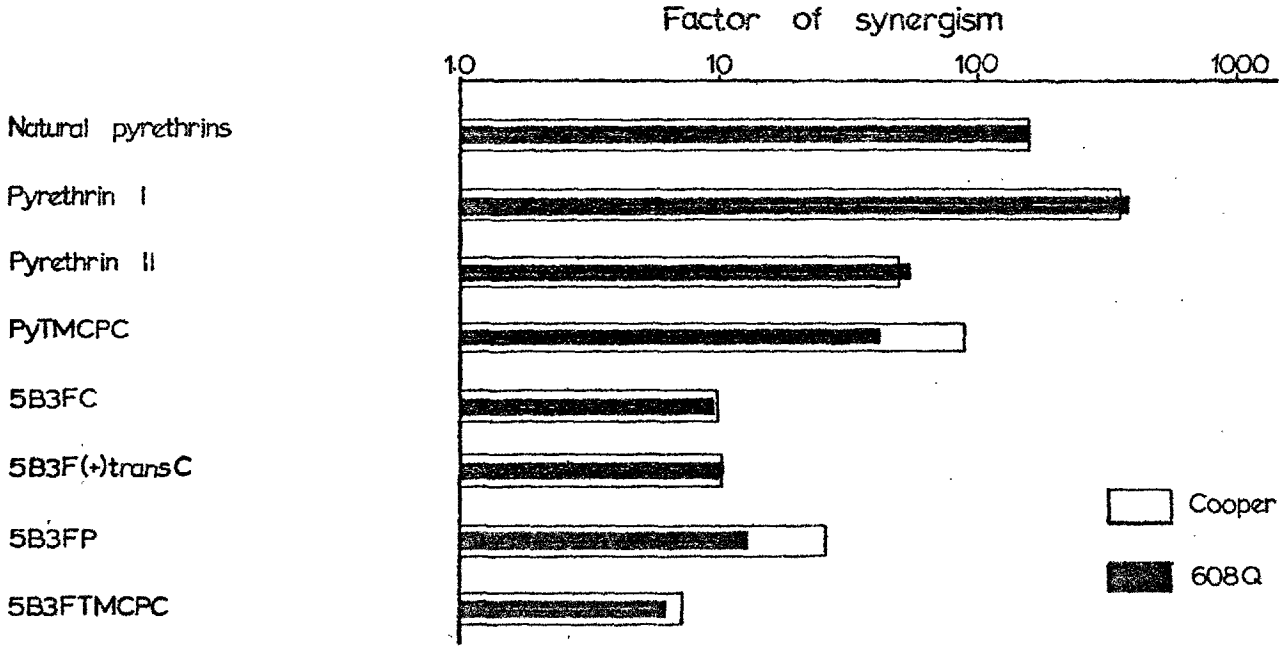
Cooper differed little from 608Q (Histogram I on page 58) in its response to all insecticides. Interstrain differences were less than two-fold and not statistically significant.

Results for the two resistant strains (Histograms II and III on pages 59 and 60) with natural pyrethrins, py I, py II, 5B3FC, 5B3F(+) trans C and 5B3FP have been reported before (Farnham 1971) and are listed in Table XX (on page 117) along with results for the two additional pyrethroids, py TMCPC and 5B3FTMCPC.

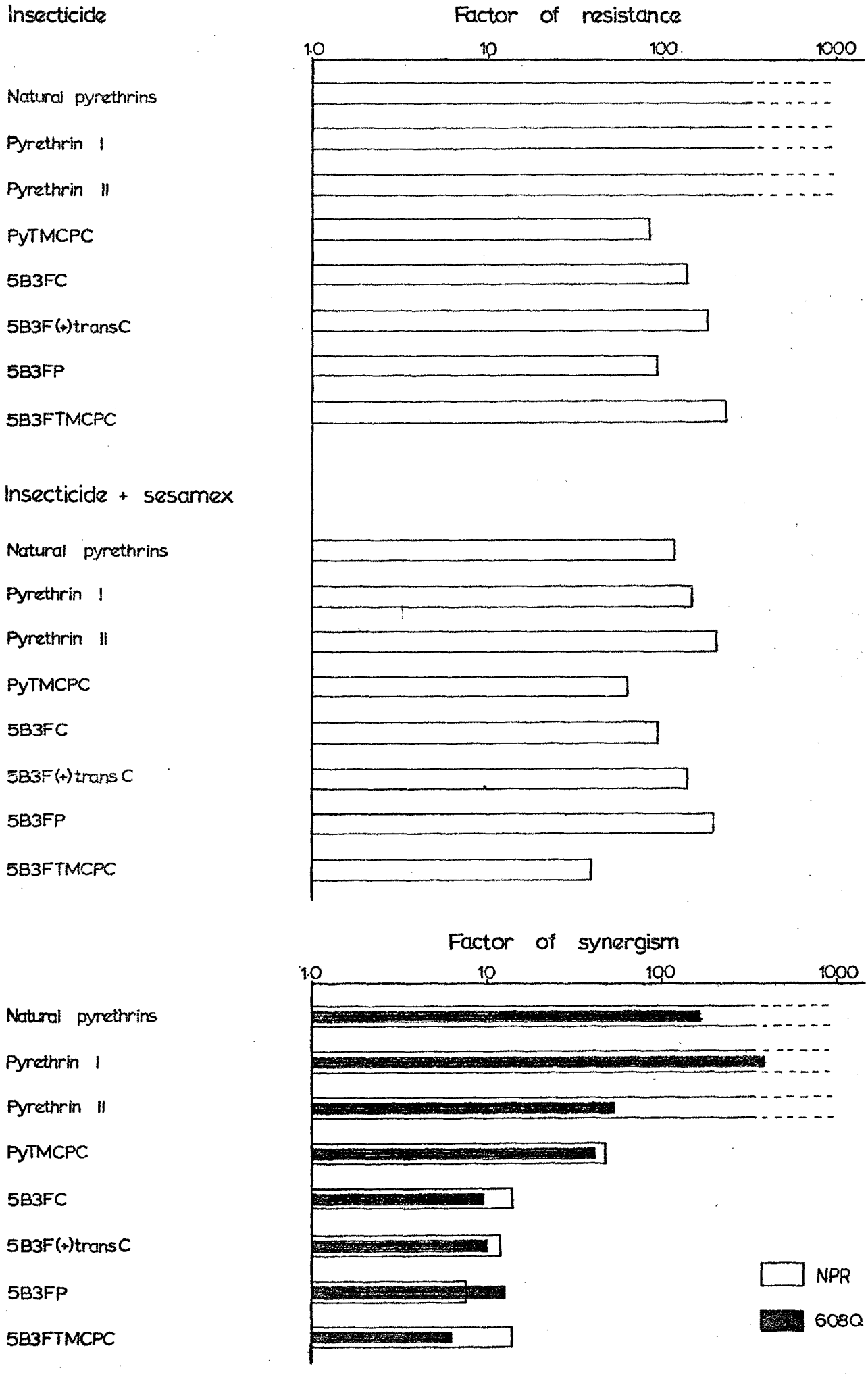
Strains 104 and NPR did not differ as much in their respons to py TMCPC as they did to other pyrethrolone esters, i.e. natural pyrethrins, py I and py II. None of the NPR was immune, and the resistance factor (RF) of 89 was only three times that for 104 (RF 34). This was because py TMCPC was the pyrethrolone ester that 104 resisted most. However as with other pyrethrolone esters, pretreatment with sesamex affected NPR more than 104 so that the interstrain difference was much reduced (RFs NPR 63, 104 48). Also 104 was much more resistant to synergised pyTMCPC

I. Histograms for Strain Cooper

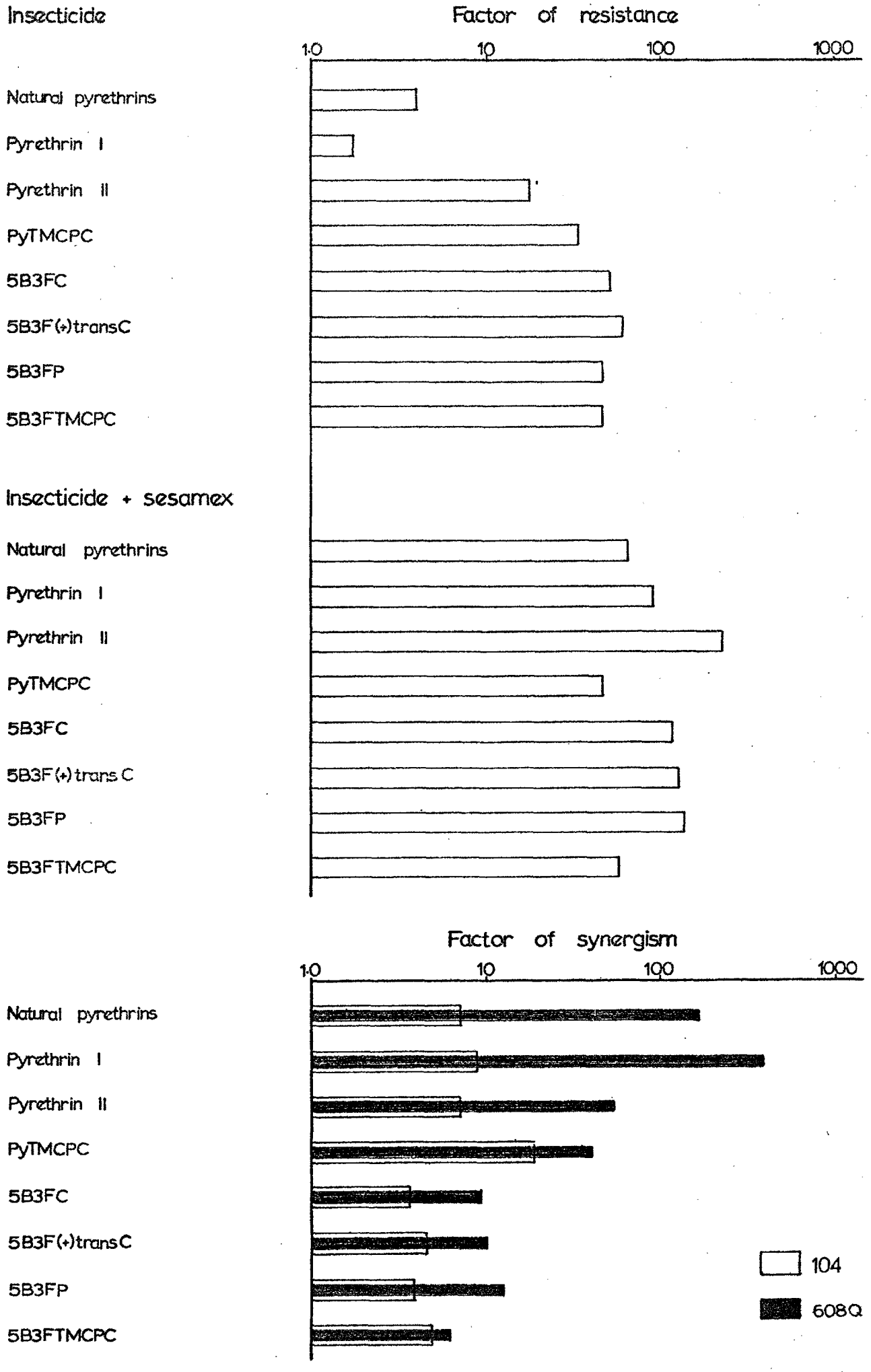




II. Histograms for Strain NPR



III. Histograms for Strain 104



than to the compound alone. Although both strains resisted 5B3FTMCPC, NPR was about five times more resistant than 104 (RF's NPR 240, 104 46). This interstrain difference was greater than that obtained with other esters of 5-benzyl-3-furylmethyl alcohol (5B3FA), but was again reduced by pretreatment with sesamex to RF's of 40 and 59 respectively.

Both strains strongly resisted DDT with more than 70% of the population immune to 50 µg/♀ fly. Both strains were heterogeneous in their response to both unsynergised and synergised Zectran, 70% of the population was immune to 50 µg/fly of Zectran alone, but after pretreatment with sesamex, only 10% were not affected by 1 µg of Zectran.

Strain 104 had lost its resistance to dieldrin during the laboratory selection period, but in NPR only 20% of the population showed any resistance and only 3% were fully homozygous for the resistance mechanism (Farnham, 1971).

2. 2. Results for (608Q x NPR)F_1

All treatments were made against (608Q x NPR)F_1

a. Pyrethroids

F_1 flies were very much less resistant than strain NPR (histograms IV on p 63) and the resistance lay between 4 and 15 except that for synergised 5B3FTMCPC (2.9). The marked difference in resistance levels between the pyrethrolone esters and their equivalent 5B3FA esters in NPR disappeared. Factors of synergism (SF) were similar to those for 608Q.

b. Other compounds

The very great resistance to DDT in NPR was reduced to x 4 in F_1 and the ld-p line was straight (see graph I on page 64).

The ld-p line for dieldrin (see graph II on page 65) showed heterogeneity. About 20% of the flies were heterozygous for dieldrin resistance whilst the remainder of the population was

susceptible. LD100 for the heterozygotes (0.8 µg/♀) was six times less than LD100 for the homozygotes.

The ld-p line for TBTA was straight and parallel to those for both parents. Resistance was intermediate.

The response of NPR and its F_1 to Zectran was heterogeneous, and both ld-p lines showed a plateau (see graph III on page 66). The response of the F_1 after pretreatment with sesamex (see graph IV on page 67) was steep and straight, and LD50 was close to the susceptible level. Sesamex also reduced resistance in NPR but failed to eliminate the plateau.

c. Conclusions

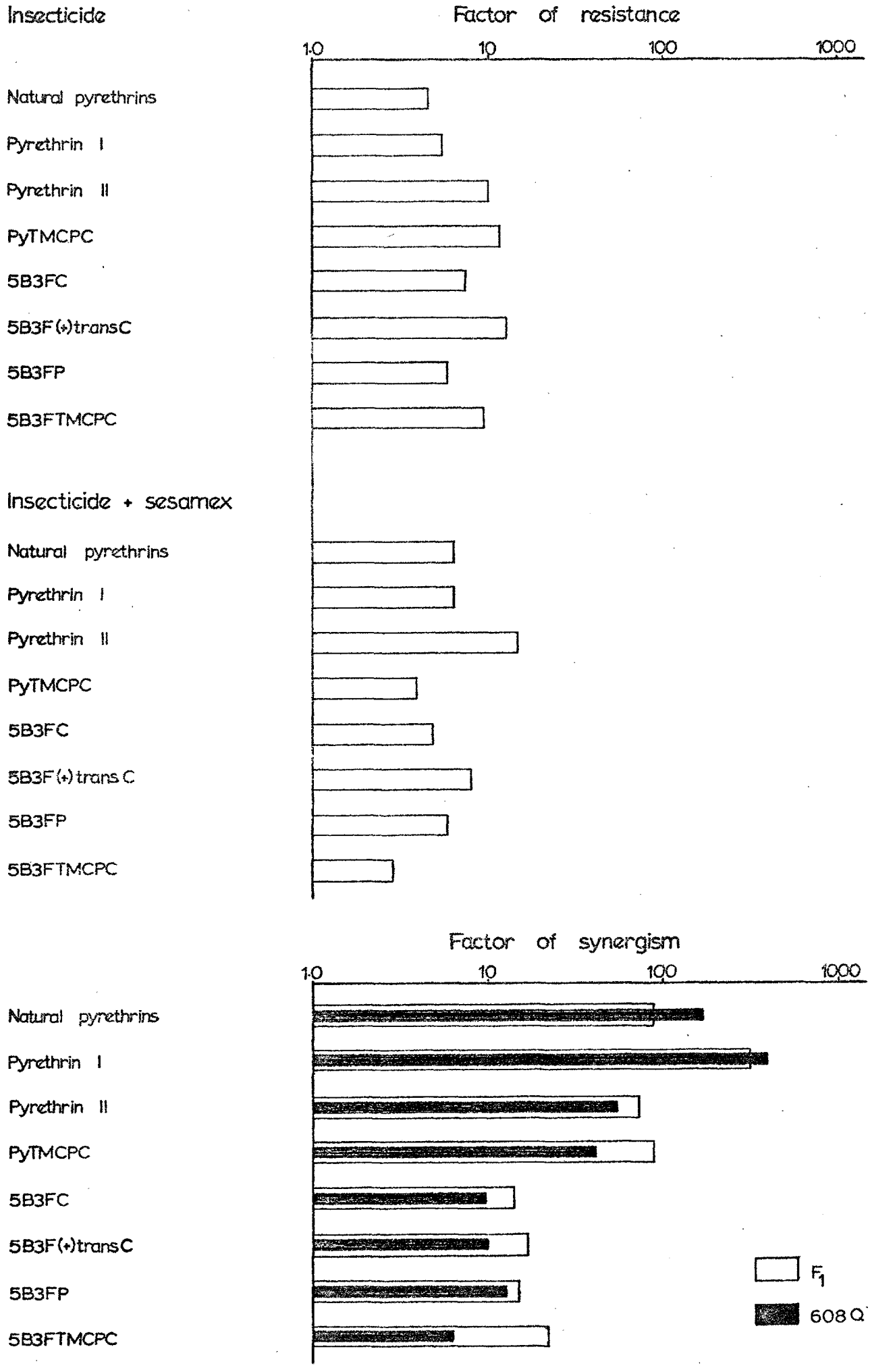
These results showed that there was no major dominant resistance factor to pyrethroids in NPR. DDT resistance was also controlled by mainly recessive factors. Zectran resistance was probably controlled by two factors, one of which was possibly dominant (which would explain the plateau in the ld-p line of the F_1). This factor could be suppressed by sesamex, to give the straight line response. Dieldrin resistance was semi-dominant.

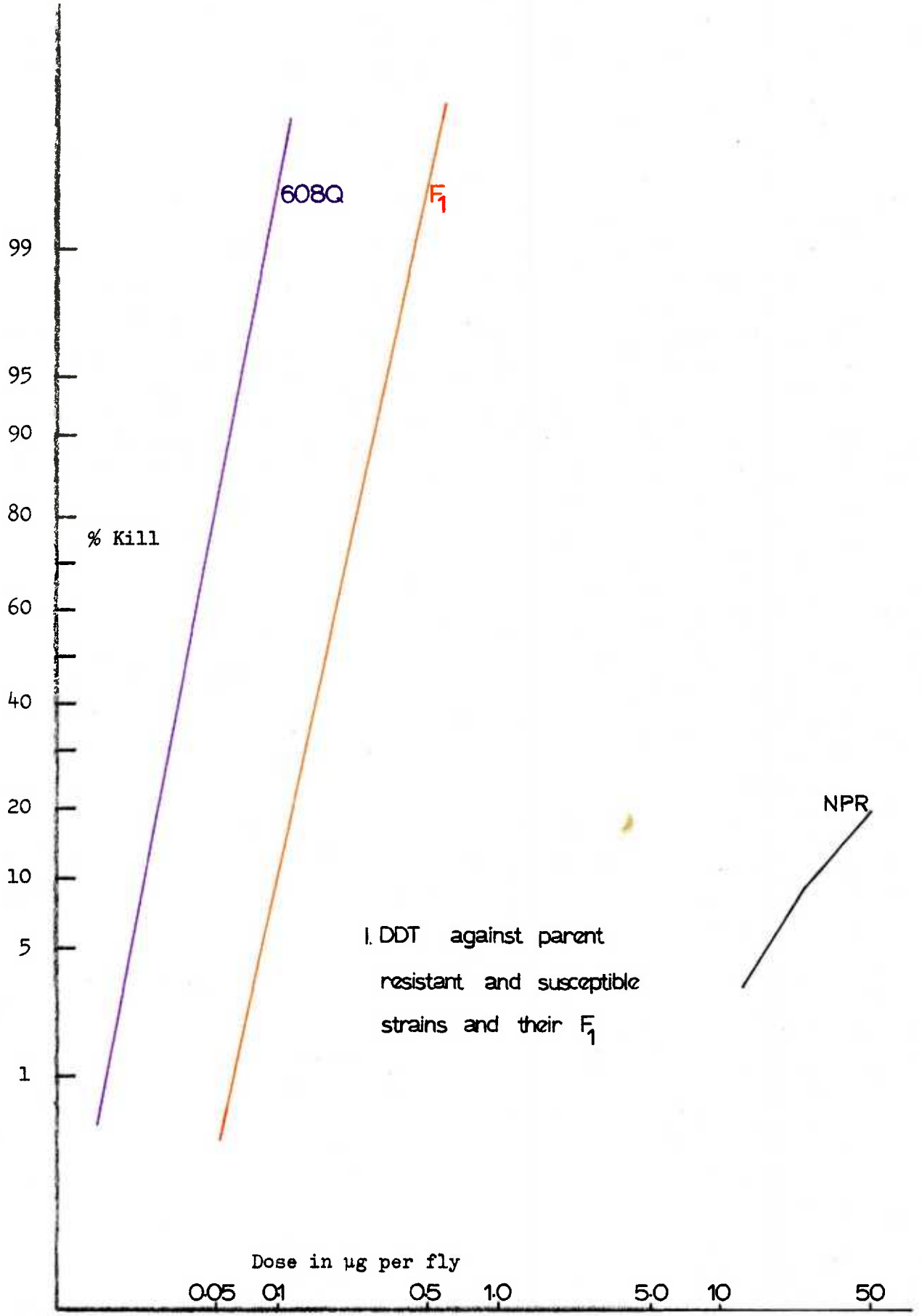
Because there were no dominant major pyrethroid resistance factors no bioassays were done at the TCF_1 stage, i.e. the next generation, since each resistant chromosome would have been in the heterozygous state, and so as susceptible as the susceptible 6080 parent.

3. Results for Testcross F_2

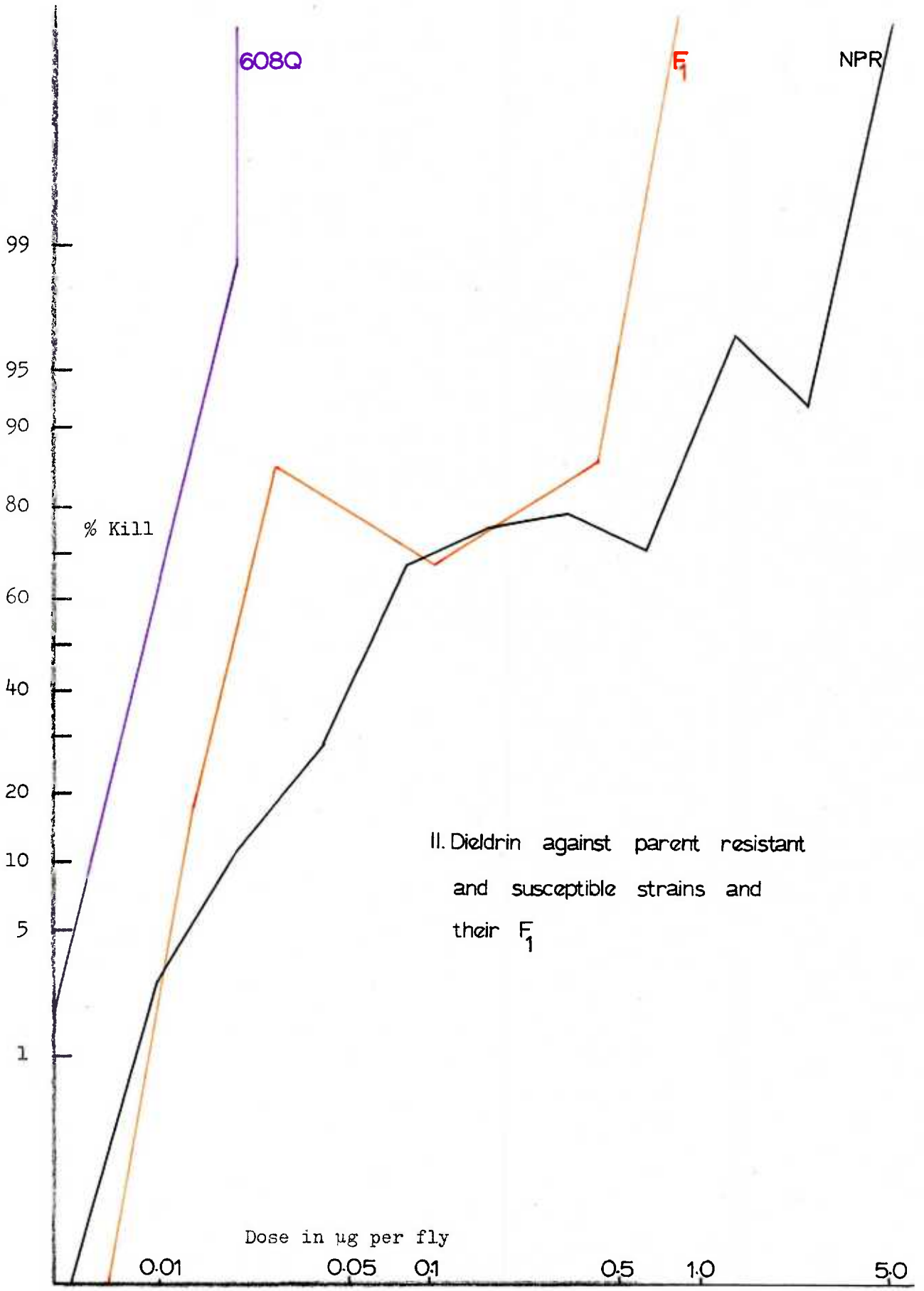
The triple marker progenies of the testcross (TC)F_1 flies were bioassayed to determine which of NPR's chromosomes bore resistance factors. TCF_1 triple marked flies had the three

IV. Histograms for F_1 of (608Q x NPR)

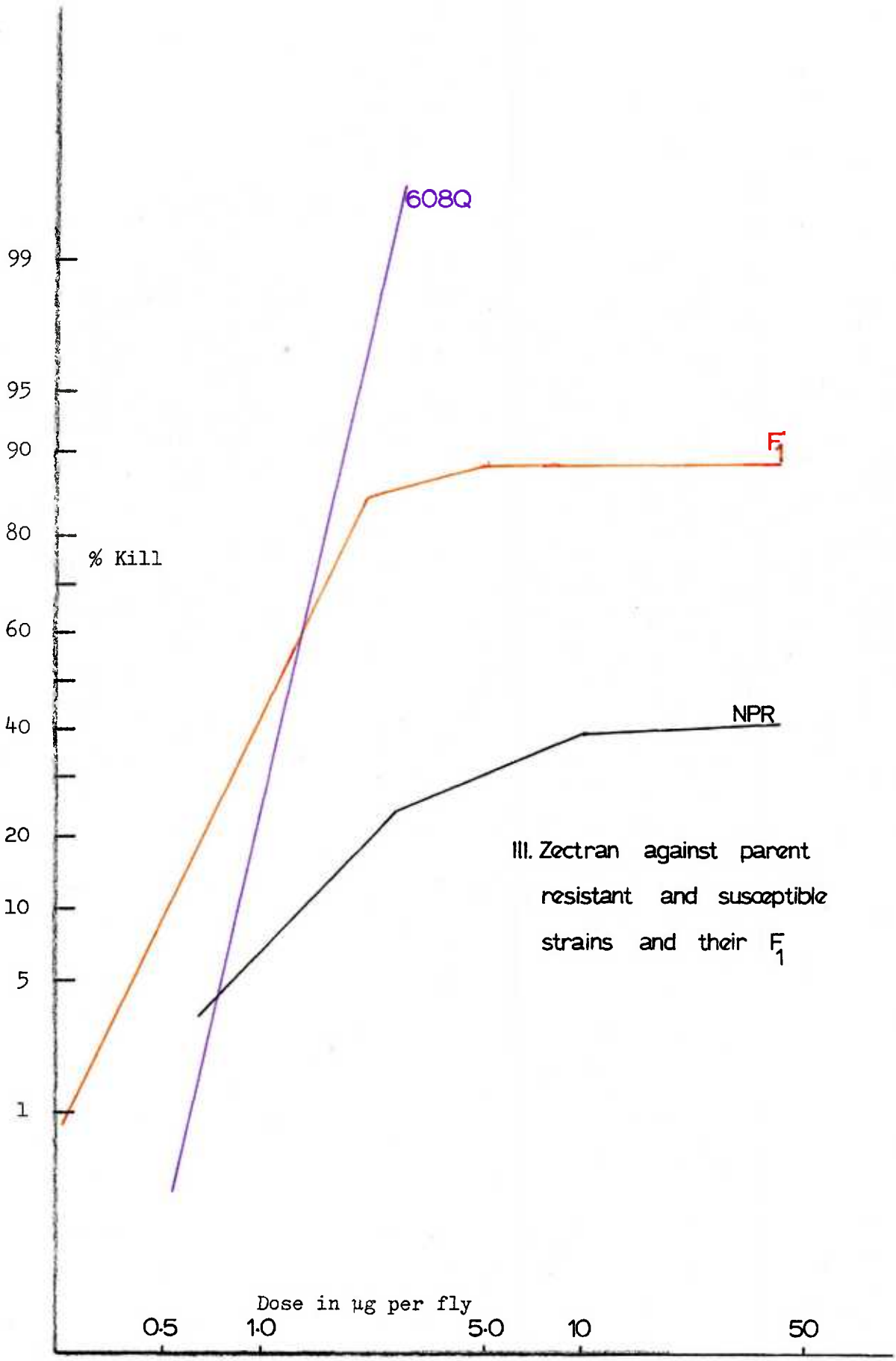




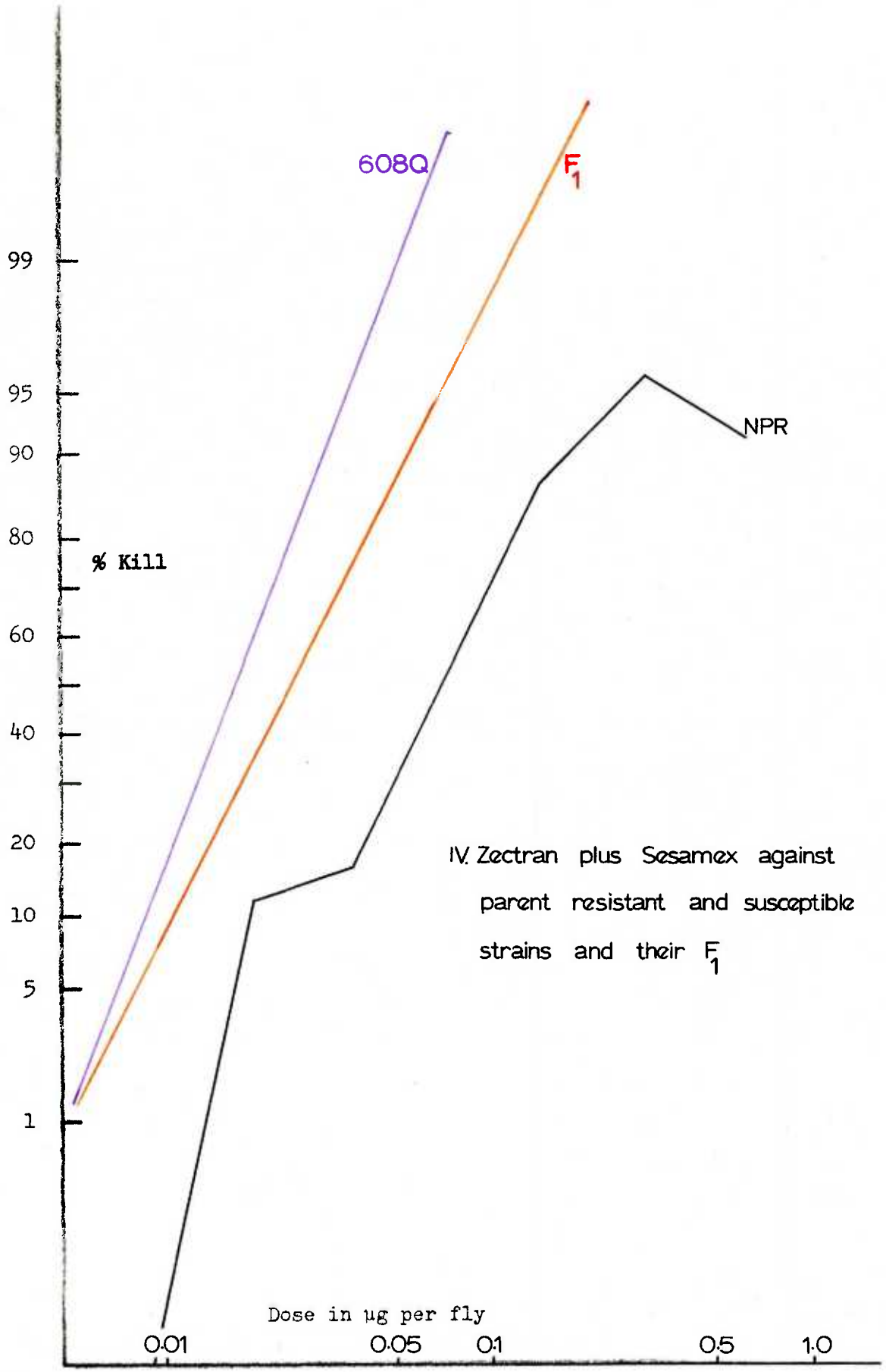
1. DDT against parent resistant and susceptible strains and their F_1



