Susceptibility of Dysdercus spp. Boisduval (Hemiptera: Pyrrhocoridae) to selected synthetic pyrethroid insecticides

By

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A C K N O W L E D G E M E N T S

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ABSTRACT

Dysdercus species are important pests of cotton because of their staining effect on cotton lint. Chemical control, using the newer generation of insecticides known as the synthetic pyrethroids, is the most important method of control of these pests on cotton in Kenya at present.

Susceptibility studies were conducted using three pyrethroids, namely cypermethrin, fenvalerate and permethrin against strains of Dysdercus fasciatus Sign., D. nigrofasciatus (Stal.), D. cardinalis (Gerst.) and D. superstitiosus (F.) collected from the field and raised in the laboratory to obtain test strains.

Laboratory observations showed that there were differences in the susceptibility levels of the four species studied. The most susceptible species to the three insecticides was D. fasciatus. On the other hand D. cardinalis and D. nigrofasciatus displayed the least susceptibility to the test insecticides.

It was also observed that there were differences in the susceptibilities of different strains of the four Dysdercus species to the chemicals tested. The most susceptible strain to all the insecticides used was that of D. fasciatus collected from Masongoleni in Machakos District. Among the D. fasciatus strains, the most resistant strain was that which was collected from Kibos. Among the rest of the species, the D. nigrofasciatus strain collected from Homabay and the D. cardinalis strain collected from Kibos were the most resistant to the test insecticides. The toxicity of the three insecticides based on the topical application technique was found to be in descending order of effectiveness: cypermethrin, fenvalerate

and permethrin.

Two species, namely D. cardinalis and D. nigrofasciatus were subjected to insecticidal selection pressure for five generations to ascertain whether resistance could be induced in them against cypermethrin, fenvalerate and permethrin.

D. cardinalis when treated with permethrin gave the highest selection response shown by a resistance factor of x2.7. Treatment of D. cardinalis and D. nigrofasciatus with cypermethrin and fenvalerate gave resistance factors of between x0.8 and x1.6. In view of the experimental evidence gathered during these studies that Dysdercus species have the propensity for development of resistance to the synthetic pyrethroids, it was considered that selection for five generations was not sufficient to induce high levels of resistance in these species. It is suggested that frequent tests be carried out on Dysdercus species in order to monitor the development of resistance in them in Kenya.

Investigations into the residual persistence of cypermethrin, fenvalerate and permethrin revealed that of the three insecticides, cypermethrin had the longest residual persistence of 21 days under field conditions at Kibos. It was also observed that for each of the three insecticides there were significant ($P<0.05$) differences in their residual persistence during the experimental period. These observations also revealed that the synthetic pyrethroid insecticides used in this study have a short residual life ranging from 7 to 21 days under field conditions. On the basis of these observations it was concluded that the field application interval for cypermethrin for the control of cotton stainers should be 21 days. On the other hand, fenvalerate and permethrin were found to perform well against these pests at application intervals of 14 days. Because of this

it was recommended that they should be applied at that interval. Data obtained in studies reported in this thesis indicated that cypermethrin had a residual toxicity by a factor of about x5 in comparison to both fenvalerate and permethrin. Cypermethrin was therefore regarded as being the most effective against Dysdercus in comparison to the other two compounds tested.

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CHAPTER ONE

1. INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

1.1.1 Early world cultivation of cotton and its introduction in East Africa

Cotton has a long history as a textile fibre. It has been used by man since the ancient times (Lewis and Richmond, 1972). Cotton textiles found in archaelogical excavations in the Indus Valley and in North Central Peru were both dated at about 3,000 BC (Berger, 1969; Lewis and Richmond, 1972).

Other historical records indicate that cotton had been a stable article of clothing in India and the East generally and that the former was the earliest region of cotton cultivation (Watt, 1907). As early as 500 BC Alexander the Great is known to have transported cotton from India to Egypt and other mediterranean countries (Merril et al., 1949). In 70 AD Pliny, the Roman naturalist, claimed that the manufacture of cotton had its origin in establishments on the banks of the Tigris and Euphrates rivers. Marco Polo (who travelled through a large portion of Asia in 1290 AD) described growth and manufacture of cotton in China (Merril et. al., 1949). It is also known that in 1492 Columbus took samples of Sea Island cotton from the Bahamas to Europe.

The most ancient introduction of cotton in Africa is said to have originated in Sind (on the continent of Asia) from where it spread eastward through the Indian subcontinent to Indonesia and China and westward to the mediterranean, the Nile Valley and Africa (Lewis and

Richmond, 1972). This cotton may have been the basis of the earliest known cotton industry in Africa in the Sudan from where it spread to West Africa (Pearson and Maxwell, 1958).

Cultivation of cotton for commercial purposes in East Africa was started early this century (Munro, 1966). Cotton extension work for the express purpose of encouraging commercial production in Kenya was started in 1908 in Nyanza Province (Anthony and Brown, 1970). Since that time, Nyanza, Western and Coast Provinces have been the traditional areas growing cotton in Kenya (Acland, 1971; Thorp, 1975).

1.1.2 *Gossypium* species of commercial importance and their uses

Cultivated cotton belongs to the genus *Gossypium* found in the family Malvaceae. Commercial varieties belong to four species namely *harbaceum*, *arboreum*, *barbadense* and *hirsutum*. The varieties are distinguished from wild species by relatively long, convoluted, spinnable seed hairs. Wild species have short, rod-shaped seed hairs that cannot be spun (Lewis and Richmond, 1972). The first two species, *G. harbaceum* and *G. arboreum*, are now cultivated almost exclusively in Asia (Pearson and Maxwell, 1958; Lewis and Richmond, 1972). *G. barbadense* constitutes the commercial crop in Peru, West Indies and Egypt while *G. hirsutum* including the so called Upland cotton forms the greater part of the crop grown in the United States, Central and South America, southern Europe, the USSR, Australia, some parts of Asia and in many African countries including Kenya (Pearson and Maxwell, 1958).

Cotton has a multiplicity of uses. Cotton lint is the most important textile fibre because it is the world's most utilised fibre as compared to the combined consumption of all other fibres such as

wool, rayon and the various types of synthetic fibres (Muller, 1962; Berger, 1969; Burkitt, 1972). The lint is especially used in the manufacture of clothing materials for man.

Cotton seeds are by-products in the production of lint. They have three economic parts namely embryos (Kernels), seed coats (hulls) and residual lint (linters). From kernels is extracted valuable edible oil which is also used in the manufacture of margarine, soap and many other products (Berger, 1969; Acland, 1971; Burkitt, 1972).

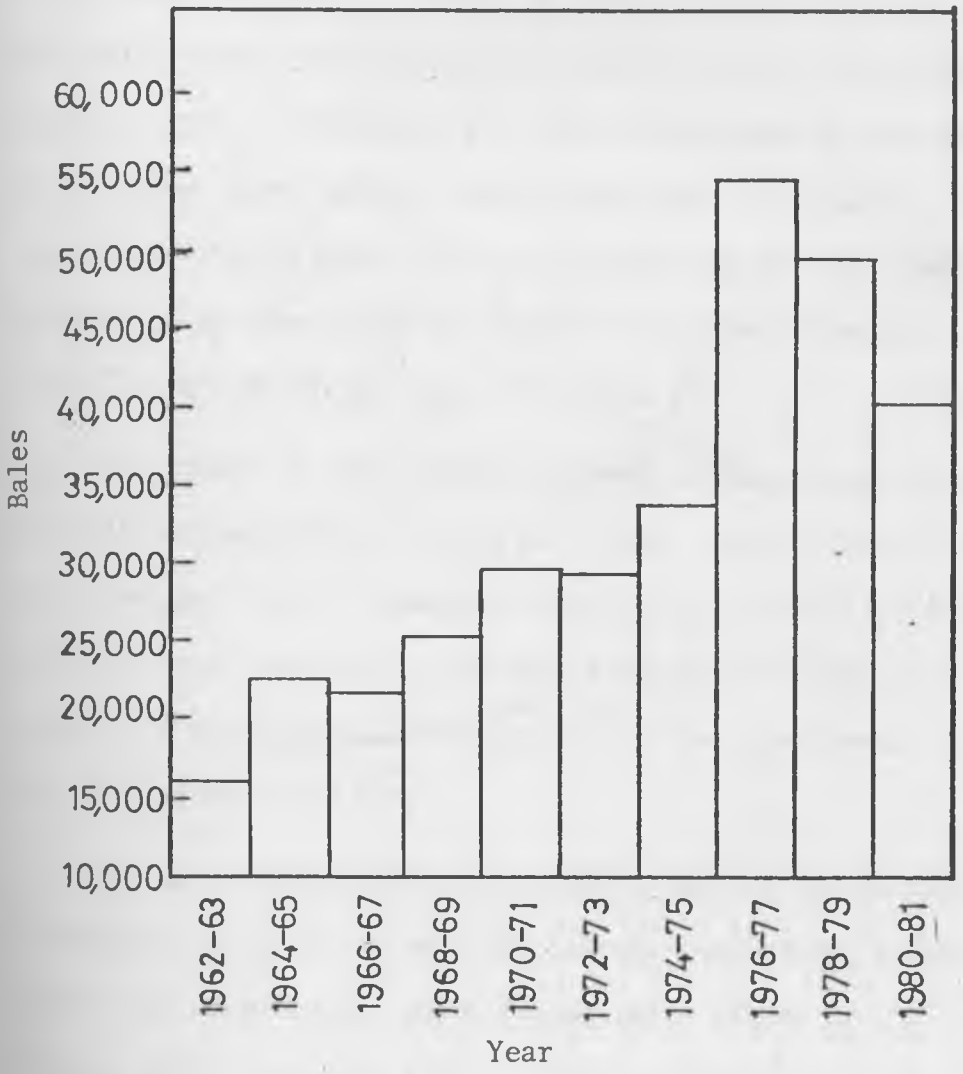
The second most important product from cotton seed is cake or meal which is used as a protein concentrate in animal rations (Berger, 1969; Burkitt, 1972). Finally, the linters are used as padding materials or as a source of cellulose in the organic chemistry industry (Berger, 1969; Burkitt, 1972).

1.1.3 Production of cotton and yield constraints in Kenya

The world production of cotton lint in 1968 was 51.8 million bales of 218 kg each (Berger, 1969). The United States produces by far the greatest proportion of the total world production followed by the USSR and China in that order (Berger, 1969). In 1970/71 Africa produced 5.8 million bales from about 28 countries (Lewis and Richmond, 1972). The most important producers in Africa are Egypt, the Sudan, Uganda, Tanzania, Zimbabwe and Mozambique in order of decreasing importance (Munro, 1966; Lewis and Richmond, 1972).

Kenya produces much less cotton than the other two East African countries (Acland, 1971). During the 1960s annual production was about 25,000 bales from approximately 80,000 ha (Acland, 1971; Fig.1). The annual production for Tanzania and Uganda for the same period was 300,000 and 370,000 bales from an area of about 500,000 to 600,000 ha

Fig. 1: Cotton production in Kenya in bales, 1962-81 (two year averages) (bale = 185 kg)



(Source: Anon., 1962-1965, 1966-1971, 1981a)