II. Dieldrin against parent resistant and susceptible strains and their F_1



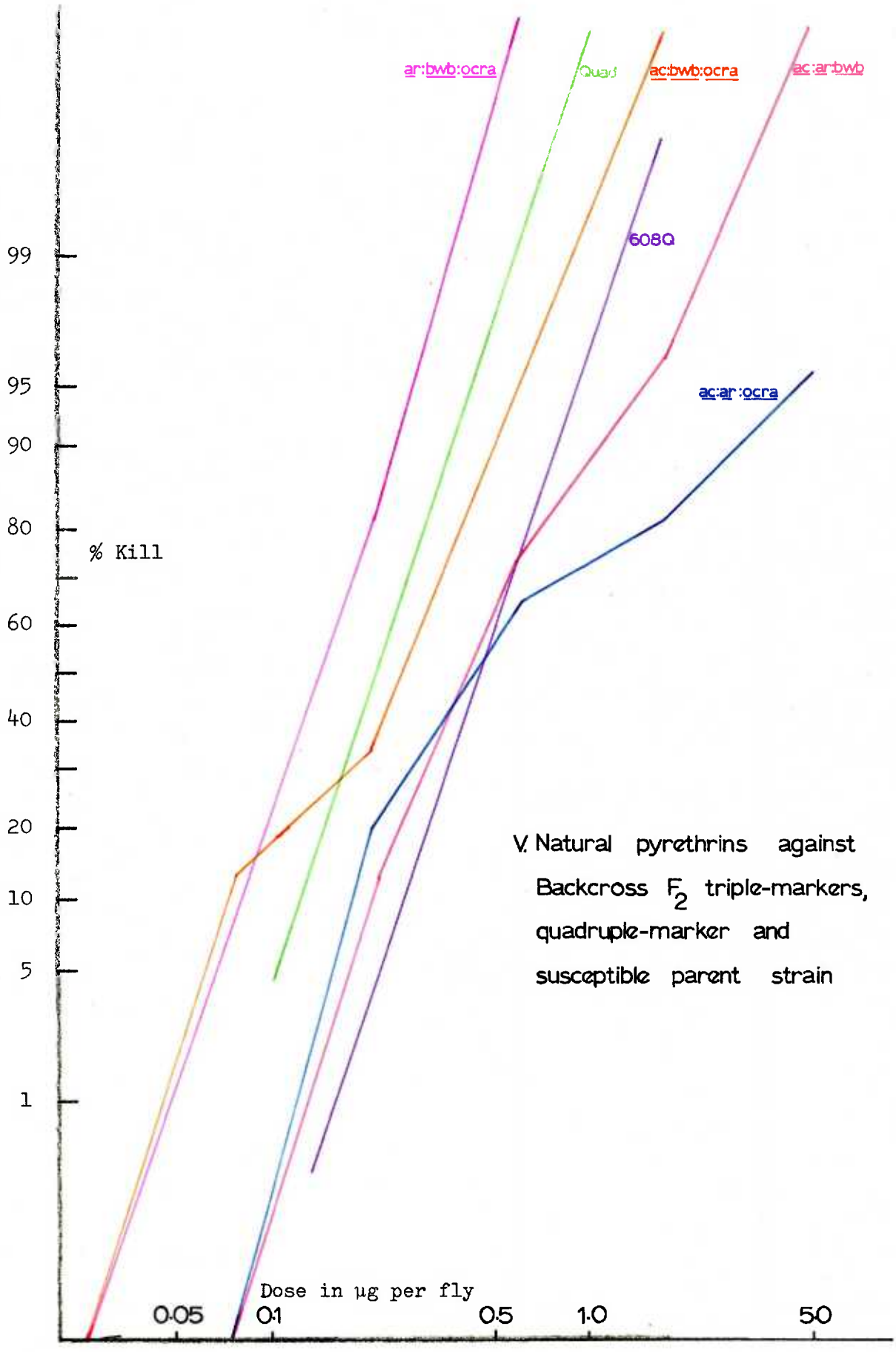
III. Zectran against parent resistant and susceptible strains and their F_1



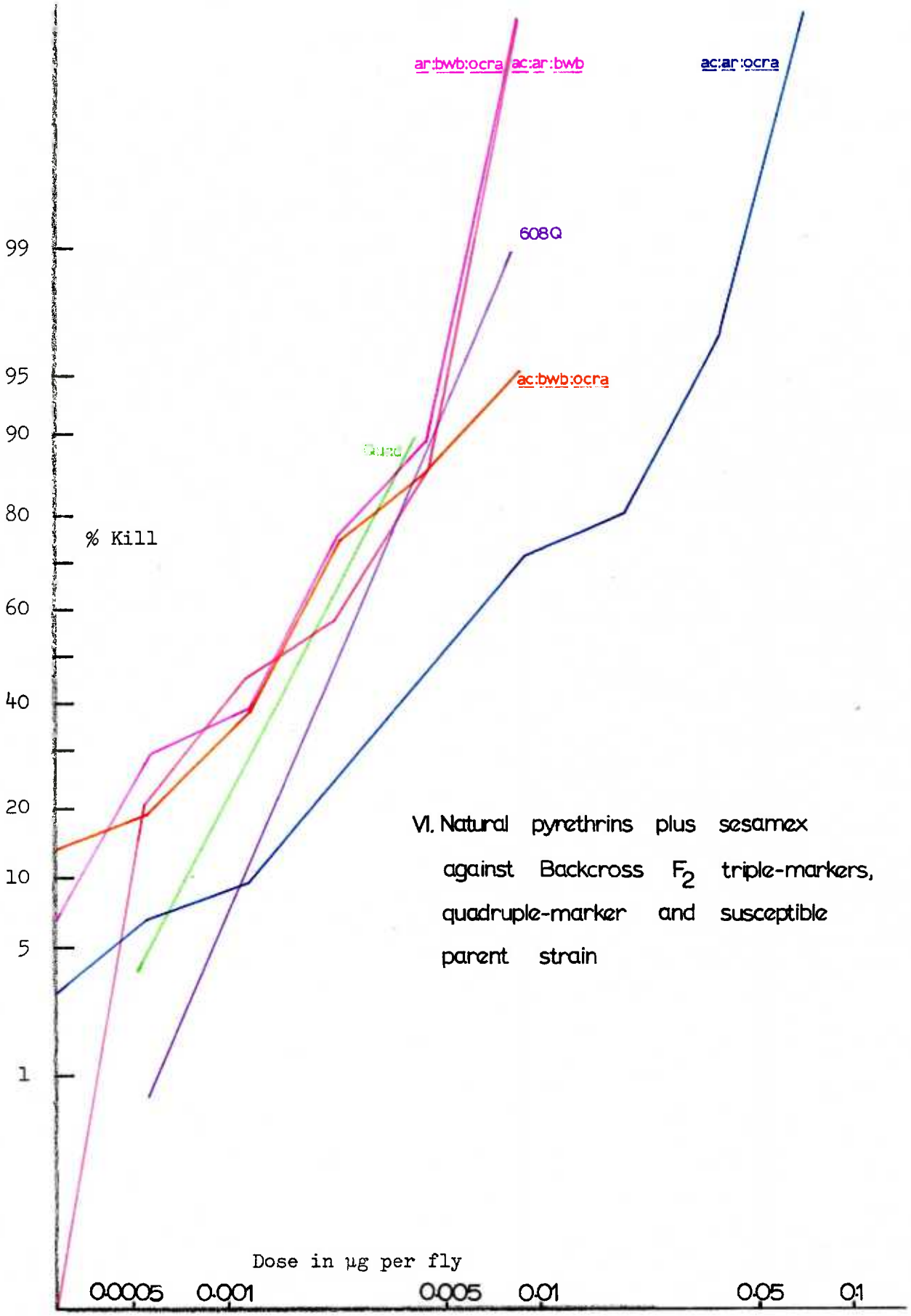
IV. Zectran plus Sesamex against parent resistant and susceptible strains and their F_1

labelled chromosomes from the susceptible 608Q parent. Chromosome IV originally unlabelled in 608Q could be derived from either parent, i.e. it was in an unknown state, whilst the other unmarked chromosome was in the heterozygous state. By inbreeding triple marker TCF_1 flies the progeny, TCF_2, consisted theoretically of individuals which were homozygous susceptible, heterozygous and homozygous resistant for each unmarked chromosome from NPR in a ratio 1:2:1 respectively. The quadruple marked homozygous susceptible flies were discarded leaving heterozygotes and homosygotes for resistance in a ratio 2:1. Therefore if the parent NPR strain was homozygous for its resistance factors a dominant factor should result in a straight ld-p line with a LD50 greater than the 608Q value, but a recessive factor should give a triple marked population at TCF_2 in which one third resists the insecticide so that there would be a plateau on the ld-p line at 67% kill. If the factor was very weak, the tolerance of the population would barely rise above the susceptible level and a change in LD100 would indicate such weakness.

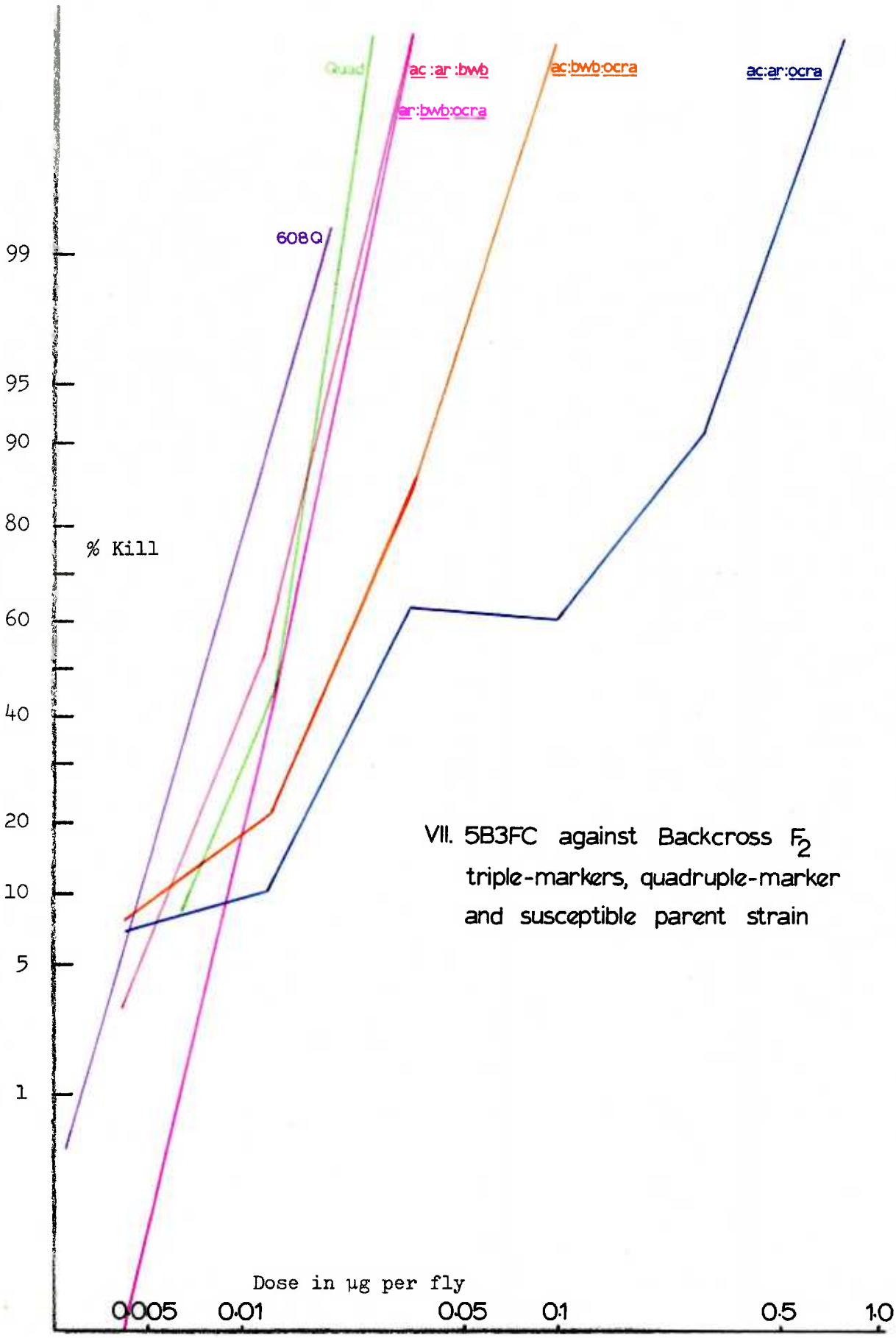
Not all the listed insecticides were tested against the four triple marked phenotypes of the TCF_2 because only limited information was expected from the results and because many insects were needed to cover the full kill range. The results are given as ld-p lines, which have been joined between points instead of constraining the points to linearity. For clarity symbols for the points have been omitted. Each phenotype was allocated a colour to make comparisons between graphs easier and the ld-p line for 608Q was included. This accounts for the difference between its ld-p line and that of the most susceptible TCF_2 phenotype. Also the TCF_2 flies were tested once only, whereas the 608Q line is a mean of a number of tests.



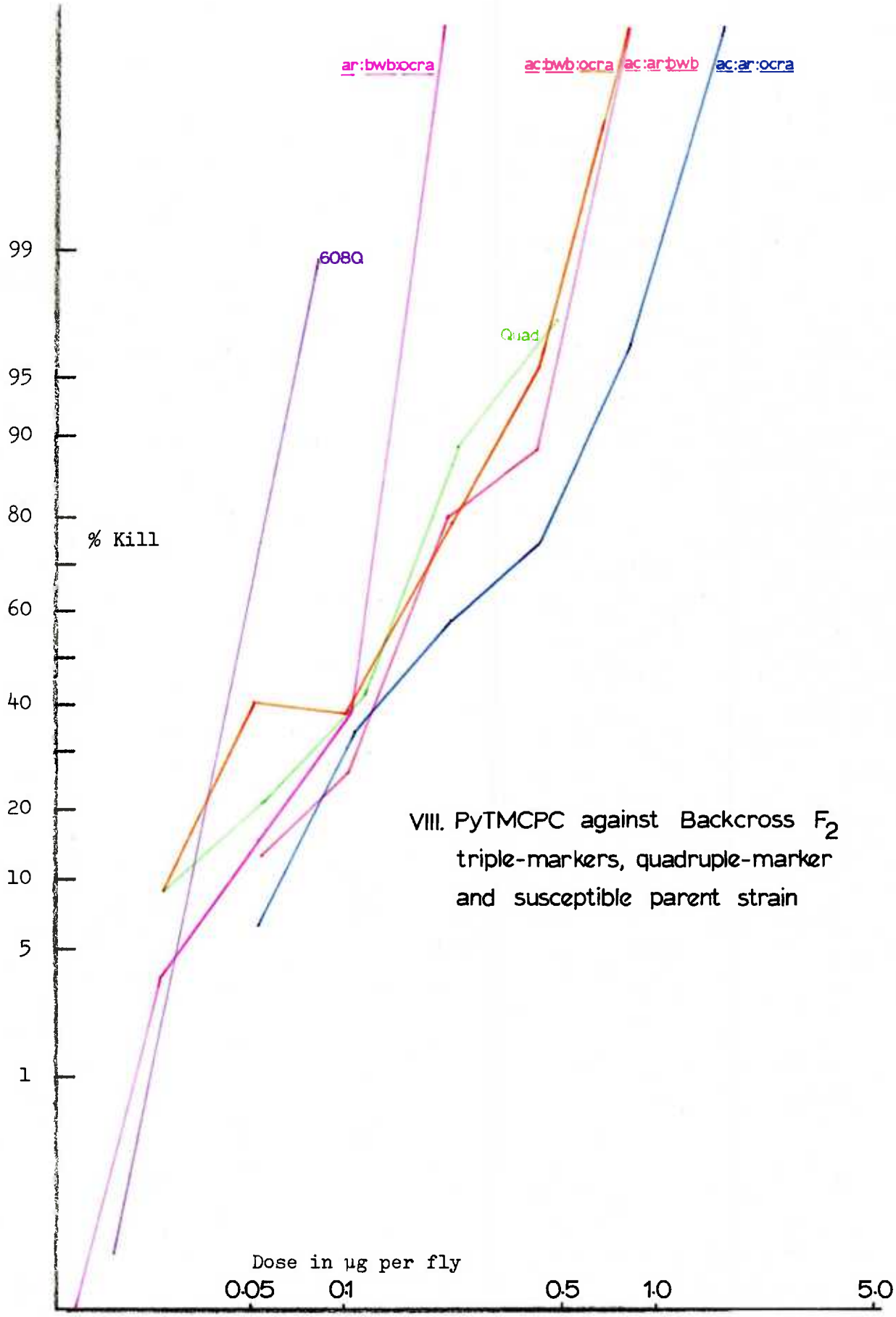
V. Natural pyrethrins against Backcross F_2 triple-markers, quadruple-marker and susceptible parent strain



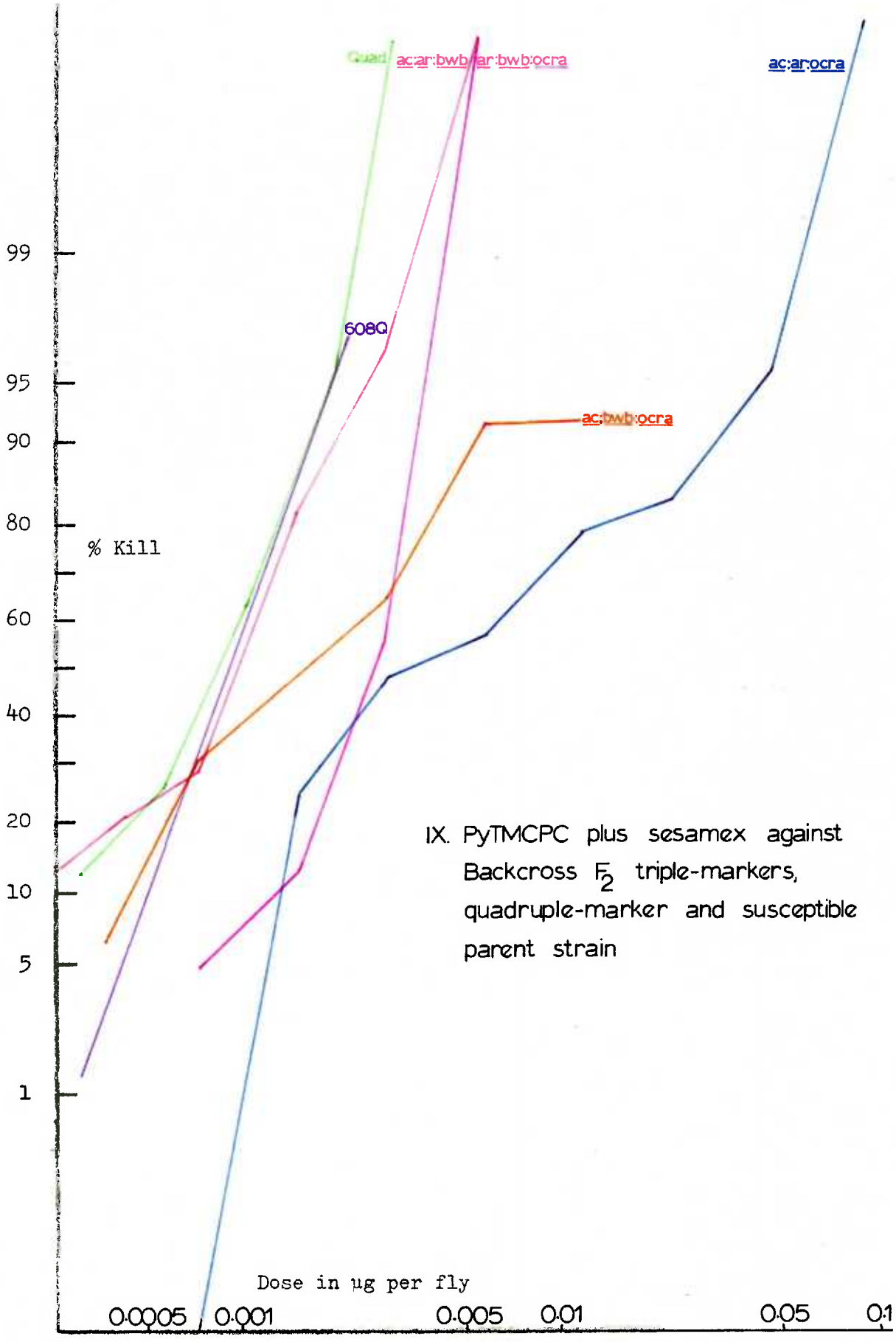
VI. Natural pyrethrins plus sesamex against Backcross F_2 triple-markers, quadruple-marker and susceptible parent strain



VII. 5B3FC against Backcross F_2 triple-markers, quadruple-marker and susceptible parent strain



VIII. PyTMCPC against Backcross F_2 triple-markers, quadruple-marker and susceptible parent strain



IX. PyTMCPC plus sesamex against Backcross F_2 triple-markers, quadruple-marker and susceptible parent strain

a. Pyrethroids

Bioassay of the TCF_2 triple marker flies with natural pyrethrins (Graph V on page 69) showed that only those with NPR's chromosome III (i.e. ac;ar;ocra phenotype) resulted in the ld-p line having a plateau. The most susceptible phenotype was ar;bwb;ocra (NPR's chromosome I). The ld-p lines for the other two phenotypes (NPR's chromosomes II and V) deviated from that for ar;bwb;ocra, showing a slight increase in LD100.

Sesamex synergised natural pyrethrins against all the phenotypes but altered the overall response (Graph VI on page 70). The phenotypes with NPR's chromosomes I and V (i.e. ar;bwb;ocra and ac;ar;bwb) were most susceptible. There was some indication of resistance on chromosome II (ac;bwb;ocra) whilst the ac;ar;ocra phenotype (NPR's chromosome III) was again the only one to give a plateau on the ld-p line.

Treatment with 5B3FC (graph VII on page 71) resulted in a similar pattern in the relative positions of ld-p lines to that obtained with synergised natural pyrethrins. Again, the ac;ar;ocra (NPR's chromosome III) phenotype included the most resistant insects and the ac;ar;bwb (V) and the ar;bwb;ocra (I) phenotypes were equally susceptible. Some ac;bwb;ocra (II) differed in tolerance at LD100 from the two susceptible phenotypes.

The other pyrethroid tested against TCF_2 flies was py TMCPC. The pattern of ld-p lines (graph VIII on page 72) was similar to that obtained with natural pyrethrins alone, not to that with 5B3FC. The most susceptible phenotype was again ar;bwb;ocra (NPR's chromosome I). The other phenotypes included individuals that resisted py TMCPC with the most resistant in the ac;ar;ocra flies (NPR's chromosome III).

Sesamex synergised py TMCPC against all phenotypes and again affected the relationship between the resulting ld-p lines (graph IX on page 73) in a similar way to those for synergised natural pyrethrins. Two phenotypes ac;ar;bwb and ar;bwb;ocra (NPR's chromosomes V and I) were susceptible whilst the other two included flies that resisted the treatment, with ac;ar;ocra (NPR's III) possibly having the most resistant flies although owing to lack of numbers, LD100 was not obtained with the ac;bwb;ocra phenotype (NPR's II).

b. Other compounds

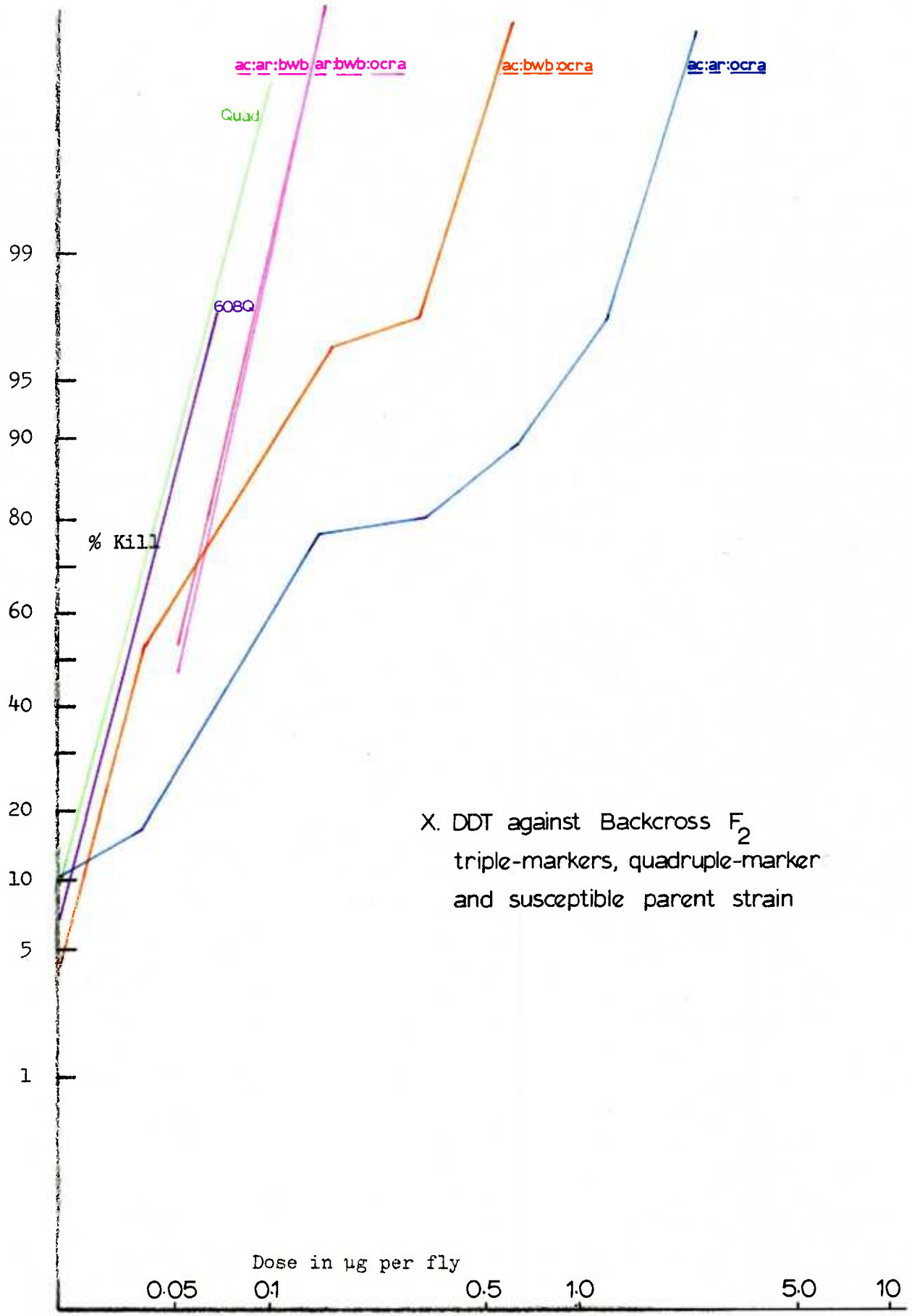
Four compounds other than pyrethroids were tested against TCF_2 flies.

With DDT (graph X on page 76) the most resistant flies had material from NPR's chromosome III (ac;ar;ocra phenotype), although some of the ac;bwb;ocra phenotype (NPR's II) were also resistant. The other two phenotypes (NPR's I and V) were susceptible.