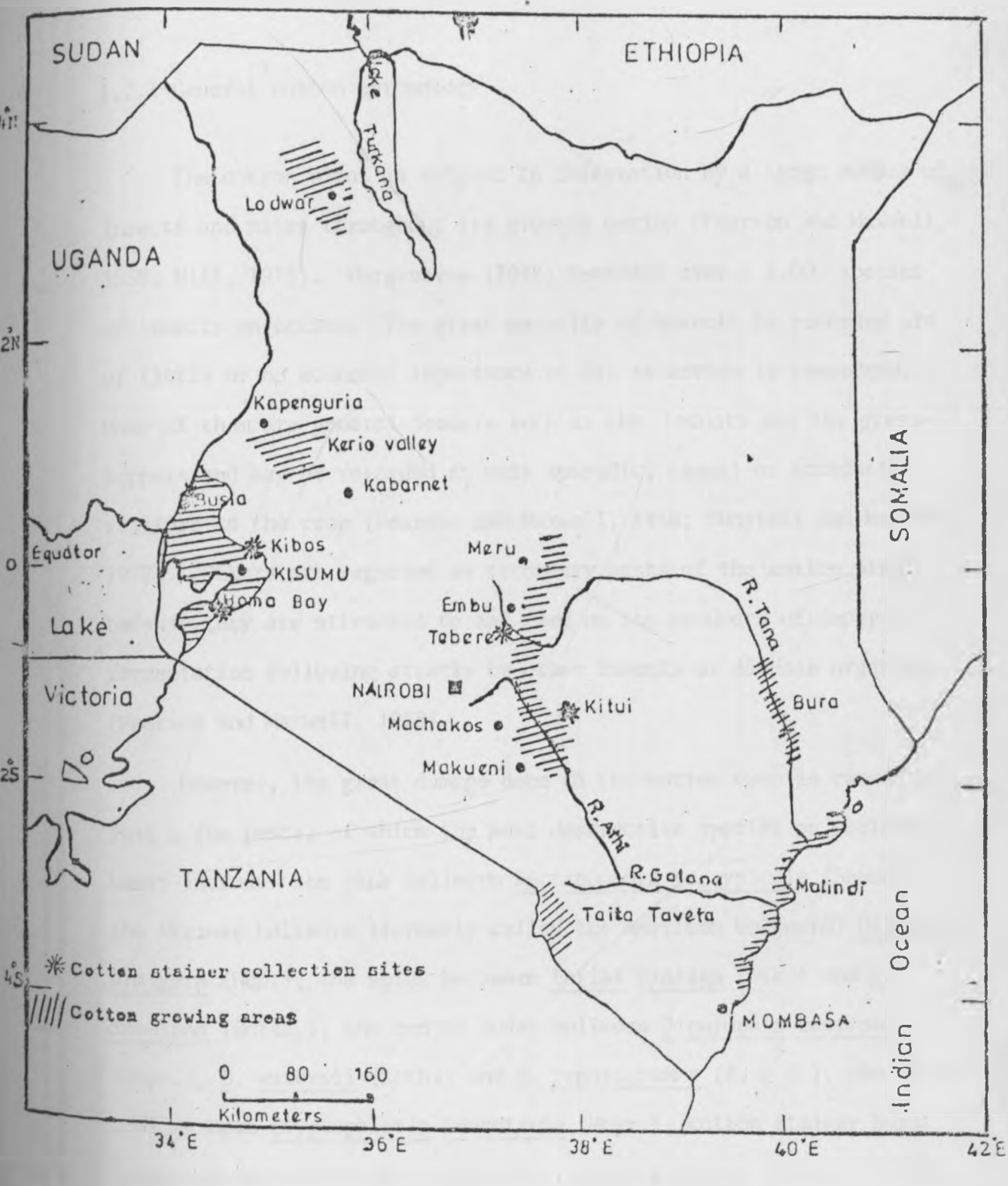
(Acland, 1971).

In Kenya, cotton is grown under both rainfed and irrigated conditions. There are three main ecological zones in which rainfed cotton is grown which are the Lake region (Nyanza and Western Provinces), the Eastern, Central and Coast Provinces (Brown et. al., 1972; Nyamasyo, 1978). Cotton in these areas is managed exclusively by small scale farmers on farm sizes of about ½ ha (Acland, 1971; Murega, 1983). Cotton has also been introduced in some parts of the Rift Valley (Kerio Valley and Turkana District) (Fig.2). Other areas where the crop is grown under irrigation include Bura and Hola irrigation schemes along the river Tana in North Eastern Province (Anon., 1984; Brown et. al., 1972; Fig. 2).

The yields of seed cotton in Kenya in most cases are low and average between 220 and 600 kg ha^{-1} under rainfed conditions (Acland, 1971; Murega, 1983). However, Brown et. al. (1972) and Acland (1971) reported that yields of up to and in excess of 3,000 kg ha^{-1} of seed cotton are obtained under irrigation and in experimental plots in research stations in Kenya.

The major bottlenecks to realising high cotton yields in this country include adverse soil and weather conditions, weeds and of significant importance, phytophagous pests (Brown et. al., 1972; Murega, 1983; Tengecho, 1984). Insect and mite pest species that attack cotton while in the field constitute a major problem to cotton growing in Kenya (Brown et. al., 1972; Murega, 1983; Murega and Khaemba, 1985a,b). Of particular significance are the cotton stainers (Dysdercus species) which infest the crop at maturity causing damage which cannot be compensated for through normal plant growth (Pearson and Maxwell, 1958; de Pury, 1968; Tengecho, 1984).

Fig. 2: Map of Kenya showing cotton growing areas



Source: Brown et. al., 1972 and personal observations.

1.2 LITERATURE REVIEW

1.2.1 General cotton entomology

The cotton plant is subject to infestation by a large number of insects and mites throughout its growing period (Pearson and Maxwell, 1958; Hill, 1975). Hargreaves (1948) recorded over a 1,000 species of insects on cotton. The great majority of insects he recorded are of little or no economic importance so far as cotton is concerned. Many of them are general feeders such as the locusts and the grasshoppers and may be regarded as only sporadic, casual or accidental visitors to the crop (Pearson and Maxwell, 1958; Tunstall and Mathews, 1972). Others are regarded as secondary pests of the cotton plant because they are attracted to and feed on the products of decay or fermentation following attacks by other insects or disease organisms (Pearson and Maxwell, 1958).

However, the great damage done to the cotton crop is caused by just a few pests, of which the most destructive species on worldwide basis include: the pink bollworm Pectinophora gossypiella (Saund), the African bollworm (formerly called the American bollworm) Heliothis armigera (Hbn.), the spiny bollworm Earias biplaga (Wik.) and E. insulana (Boisd.), the red or Sudan bollworm Diparopsis castanea (Hmps.), D. watersii (Roths) and D. tephragramma (B. & B.), the false codling moth Cryptophlebia leucotreta (Meyr.), cotton stainer bugs Dysdercus species Boisd., Lygus spp., Aphis gossypii (Glov.), thrips, cotton whitefly Bemisia tabacci (Genn.), the armyworms Spodoptera spp. and the spidermites, Tetranychus species (Linn.) (Pearson and Maxwell, 1958; Stapley and Gayner, 1969; Presley, 1972; Tunstall and Mathews, 1972).

Of the afore mentioned species, those that are economically important on the African continent are Dysdercus species, H. armigera, P. gossypiella, E. biplaga and E. insulana (Stapley and Gayner, 1969; Presley, 1972; Hill, 1975). Dysdercus, comprising of ten species in the African continent (Tengecho, 1984) are important pests in all the areas where cotton is grown in the continent (Pearson and Maxwell, 1958; Rens, 1977; Duviard, 1977; Quaison-Sackey and Kwofie, 1978; Hill, 1975).

In Kenya Dysdercus species are recognised as major pests of cotton in all parts of the country where cotton is grown (Muthamia, 1971; Brown, et. al., 1972; Rens, 1977; Tengecho, 1984). They are also reported to be particularly damaging in Eastern and Central Provinces where in some years, the bugs can cause heavier crop losses than any other cotton pests (Rens, 1977). Five species of cotton stainers have been recorded in Kenya (La Croix, 1966; Crowe, 1967, 1971; Muthamia, 1971). These are D. intermedius (Dist.), D. nigrofasciatus, D. fasciatus, D. cardinalis and D. superstitiosus.

The species D. intermedius is rare in occurence and has only been recorded around Mtwapa in Coast Province of Kenya (La Croix, 1966). In Nyanza Province, the dorminant species of cotton stainers are D. superstitiosus and D. nigrofasciatus. In Eastern and Central Provinces, D. cardinalis and D. fasciatus are the dorminant species (Crowe, 1967, 1971; Muthamia, 1971). In Coast Province, D. cardinalis is reckoned to be the most important species followed by D. fasciatus (Muthamia, 1971; Rens, 1977; Tengecho, 1984). Only four species namely D. nigrofasciatus, D. fasciatus, D. superstitiosus and D. cardinalis were used in studies reported here. These species were chosen because they are predorminant and widely distributed in all the cotton growing areas of Kenya (Muthamia, 1971).

1.2.2 Damage caused by cotton stainers to cotton

The considerable damage caused to cotton by cotton stainers has enabled these insects to be regarded as major pests of the crop (Pearson and Maxwell, 1958). They infest the crop during its early boll formation and for this reason, they are generally regarded as late season pests (Pearson and Maxwell, 1958; Muthamia, 1971). The stainers attack bolls causing considerable damage to them and their contents (Pearson and Maxwell, 1958). The damage caused to the bolls by the stainers can be categorised into two types namely, primary and secondary (Pearson and Maxwell, 1958).

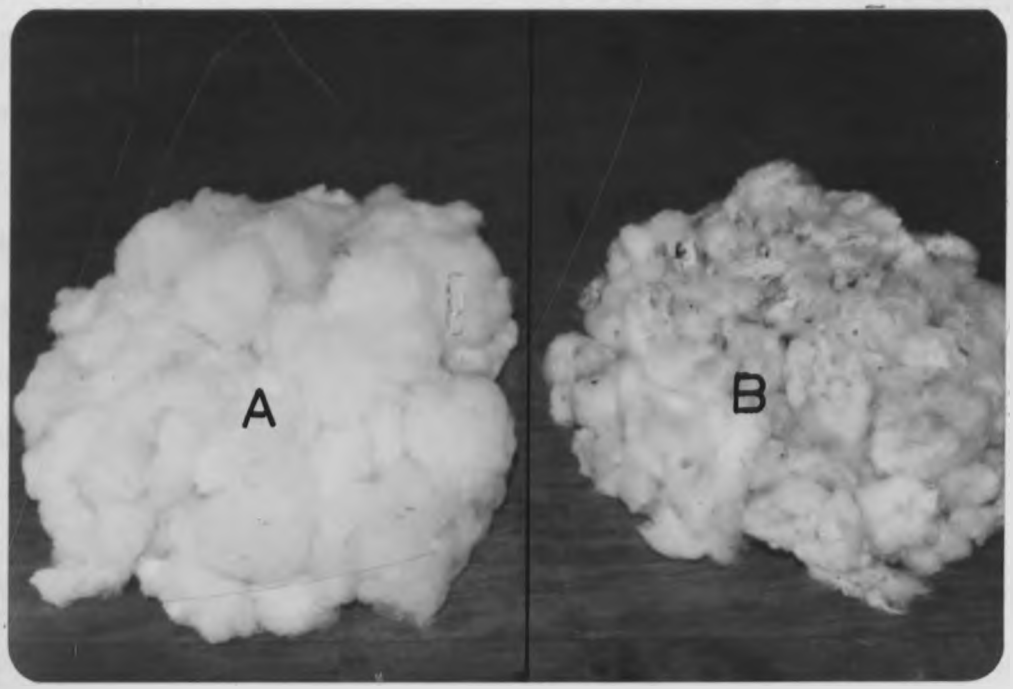
Primary damage is caused when developing seeds inside green bolls are killed due to the sucking activities of the cotton stainers. It also leads to reduction of the viability of the seeds and destruction of the embryo hence its quality as a source of oil and cotton seed cake is lowered. Besides, some bolls may shed, become stunted or open prematurely and their lint does not fluff out as in unattacked bolls (Rens, 1977).

Secondary damage is caused when the punctures created by cotton stainers are used for entry into the cotton bolls by other organisms to infect the damaged developing seeds. For example, the fungus of the genus Nematospora is known to be transmitted in attacked bolls which leads to the discoloration of lint (Plate 1) thereby lowering its value (Pearson and Maxwell, 1958; Quaison-Sackey and Kwofie, 1978).

1.2.3 Control of cotton stainers

Possible means of control of Dysdercus have been discussed by several workers (Pearson and Maxwell, 1958; La Croix, 1966; Tunstall

Plate 1: A photograph of cotton lint showing unstained lint (A) and lint stained (B) by Nematospora species.



A = unstained lint; B = stained lint

and Mathews, 1966; Muthamia, 1971; Brown, et. al., 1972; Hill, 1975). Methods of control are conveniently classified into biological and cultural on the one hand and chemical on the other.

A number of Reduviid bugs, Tachnid flies and parasitic wasps have been recorded as predators and parasites of Dysdercus species (Pearson and Maxwell, 1958; Davidson, 1964; Sweeney, 1960; Rens, 1977).