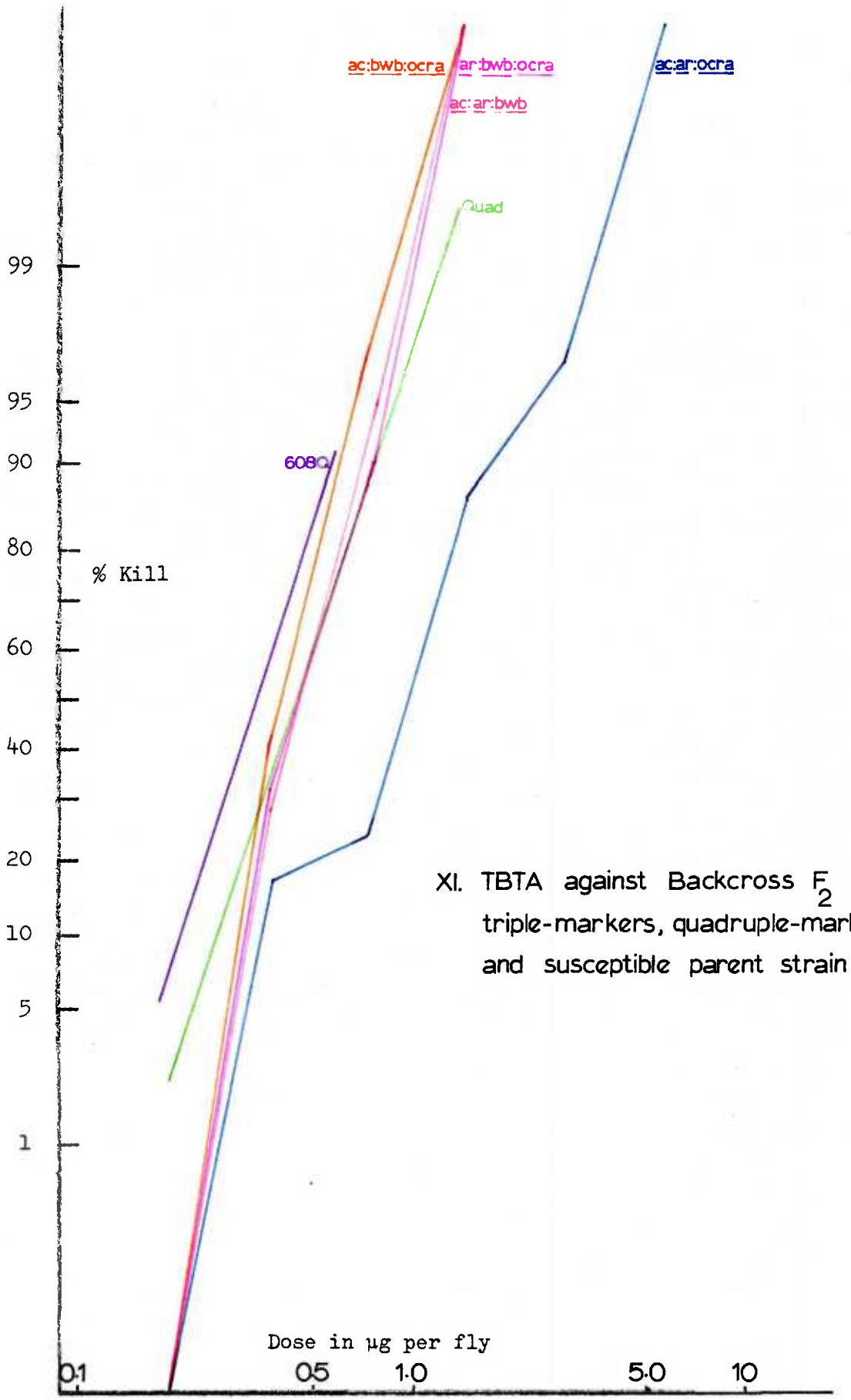
Only flies with NPR's chromosome III (ac;ar;ocra) resisted TBTA (graph XI on page 77). All four phenotypes had some individuals that resisted dieldrin (graph XII on page 78). Zectran resistance was located (graph XIII on page 79) on NPR's chromosome II (ac;bwb;ocra) and III (ac;ar;ocra) with the former phenotype including individuals that were immune to 50 µg.

c. Summary

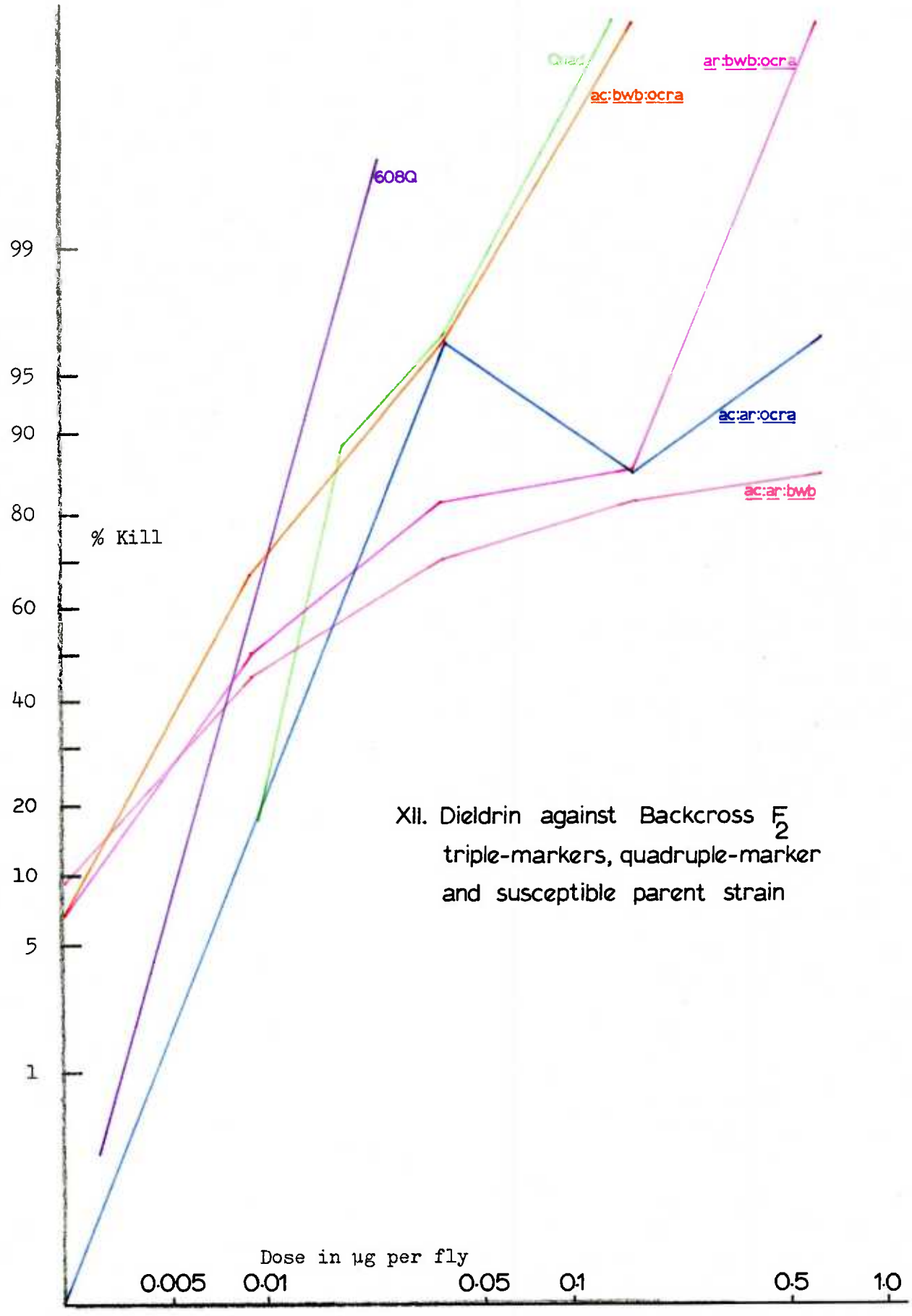
The results of bioassay of TCF_2 flies may be summarised, and Table XVI (p 80) shows with which of NPR's chromosomes resistance is associated.



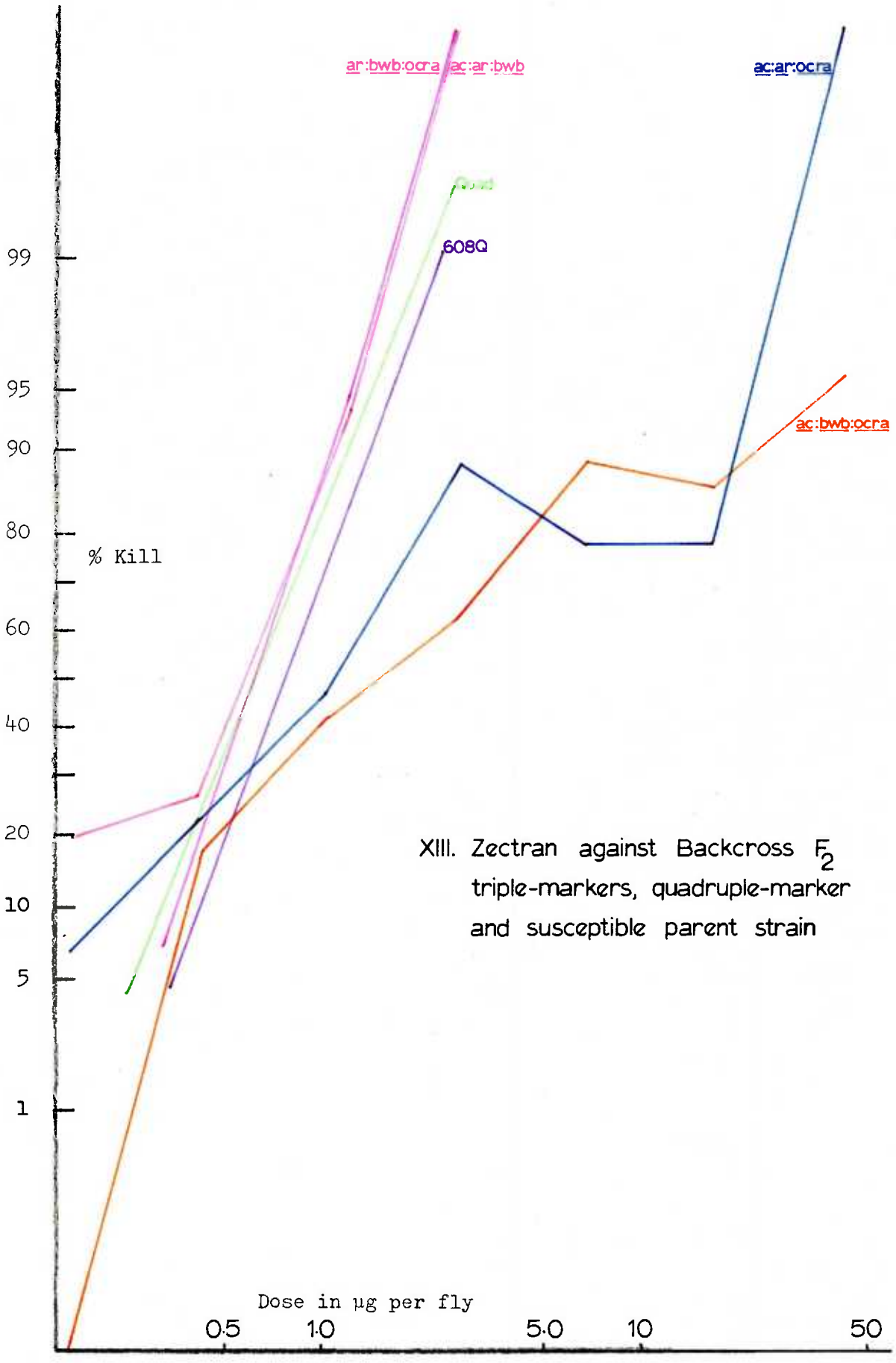
X. DDT against Backcross F_2 triple-markers, quadruple-marker and susceptible parent strain



XI. TBTA against Backcross F_2 triple-markers, quadruple-marker and susceptible parent strain



XII. Dieldrin against Backcross F_2 triple-markers, quadruple-marker and susceptible parent strain



XIII. Zectran against Backcross F_2 triple-markers, quadruple-marker and susceptible parent strain

Table XVI showing which of NPR's chromosomes bore resistance, based on results of bioassay of triple and quadruple marker flies from the TCF_2.

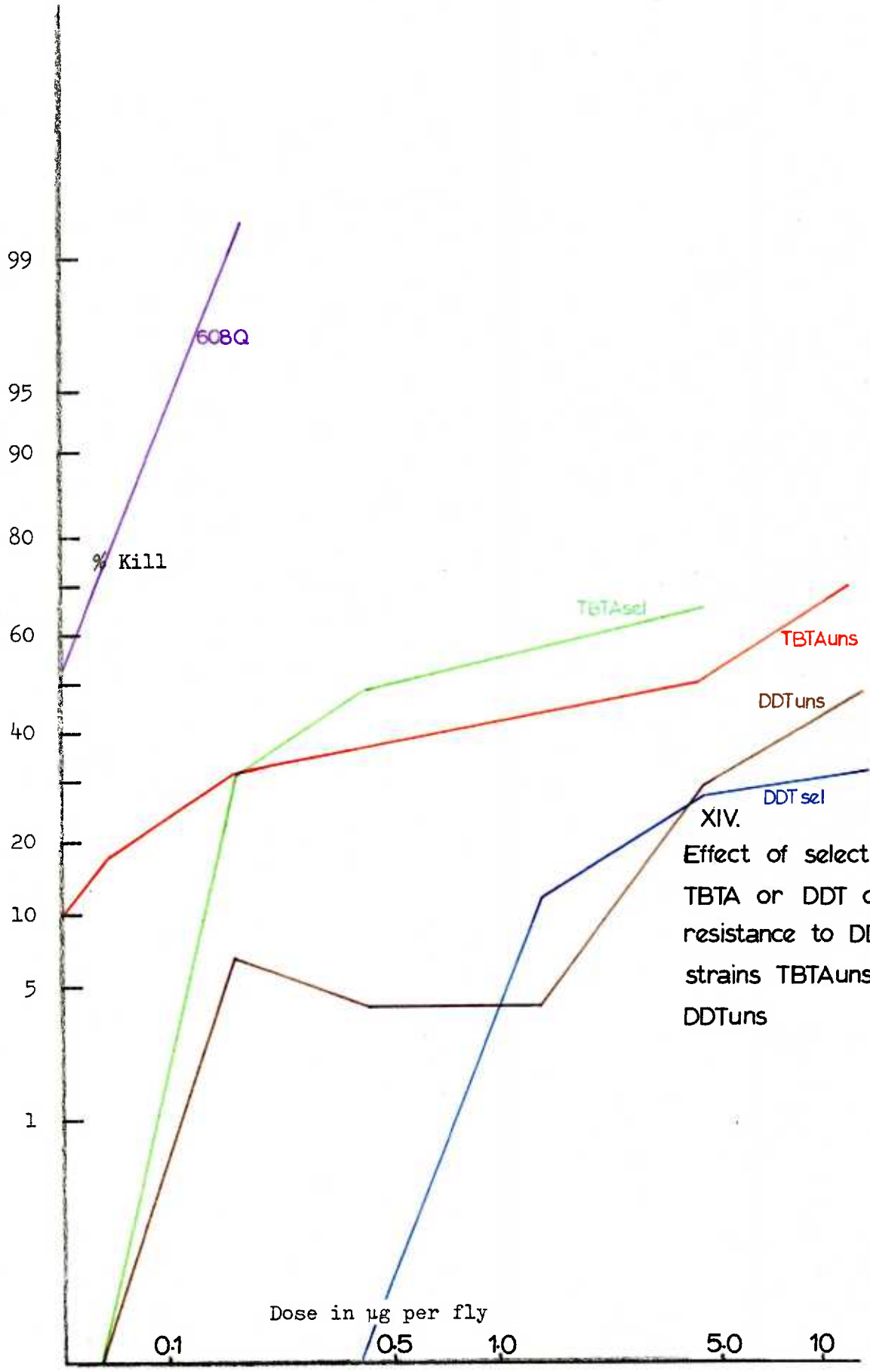
	Chromosome from NPR				
	I	II	III	IV	V
natural pyrethrins		+	+		+
natural pyrethrins + sesamex		+	+		
py TMCPC		+	+		+
py TMCPC + sesamex		+	+		
5B3FC		+	+		
DDT		+	+		
TBTA			+		
dieldrin				?	
Zectran		+	+		

4. Changes in response to insecticides during the isolation of the factors on chromosome III

During the isolation of chromosome III from NPR and the separation of its two resistance mechanisms, the strains (see genetical section, page 46 et seq.) were bioassayed before and after selecting with DDT and TBTA to demonstrate the effect of selection on the cross-tolerance to natural pyrethrins, 5B3FC and to the selecting agents. The four strains used were called TBTA uns (selected once with TBTA), TBTA sel (selected twice with TBTA), DDT uns (selected once with DDT) and DDT sel (selected twice with DDT).

a. Response to DDT, 24 hours after treatment

The response of TBTA uns to DDT was heterogeneous (graph XIV page 81). About 30% of the population was fully susceptible.



XIV.
Effect of selection with TBTA or DDT on resistance to DDT in strains TBTAuns and DDTuns

Kill increased gradually with increased dose, but 25% survived 5 µg. The second selection with TBTA, resulting in strain TBTA sel, almost fully eliminated the susceptible part of the population, but it remained heterogeneous, with almost 40% surviving 15 µg DDT.

Less than 5% of the DDT uns flies were susceptible or slightly resistant to DDT. The remainder of the population was very resistant. Selection with DDT (to produce strain DDT sel) eliminated the less resistant individuals and over 70% survived 15 µg DDT.

b. Response to TBTA

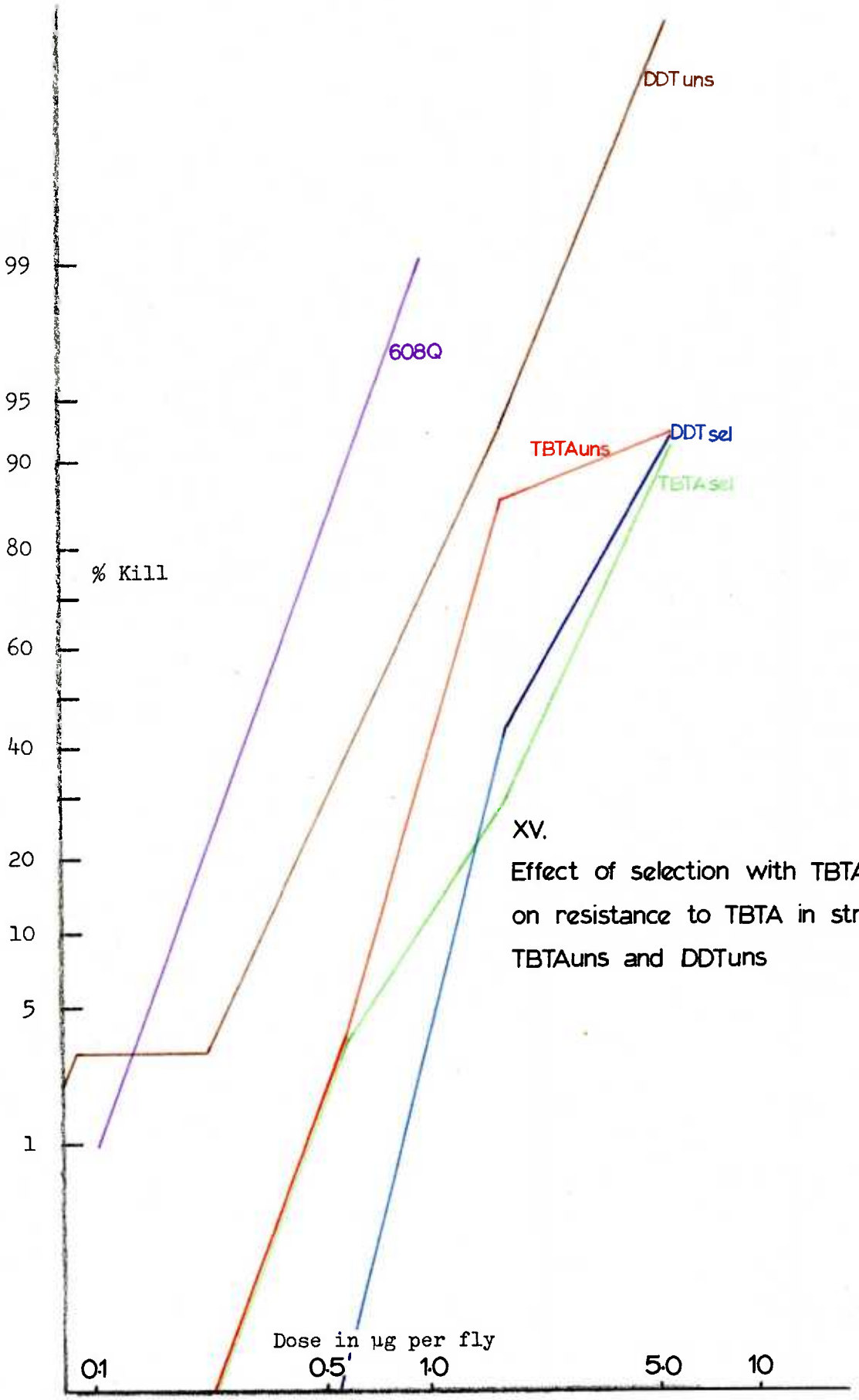
When strain TBTA uns, heterogenous for resistance to TBTA, was selected with TBTA, the ld-p line of its progeny (TBTA sel) (graph XV on page 83) was steeper and straighter, and lay close to that for NPR, indicating that the selection had killed almost all the heterozygotes.

The large portion of DDT uns susceptible to TBTA was eliminated by selecting with DDT, and the strain DDT sel was as resistant to TBTA as TBTA sel and NPR.

These results imply that resistance to TBTA was controlled by a factor that may be selected for with either TBTA or DDT, but that the major DDT resistance mechanism may be selected for only with DDT.

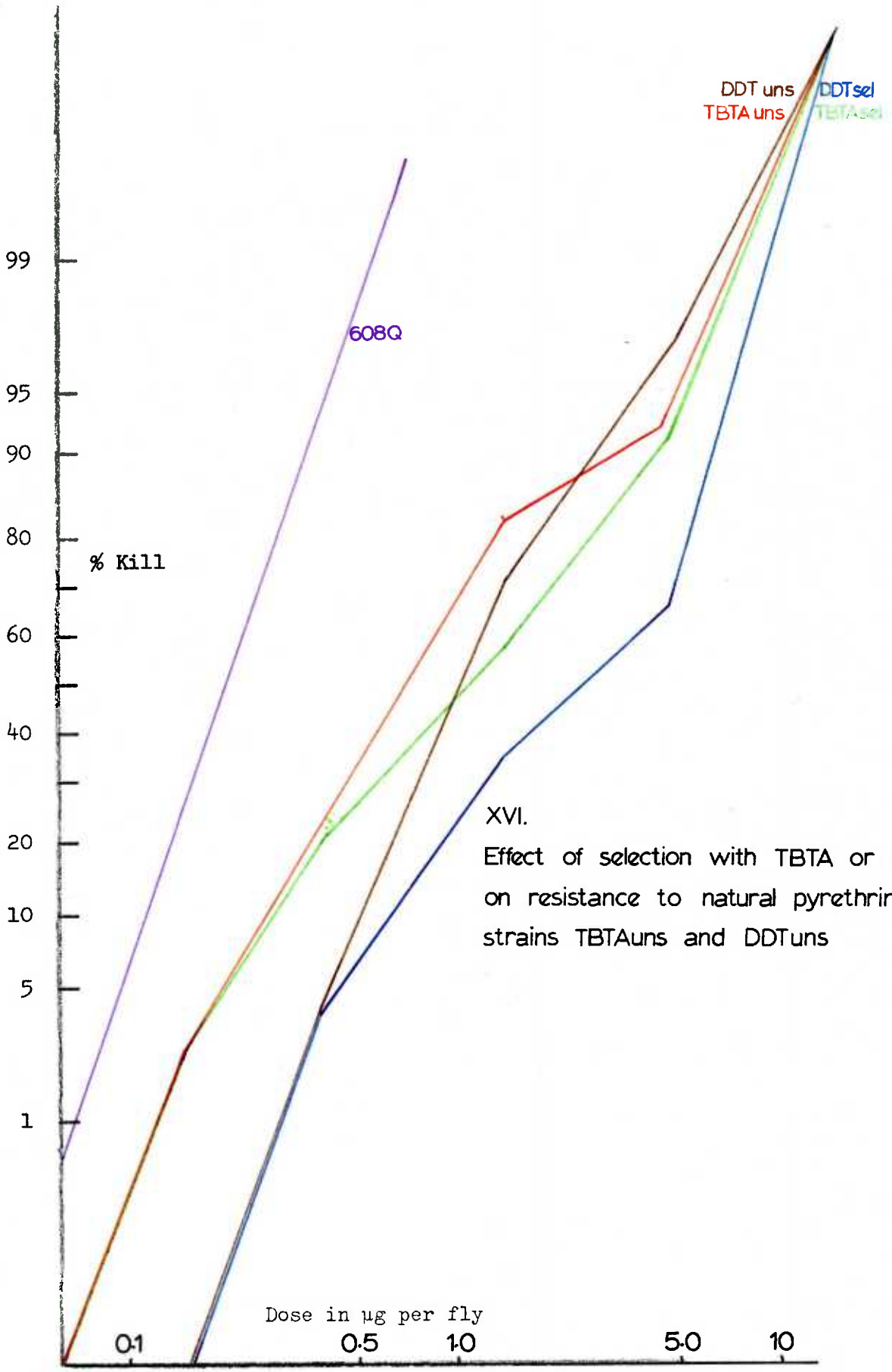
c. Response to pyrethroids

Selection with either DDT or TBTA failed to suppress the heterogeneity of response to natural pyrethrins (graph XVI on page 84) or to 5B3FC (graph XVII on page 85) in the four strains, but selection with DDT eliminated more susceptible or heterozygous insects than selection with TBTA. As a result, DDT



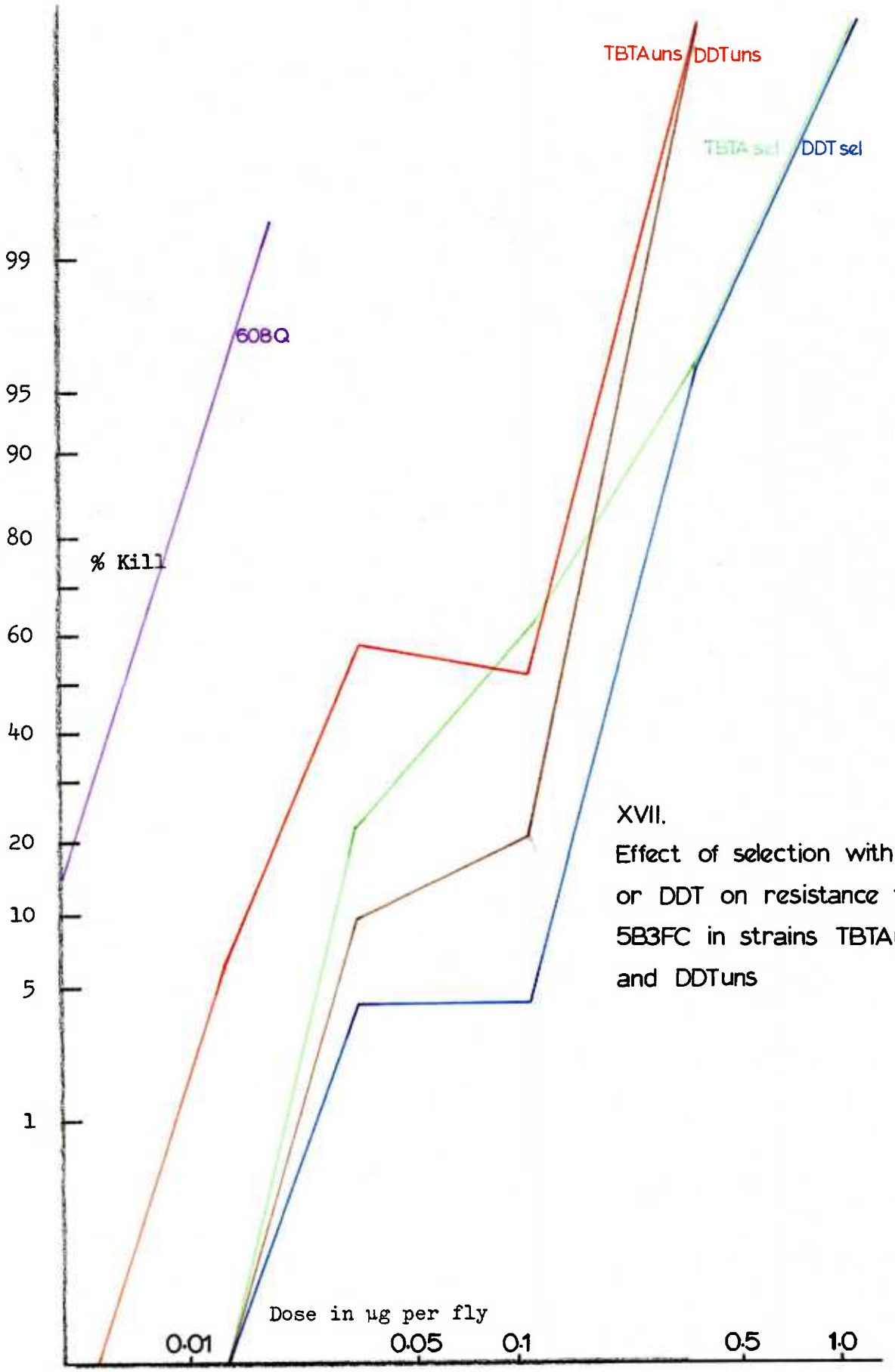
XV.

Effect of selection with TBTA or DDT on resistance to TBTA in strains TBTAuns and DDTuns



XVI.

Effect of selection with TBTA or DDT on resistance to natural pyrethrins in strains TBTAuns and DDTuns



XVII.
Effect of selection with TBTA or DDT on resistance to 5B3FC in strains TBTAuns and DDTuns

selected strains were more resistant against the two compounds.

These results confirm that resistance to pyrethroids on NPR's chromosome III is linked with resistance to DDT and to TBTA, and suggest that it is more likely to be either close to the DDT resistance factor or to be one and the same factor because, like the DDT resistance factor, it is selected for more by DDT than by TBTA.

Because both selected populations were heterogeneous in their response to all treatments, except TBTA, further genetical manipulation was necessary to isolate the two mechanisms and this is described elsewhere (page 46 et seq.).