Sweeney (1960) conducted laboratory and field experiments in Rhodesia and Malawi which indicated that nymphal stages of Phonoctonus nigrofasciatus Stal. were quite effective in controlling Dysdercus species. However, he concluded that these predators were never sufficiently abundant in cotton crops to effectively control the stainers. Records of parasitism of Dysdercus by Tachnids in Africa indicate that only low levels occur. Because of this Pearson and Maxwell (1958) concluded that Tachnids exerted only slight control of the stainers.

Cultural methods have also been used effectively to minimise stainer infestations in cotton fields. These include time of planting, close season, sanitation and regular weedings (Brown, et. al., 1972; Rens, 1977; van Emden, 1977). These workers have further reported that effective control of Dysdercus and other cotton pests can only be achieved by the integration of cultural control methods and chemical control.

One of the most important methods of Dysdercus control is by spraying insecticides (Mathews, 1966; Presley, 1972; Rens, 1977; Hill, 1975). Mathews (1966) working in Malawi demonstrated that chemical control when accompanied by a high standard of crop husbandry resulted in increased yield and cleanliness of seed cotton. Chemical control is also reported to be the most effective method of control for

Dysdercus species in Kenya (Brown, et. al., 1972; Rens, 1977; Murega, 1983).

Before the synthetic pyrethroid insecticides were recommended for use by farmers on cotton in Kenya (Anon., 1979), the standard insecticides used then against Dysercus and other pests were DDT, carbaryl and Dimethoate (Brown, et. al., 1972; Rens, 1977). The use of DDT was restricted because of its undesirable effects on the environment (Anon., 1981b). Furthermore, the frequent use of DDT and Carbaryl early in the season often led to severe spidermite insfestations (Brown, et. al., 1972; Bohlen, 1973). These reasons, together with the fact these insecticides could not effectively control the entire pest complex of cotton (Brown, et. al., 1972) led to continued search for other more suitable and effective insecticides - hence the synthetic pyrethroids.

1.2.4 The development of resistance in pest species

Whenever and wherever pesticides have been used, problems have arisen due to a growing number of instances of loss of potency of a pesticide within a few years - sometimes within months - on account of the development of resistance in pests (Carson, 1958; Brown, 1961; FAO, 1969). Resistance in this context infers the loss of or decreased control of a pest's population resulting from the use of chemicals for their control.

The development of resistance has been documented for many species such as houseflies, cattle ticks and mosquitoes (Busvine, 1971; Gonzalez, 1976; Sawicki, 1979). Resistance has also been demonstrated in numerous laboratory experiments in which different species have been subjected to insecticide selection pressure (Brown and Pal, 1971a,b;

Georghiou and Taylor, 1977a,b; Nyamasyo, 1978; Nyamasyo and Karel, 1982).

Resistance is a problem not only in medical entomology but also in insects of agricultural importance in which it first appeared in 1908 and by 1958, at least about 30 species that feed on plants had become resistant. By 1969 there were about 130 species known to be resistant to chemicals (Brown, 1969). By 1980, this number had risen to 428 species of which there were 261 species of agricultural importance (Fargash, 1984). In 1978, one cotton stainer species, Dysdercus fasciatus was reported to be resistant to Carbaryl in Kenya (Nyamasyo, 1978; Nyamasyo and Karel, 1982).

The main problem related to the use of chemical pesticides is that of the development of resistance by one pest species to a number of related (or unrelated) chemicals which have never been applied for the control of that species. This phenomenon, which is known as cross resistance has been demonstrated in many pest species (Gonzalez, 1976; Fargash, 1984). For instance, in the control of the green rice leafhopper, Nephotettix species, malathion resistance was induced by fenitrothion selection and in turn malathion induced resistance against phenthoate (Gonzalez, 1972). In cross resistance, a single resistance mechanism occurs and protects against the selecting agent (or compounds).

When two or more resistance mechanisms are present in a pest species, the resistance is either multiple or multiplicate (Sawicki, 1979). Multiple resistance is the term used when the mechanisms present are distinct each protecting against a different group of insecticides. On the other hand, multiplicate resistance implies that two or more resistance mechanisms protect the insect against the same poison (Brown and Pal, 1971a,b; Crow, 1957; Gonzalez, 1972; Sawicki, 1979)

1.2.5 Justification and objectives of the present study

Cotton stainers are a major constraint to realising high cotton yields. Besides, staining of lint by Nematospora transmitted by these pests lowers its quality and as such fetches a lower market value than unstained cotton. This fact has constantly defeated the government's efforts to produce sufficient cotton lint to meet the growing domestic demand estimated at 200,000 bales yr^{-1} during the 1979/83 development plan (Tengecho, 1984). To date the target has not been achieved and production has stagnated at about 30,000 bales yr^{-1} while farm yields continue to remain low at 600 kg ha^{-1} (Murega, 1983; Tengecho, 1984).

One of the reasons why cotton stainers cannot be effectively controlled by farmers is the haphazard introduction of many new chemicals for field application against these insects before laboratory tests are done to determine their toxicities. Many workers, among them Busvine (1958, 1971) and Brempong-Yeboah et al. (1982) have demonstrat the importance of testing newly introduced insecticides in the laboratory before their recommendation for field application against pest species. In this way the toxicity levels of concerned insecticides are quantified to indicate their LC_{50s} and LC_{95s} (Busvine, 1958, 1971; Gonzalez, 1976).

By screening chemicals in the laboratory, Nyamasyo (1978) demonstrated the development of resistance in Dysdercus fasciatus against carbaryl. This stimulated interest into the establishment of the susceptibility levels of Dysdercus species collected from various sites in Kenya to selected synthetic pyrethroids, namely cypermethrin, fenvalerate and permethrin. These insecticides are currently used for the control of cotton stainers. It was hoped that studies of the type reported in this thesis will throw light on the effectiveness of the

current synthetic pyrethroids against the cotton stainers, which are major pests of cotton in Kenya. For instance, the data obtained would form a useful basis upon which reference could be made to monitor the development of resistance, if any, in cotton stainers.

It has been previously shown that when populations of insects are subjected to insecticidal selection pressure, there is a corresponding increase in the number of resistant individuals since heterozygous and homozygous susceptible genotypes are eliminated (Crow, 1957; Brown, 1960; Nyamasyo and Karel, 1982). No such previous work has been carried out in the laboratory in Kenya on cotton stainers under the selection pressure of synthetic pyrethroids. Therefore part of the studies reported here were designed to obtain this information.

The other objective of this study was to select for resistance with doses of cypermethrin, fenvalerate and permethrin equivalent to LC_{50} - LC_{70} over five generations of the test insects. The information obtained from such a study would reveal the rate at which Dysdercus species are likely to develop, if any, resistance to the synthetic pyrethroids. This information would be important in deciding on appropriate management techniques for these pests.

The residual persistence is a valuable characteristic of any insecticide since it is this property that enables the compound to control immigrant individuals that were not exposed to it during its application (Bohlen, 1973; Spielberger et. al., 1979). The residual persistence of the synthetic pyrethroids currently used by cotton farmers in Kenya were determined by spraying the crop in field and subsequently counting at known intervals live insects (Anon., 1978, 1979). There are many variables in the field situation that would interfere with the effectiveness of insecticides once applied (Busvine, 1971; Anon., 1979). This is likely to lead to inaccurate

determination of the residual properties of a compound. In the present study more accurate techniques were developed to accurately assess the residual properties of the test insecticides.

CHAPTER TWO

2.1 MATERIALS AND METHODS

2.1.1 General Procedure

Four of the five Dysdercus species occurring in Kenya were used as the test material. These species were D. fasciatus, D. nigrofasciatus, D. cardinalis and D. superstitiosus. Adult insects from which laboratory colonies were established were collected from the field in six widely separated areas of Kenya. These areas were Kibos and Homabay in Nyanza Province, Busia in Western Province, Kitui and Kibwezi (Masongoleni) in Eastern Province and Mwea-Tebere in Central Province.

The insects were reared in the laboratory for 2-3 generations to obtain laboratory strains for use in experiments reported here. Before use for bioassays, the insects were standardised in terms of age, sex, stage of development and condition of nutrition. Laboratory colonies were maintained at room temperature of 29°C (range: 28-30°C) and RH of 70% (range: 60-80%) under a 12:12 hour light regime. The temperatures were maintained within the stated limits by using an electric heater (model Metway Watts 2000) and an electric fan (model KDK type G 40 BK 40cm). The RH was maintained by the fan which blew hot air over a water bath contained in a shallow basin.

The nymphs were reared in kilner jars measuring 8.2 cm in diameter and 15 cm high (Plate 2; Fig. 3). The adults were kept in large tin cages measuring 23cm in diameter and 13cm high covered with a muslin cloth weighed down with a metal ring (Plate 3; Fig. 4).

Freshly laid eggs were collected and put into the kilner jars, the bottoms of which were lined with Whatman No. 1 filter papers

Fig. 3: Diagramatic representation of the cage used in rearing Dysdercus nymphs:

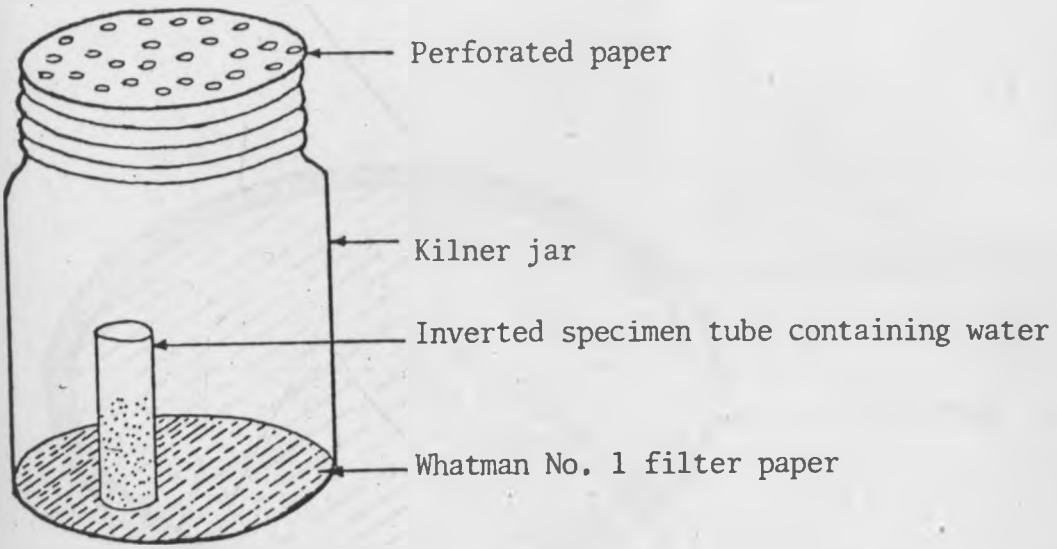


Plate 2: Photograph of kilner jar used in rearing Dysdercus nymphs.

T = Inverted specimen tube containing water

Fig. 4: Diagramatic representation of the cage used in this study to rear Dysdercus adults.