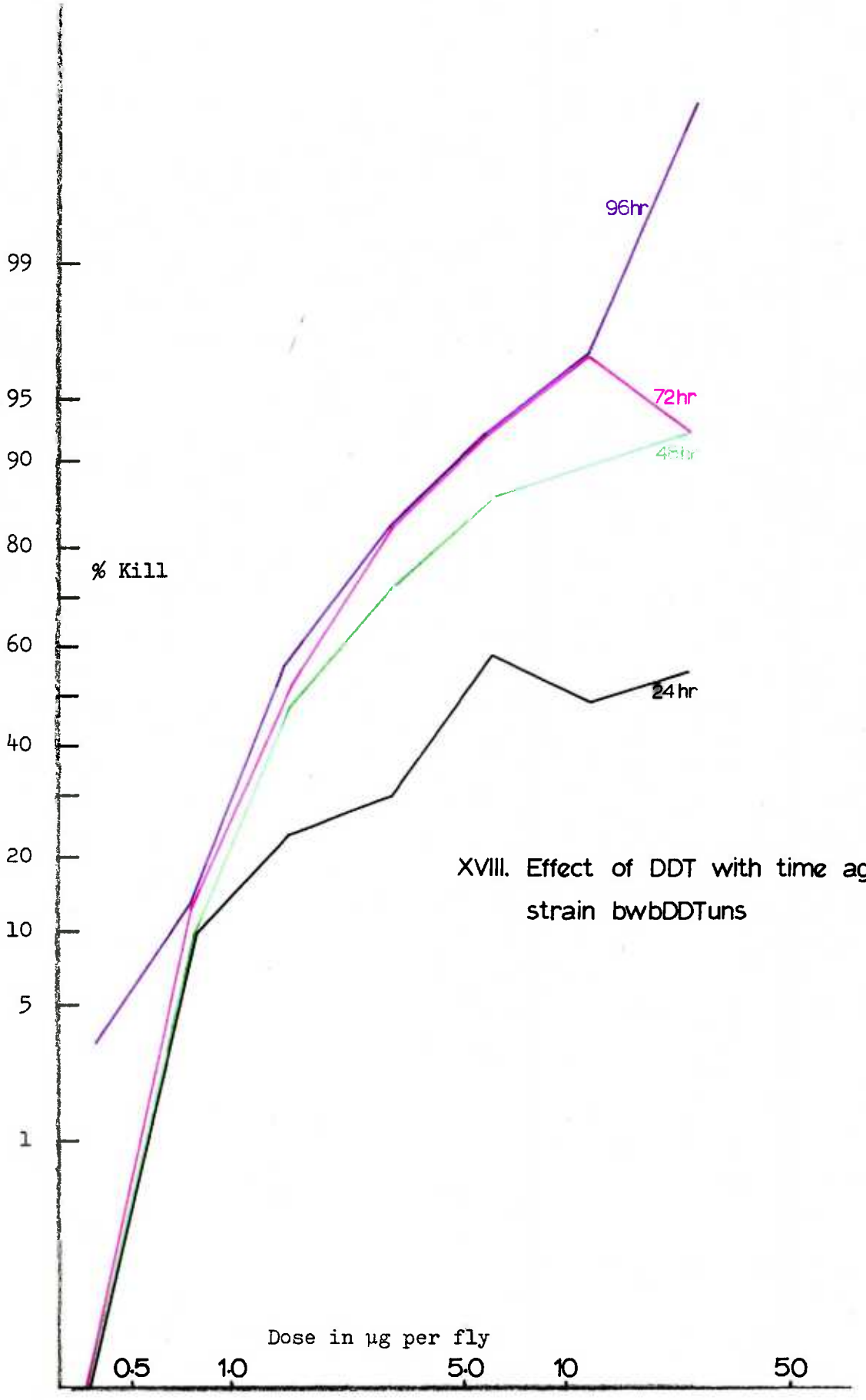
d. Resistance of bwb DDT uns to DDT

The bwb DDT uns population was tested at F_4 with DDT and kill was assessed 24 hours after treatment and at 24 hour intervals for four days after which no further kill other than control mortality was observed. Kill increased with time (graph XVIII on page 87), and this increase can be compared with later results for the isolated resistance mechanisms of chromosome II in strains 348 (graph XXIV on page 100) and 314 (graph XXV on page 102) and with the combination in strain 331 (graph XXVI on page 105).

No part of the bwb DDT uns was fully susceptible to DDT, but the strain was not homogeneous in its response to DDT. There was a great change during the first 48 hours after treatment, after which the change was much smaller as kill end-point was approached.

5. Results for isolated strains

The strains that were isolated by genetical techniques from the cross 608Q x NPR were bioassayed with all insecticides



XVIII. Effect of DDT with time against strain bwbDDTuns

to obtain data on the resistance spectrum of each factor in turn. Detailed LD50 values, factors of synergism and resistance are given in Table XX on page 117). Results for pyrethroids are also expressed graphically and/or as histograms. Detailed pyrethroid results for 6080 are also listed separately on the fold-out Table XIX on page 116 for ease of comparison with the various histograms.

a. Results for 420 (NPR's chromosome I - Histogram V on page 89)

420 was homozygous for markers ar;bwb;ocra but heterozygous for ac and hence for chromosome I from NPR. The ld-p lines were steep and straight for all insecticides, and the strain was as susceptible as, and had SFs similar to, those of 6080.

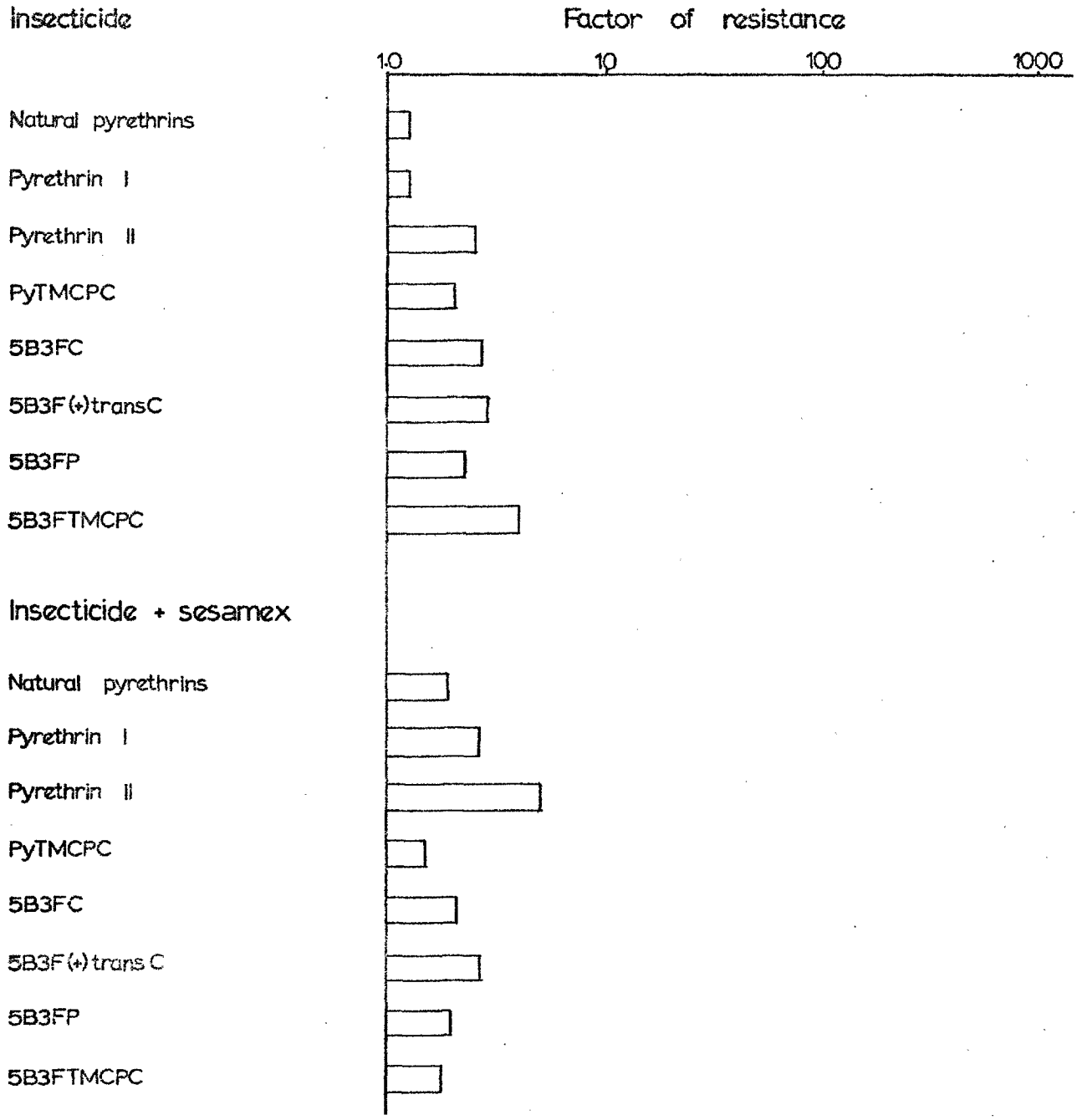
The knock-down effect of natural pyrethrins similar to 6080 was observed but not measured.

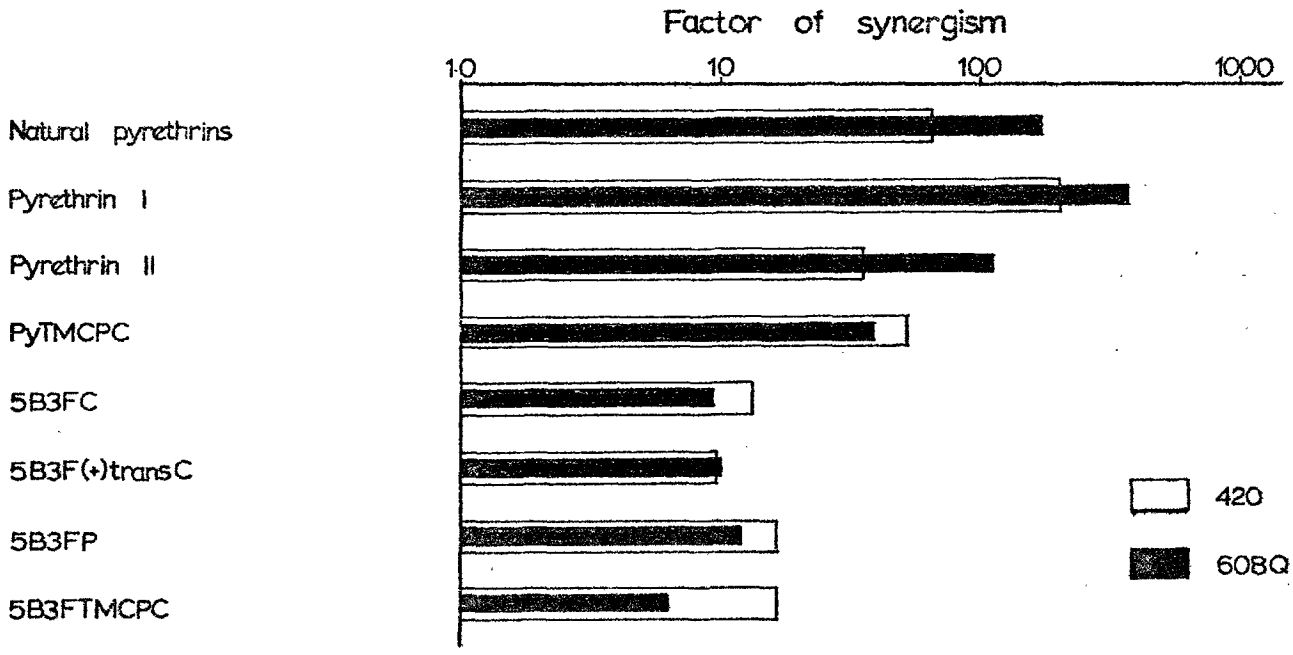
b. Results for 381 (NPR's chromosome II - Histogram VI on page 90

381 was homozygous for ac;bwb;ocra and for lack of ar indicating that the substitution of chromosome II from NPR into 6080 had been successful. However, bioassay results showed that 381 was heterogeneous in its response to synergised natural pyrethrins (graph XIX on page 91) and to synergised pyrethrolone esters, (graphs XX, XXI, XXII on pages 92, 93, 94). This was shown by the ld-p lines not being straight. Even when they were constrained to linearity as they were for estimating RFs and SFs for the histograms (see page 90) resistance to these synergised pyrethroids was much more than when unsynergised. For example, the RF for py II was 3.4 and for synergised py II was 14. The knock-down effect of natural pyrethrins was observed but not measured.

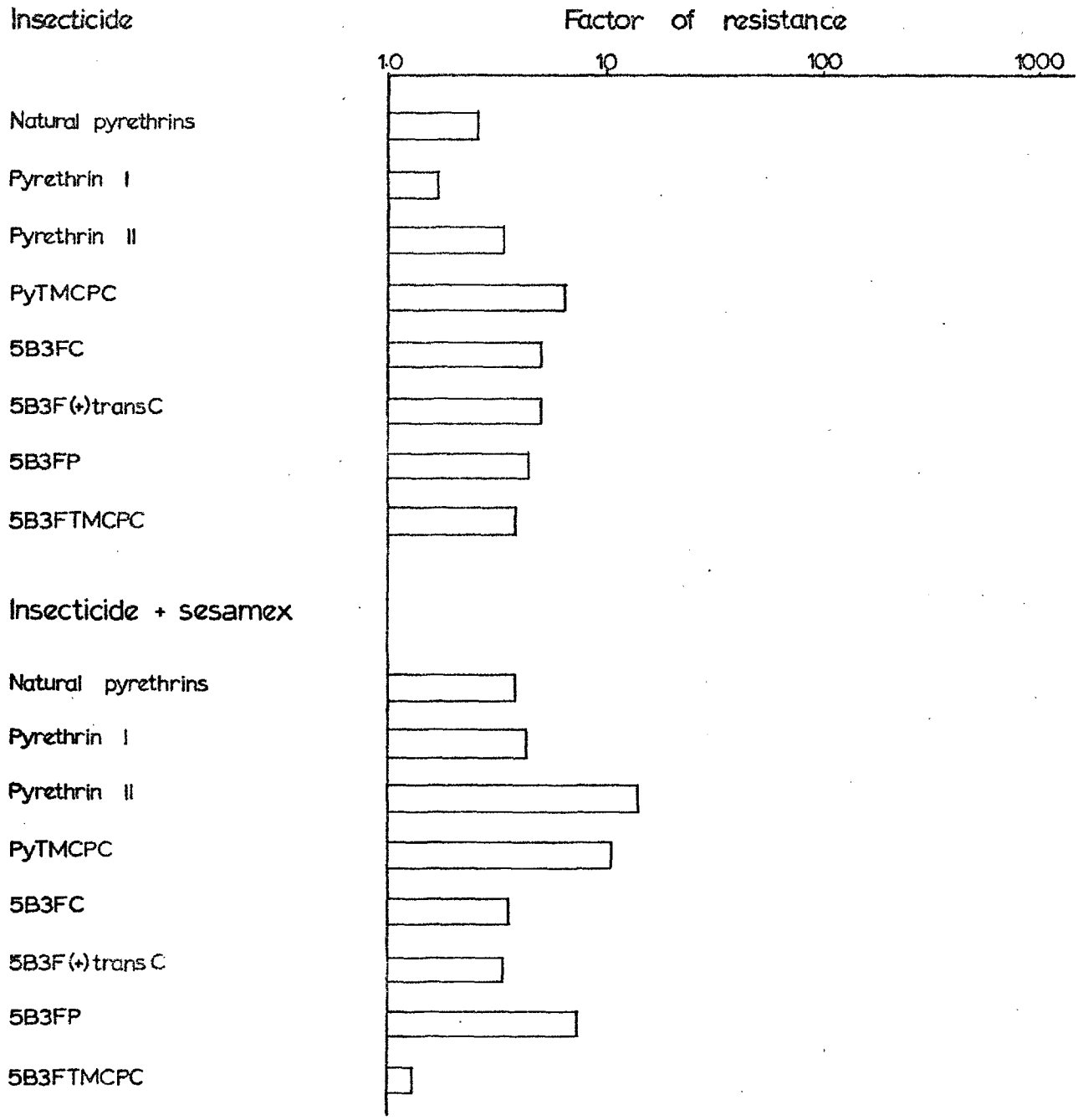
381 was susceptible to TBTA and dieldrin, was homogeneous in its response to DDT which it resisted (RF 2.0) but was hetero-

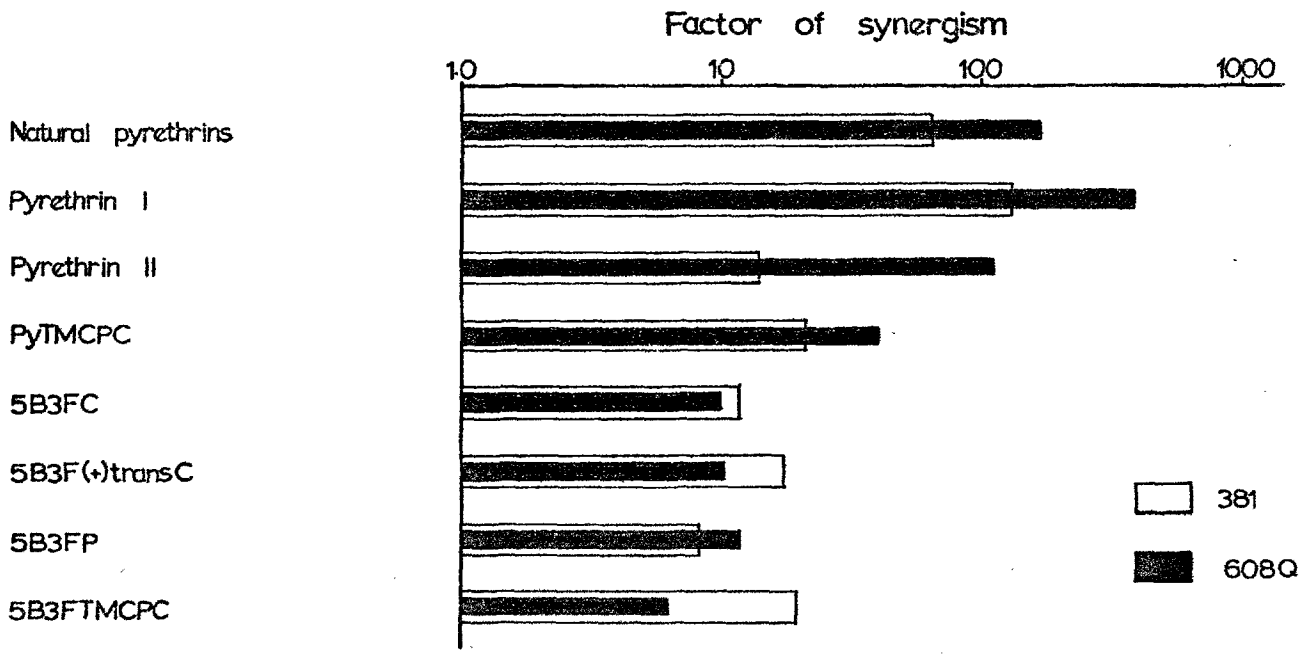
V. Histograms for Strain 420

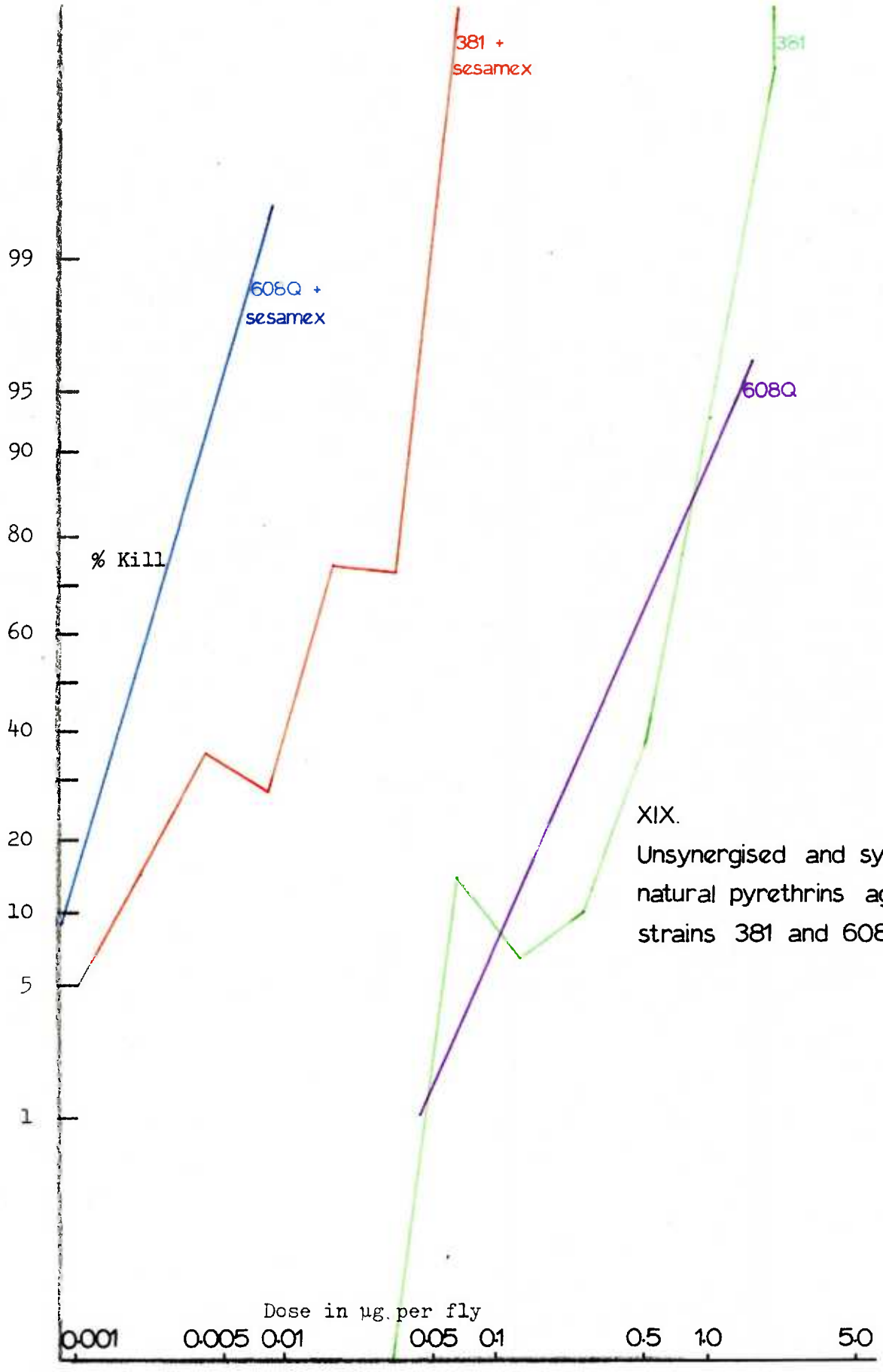




VI. Histograms for Strain 381

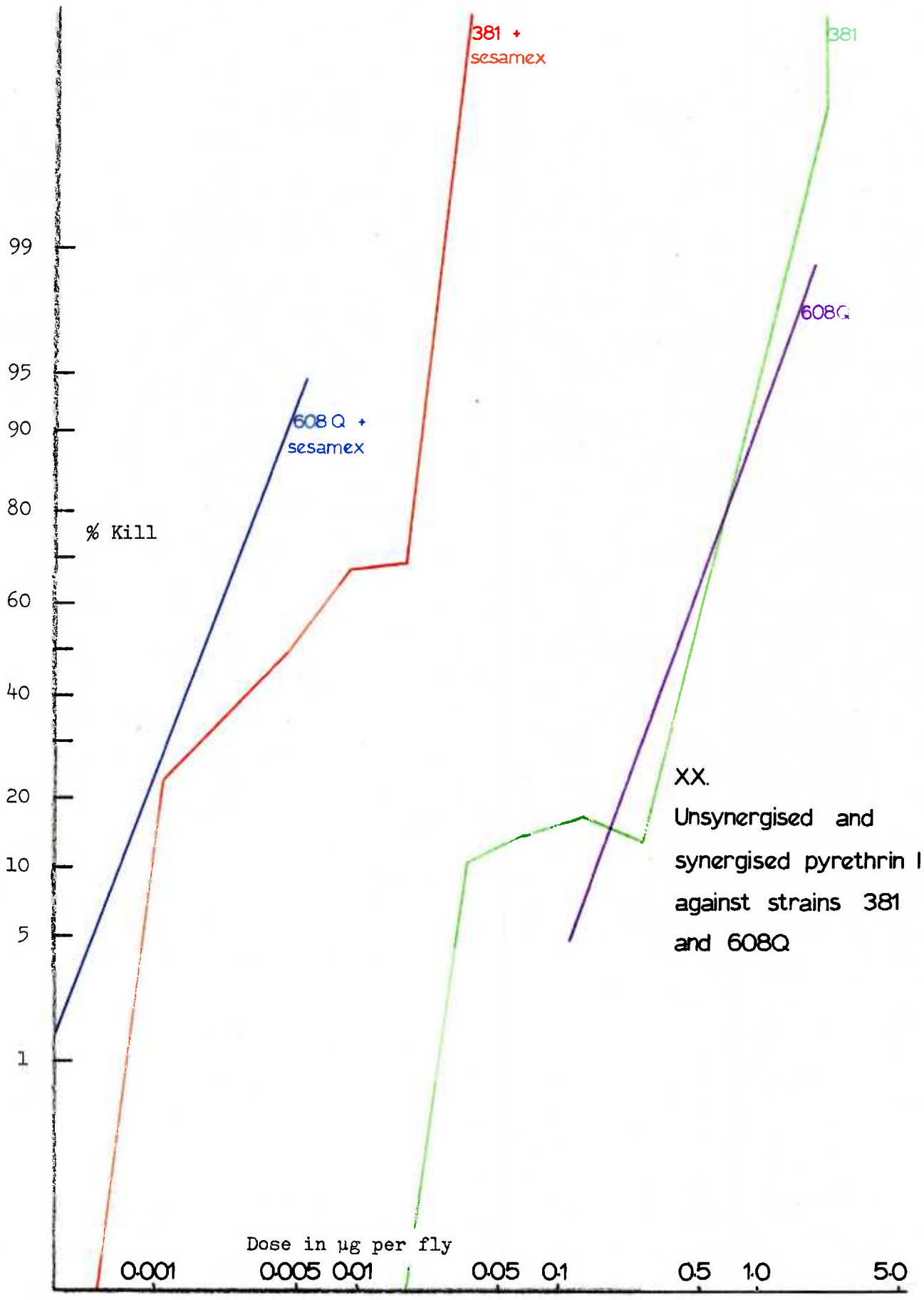




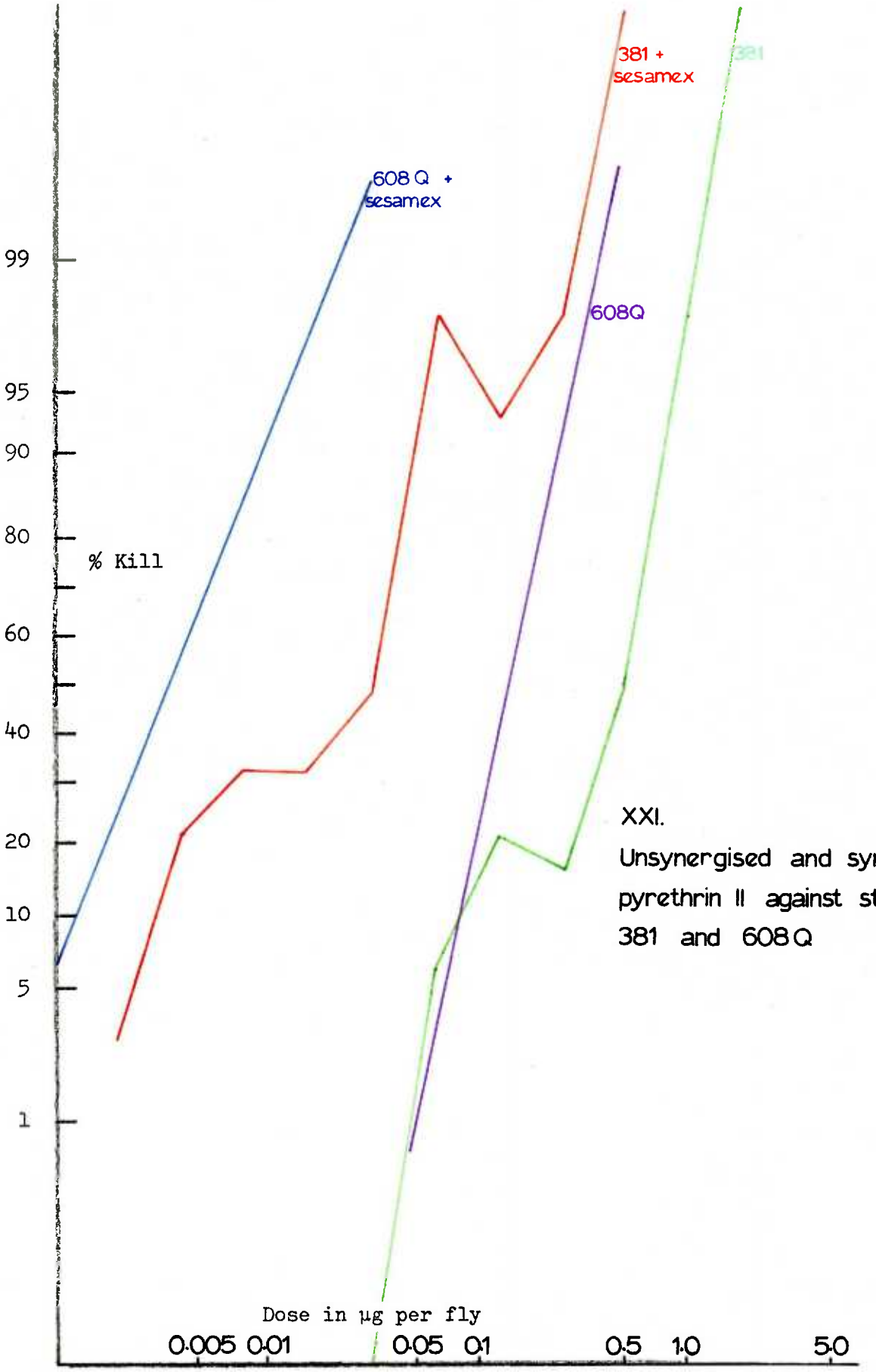


XIX.

Unsynergised and synergised natural pyrethrins against strains 381 and 608Q

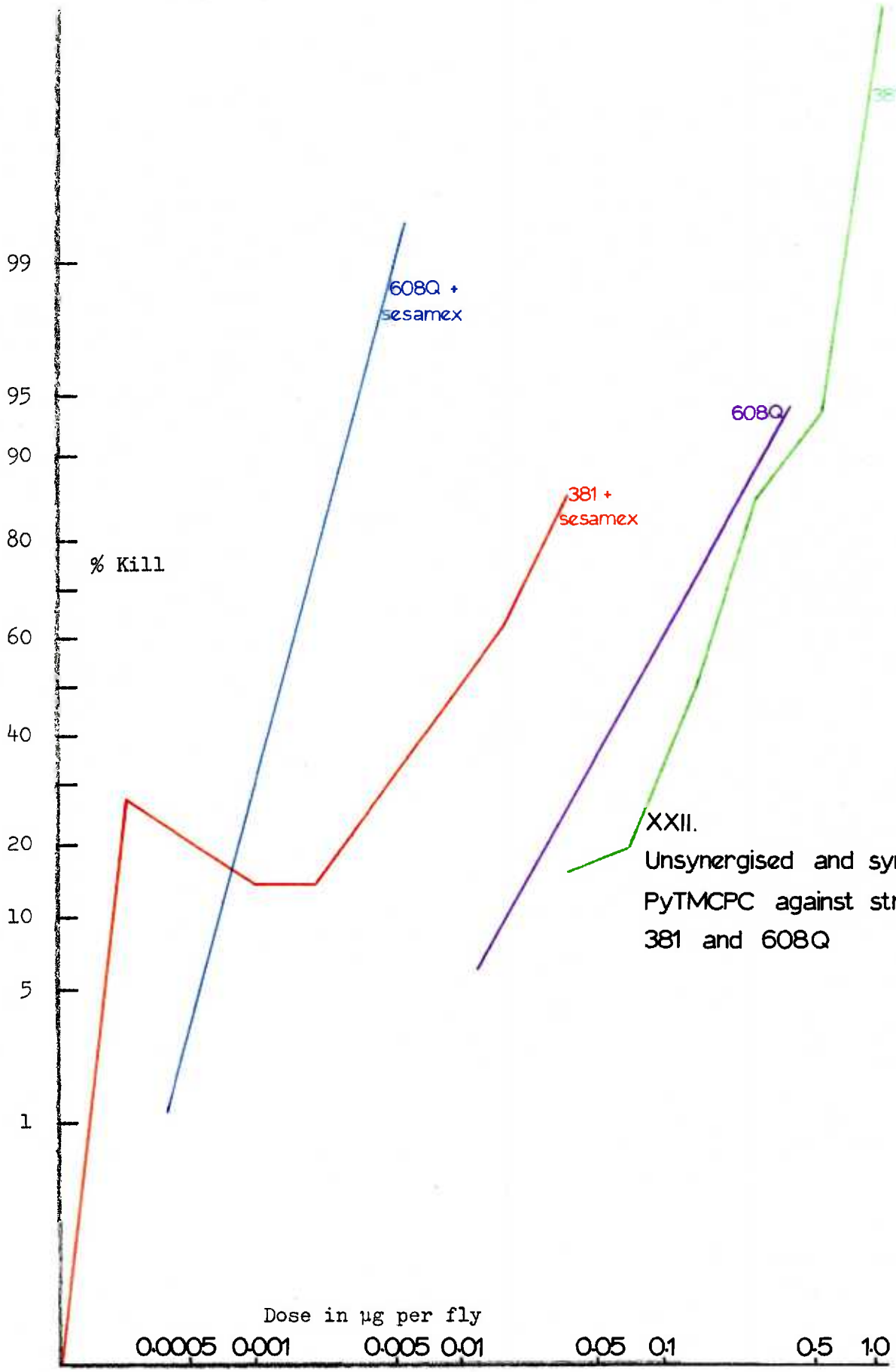


XX.
Unsynergised and synergised pyrethrin I against strains 381 and 608Q



XXI.

Unsynergised and synergised pyrethrin II against strains 381 and 608 Q



XXII.
Unsynergised and synergised PyTMCPC against strains 381 and 608Q

geneous to Zectran, with 20% of the population resistant (graph XXIII on page 96). Sesamex synergised Zectran, but again the response was heterogeneous, and the population still resisted the insecticide.

c. Results for 200 (NPR's chromosome III - Histogram VII on p 97)

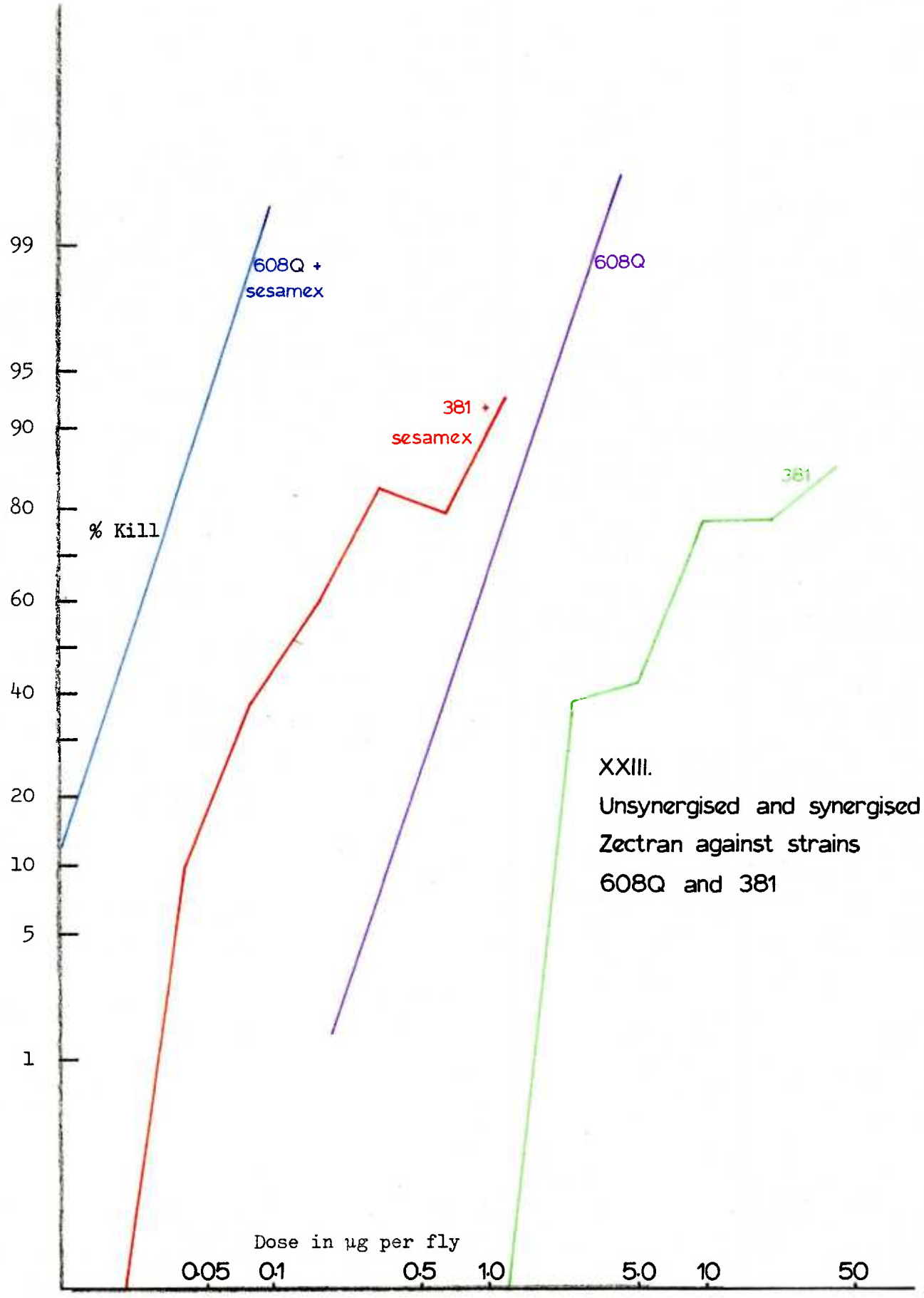
200 was homozygous for ac;ar;ocra and for lack of bwb indicating the successful substitution from NPR of chromosome III, but failed to show the resistance to pyrethroids or DDT which had been demonstrated in tests on TCF_2 ac;ar;ocra flies. RFs of less than two for all pyrethroids without or with the synergist indicated that the strain was susceptible. SFs were as for 608Q. The knock-down effect of natural pyrethrins was slower than for 608Q.

The ld-p lines were steep and straight for all other compounds except Zectran. Although the majority of the flies were susceptible to Zectran, 20% survived 40 µg/♀. Pretreatment with sesamex eliminated resistance completely and the ld-p line of 200 was then very close to that for 608Q (RF 1.2).

200 resisted TBTA (RF 6) strongly, as did NPR (RF 17) but was susceptible to dieldrin.

d. Results for 348 (NPR's chromosome III - Histogram VIII, p 98)

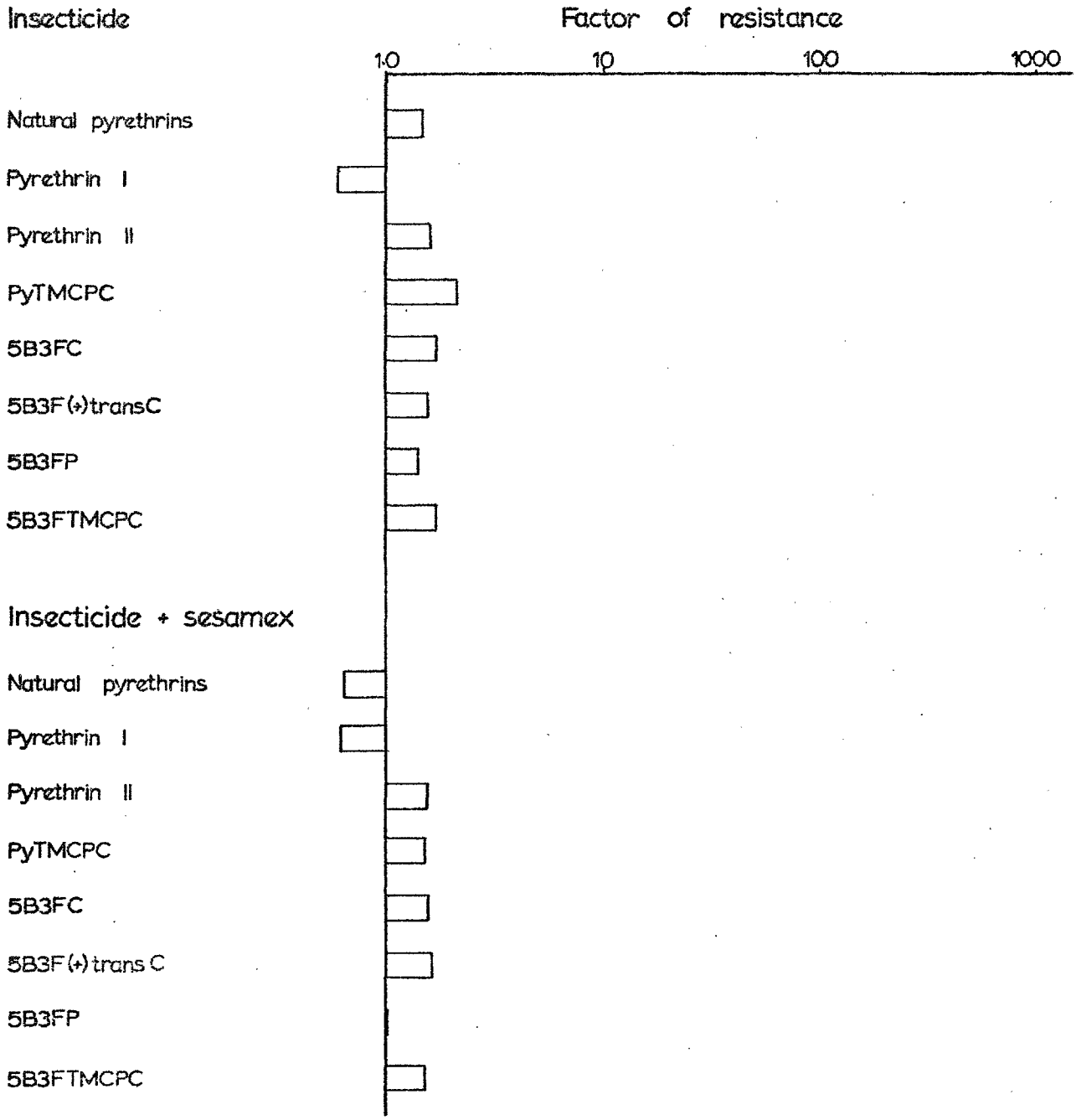
348 was homozygous for ac;ar;bwb;ocra, but in the genetical isolation of this strain (page 46), some chromosomal material from NPR's chromosome III was linked to the bwb marker by crossing over. The strain was homogeneous in its response to all insecticides at kill end-point. It resisted pyrethroids without or with sesamex, but was more resistant to synthetic esters (including py TMCPC) than to py I, py II or natural pyrethrins. For example the RF for py I was 1.9 and for bioresmethrin was 17. Although

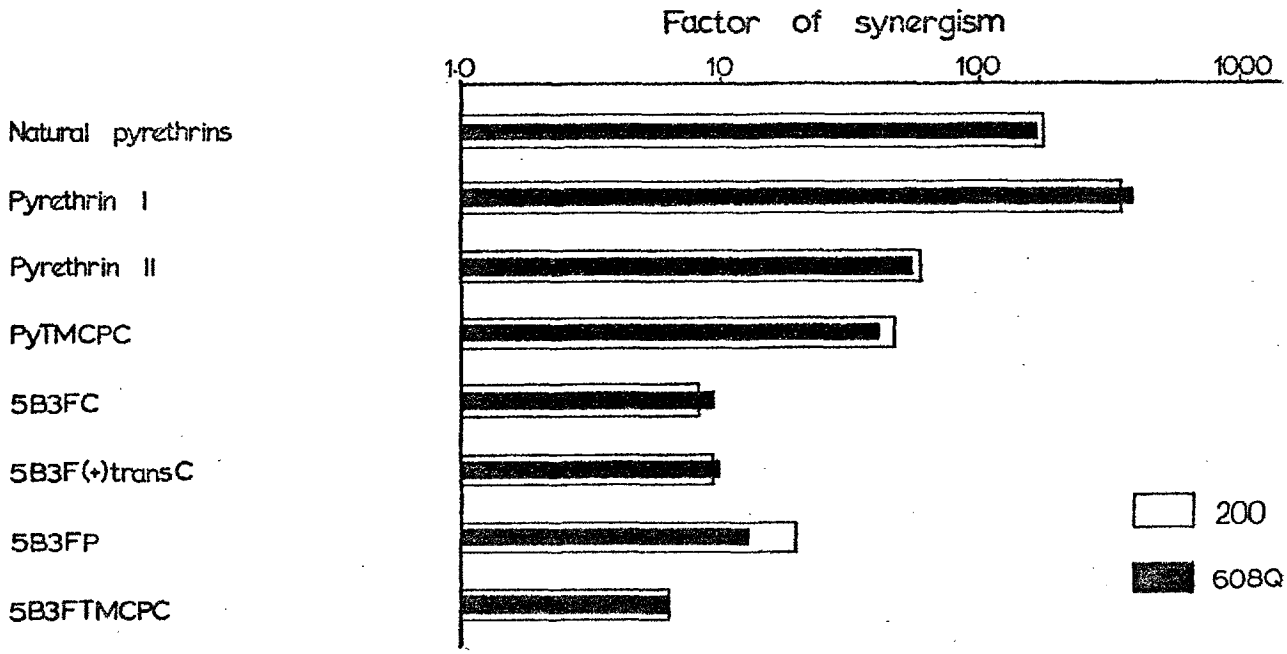


XXIII.

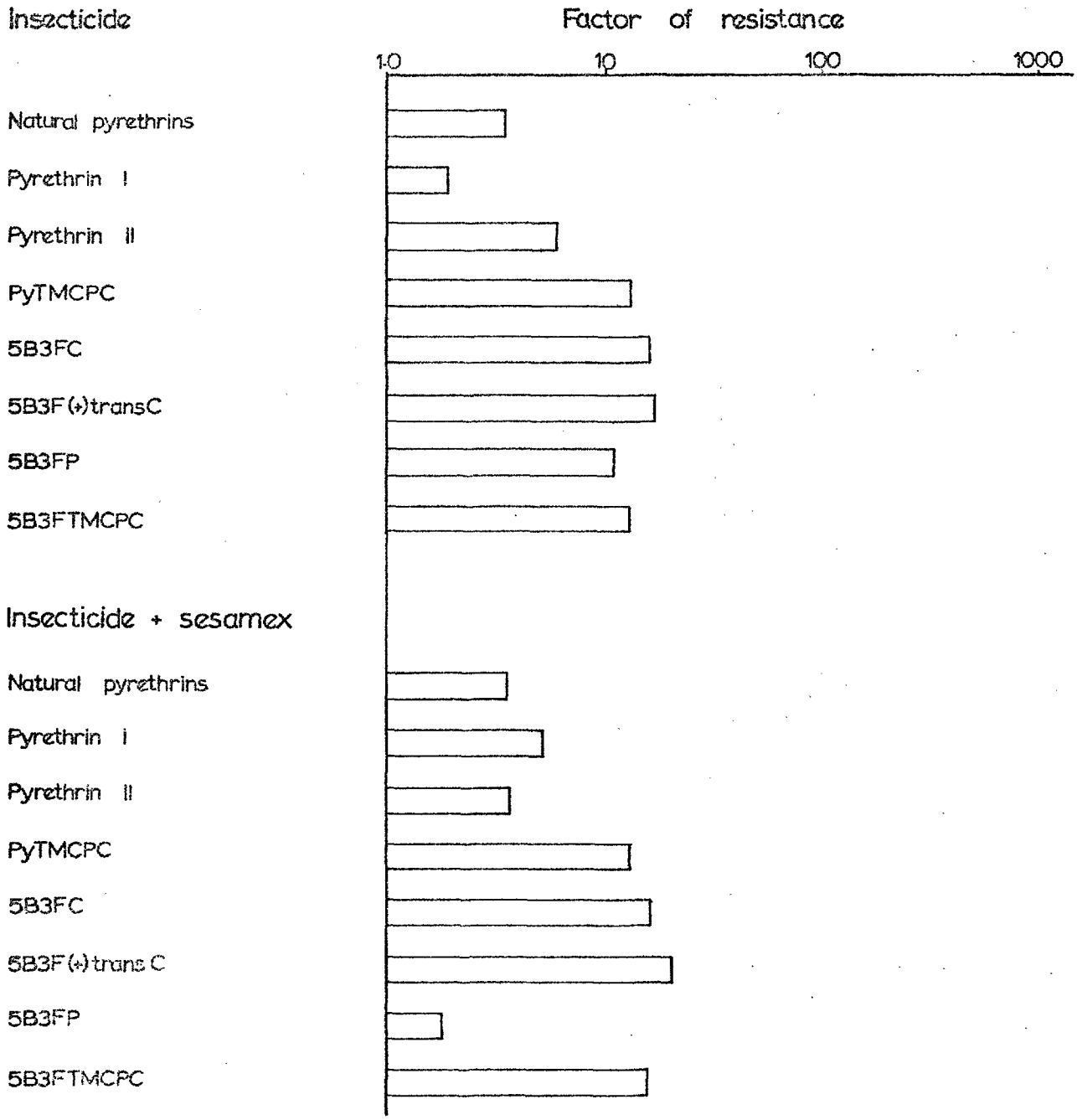
Unsynergised and synergised Zectran against strains 608Q and 381

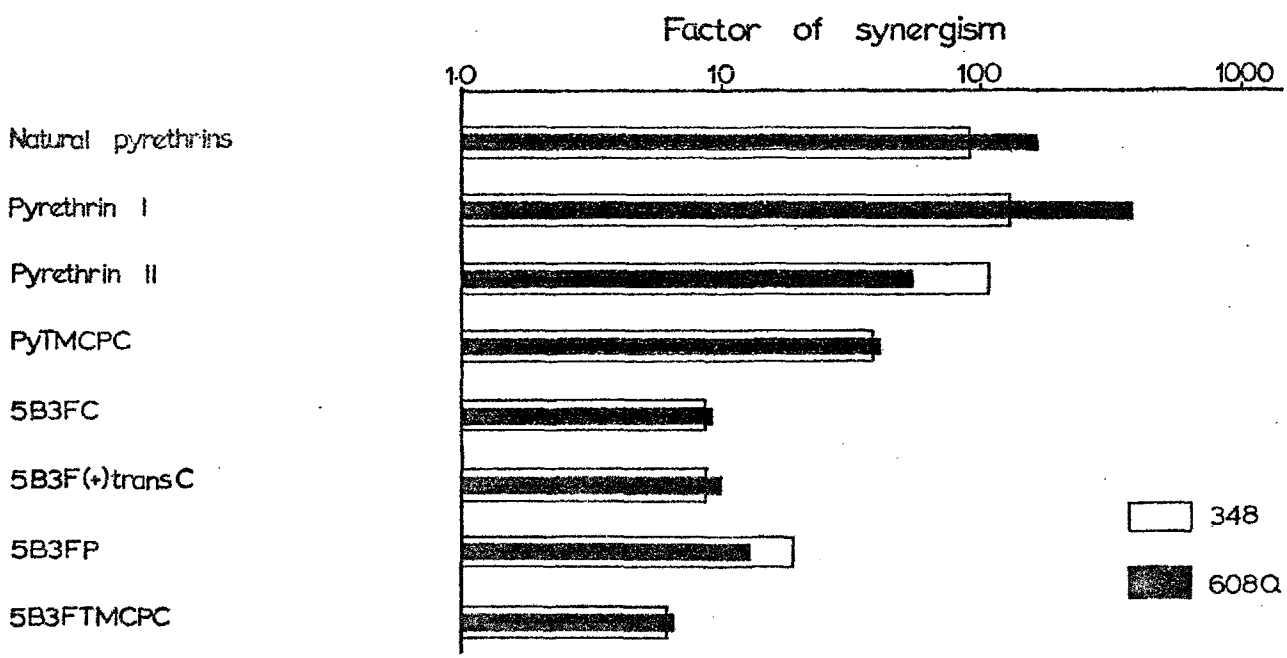
VII. Histograms for Strain 200





VIII. Histograms for Strain 348





not measured, the observed knock-down effect of natural pyrethrins was very much delayed in comparison with 608Q.

348 strongly resisted DDT at kill end-point (RF 17) but the time taken to reach this point was about 96 hours. This is shown in graph XXIV on page 100. This slow response may be compared with the result for DDT against bwb DDT uns (graph XVIII page 87). Unlike 348, bwb DDT uns was not homogeneous in its response to DDT. It was homozygous for the mechanism of resistance that was isolated in 348, so LD_0 was the same for both strains. However it was also heterozygous for pen, isolated in 314, and this enhanced resistance to DDT in part of the bwb DDT uns population, so that the LD100s for the two strains were not the same.

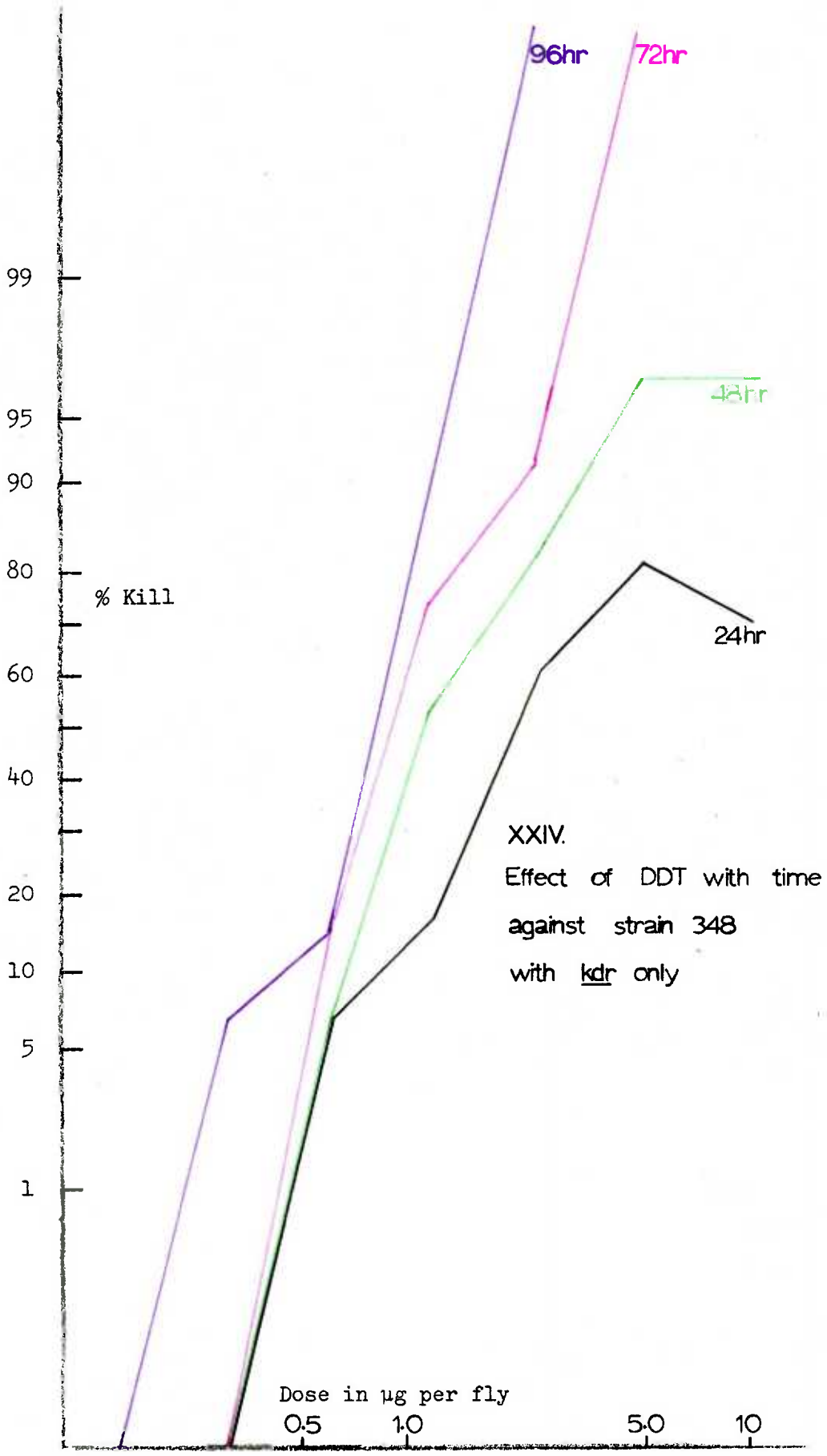
348 was susceptible to the other insecticides tested, including Zectran.

e. Results for 314 (NPR's chromosome III - Histogram IX, p 101)

314 was homozygous for ac;ar;bwb;ocra but like 348 had material from NPR's chromosome III linked to bwb. The strain was homozygous in its response and susceptible to all pyrethroids, but was slightly resistant after pretreatment with sesamex, and SFs were smaller than for 608Q. The observed knock-down of natural pyrethrins was delayed even though the flies were susceptible to kill with pyrethroids.

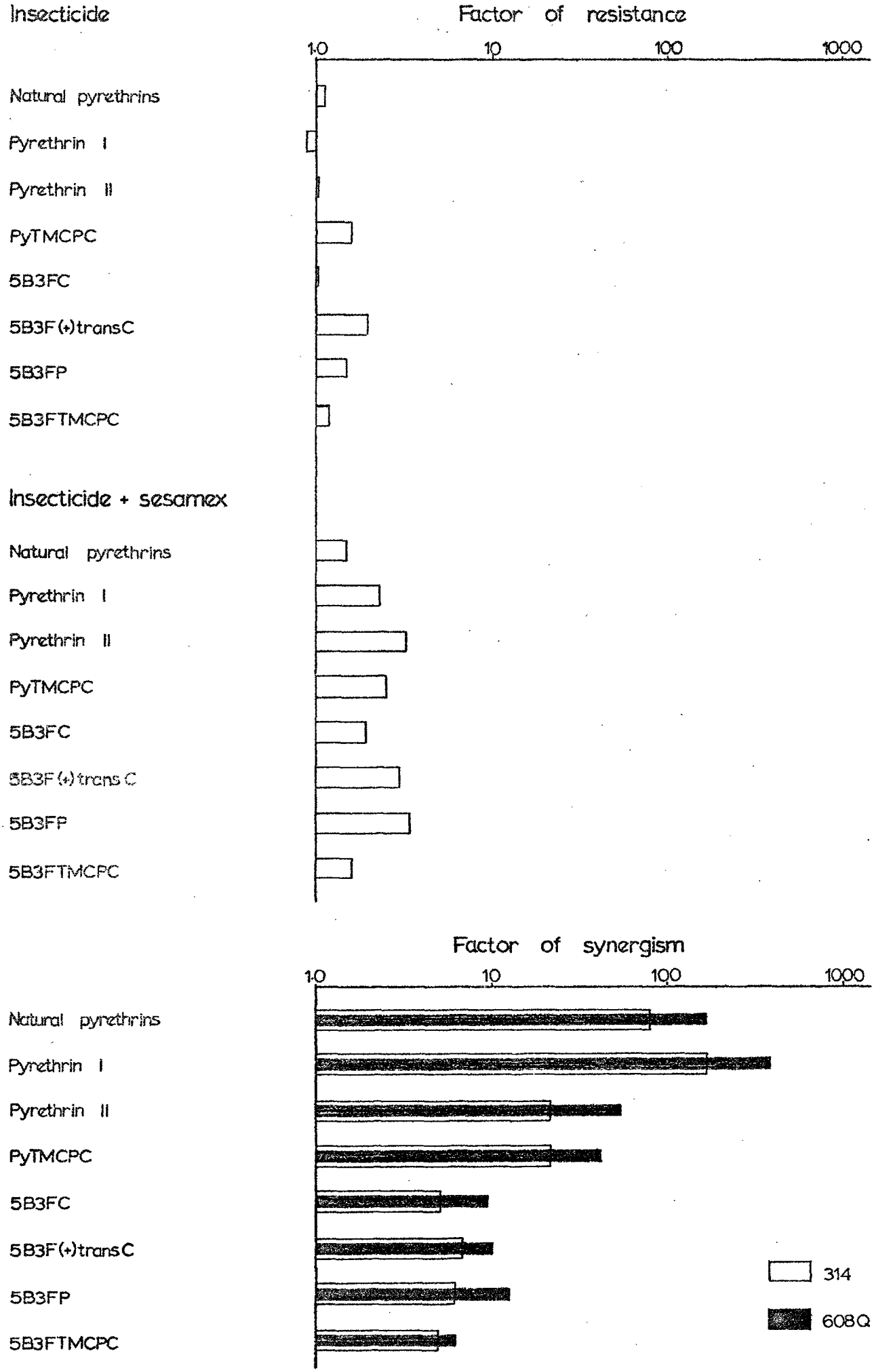
Response to DDT was homogeneous. The final LD50, slightly greater than that for 608Q (RF 2.3), occurred only 48-72 hours after treatment whereas kill end-point for DDT against 608Q was reached within 24 hours. The increased kill between 24 hours and 72 hours was little (graph XXV on page 102).