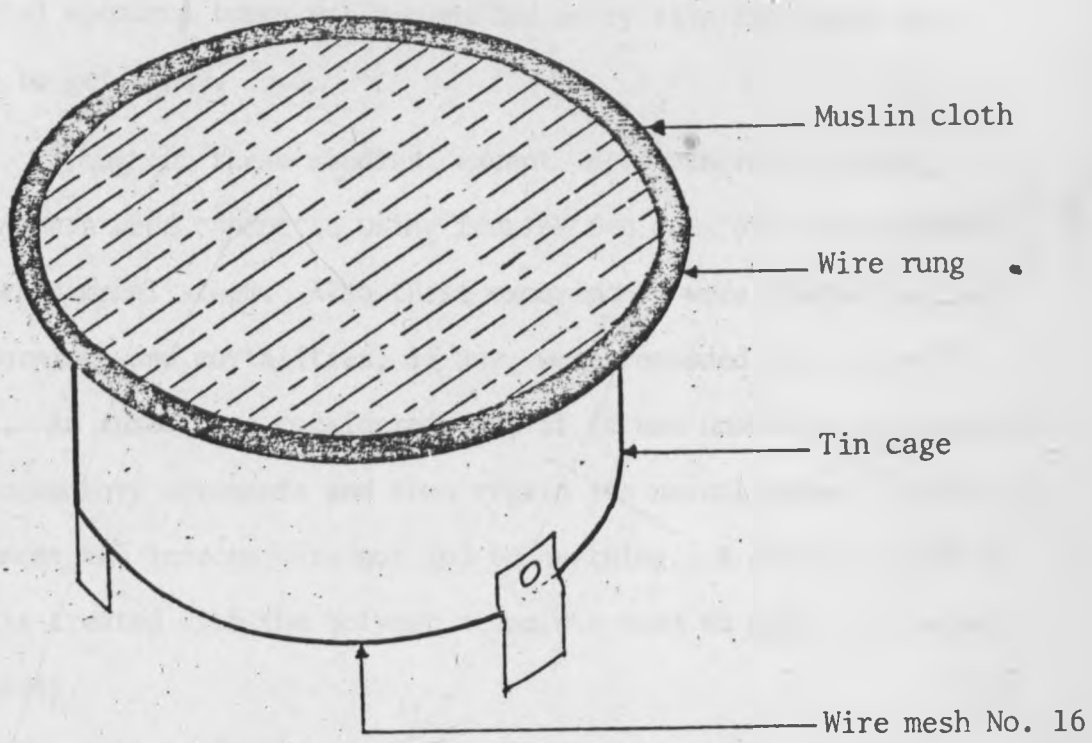


Plate 3: Photograph of cage used in this study to rear Dysdercus adults.

while the mouths of the jars were sealed with perforated paper to provide ventilation for the eggs and subsequently for the nymphs. A diet, which consisted of wet cotton seeds and a constant supply of water was given to the nymphs and adults. The cotton seed was changed every other day while the water which was contained in inverted specimen tubes was replenished every time the tubes were about to get empty.

Throughout these studies, except where otherwise stated, experiments were conducted using females 5-6 days old belonging to the 5th nymphal stage. Also these experiments were carried out in the mornings and mortalities, if any, were recorded once every 24 hours. An insect was considered dead if it was unable to co-ordinate its locomotory movements and thus regain its normal stance. After treatment the insects were not fed to anything. A control batch of insects treated with the solvent alone was used to check for natural mortality.

2.1.2 Experiment 1. Identification of a suitable solvent for the synthetic pyrethroids used in bioassays against Dysdercus species.

The objective of this experiment was to identify a suitable solvent for dissolving and diluting cypermethrin, fenvalerate and permethrin for their bioassay studies against cotton stainers. The criterion used for choosing a suitable solvent was its lethal effect on the test insects. The most suitable solvent for the three insecticides was thefore one with the least lethal effect on the test insects.

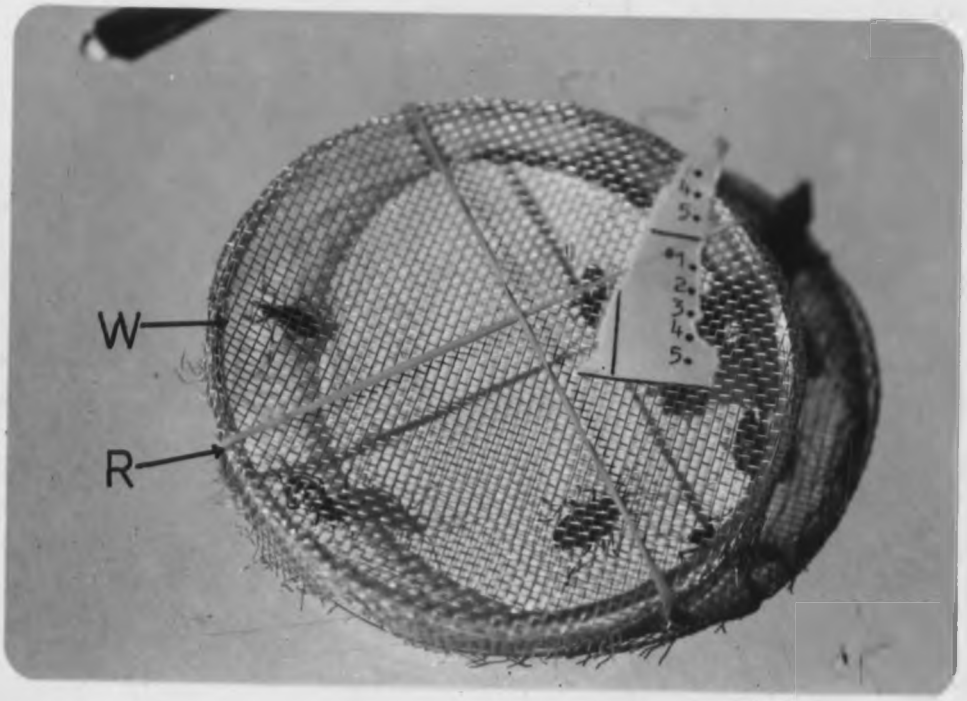
The solvents that were tested in a bid to find a suitable one for the insecticides were ordinary motor engine oil (GTS super multi-grade S.A.E. 20W/50), acetone and Isobutyl methyl ketone. The suitability of these compounds was assessed by treating the test insects with 1 µl of each of them on the caudal abdominal tergites of these insects. For each compound, 100 5th nymphal instar insects were treated. After treatment the insects were left in petri-dishes (Plate 4) for 24 hours at the end of which they were examined to count those that had died. This experiment was repeated three times.

The data collected were expressed as per cent dead insects. These data were subjected to the Arcsine transformation before they were statistically analysed for significant F-values. Duncan's New Multiple Range Test was applied to test for significance in all those means which were significantly different from each other.

2.1.3 Experiment 2. Determination of the susceptibility of various Dysdercus species collected from different parts of Kenya to cypermethrin, fenvalerate and permethrin

The objective of these experiments was to determine the susceptibility of Dysdercus species namely D. fasciatus, D. nigrofasciatus, D. cardinalis and D. superstitiosus to cypermethrin, fenvalerate and permethrin. The technical grade materials of these insecticides (cypermethrin, 73.8%; fenvalerate, 95.4%; permethrin, 92.7%) were diluted with ordinary motor engine oil. These were diluted to geometric concentration series within the following concentration ranges: cypermethrin, 1.0 to 0.028 mg/ml (0.1-0.00 28%) (Table 1a); fenvalerate, 2.93 to 0.201 mg/ml (0.3 - 0.02%) (Table 1b)

Plate 4: Treated insects confined in petri-dish.