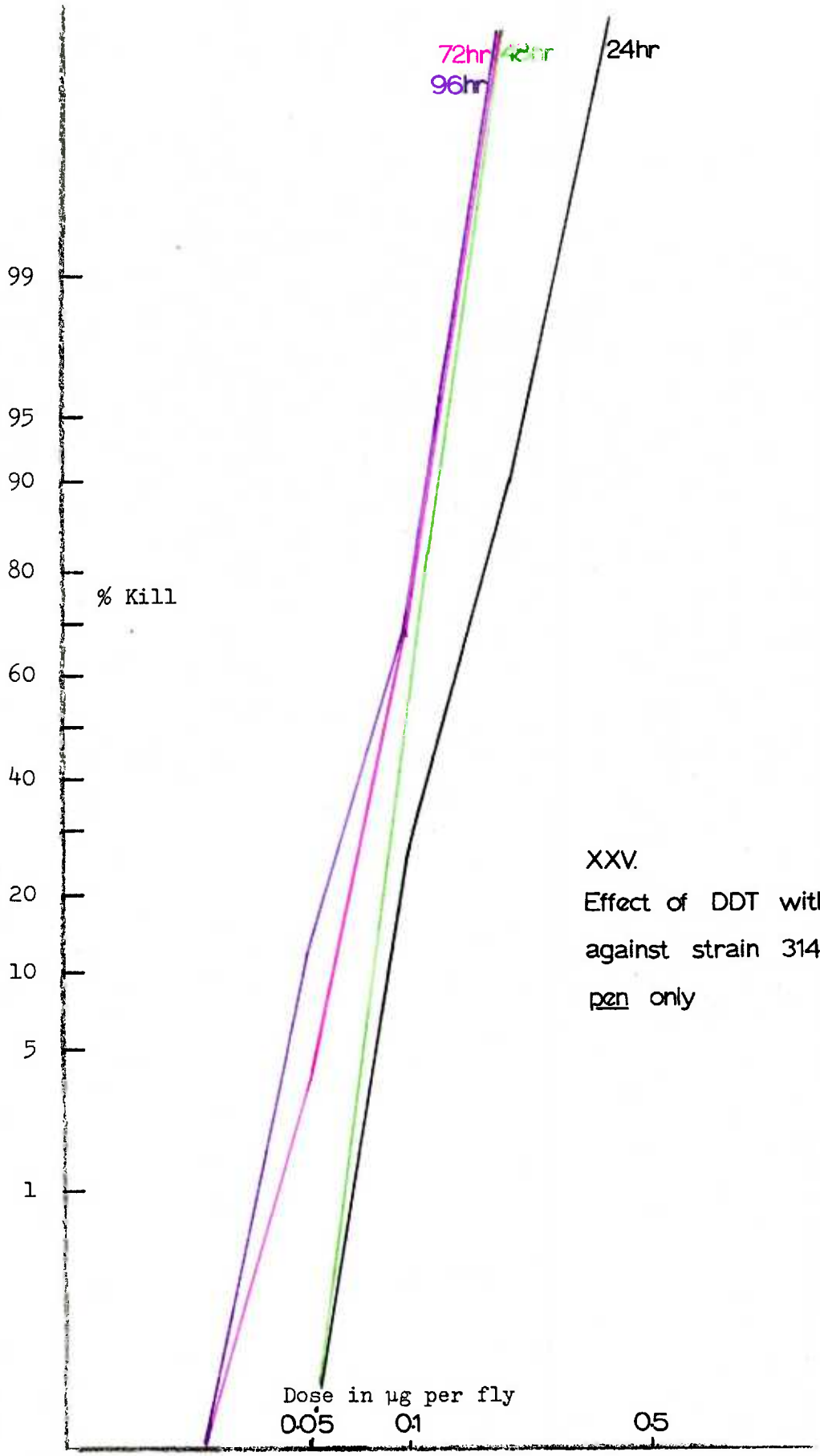
314 strongly resisted TBTA (RF 9.2), but was susceptible to dieldrin (RF 1.3), although the time taken to reach kill end-point



XXIV.
Effect of DDT with time against strain 348 with kdr only

Histograms for Strain





XXV.

Effect of DDT with time against strain 314 with pen only

was slightly delayed compared with 608Q. With Zectran 93% of ♀ 314 flies were killed by 40 µg, but most of the population was as susceptible as 608Q. This resistance was suppressed by pretreatment with sesamex and the resulting ld-p line lay close to that for 608Q (RF 1.3), as in strain 200 and (608Q x NPR)F_1. A dominant factor for resistance to Zectran affected by sesamex was probably present in the heterozygous condition in 200, 314 and NPR.

f. Results for 331 (NPR's chromosome III - Histogram X on page 104)

331 was homozygous for ac;ar;bwb;ocra and was the third strain isolated with chromosomal material from NPR's chromosome III linked to bwb.

331 was the most resistant strain to pyrethroids and DDT. The RFs for all pyrethroids except py I (RF 4.3), either without or with sesamex were 10 or more. The SFs like those for 314 were less than for 608Q, but pyrethrolone esters were still strongly synergised. The knock-down effect of natural pyrethrins was greatly delayed.

331 resisted DDT (RF 40), kill end-point occurred only 96 hours after treatment (graph XXVI on page 105). Resistance was much greater than in 200, 314 or 348 which also had some of the chromosome III material from NPR.

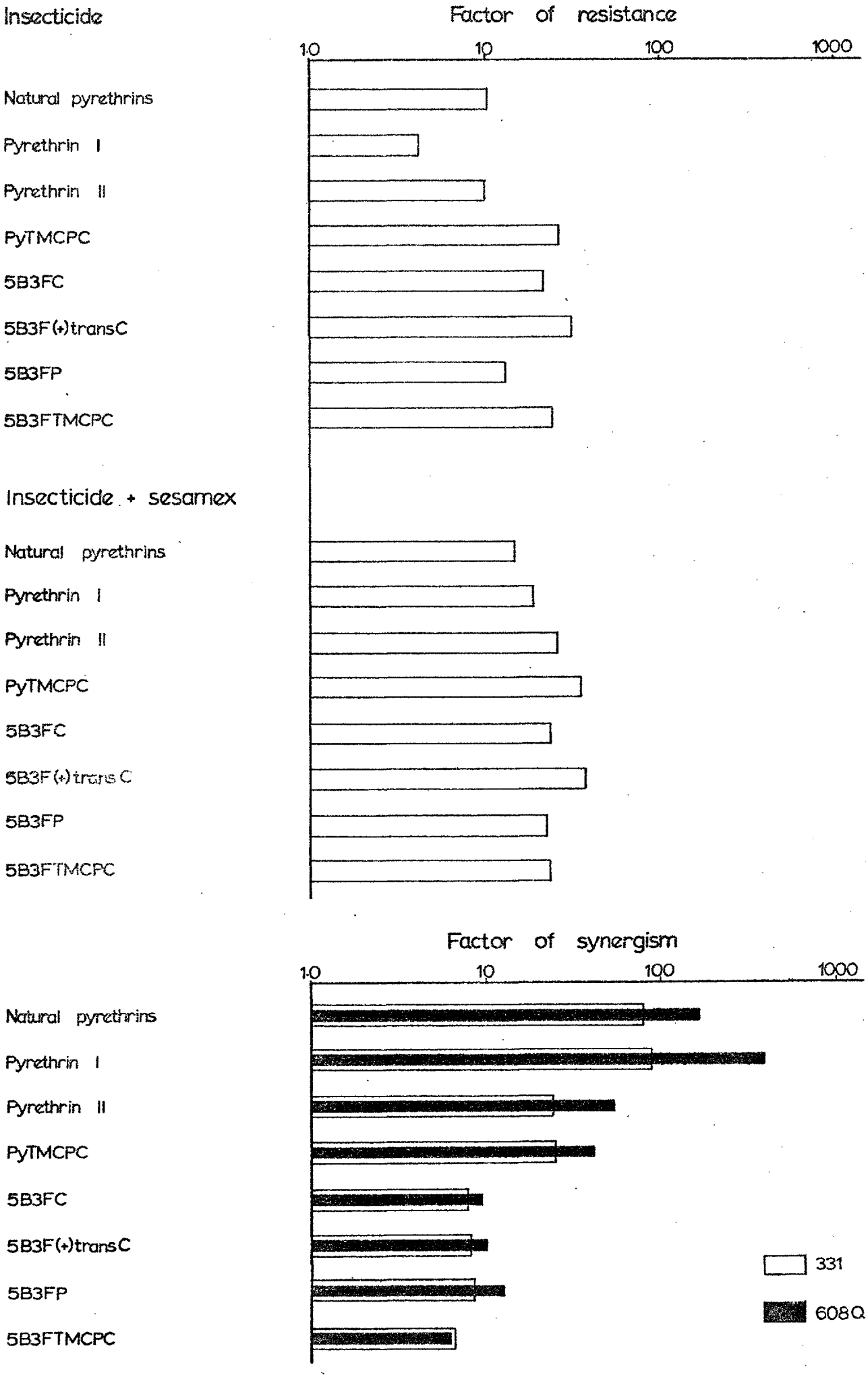
Like 314 and 200, 331 resisted TBTA (RF 8.9) and was heterogeneous in its response to Zectran (40 µg/♀ killing 97%). Pretreatment with sesamex eliminated this resistance completely.

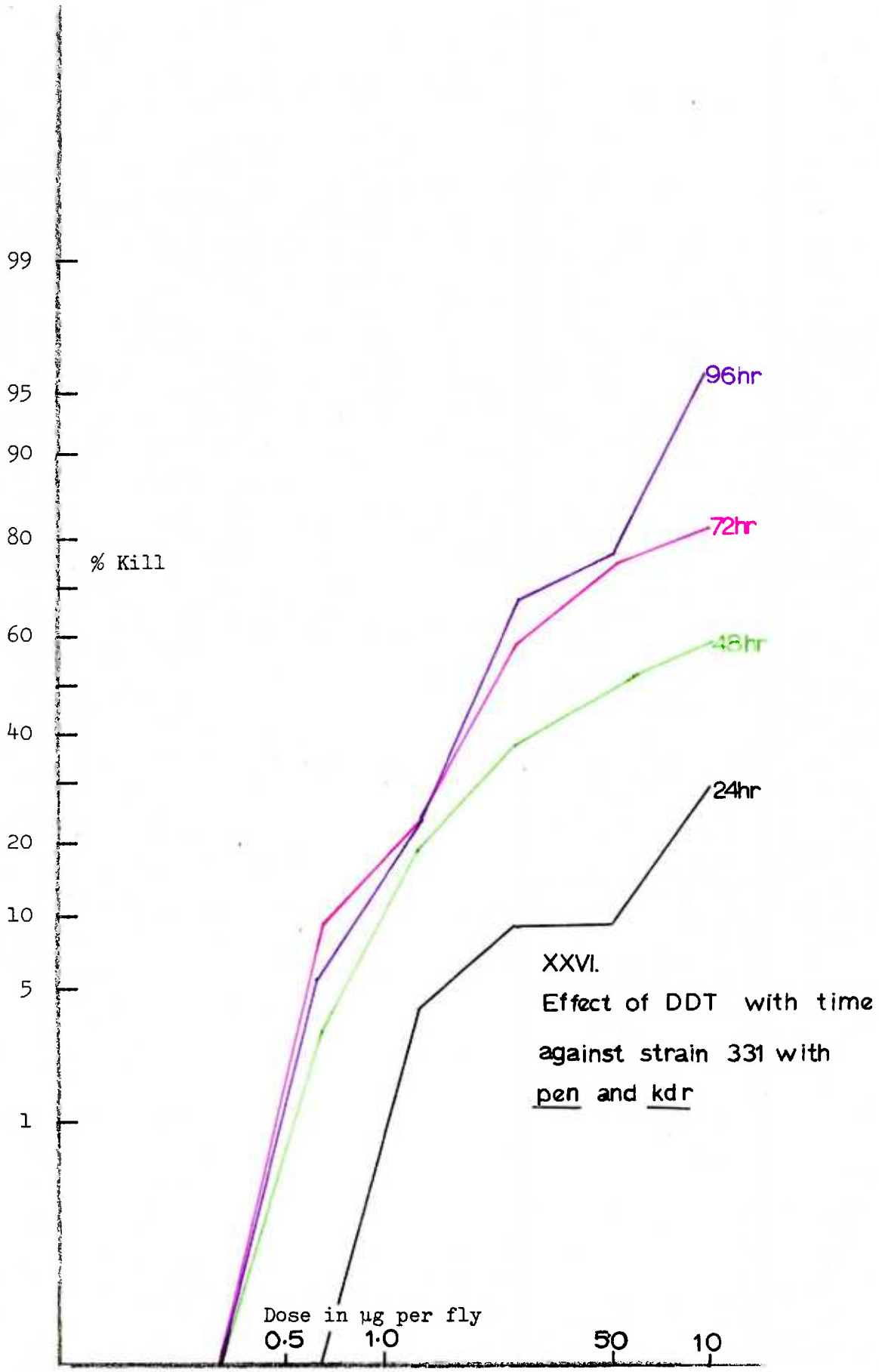
The effects of dieldrin were delayed, but like 314, 331 was susceptible at kill end-point.

g. Results for 213Q (NPR's chromosome IV - Histogram XI on p 106)

213Q was homozygous for ac;ar;bwb;ocra with the chromosomal

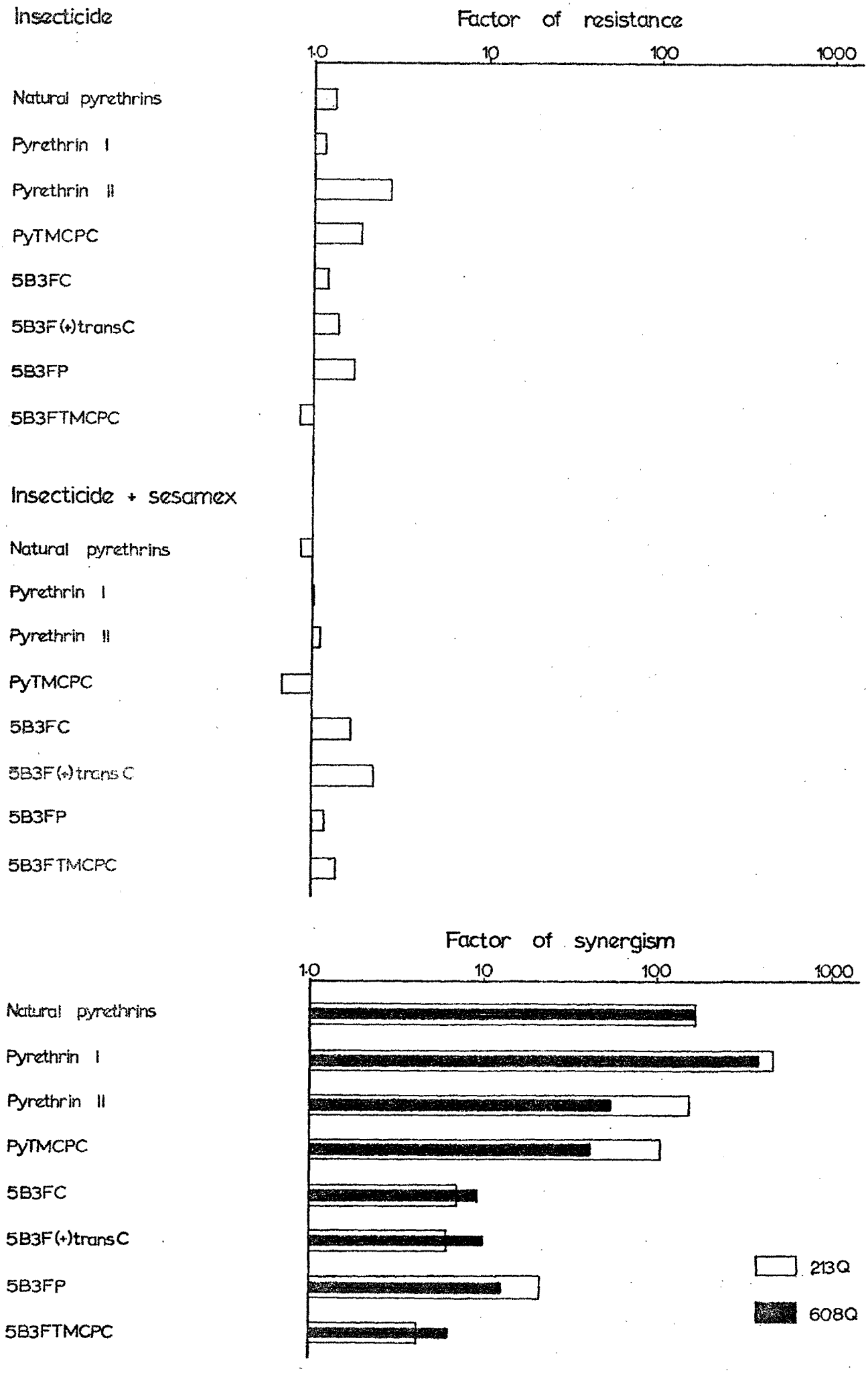
X. Histograms for Strain 331





XXVI.
Effect of DDT with time against strain 331 with pen and kdr

XI. Histograms for strain [illegible]



material from NPR's chromosome IV in the heterozygous state. It was susceptible to all pyrethroids either without or with sesamex, although there was possibly some resistance to py II (RF 2.8). Synergism was as for the susceptible strains, and the knock-down effect of natural pyrethrins was not delayed.

213Q was also susceptible to all other insecticides tested except dieldrin to which it was heterogeneous.

h. Results for 213QDR (NPR'S chromosome IV - Histogram XII, p 108)

213QDR derived from 213Q by selection with dieldrin was homogeneous at kill end-point for dieldrin resistance (RF 750) 96 hours after treatment.

It responded to all other insecticides like 608Q.

i. Results for 85 (NPR's chromosome V - Histogram XIII, p 109)

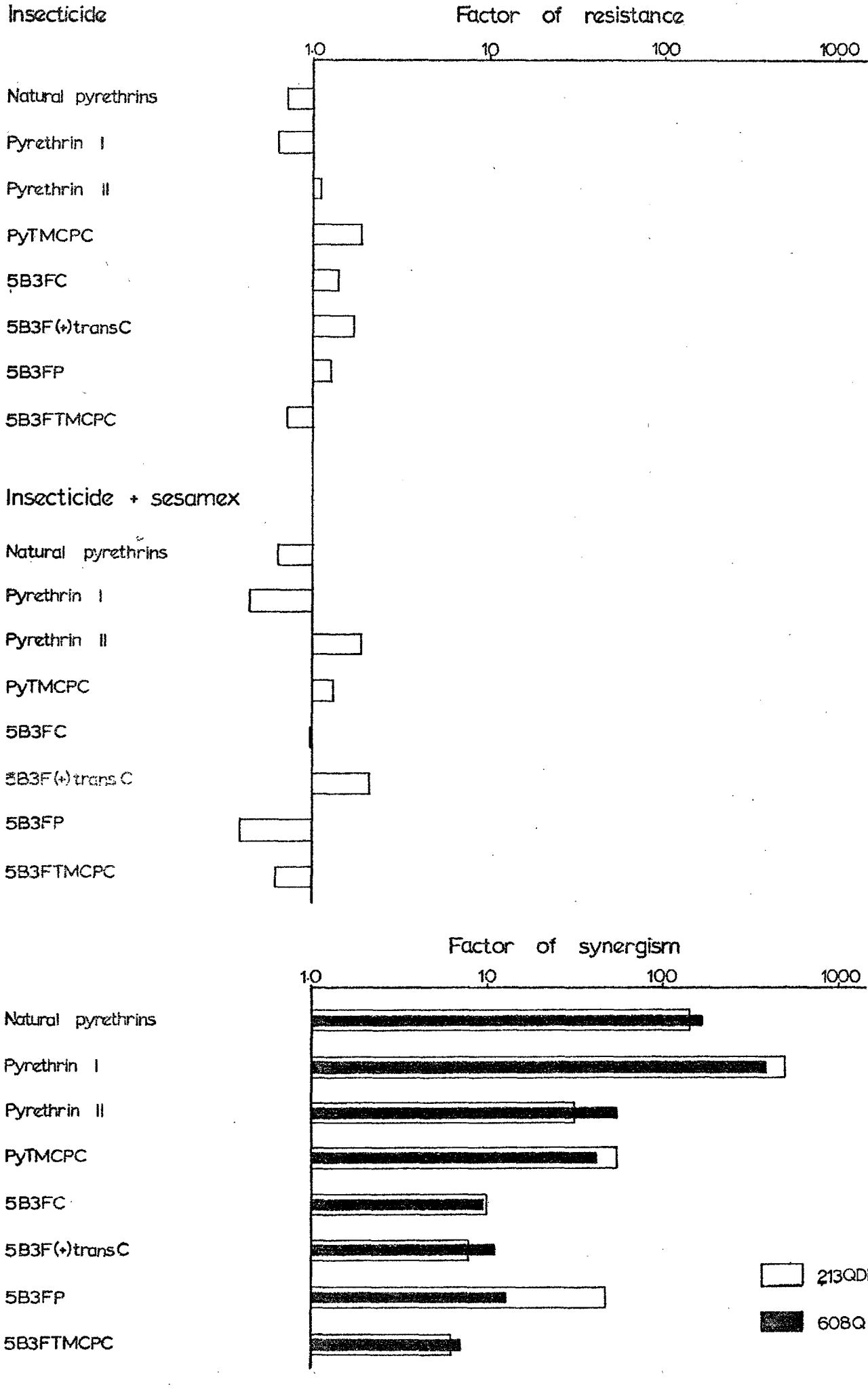
85 was homozygous for ac;ar;bwb; and for lack of ocra, indicating that chromosome V from NPR had been successfully substituted into 608Q.

85 only resisted esters of pyrethrolone. Resistance was small (RFs 2-5), but after pretreatment with sesamex resistance was eliminated. SFs were very great (1750 for py I). This mechanism did not affect 5B3FA esters, nor was there greater synergism of them than with 608Q.

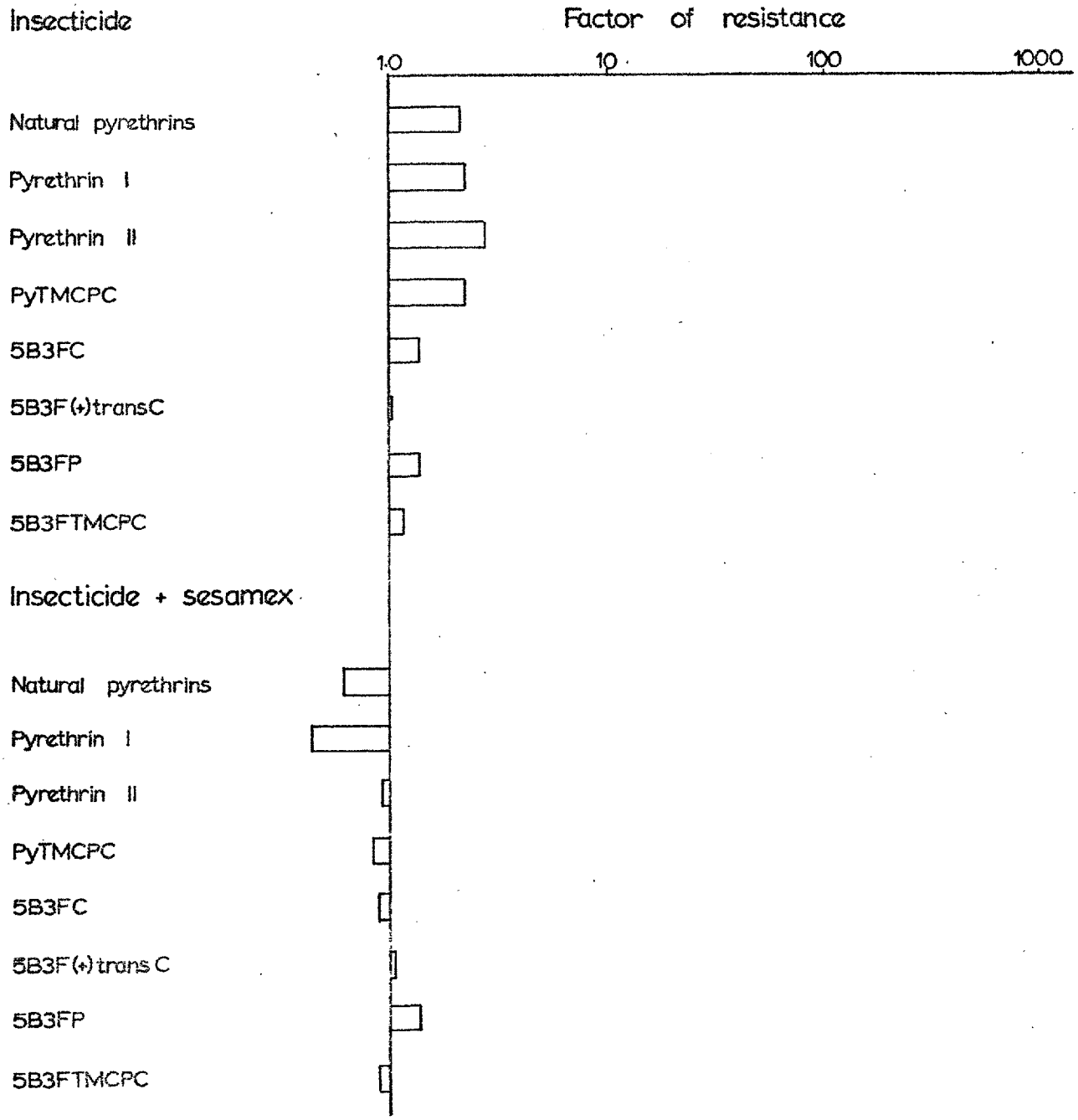
Although the knock-down effect of natural pyrethrins was observed, the comparison between the smallest dose to cause the effect in 608Q and 85 was not made.

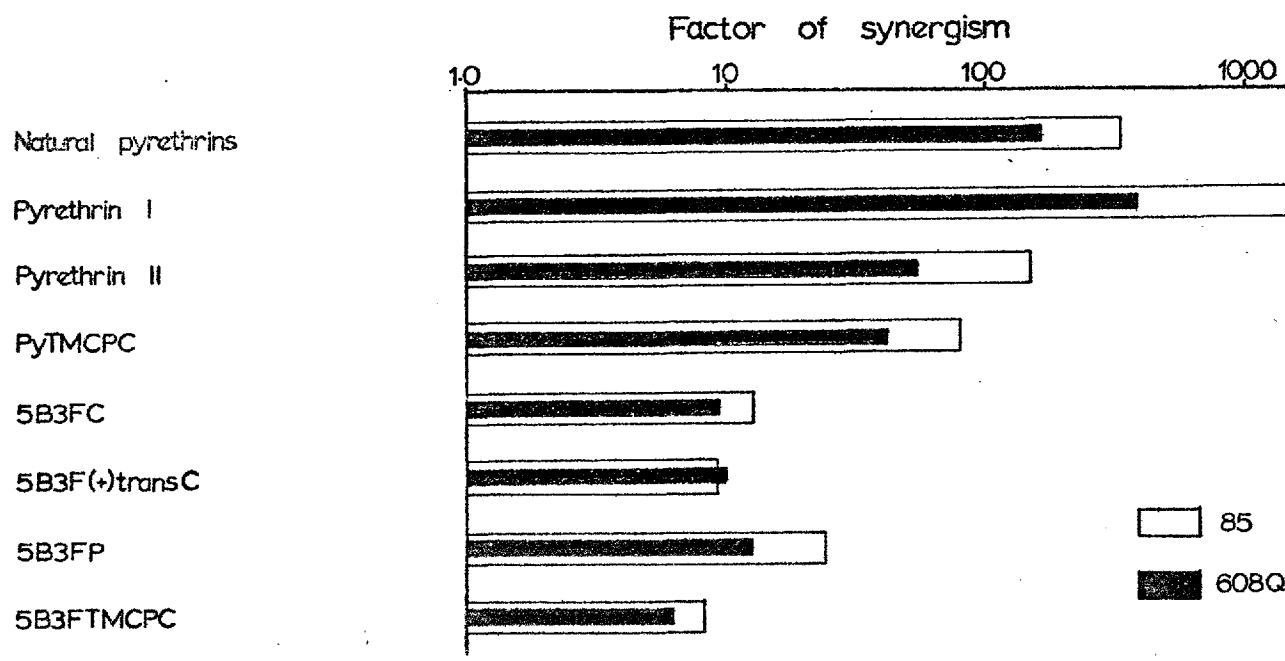
Because in preliminary tests on 85, the resistance to natural pyrethrins expected from bioassays of TCF_2 ac;ar;bwb flies (page 74) was thought to be missing, another attempt was made to isolate a strain homozygous for lack of ocra (see genetical results, page 52).

XII. Histograms for Strain 213QDR



XIII. Histograms for Strain 85





j. Results for 389 (NPR's chromosome V - Histogram XIV, p 111)

389 was homozygous for ac;ar;bwb and for lack of ocra. Like 85 it had chromosome V from NPR substituted into 6080.

In spite of the selection procedure used during the isolation of 389 (see page 52) it resembled 85 in its cross-resistance spectrum, and resisted only esters of pyrethrolone. This resistance could be completely suppressed by sesamex. The observed rate of knock-down with natural pyrethrins was similar to 85.

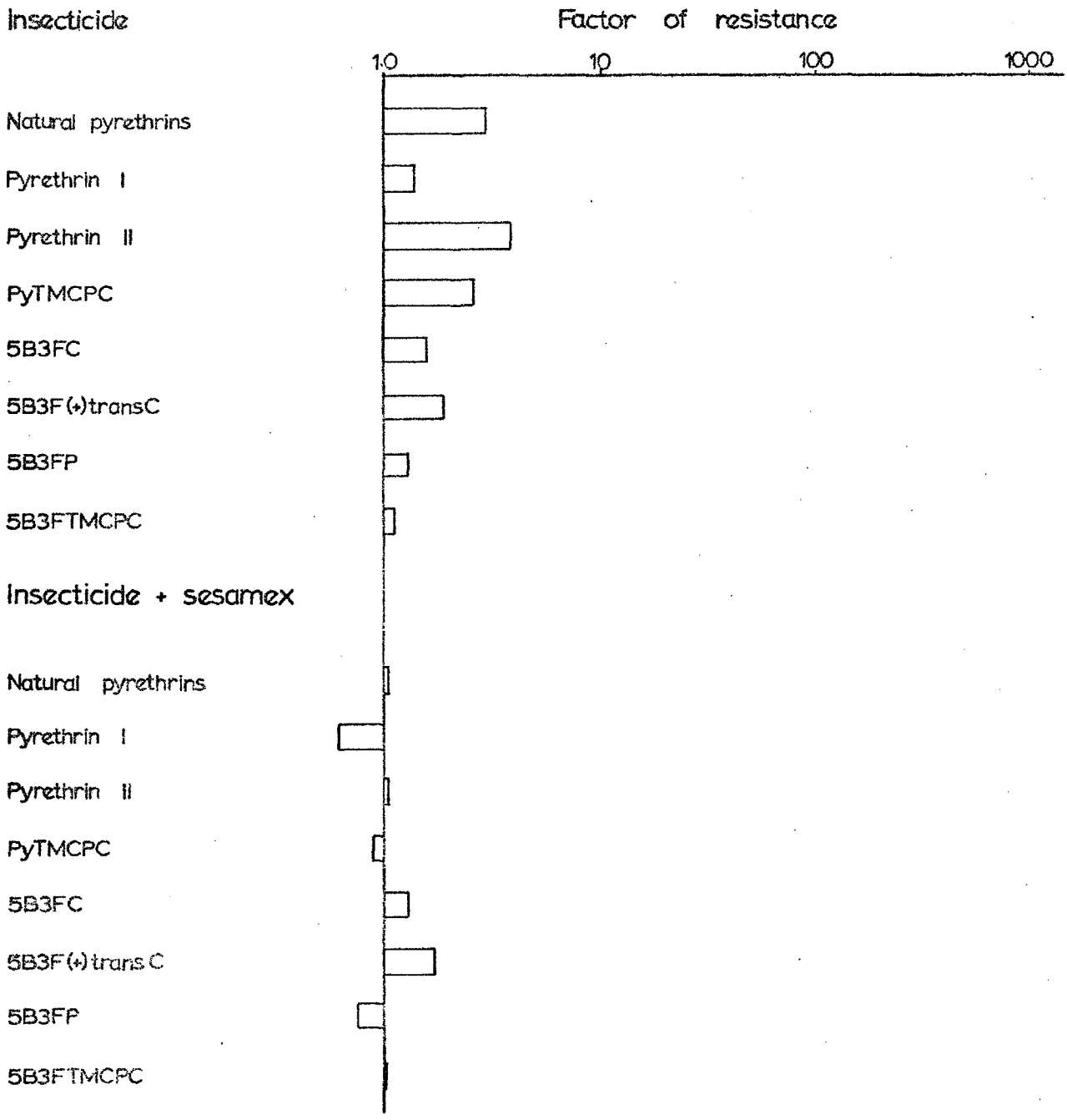
k. Conclusions

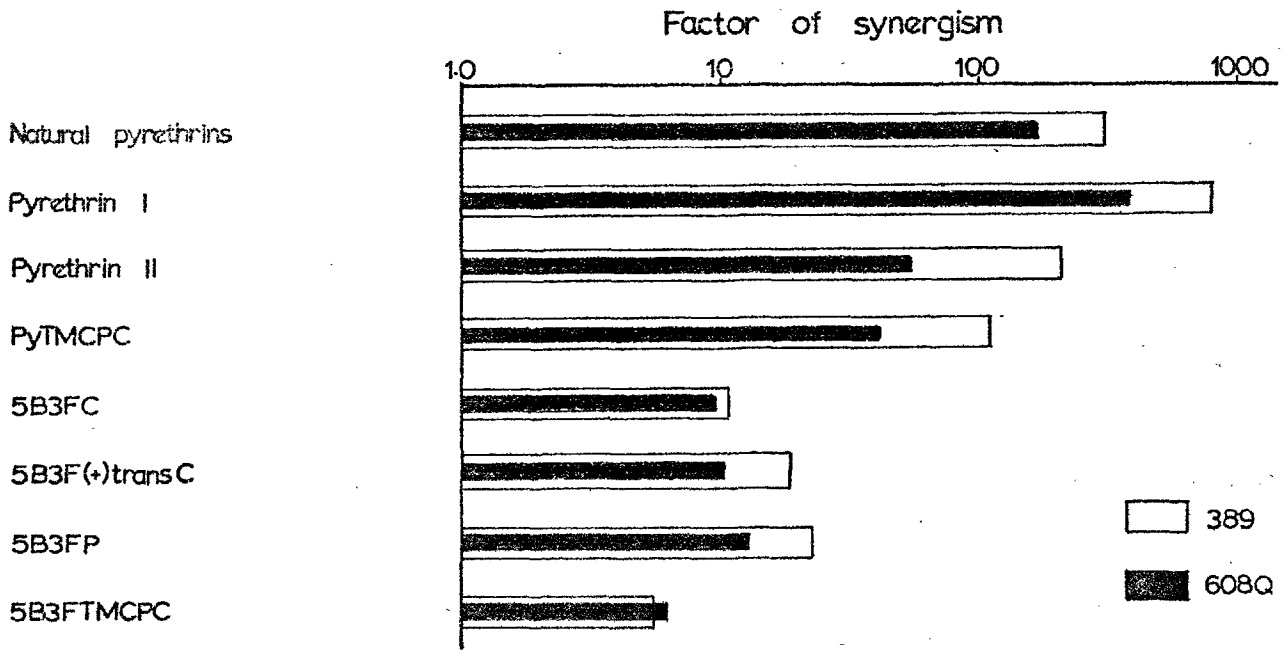
The results of bioassays on individual strains are summarized in Table XVII on page 114 , which shows which of NPR's chromosomes bear the isolated resistance factors. The results confirm the predictions made from the results of the bioassays of the TCF_2 triple and quadruple marker flies which were given in Table XVI on page 80. The names given to the various factors are listed in Table XVIII, p 115.

NPR's chromosome I had no major resistance mechanism that could be detected at the TCF_2 stage where the ar;bwb;ocra phenotype was the most susceptible to all pyrethroids. There was slight resistance in 420 to unsynergised and synergised 5B3FA esters (RF 2-4) which might have been due to vigour tolerance, as NPR's chromosome I was never isolated in the homozygous state. There was no resistance to any other insecticide.

NPR's chromosome II, isolated in 381, was not homogeneous for pyrethroid or Zectran resistance. It bore strong resistance to synergised pyrethrolone esters and to synergised natural pyrethrins, but less to synergised and unsynergised 5B3FA esters and small resistance to natural pyrethrins, py I, py II and

XIV. Histograms for Strain 389





py TMCPC. With the latter four insecticides, SFs were much smaller than those for 608Q but SFs were similar to those for 608Q for the 5B3FA esters. This factor was named R_{381}

The resistance to Zectran on NPR's chromosome II was also not homogeneous. It was affected by sesamex but not completely suppressed. It is not known whether the pyrethroid and Zectran resistance mechanisms on this chromosome are allelic or only linked. DDT resistance was also present on this chromosome, but in the homozygous state. Apart from noting its presence no other attempts to identify it were made.

Three resistance factors were located on NPR's chromosome III. The major factor, called kdr-NPR gave resistance to all pyrethroids and to DDT. It delayed the onset of poisoning symptoms with pyrethroids and DDT but not with dieldrin. SFs were similar to 608Q indicating that the mechanism was unaffected by sesamex.

The second factor, Pen, gave resistance to TBTA, but none to pyrethroids at kill end-point. However there was small resistance (RF 2-4) to all synergised pyrethroids which was noted in 314, but not in 200. Like kdr-NPR, Pen delayed the onset of symptoms of poisoning to pyrethroids and DDT, but also to dieldrin

The third factor on chromosome III in NPR which gave resistance to Zectran was not isolated in the homozygous state, and was found only in those strains with Pen, i.e. 200, 314 and 331, but not in 348. The resistance could be completely suppressed by sesamex.

The two major pyrethroid resistance factors on NPR's chromosome III together, as in 331 resulted in very strong resistance to all pyrethroids (RFs >5) and to DDT (RF 40). Although Pen gave no resistance to pyrethroids, the resistance

of kdr-NPR was increased by its presence, e.g. RFs for natural pyrethrins for Pen 1.1, for kdr-NPR 3.5, for Pen and kdr-NPR together 10.4. However, as with Pen alone in 314, SFs in 331 for all pyrethroids were lower than those for the isolated kdr-NPR.

NPR's chromosome IV has only dieldrin resistance which gave very strong resistance when in the homozygous state.

NPR's chromosome V has resistance to natural pyrethrins and the pyrethrolone esters. This resistance could be completely suppressed by sesamex, and the factor was called py-ses.

Table XVII Showing the resistance associated with those strains isolated from the cross (NPR x 608Q) and its extent

Chromosome from NPR	I	II	III	III	III	III	IV	IV	V	V
Strain	420	381	200	314	348	331	213Q	213QDR	85	389
Pyrethrolone esters	0	+	0	0	+	++	0	0	+	+
+ sesamex	0	++	0	+	++	+++	0	0	0	0
5B3FA esters	+	+	0	0	++	+++	0	0	0	0
+ sesamex	+	+	0	+	++	+++	0	0	0	0
Zectran	0	H	H	H	0	H	0	0	0	0
+ sesamex	0	H	0	0	0	0	0	0	0	0
DDT	0	+	0	0+	+++	+++	0	0	0	0
TBTA	0	0	++	++	0	++	0	0	0	0
Dieldrin	0	0	0	0	0	0	H	+++	0	0
Penetration	0	0	+	+	0	+	0	0	0	0

+++ greater than 15
++ 5-15
+ 2-4
0 less than 2
H heterozygous

Table XVIII Names of pyrethroid resistance factors isolated from NPR.

Strain	Phenotype	Chromosome from NPR	Name of resistance factor
381	ac;bwb;ocra	II	R_{381}
314	ac;ar;bwb;ocra	III	Pen
348	ac;ar;bwb;ocra	III	kdr-NPR
85	ac;ar;bwb	V	py-ses

Table XIX. LD50s for 608Q

Insecticide	LD50 in µg/♀
Natural pyrethrins	0.24 ± 0.011
Pyrethrin I	0.30 ± 0.020
Pyrethrin II	0.14 ± 0.0090
PyTMCPC	0.038 ± 0.0017
5B3FC	0.0081 ± 0.00028
5B3F(+)transC	0.0035 ± 0.00016
5B3FP	0.019 ± 0.0012
5B3FTMCPC	0.0046 ± 0.00024
Insecticide + sesamex	
Natural pyrethrins	0.0023 ± 0.00019
Pyrethrin I	0.00082 ± 0.000068
Pyrethrin II	0.0023 ± 0.00021
PyTMCPC	0.0011 ± 0.000060
5B3FC	0.00090 ± 0.000053
5B3F(+)trans C	0.00034 ± 0.000021
5B3FP	0.0013 ± 0.000081
5B3FTMCPC	0.00073 ± 0.000047

Table XX Table of Results for individual strains

Phenotype	ac;ar;bwb;ocra		+;+;+;+;+;+			+;+;+;+;+;+			+;+;+;+;+;+			+;+;+;+;+;+			ac/+;ar;bwb;+;ocra			ac;ar;bwb;+;ocra			ac;ar;+;+;ocra		
Strain	608Q		Cooper			NPR			104			[608Q x NPR]F1			420			381			200		
INSECTICIDE	LD50	SF	LD50	SF	RF	LD50	SF	RF	LD50	SF	RF	LD50	SF	RF	LD50	SF	RF	LD50	SF	RF	LD50	SF	RF
Natural pyrethrins	0.24		0.19		0.79	∞			0.97		4.0	1.1		4.6	0.30		1.3	0.59		2.5	0.37		1.5
+ sesamex	0.0023	165	0.0010	168	0.43	0.28		120	0.15	7.2	65	0.015	86	6.5	0.0046	63	2.0	0.0083+	61	4.0	0.0015	180	0.65
5B3F C	0.0081		0.010		1.2	1.1		140	0.42		52	0.060		7.4	0.023		2.8	0.0426		5.2	0.014		1.7
+ sesamex	0.00090	9.3	0.00079	9.9	0.88	0.084	14	93	0.11	3.7	120	0.0044	14	4.9	0.0019	13	2.1	0.00337	11	3.7	0.0016	8.1	1.8
Pyrethrin I	0.30		0.33		1.1	∞			0.52		1.7	1.7		5.7	0.39		1.3	0.52		1.73	0.18		0.60
+ sesamex	0.00081	389	0.0011	352	1.4	0.125		150	0.076	8.7	94	0.0053	320	6.5	0.0022	191	2.7	0.0036+	102	4.4	0.00051	356	0.63
Pyrethrin II	0.14		0.21		1.5	∞			2.5		18	1.4		10	0.37		2.6	0.47		3.4	0.23		1.6
+ sesamex	0.0023	54	0.0043	50	1.9	0.48		210	0.52	7.1	230	0.035	75	15	0.012	35	5.2	0.0326+	14	14.1	0.0041	60	1.8
Py TNCPC	0.038		0.072		1.9	3.4		89	1.3		34	0.44		12	0.080		2.0	0.25		6.6	0.078		2.1
+ sesamex	0.0011	42	0.00084	86	0.76	0.069	48	63	0.053	19	48	0.0043	93	359	0.0016	50	1.5	0.012+	20	11	0.0017	48	1.5
5B3F (+) trans C	0.0035		0.0054		1.5	0.66		190	0.21		60	0.044		13	0.010		2.9	0.0176		5.0	0.0064		1.8
+ sesamex	0.00034	10	0.00057	10	1.7	0.049	12	140	0.044	4.6	130	0.0027	17	7.9	0.00096	9.2	2.7	0.00119	17	3.5	0.00056	9.3	1.6
5B3F P	0.019		0.027		1.6	1.8		95	0.90		47	0.11		5.8	0.042		2.2	0.081		4.3	0.027		1.4
+ sesamex	0.0013	13	0.0011	25	0.85	0.26	7.7	200	0.18	3.9	140	0.0076	15	5.8	0.0027	16	2.1	0.0102	8	7.8	0.0013	20	1.0
5B3F TMCPC	0.0046		0.0063		1.4	1.1		240	0.21		46	0.043		9.4	0.020		4.3	0.017		3.7	0.0079		1.7
+ sesamex	0.00073	6.2	0.00077	7.2	1.1	0.029	14	40	0.043	4.9	59	0.0021	23	2.9	0.0013	16	1.8	0.00091	18	1.2	0.0011	6.4	1.5
Zectran	0.86		0.71		0.83	∞			1.02		1.2	2.3+		2.7	1.4		1.6	4.3+		5.0	2.2+		2.6
+ sesamex	0.019	52	0.011	77	0.60	0.019+		1.0	0.072		3.8	0.025	50	1.3	0.030	47	1.6	0.1+	43	5.0	0.023	100	1.2
TBTA	0.24		0.39		1.6	4.0		17	2.5		10	0.71		3.0	0.62		2.6	0.6		2.5	1.35		5.6
DDT	0.047		0.033		0.70	∞			∞			0.15		3.2	0.035		0.74	0.094		2.0	0.093		2.0
Dieldrin	0.0081		0.0083		1.0	0.084+		10	0.032		3.9	0.020		2.5	0.012		1.5	0.010		1.4	0.011		1.4

All LD50 values are weighted means of two or more bioassays. The SFs are weighted mean values from tests in which both unsynergised and synergised insecticide are included. This accounts for the apparent discrepancy between LD50s for unsynergised and synergised insecticide and their SF value.

Phenotype	ac;ar;bwb;+;ocra			ac;ar;bwb;+;ocra			ac;ar;bwb;+;ocra			ac;ar;bwb;DR/+;ocra			ac;ar;bwb;Dr/Dr;ocra			ac;ar;bwb;+;+			ac;ar;bwb;+;+		
Strain	314			348			331			213Q			213Q DR			85			389		
INSECTICIDE	LD50	SF	RF	LD50	SF	RF	LD50	SF	RF	LD50	SF	RF	LD50	SF	RF	LD50	SF	RF	LD50	SF	RF
Natural pyrethrins	0.27		1.1	0.84		3.5	2.5		10.4	0.30		1.3	0.17		0.71	0.51		2.1	0.70		2.9
+ sesamex	0.0034	80	1.5	0.0086	99	3.7	0.035	79	15	0.0019	163	0.83	0.0015	140	0.65	0.0014	360	0.61	0.0025	320	1.1
5B3F C	0.0083		1.0	0.13		16	0.18		22	0.010		1.2	0.011		1.4	0.011		1.4	0.013		1.6
+ sesamex	0.00175	5.1	1.9	0.014	8.9	16	0.022	7.7	24	0.0016	6.1	1.8	0.00087	10	0.97	0.00082	13	0.91	0.0012	11	1.3
Pyrethrin I	0.26		0.87	0.57		1.9	1.3		4.3	0.35		1.2	0.19		0.63	0.65		2.2	0.42		1.4
+ sesamex	0.0019	164	2.3	0.0043	131	5.3	0.015	87	19	0.00081	477	1.0	0.00036	520	0.44	0.00036	1750	0.44	0.00049	830	0.6
Pyrethrin II	0.14		1.0	0.86		6.1	1.4		10	0.39		2.8	0.15		1.1	0.38		2.7	0.54		3.9
+ sesamex	0.0075	21	3.3	0.0084	106	3.7	0.059	24	26	0.0025	155	1.1	0.0044	32	1.9	0.0026	150	1.1	0.0025	210	1.1
Py TMCPC	0.061		1.6	0.50		13	1.04		27	0.073		1.9	0.074		1.9	0.084		2.2	0.10		2.6
+ sesamex	0.0027	21	2.5	0.014	39	13	0.039	25	35	0.00072	107	0.65	0.0014	54	1.3	0.00093	81	0.85	0.00099	102	0.90
5B3F (+) trans C	0.0071		2.0	0.060		17	0.11		31	0.0049		1.4	0.0061		1.7	0.0035		1.0	0.0065		1.8
+ sesamex	0.00103	6.9	3.0	0.0073	8.9	21	0.013	8.2	38	0.00079	6.3	2.3	0.00073	7.8	2.1	0.00036	9.9	1.1	0.00059	10.9	1.7
5B3F P	0.028		1.5	0.21		11	0.24		13	0.033		1.7	0.025		1.3	0.027		1.4	0.022		1.2
+ sesamex	0.0044	6.2	3.4	0.011	19	8.5	0.028	8.8	22	0.0016	21	1.2	0.00050	47	0.38	0.0018	24	1.4	0.0010	23	0.7
5B3F TMCPC	0.0056		1.2	0.061		13	0.11		24	0.0037		0.81	0.0033		0.72	0.0054		1.2	0.0040		0.87
+ sesamex	0.0012	5.0	1.6	0.012	5.8	16	0.017	6.7	23	0.0010	4.2	1.4	0.00045	7.1	0.62	0.00066	8.2	0.90	0.00072	5.4	1.0
Zectran	0.94 +		1.1	1.06		1.2	1.4+		1.6	0.64		0.75	0.80		0.93	0.55		0.64	0.59		0.69
+ sesamex	0.025	37	1.3	0.016	64	0.85	0.028	52	1.5				0.026	32	1.4	0.018	30	0.95	0.016	36	0.84
TBTA	2.21		9.2	0.49		2.0	2.13		8.9	0.47		2.0	0.40		1.7	0.43		1.8	0.46		1.9
DDT	0.11		2.3	0.81		17.2	1.9		40.4	0.040		0.85	0.027		0.57	0.069		1.5	0.038		0.81
Dieldrin	0.015		1.9	0.012		1.4	0.013		1.6	0.040+		4.9	6.1		750	0.0073		0.90	0.0079		0.98

+Strain heterogeneous in response

LD50s in $\mu g/\frac{0}{+}$ fly

DISCUSSION

1. Introduction

The relatively small number of studies of the genetics of resistance to insecticides in strains with polyfactorial resistance (Tsukamoto and Suzuki, 1964, 1966; Sawicki and Farnham, 1968b) has clearly shown that the problem is complex, involving not only a series of factors or genes which confer resistance to specific groups of insecticides, but also factors conferring multiplicate resistance. These have been reviewed by Busvine (1970) Sawicki (1973) and Tsukamoto (1969). The present work shows that resistance in a natural pyrethrins selected strain of houseflies, NPR, is also polyfactorial and very complex.

None of the four factors of resistance to pyrethroids (see Tables XVII and XVIII on pages 114 and 115) was of paramount importance, which suggests that there must be strong interaction between some if not all mechanisms involved, i.e. there is multiplicate resistance to pyrethroids. At the same time, there is multiple resistance affording some protection for NPR against such diverse compounds as DDT, dieldrin and Zectran.

However this type of pyrethroid resistance has now been superseded by houseflies on Danish farms (Keiding, 1973) where much stronger resistance, particularly to bioresmethrin and synergised natural pyrethrins has occurred (Farnham, to be published). This demonstrates that in spite of severe laboratory selection pressure, field resistance has overtaken laboratory resistance and implies that extrapolation of laboratory results to field conditions must be interpreted with caution.

2. Genetical

Predicting the extremely complex genetical nature of strains and their substrains is difficult from the study of the very limited number of genes under consideration in this work. Several difficulties were encountered during genetical isolation of the resistance factors from the pyrethrins selected NPR. Although NPR, like the susceptible quadruple marker strain 608Q, was extremely fertile, the skewed segregation of the markers and resistance factors in the testcross progeny, and poor fertility in sub-strains from single pair matings of TCF_2 flies indicated that combinations of resistance factors and/or markers greatly affected breeding and fertility.

"Good" mutants are those which (a) do not overlap the normal phenotype (i.e. good expressivity) (b) have good viability and (c) generally conform to expectations in Mendelian segregation (i.e. good penetrance). Likely mutants to satisfy these criteria are those that affect the colour of eyes and body, the form of wings, enzyme abnormalities and insecticide resistance (Milani, 1967). The mutants under consideration in the present work, i.e. visible markers and resistance factors fit this description.

Inbreeding a population can result in lowering the load of hidden heterozygosity (Milani 1967). However, the populations of NPR and 608Q were selected for good fertility and presumably for desirable qualities which facilitated the task of maintaining them, during normal laboratory rearing before the initial cross, whilst in F_1 (NPR x 608Q), heterosis reduced or nullified the effects of parental inbreeding.

In spite of the hybrid vigour of this F_1 and the fertility of the quadruple marked parent, the testcross progeny recombinants

608Q x F_1 (NPR x 608Q) did not appear in the expected 1:1:1...:1 ratio (χ^2 = 207 for 15 degrees of freedom) (see Table XXI, page 122). Visible mutants were less frequent than their normal phenotype. For practical reasons, the equivalent ratio for resistant mutants could not be measured at this stage but possibly each deviated from the expected 1:1 ratio also.

Hiroyoshi (1961) interpreted deviations from expected ratios as being due to reduction in the number of flies of particular mutant classes. Lanna and Franco (1961) also observed that the number of mutants is always less than expected but that the proportions differ significantly between cultures, i.e. each mutant can behave differently in different crosses. Milani (1967) suggested that this deficiency in mutants may be caused by factors acting independently, cumulatively or interacting in various ways. However, fitness of a genotype in mixed culture is not predictable from its fitness in pure culture (Bhalla and Sokal, 1964). This makes comparison between results obtained in the present work and those of other authors impossible and explanations of any such differences meaningless. Even the genetical results of the analysis of the diazinon selected strain SDA (Sawicki and Farnham, 1968b) where the quadruple marked susceptible stock, 2345SRS, was similar to that used in this work, cannot be compared.

Further difficulties expressed as loss of fertility were encountered in trying to breed strains from single pair matings. Milani and Franco (1960) have shown that there is a reduction in fertility in inbreeding experiments where there was no selection for fertility. Sawicki (1970) met with similar problems when trying to synthesise a strain with the resistance factors Pen and gene a.

Table XXI List of frequency of visible marker recombinants at TCF_1

Phenotype	Numbers ♀	Numbers ♂	Total	Actual ratio	Estimated* ratio
+;+;+;+	83	102	185	1.0	1.0
ac;+;+;+	79	84	163	0.88	0.78
+;ar;+;+	70	79	149	0.81	0.71
+;+;bwb;+	64	75	139	0.75	0.68
+;+;+;ocra	59	75	134	0.72	0.71
ac;ar;+;+	52	62	114	0.62	0.55
ac;+;bwb;+	54	60	114	0.62	0.53
ac;+;+;ocra	58	57	115	0.62	0.55
+;ar;bwb;+	41	51	92	0.50	0.48
+;ar;+;ocra	46	70	116	0.63	0.50
+;+;bwb;ocra	39	59	98	0.53	0.48
ac;ar;bwb;+	36	42	78	0.42	0.38
ac;ar;+;ocra	34	35	69	0.37	0.39
ac;+;bwb;ocra	37	37	74	0.40	0.38
+;ar;bwb;ocra	39	35	74	0.40	0.34
ac;ar;bwb;ocra	23	16	39	0.21	0.27

*Estimated ratio calculated from fertility factor for individual markers in total population

Similar difficulties arose in this work. Substrains with the triple marker combination ar;bwb;ocra designed to isolate NPR's chromosome I never bred true for these visible mutants (see Table VI on page 43). From 150 single pairs, none yielded a pure stock. The major cause was infertility (33% lost) at the single pair stage or its F_1. This was obviously due to some deleterious factor associated with chromosome I due to its incompatability with the genetic background of 608Q. Whether the cause was a lethal effect on NPR's chromosome I or the removal of some "desirable quality" from 608Q due to substitution of its chromosome I causing the reduced fertility or the incompatability of NPR's chromosome I with the 608Q background could not be decided without a more detailed genetic investigation beyond the scope of this work.

The mutant combination ac;bwb;ocra never bred true for resistance factors on NPR's chromosome II (see Table VII on page 44) although 7% of the 65 single pairs gave phenotypically pure substrains. This was close to the expected success rate for single pairs (1 in 9 or 11%). Lack of homozygosity for resistance suggested possible heterozygosity of the factors in NPR and the possibility that the factor is itself a recessive lethal or closely linked to one.

With the other mutant combinations, segregations were more normal. Progenies of single pairs (see Table XIII on page 53) with the combination ac;ar;bwb with NPR's chromosome V remained homozygous for both markers and the resistance factor py-ses for at least 20 generations. The combination ac;ar;ocra with NPR's chromosome III which remained homozygous for the markers and for Pen lost kdr-NPR within six generations. The loss of

kdr-NPR from this substrain was probably due to the heterosygosity of one of the parents at the single pair stage, indicating the possibility of heterozygosity of this factor in the NPR parent of the original cross.

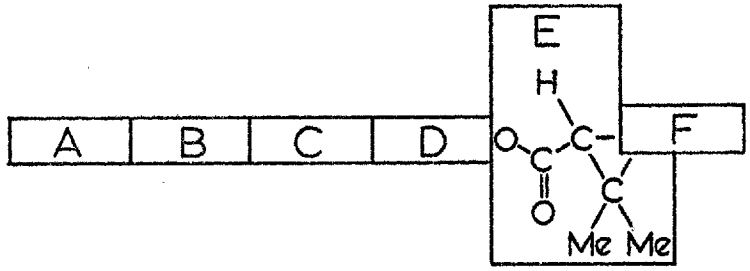
3. Structure toxicity of pyrethroid insecticides

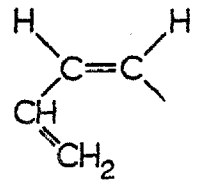
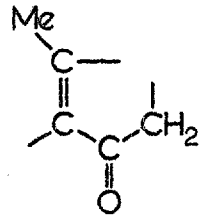
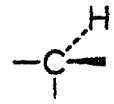
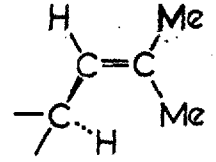
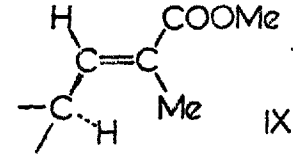
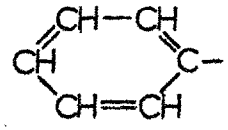
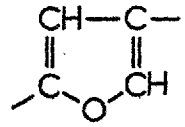
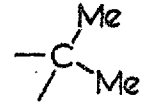
In discussing the relationship between chemical structure and insecticidal activity in pyrethroids, Elliott (1969, 1970) said that potent insecticides must combine appropriate physical and chemical properties to reach and be effective at the site of action. This entails among others, the ability to resist detoxification and the presence of certain features of the pyrethroid molecule which enables it to adopt a conformation that presents specific groups to the complementary substrate at the site of action, thus making it toxic. These may be methyl groups in the acid and the methylene group in the alcohol side chain arranged in a particular relation to one another.

The basic unit, present in all pyrethroids is the unit E in Figure IX, page 125. It can be substituted in various ways in the cyclopropane ring by unit F. In the present work, unit F was one of three substituents VIII, IX and X (Figure IX) to give respectively chrysanthemic, pyrethric and 2,2,3,3-tetramethylcyclopropanecarboxylic acid. The structure of group F is not critical for insect toxicity, but influences the reactivity and physical properties of the molecule, and partly determines the ease with which compounds are detoxified in insects and mammals (Casida et al., 1971; Yamamoto et al., 1969).

The units A, B, C and D determine the nature of the alcohol. Esters of two alcohols were used in the present work, namely pyrethrolone and 5-benzyl-3-furylmethyl alcohol (5B3FA).

IX. Structural requirements for pyrethrin-like activity (after Elliott, 1970)



	A	B	C	D		F	
pyrethrolone	 I		 III	 IV		 VIII	chrysanthemic acid
						 IX	pyrethric acid
5B3FA	 V	II	 VI	$-CH_2-$ VII		 X	2,2,3,3-tetramethylcyclo-propane carboxylic aci

In pyrethrolone esters, the unit D (IV in Figure IX) is an asymetric methine group of the cyclopentenolone ring, the rest of which (III) becomes unit C but in 5B3FA esters the O-acyl link is to the CH_2 group (VII), so with this alcohol, there is no centre of assymetry. Unit C is the furan (VI) in 5B3FA esters.

The O-acyl bond in the ester linkage between units E and D must adopt a position approximately parallel to the plane through units B, C and D for the molecule to have insecticidal activity.

The unit B in both alcohols is a CH_2 group (II) attached to unit A, which is a centre of unsaturation that can adopt a position skew to the plane defined by units B, C and D. In pyrethrolone, unit A (I) is a conjugated system of double bonds while in 5B3FA it is an aromatic ring (V).

Thus the six pure pyrethroids used in this work varied at units A, C, D and F, with units B and E common (Figure IX). The number of variables in the nature of the groups was too many to establish a comprehensive account of the structure toxicity relationships in susceptible and resistant flies, but some conclusions can be drawn. The ranking order (Table XXII page 127) which is the decreasing order of potency of the pyrethroids, for strain 608Q shows that 5B3FA esters are more toxic than their pyrethrolone equivalent, and the overall range of toxicities is 85-fold between the most toxic (5B3F(+)trans C) and the least toxic (py I).

Sesamex has comparatively little effect on the order (Table XXIII on page 128), but the overall range is reduced to 7-fold, because pyrethrolone esters are much more strongly synergised than are 5B3FA esters (Table XVIV on page 129). The inference is that this must be due to differences on the

Table XXII Ranking order for pyrethroids alone against strains 608Q, NPR and 104 listing the compounds in decreasing order of potency

Rank	608Q Compound	608Q LD50	NPR Compound	NPR LD50	104 Compound	104 LD50
1	5B3F(+)trans C	0.0035	5B3F(+)trans C	0.66	5B3F(+)trans C	0.21
2	5B3FTMCPC	0.0046	5B3FTMCPC	1.1	5B3FTMCPC	
3	5B3FP	0.019	5B3FP	1.8	py I	0.52
4	py TMCPC	0.038	py TMCPC	3.4	5B3FP	0.90
5	py II	0.14	py I)		py TMCPC	1.3
6	py I	0.30	py II)	∞	py II	2.5

Table XXIII Ranking order for synergised pyrethroids against strains 608Q, NPR and 104 listing the compounds

	608Q		NPR		104	
Rank	Compound	LD50	Compound	LD50	Compound	LD50
1	5B3F(+)trans C	0.00054	5B3F TMCPC	0.029	5B3F TMCPC	0.043
2	5B3F TMCPC	0.00073	5B3F(+)trans C	0.049	5B3F (+)trans C	0.044
3	py I	0.00081	py TMCPC	0.069	py TMCPC	0.053
4	py TMCPC	0.0011	py I	0.125	py I	0.076
5	5B3FP	0.0013	5B3FP	0.26	5B3FP	0.18
6	py II	0.0023	py II	0.48	py II	0.52

Table XXIV Ranking order for synergistic factors (SFs) for pyrethroids against strains 6080, NPR and 104, listing the compounds in decreasing order for their SFs

Rank	6080 Compound	SF	NPR Compound	SF	104 Compound	SF
1	py I	390	py I)		py TMCPC	19
2	py II	54	py II)		py I	8.7
3	py TMCPC	42	py TMCPC	48	py II	7.1
4	5B3FP	13	5B3F TMCPC	14	5B3F TMCPC	4.9
5	5B3F(+)trans C	10	5B3F(+)trans C	12	5B3F(+)trans C	4.6
6	5B3F TMCPC	6.2	5B3FP	7.2	5B3FP	3.9

Table XXV Ranking order for resistance factors (RFs) for pyrethroids against strains NPR and 104, listing the compounds in increasing order for their RFs

	pyrethroids alone				synergised pyrethroids			
	NPR		104		NPR		104	
Rank	Compound	RF	Compound	RF	Compound	RF	Compound	RF
1	py TMCPC	89	py I	1.7	5B3FTMCPC	40	py TMCPC	48
2	5B3FP	95	py II	18	py TMCPC	63	5B3FTMCPC	59
3	5B3F(+)trans C	190	py TMCPC	34	5B3F(+)trans C	140	py I	94
4	5B3F TMCPC	240	5B3F TMCPC	46	py I	150	5B3F(+)trans C	130
5	py I)		5B3FP	47	5B3FP	200	5B3FP	140
6	py II)		5B3F(+)trans C	60	py II	210	py II	230

alcohol side and the synergist suppresses attack on pyrethrolone much more strongly than on 5B3FA.

For NPR (Table XXII, page 127) the ranking order is the same as 608Q, with a wide range between the most and least toxic pyrethroid, but for 104 the list shows two differences. Py I is almost as potent as 5B3F(+)trans C and the overall range is only 12-fold. All pyrethroids are more strongly resisted by NPR than by 104 (Table XXV, p 129) with immeasurable RFs for py I and py II which 104 resists weakly. The previously postulated "extra" mechanism py b (= py-ses on chromosome V) in NPR (Farnham, 1971) was little affected by py TMCPC which is not only the most toxic pyrethrolone ester but also the pyrethroid that NPR resists least. Possibly the inability of py b to degrade py TMCPC as readily as py I and py II was influenced by the absence from the acidic part of the molecule of a side-chain in group F. Elliott (1971) has suggested that there is some "interaction" between properties of acid and alcoholic components of pyrethroids which determines the susceptibility of the molecule to detoxification, i.e. that the molecule should be viewed as a whole and not as two associated entities.

After pretreatment with sesamex, the resistant strains surprisingly gave identical ranking orders (Table XXIII, p 128) and the maximum differences in LD50s between the least and most toxic compounds are small, (x 17 for NPR, x 12 for 104). This suggests that NPR has the same factors of resistance as 104 plus the factor py-ses which is fully inhibited by sesamex.

The results are difficult to interpret in structure toxicity terms because of the limited range of pyrethroids used in this work. Also, active sites on the molecule essential for toxicity need not necessarily be the point of detoxification. Since the stereochemistry is so important slight alterations may

result in an active site now no longer correctly orientated at the site of action of the insect. A wider range of compounds could elucidate further the essential molecular features and the nature of the relationship between structure and toxicity in these strains.

4. DDT resistance

Resistance to DDT in NPR was detected only on chromosomes II and III. Strain NPR lacked the factor called ses (Sawicki and Farnham, 1968b) which is also called DDT-md (Oppenoorth and Houx, 1968) which is the sesamex suppressible factor on chromosome V, and strong Deh, the DDT dehydrochlorinase factor on chromosome II, because neither sesamex nor FDMC singly or together had any effect on the resistance of NPR or 104 to DDT (Farnham, 1971).

Factor kdr-NPR on chromosome III in NPR, the major mechanism isolated, conferred only 17-fold resistance to DDT. Factor Pen, delayed penetration, which was also on chromosome III conferred only 2-3-fold resistance and an unidentified factor on chromosome II called r-II-DDT conferred 4-fold resistance. Factor kdr-NPR gave resistance to DDT and pyrethroids, was completely recessive, and when homozygous greatly delayed both the initial knock-down and the time taken to reach kill end-point, both with DDT and pyrethroids. This same factor may have been selected by Busvine (1953) who showed that selection for knock-down resistance with DDT could induce resistance to pyrethroids. However, kdr-NPR differed from other knock-down resistance factors in conferring only relatively weak resistance to kill to DDT at kill end-point, whereas other workers assumed that knock-down resistance conferred virtual immunity. This assumption arose because Milani (1954b) failed to exclude Deh from his strain bwb-kdr, and Deh was

later shown to greatly decrease kill and knock-down when associated with kdr (Grigolo et al., 1965). Plapp and Hoyer (1967) examined the effects of kdr-0 only for 24 hours, in a strain that was not fully homozygous and may have included flies with TY-III. This translocation was the cause of the failure to isolate kdr-0 in the homozygous state (Milani, 1954b).

Differences in technique (topical application in the present work, contact with deposits in previous works) is also likely to cause differences in observed resistance levels. However, most recent work (Farnham, to be published) has shown that kdr-NPR, kdr and kdr-0 are most likely to be very close alleles and may well be homologous with the factor responsible for decreased nerve sensitivity to DDT (Tsukamoto et al., 1965), which also delays knock-down and provides resistance to DDT, and is also recessive.

The mechanism controlled by factor r-II-DDT on chromosome II is not known, but may be intermediate Deh which confers little or no resistance to DDT (Oppenoorth, 1963) and which may have been detected by Gil et al. (1968) in strain P_2/sel, a strain derived from 213ab, the parent of NPR and 104.

5. Dieldrin resistance

Dieldrin resistance in NPR was detected on chromosome IV only. The factor provided 750-fold resistance to dieldrin but none to any other insecticides used in the present work. NPR was heterogeneous for this incompletely dominant factor which was probably the same as DR_4 in SKA (Sawicki and Farnham, 1968a) later called Dld (Sawicki and Farnham, 1968b). Although factor Pen (chromosome III) greatly delayed penetration of ^{14}C-dieldrin and the onset of poisoning symptoms, it did not appreciably influence LD50 at kill end-point (RF x 2).

These results are remarkably similar to those for the diazinon selected strain SKA where comparable techniques of isolation and bioassay were used to measure resistance. Strain 213ab had probably been in contact with dieldrin at some stage in the field. Dld has persisted at a low frequency for 15 years in the absence of selection with a cyclodiene, and is an example of an excellent mutant gene which could be used to label chromosome IV. This contrasts with strain 104 where dieldrin resistance was lost. This was probably due to reducing the population on occasions to small numbers by strong selection with 5B3FC, so that survivors were no longer representative of the total background genetical material originally found in the parent 213ab strain.

6. Zectran resistance

Strain NPR was heterogeneous in its response to Zectran with approximately 20% of the population within the susceptible dose range, but with 55% surviving 50 µg/♀. Two resistance mechanisms were detected, one each on chromosomes II and III but neither was isolated in the homozygous state. Carbamate resistance has been detected in various strains of flies on chromosomes II and III (Georghiou and Garber, 1965; Hoyer et al., 1965; Kasai and Ogita, 1965; Plapp and Hoyer, 1967) as well as on V (Tsukamoto et al., 1968), and is very common in all resistant Danish strains (Keiding, 1973).

Bioassay of the F_1 (NPR x 608Q) indicated that much of the resistance was dominant and could be suppressed by sesamex. r-II-Zect on chromosome II which gave no complete kill at 50 µg/♀ was not suppressed by sesamex. It was probably recessive because F_1 (NPR x 608Q) gives complete kill when the flies are synergised with sesamex. r-III-Zect on chromosome III which also gave very

strong resistance to Zectran could be inhibited by sesamex.

7. Pyrethroid resistance

The natural pyrethrins-selected strain of houseflies NPR strongly resisted natural pyrethrins (80% survive 50 µg/♀) and individual pyrethrolone esters (Table XX on page 117). These were well synergised by sesamex against both NPR and susceptible flies. Like NPR, the 5B3FC selected strain 104 resisted 5B3FA and synergised pyrethrolone esters, but weakly resisted pyrethrolone esters alone. From these results, Farnham (1971) concluded that resistance in NPR was controlled by three factors, py a, py b and Pen. Pen and py a were common factors of resistance for all pyrethroids, but py b was sesamex inhibitable affecting esters of pyrethrolone and closely related alcohols but not esters of 5B3FA. Farnham also postualted that py a which provided resistance to pyrethroids alone and strong resistance to synergised pyrethrolone esters was similar to kdr (Milani, 1954) or kdr-0 (Plapp and Hoyer, 1967) because of its cross-resistance to DDT.

However, the present work has shown that pyrethroid resistance is controlled by four mainly recessive genes. The tolerance of F_1 (NPR x 6080) was approximately 8 x to all pyrethroids. These factors are R_{381} (on chromosome II), kdr-NPR (III), Pen (III) and py-ses (V). kdr-NPR, Pen and py-ses were isolated in the homozygous state but all attempts to similarly isolate R_{381} failed. Since this isolation involved the replacement of individual chromosomes of the multi-marker susceptible strain by resistant chromosomes from NPR, the properties of resistance factors borne on each chromosome were superimposed on the back-ground of the susceptible strain. This particularly influenced the results of tests for synergism where the effect of sesamex on resistance mechanisms was superimposed on the normal

synergism for the susceptible strain. This meant that SFs for a mechanism unaffected by sesamex was the same as for the susceptible strain while deviation from susceptible SF values was an indication of an effect of the synergist on the factor(s) involved.

a. Factor kdr-NPR

kdr-NPR, located on chromosome III provided resistance to all pyrethroids, was more effective against 5B3FA esters than their pyrethrolone equivalents and was unaffected by sesamex. No work was done on its mode of action but the delayed knock-down effects by pyrethroids and cross-resistance to DDT suggest that it is either kdr (Milani, 1954) or kdr-0 (Plapp and Hoyer, 1967). Recent studies have shown that kdr, kdr-0 and kdr-NPR give similar cross-resistance patterns and are most probably allelic (Farnham to be published). It is also possible that kdr-NPR resembles the mechanism of resistance to DDT through reduced nerve sensitivity isolated on chromosome III from strain JIR by Tsukamoto et al. (1965) Its mode of action is unknown but may be reduced penetration to the primary sites of action, which Burt and Goodchild (1971), working with Periplaneta americana have suggested are within the ganglia rather than peripheral nerve axons or connections of the central nervous system. This reduced penetration probably delays the time between initial contact of the insecticide and kill, enabling degradation processes to detoxify the poison, and this provides resistance. If the degradation process is inhibitable by sesamex the observed SFs should be the same as for the susceptible strain, as was found in this work.

b. Factor py-ses

The mechanism py-ses on chromosome V affects only pyrethrolone esters, giving approximately 4-fold resistance completely suppressed by sesamex and is similar to py b postulated previously (Farnham, 1971).

Because the mechanism is strongly synergised and affects esters of pyrethrolone only, it may involve an oxidative attack on the pyrethrolone moiety. Oxidative detoxification has been suggested by Chang and Kearns (1964), who postulated that the sesamex inhibitable degradation of py I and cinerin I in susceptible houseflies was initiated on the keto-alcohol moiety without specifying the site involved. Mammals detoxify py I by oxidation of the terminal methyl group (Elliott et al., 1972) and this may also be the site of attack in insects. In 5B3FA esters in which this side chain is replaced by a benzene ring synergism is small and py-ses ineffective.

Oxidative metabolism by py-ses may also involve the terminal methyl group on the acid side of the ester linkage (Yamamoto and Casida, 1966) but this is less likely because only pyrethrolone esters are resisted and synergised by strains with py-ses.

c. Factor R_{381}

The third factor, R_{381} on chromosome II gives some resistance to all pyrethroids, particularly 5B3FA esters but strong resistance to synergised pyrethrolone esters. Its mode of action is unknown but could be similar to the ester cleavage suggested for bioneopynamin in susceptible flies, a mechanism little affected by sesamex, but greatly affected by MB.16388 (Yamoto and Suzuki, 1972). It seems that in the absence of sesamex, most of the degradation occurs on the alcoholic side-chain with little ester cleavage, but when mfo (mixed function oxidase) metabolism is blocked by sesamex, ester cleavage (i.e. R_{381}) becomes important and this is why strains with this factor resist synergised pyrethrolone esters. If factor R_{381} is the esterase that cleaves the ester linkage in pyrethroids it should be selected for by both 5B3FC which is cleaved more easily than pyrethrins (Elliott

personal communication) or by pyrethrins/piperonyl butoxide where the synergist blocks the mfo and thus be present in both strains NPR and 104.

d. Factor Pen

The fourth factor Pen on chromosome III gave only small resistance (approximately x 2) to all pyrethroids but slightly more after pretreatment with sesamex. It also delayed their knock-down effects, and delayed penetration of ^{3}H-pyrethrin I and ^{14}C-dieldrin (Farnham, 1973). A similar factor from various strains, e.g. from SKA (Sawicki and Farnham, 1968b), and Cal-PR (Hoyer and Plapp, 1967) delays penetration of many insecticides. Delayed penetration also occurred for ^{14}C-pyrethrin I in strain P_2sel, a substrain of 213ab (Fine et al., 1963), the parent strain of NPR.

Despite greatly delaying penetration of pyrethroids, Pen gave surprisingly little resistance. The rate of loss of ^{3}H-pyrethrin I from the cuticle suggests that the internal build-up in susceptible flies must be rapid while strong synergism suggests rapid degradation. A decrease in rate of entry of the insecticide should therefore lead to a decrease in susceptibility because of the greater opportunity to detoxify the incoming insecticide. In spite of this, LD50s for flies with Pen were remarkably similar to those for susceptible flies, i.e. cuticular penetration is not the rate determining step in the poisoning process. If this is so, then the sesamex inhibitable metabolic process in susceptible insects must become exhausted and not regenerate itself.

e. Interactions

Although 80% of ♀ NPR flies were immune to 50 µg/♀ natural pyrethrins, not one isolated factor provided more than 4-fold

resistance. This loss of tolerance on analysing resistance systems by isolating mechanisms occurred with other polygenic systems (Sawicki and Farnham, 1968b; Georghiou, 1969) and has been explained on the basis of interaction between individual factors. It has been confirmed by resynthesising complex systems from isolated factors (Hoyer and Plapp, 1968; Sawicki, 1970; Hoyer and Plapp, 1971; Georghiou, 1971). In this work on NPR, several interactions were possible and some were observed.

(i) Interactions involving Pen.

Pen, which was isolated in this work, can act as a modifier by greatly enhancing the effect of other factors. However, the effect of any combination is unpredictable, e.g. Pen, in combination with the factors on chromosome II, gene a and gst, boosted resistance to chlorthion ethyl (from 40 to 1000) but had much less effect on chloroxon ethyl (from 12 to 28) probably due to the latter's more rapid penetration (Sawicki, 1970) indicating that Pen is not equally effective with all insecticides.

Pen had little effect on the resistance of kdr-NPR, e.g. it increased resistance to py II from 6 to 10, and in general the increase was less than 2-fold. Even resistance to DDT increased only from 17 to 40. This contrasted with the results of Hoyer and Plapp (1971) who synthesised a strain with tin (= Per) and kdr-0 (= kdr-NPR) and found in a residue test that the LC50 for DDT increased from 558 to 14800 µg per jar, i.e. 27-fold. However this result may have been influenced by the treatment technique and by measuring 24 hour mortality only. The residue technique of bioassay relies on passive pick-up of insecticide from glass onto the insect, and probably involves lateral flow around the cuticle, making actual rate of pick-up of poison much slower than in the topical application technique, where the

insecticide is actively applied directly to the cuticle. If cuticular penetration can be delayed by Pen, lateral spread may be likewise affected. Also as this work shows (graphs XXIV, XXV, XXVI on pages 100, 102 and 105) the time taken to reach kill end-point with DDT can be extended when either Pen or kdr-NPR are involved, making 24 hour mortality observations of limited value.

Cuticular penetration should not greatly affect resistance if kdr-NPR is not a detoxification system but instead reduces penetration into the nervous site of action and is the rate determining step in the poisoning process.

Pen may also delay penetration of sesamex which could lower SFs for all pyrethroids. Smaller SFs were found not only in the strain with Pen only but also in the strain with Pen and kdr-NPR, and both strains resisted all synergised pyrethroids slightly more strongly than the pyrethroids alone.

(ii) Interactions between other pyrethroid resistance factors

Although other combinations of homozygous factors from NPR were not synthesised, further conclusions on interaction may be drawn by comparing the response of strains NPR and 104 to natural pyrethrins.

104 resisted natural pyrethrins only 4-fold, but 80% NPR survived even 50 µg/Q. Farnham (1971) has suggested that the major difference between NPR and 104 is that the latter strain lacks the factor he called py b. This factor, which could be inhibited by sesamex very greatly resembled factor py-ses. However, singly, py-ses gave only three-fold resistance to natural pyrethrins, although the difference between NPR and 104 was very much larger. This infers that py-ses has a very much greater effect when in combination with the other pyrethroid

resistance factors than it does on its own.

If the major difference between NPR and 104 was due to lack of py-ses in 104, its major factors are Pen, kdr-NPR and R_{381}. Pen and kdr-NPR provide resistance to all pyrethroids, but not to synergised pyrethrolone esters to the extent observed in 104, which infers the involvement of another factor. R_{381}, which was not fully characterised because it was not isolated in the homozygous state, gave reduced SFs, and so could be the third factor in 104.

(iii) Interaction between other resistance factors from NPR.

The very strong resistance of NPR to DDT also could not be accounted for on the basis of Pen and kdr-NPR, which in combination gave only 40-fold resistance. Farnham (1971) suggested that strong DDT dehydrochlorinase (Deh) and gene ses were not major factors in NPR, but the weak factor on chromosome II called r-II-DDT (RF 2) could be weak DDT-ase (Oppenoorth, 1965). Grigolo et al., (1975) have shown that such a factor in combination with kdr (= kdr-NPR) could confer very strong resistance to DDT, again due to interaction. Such an interaction, coupled with Pen could account for the partial immunity of NPR to 50 µg/♀ DDT.

Thus there is probably strong interaction between the various factors in NPR to account for its strong resistance. This could be confirmed by synthesising strains from individual factors, a scheme which has practical difficulties when applied to other resistance systems (Sawicki, 1970, 1973b; Georghiou, 1971).

CONCLUSIONS

Resistance to pyrethroids in houseflies is complex. It involves a number of apparently unrelated mechanisms whose role had to be assessed from the interpretation of their cross-resistance spectra because their mode of action is still unknown. The

conclusions as to how they confer resistance are therefore tentative.

The four mechanisms of resistance individually confer small or negligible resistance, and yet jointly prevent the insects from dying of pyrethroid poisoning in the following way:-

The penetration delaying mechanism, Pen, slows the entry of pyrethroids into the fly and this protects it from rapid knock-down. If the penetrating pyrethroid is a pyrethrolone ester it is attacked by the system controlled by py-ses which probably oxidises a susceptible site in the pyrethrolone moiety, perhaps the pentadienyl side chain. Synthetic pyrethroids which lack this group are unaffected by py-ses. However both synthetic and natural pyrethroids may be cleaved by an esterase controlled by R_{381}, which confers slight but measurable resistance. When the remaining unmetabolised pyrethroid reaches the nervous system its entry may be delayed by kdr-NPR and thus may be attacked further while remaining outside the CNS. Thus although any one resistance mechanism confers little resistance, the combined effect is likely to be large, and this agrees with experimental data.

When flies with these mechanisms of resistance are treated with either synthetic pyrethroids or mixtures of pyrethrins plus methylene dioxylphenyl synergists, system py-ses is either inoperative or inhibited, and is thus ineffective and unselected. This is probably why strain 104, selected with 5B3FO, and the parent field strain 213ab were not very resistant to unsynergised pyrethrins.

This tentative model fits the experimental data and suggests why NPR flies resist synthetic pyrethroids less. However latest reports show that this type of pyrethroid resistance, originally the major system has now been surpassed by more powerful mechanisms in the field.

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APPENDIX Table of LD50 values with their standard errors for all strains

	608Q		Cooper		NPR		104		F1		420		381		200	
	LD50	SE	LD50	SE	LD50	SE	LD50	SE	LD50	SE	LD50	SE	LD50	SE	LD50	SE
Natural pyrethrins	0.24	0.011	0.19	0.0050	∞		0.97	0.035	1.1	0.044	0.30	0.024	0.59	0.020	0.37	0.015
+ sesamex	0.0023	0.00019	0.0010	0.000077	0.28	0.014	0.15	0.0044	0.015	0.0011	0.0046	0.00036	0.0083	0.00045	0.0015	0.00011
5B3F C	0.0081	0.00028	0.010	0.00024	1.1	0.084	0.42	0.015	0.060	0.0024	0.023	0.0013	0.043	0.0022	0.014	0.00089
+ sesamex	0.00090	0.000053	0.00079	0.000043	0.033	0.0045	0.11	0.0046	0.0044	0.00025	0.0019	0.00017	0.0034	0.00019	0.0016	0.000099
Pyrethrin I	0.30	0.020	0.33	0.0097	∞		0.524	0.076	1.66	0.091	0.39	0.030	0.52	0.0206	0.18	0.012
+ sesamex	0.00082	0.000068	0.0011	0.000064	0.13	0.0072	0.232	0.0057	0.0053	0.00041	0.0022	0.00020	0.00356	0.000192	0.00051	0.000050
Pyrethrin II	0.141	0.0090	0.21	0.0069	∞		2.5	0.20	1.4	0.10	0.37	0.022	0.47	0.0183	0.23	0.012
+ sesamex	0.0023	0.00021	0.0043	0.00033	0.48	0.061	0.52	0.039	0.035	0.0024	0.012	0.0019	0.033	0.0019	0.0041	0.00032
Py TMCPC	0.038	0.0017	0.072	0.0032	3.4	0.20	1.3	0.057	0.435	0.021	0.066	0.0071	0.18	0.013	0.078	0.0058
+ sesamex	0.0011	0.000060	0.00084	0.00012	0.069	0.0070	0.053	0.0039	0.0043	0.00037	0.0017	0.00017	0.0082	0.00069	0.0017	0.000081
5B3F (+) trans C	0.0035	0.00018	0.0054	0.00016	0.66	0.035	0.21	0.0078	0.044	0.0026	0.010	0.00062	0.018	0.00089	0.0064	0.00035
+ sesamex	0.00034	0.000021	0.00057	0.000030	0.049	0.0034	0.044	0.0026	0.0027	0.00015	0.00096	0.000069	0.0012	0.00011	0.00056	0.000044
5B3F P	0.019	0.0012	0.027	0.0045	1.80	0.11	0.90	0.039	0.11	0.0052	0.042	0.0031	0.081	0.0055	0.027	0.0018
+ sesamex	0.0013	0.000081	0.0011	0.000073	0.26	0.025	0.18	0.0095	0.0076	0.00062	0.0027	0.00023	0.010	0.00097	0.00126	0.000095
5B3F TMCPC	0.0046	0.00024	0.0063	0.00024	1.1	0.070	0.21	0.0090	0.043	0.0021	0.020	0.0015	0.017	0.0010	0.0079	0.00048
+ sesamex	0.00073	0.000047	0.00077	0.000049	0.029	0.0019	0.043	0.0027	0.0021	0.00010	0.0013	0.000084	0.00091	0.000067	0.0011	0.000074
Zectran	0.86	0.036	0.71	0.032	∞		1.02	0.078	2.3	0.14	1.4	0.10	5.0	0.35	2.2	0.26
+ sesamex	0.019	0.0012	0.011	0.00069	0.019	0.0023	0.072	0.0050	0.025	0.0023	0.030	0.0025	0.10	0.0078	0.023	0.0013
TETA	0.24	0.021	0.39	0.015	4.0	0.33	2.5	0.14	0.71	0.038	0.62	0.031	0.72	0.067	1.35	0.085
DDT	0.047	0.0033	0.033	0.0012	∞		∞		0.15	0.0064	0.035	0.0022	0.092	0.0044	0.093	0.0074
Dieldrin	0.0081	0.00037	0.0083	0.000042	0.084	0.0071	0.032	0.0012	0.020	0.0021	0.012	0.00062	0.0089	0.00066	0.011	0.00057

APPENDIX (contd)

Table of LD50 values with their standard errors for all strains

	314		348		331		213Q		213Q DR		85		389	
	LD50	SE	LD50	SE	LD50	SE	LD50	SE	LD50	SE	LD50	SE	LD50	SE
Natural pyrethrins	0.27	0.013	0.84	0.035	2.48	0.12	0.30	0.014	0.17	0.0091	0.52	0.022	0.70	0.039
+ sesamex	0.0034	0.00022	0.0086	0.00052	0.035	0.0025	0.0019	0.00020	0.0015	0.000037	0.0014	0.00013	0.0025	0.00019
5B3F C	0.0083	0.00050	0.132	0.0068	0.18	0.0097	0.010	0.00036	0.011	0.00067	0.011	0.00058	0.0125	0.00064
+ sesamex	0.0018	0.00012	0.014	0.00079	0.022	0.0012	0.0016	0.000087	0.00087	0.000070	0.00082	0.000057	0.0012	0.000076
Pyrethrin I	0.26	0.014	0.57	0.033	1.34	0.079	0.35	0.021	0.19	0.012	0.65	0.045	0.42	0.026
+ sesamex	0.0019	0.00015	0.0043	0.00030	0.015	0.0011	0.00081	0.000053	0.00036	0.000031	0.00036	0.000029	0.00049	0.000036
Pyrethrin II	0.14	0.0091	0.86	0.057	1.41	0.10	0.39	0.022	0.15	0.0079	0.38	0.023	0.54	0.032
+ sesamex	0.0075	0.00053	0.0084	0.00065	0.059	0.0041	0.0025	0.00029	0.0044	0.00039	0.0026	0.00024	0.0025	0.00025
Py TMCPC	0.061	0.0037	0.50	0.031	1.04	0.053	0.072	0.0076	0.074	0.0059	0.084	0.0067	0.10	0.0073
+ sesamex	0.0027	0.00015	0.014	0.00085	0.039	0.091	0.00072	0.000049	0.0014	0.000087	0.00093	0.000052	0.00099	0.000076
5B3F (+) trans C	0.0071	0.00037	0.060	0.0034	0.11	0.0055	0.0049	0.00028	0.0061	0.00061	0.0035	0.00022	0.0063	0.00032
+ sesamex	0.00103	0.000057	0.0073	0.00052	0.013	0.00074	0.00079	0.000046	0.00073	0.000047	0.00036	0.000022	0.00059	0.000040
5B3F P	0.028	0.0016	0.212	0.015	0.24	0.013	0.033	0.0023	0.025	0.0017	0.027	0.0031	0.022	0.0017
+ sesamex	0.0044	0.00032	0.0109	0.00073	0.028	0.0016	0.0016	0.00012	0.00050	0.000044	0.0018	0.00020	0.0010	0.000083
5B 3F TMCPC	0.0056	0.00029	0.061	0.0032	0.11	0.0061	0.0037	0.00026	0.0033	0.00020	0.0056	0.00041	0.0040	0.00021
+ sesamex	0.0012	0.000073	0.0115	0.00070	0.017	0.00096	0.0010	0.000050	0.00045	0.000028	0.00066	0.000036	0.00072	0.000046
Zectran	0.94	0.072	1.06	0.070	1.4	0.12	0.64	0.041	0.80	0.055	0.55	0.040	0.59	0.035
+ sesamex	0.025	0.0016	0.016	0.00085	0.028	0.0020			0.026	0.0019	0.018	0.0014	0.016	0.00086
TBTA	2.21	0.12	0.49	0.029	2.13	0.15	0.47	0.037	0.40	0.024	0.43	0.022	0.46	0.024
DDT	0.11	0.0064	0.81	0.042	1.9	0.12	0.040	0.0021	0.027	0.0021	0.069	0.0039	0.038	0.0023
Dieldrin	0.015	0.00098	0.012	0.00072	0.013	0.00065	0.040	0.0027	6.1	0.56	0.0073	0.00046	0.0079	0.00055