W = Wire gauze R = Rubber band

Table 1a: Dilution chart for cypermethrin 73.8% technical grade material used in the bioassay.

Insecticide	stock solution	dilution in geometric series	
		ml*	mg/ml
		2.5	1.0
		2.0	0.8
		1.6	0.64
		1.28	0.512
		1.0	0.409
cypermethrin	0.68 ml of	0.82	0.328
	insecticide dissolved	0.7	0.262
	in 50ml of oil to	0.5	0.210
	give 10 mg/ml	0.4	0.167
		0.3	0.134
		0.27	0.107
		0.21	0.086
		0.17	0.069
		0.13	0.055
		0.11	0.044
		0.09	0.035
		0.07	0.028

Arrow denotes dilution from highest to lowest concentration (1.0 to 0.028 mg/ml). ml* denotes volume in millilitres that was taken from stock solution and made to 25ml with oil in volumetric flasks.

Table 1b: Dilution chart for fenvalerate 95.4% technical grade material used in the bioassay.

Insecticide	stock solution	dilution in geometric series	
		ml*	mg/ml
		3.7	2.93
		2.9	2.344
		2.3	1.875
		1.9	1.5
		1.5	1.2
fenvalerate	1.05 gm of insecticide	1.2	0.96
	dissolved in 50ml	0.96	0.768
	of oil to give	0.8	0.614
	20 mg/ml	0.6	0.491
		0.5	0.393
		0.4	0.315
		0.3	0.252
		0.25	0.201

Arrow denotes dilution from highest to lowest concentration. (2.93 to 0.201 mg/ml). ml* denotes volume in millilitres that was taken from stock solution and made to 25 ml with oil in volumetric flasks.

and permethrin, 2.4 to 0.262 mg/ml (0.23 - 0.026%) (Table 1c).

Before the diluted insecticidal solutions were applied to insects, they were first dissolved in Diethyl ether in the ratio 1:3 in order to facilitate ease of flow of the solution through a dispensing stainless steel hypodermic needle, gauge 20, which was attached to an insulin syringe. A manual microdroplet applicator shown in Plate 5 was used to operate the insulin syringe.

In these experiments, insects were individually treated with droplets of 1µl of each of the three insecticides on the caudal abdominal tergites. The procedure adopted for each insecticide was to treat 50 insects which were then kept in batches of 10 replicated five times. Depending on the availability of the test insects, 4-6 concentrations in geometric series of each insecticide were evaluated for effectiveness against cotton stainers. For each insecticide, the experiments were repeated three times.

For this experiment, the data collected were the number of insects killed at various insecticidal concentrations. These data were subjected to probit analyses to ascertain the lethal concentrations at 50 and 95% mortality. These were the doses of the test compounds that killed 50 and 95 per cent of the individuals in test populations of the insects.

2.1.4 Experiment 3. Induction of resistance in cotton stainers using cypermethrin, fenvalerate and permethrin.

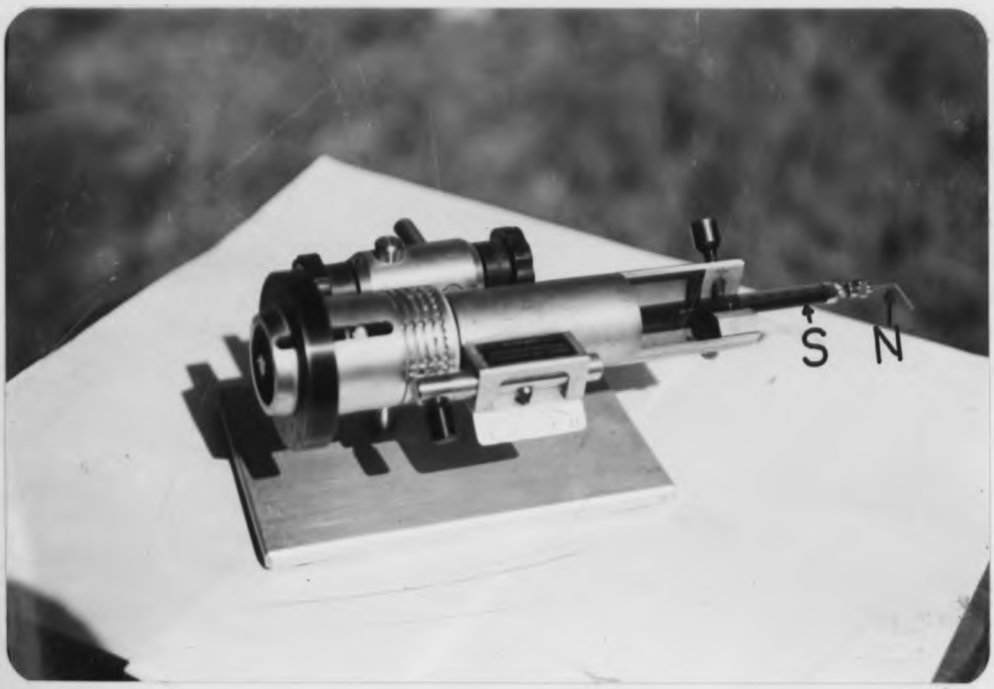
The objective of this experiment was to ascertain whether it was possible to induce resistance in cotton stainers by subjecting them to selection pressure of cypermethrin, fenvalerate and permethrin over 5 generations of the test insects. In this experiment, 5th

Table 1c: Dilution chart for permethrin 92.7% technical grade material used in the bioassay

Insecticide	stock solution	dilution in geometric series	
		ml*	mg/ml
		6.1	2.441
		4.9	1.953
permethrin	0.5 ml of insecticide	3.9	1.563
	dissolved in 50 ml	3.1	1.25
	of oil to give	2.5	1.0
	10 mg/ml	2.0	0.8
		1.6	0.64
		1.1	0.512
		1.0	0.410
		0.8	0.328
		0.7	0.262

Arrow denotes dilution from highest to lowest concentration (2.441 to 0.262 mg/ml). ml* denotes volume in millilitres that was taken from stock solution and made to 25 ml with oil in volumetric flasks.

Plate 5: Arnold's Hand-operated microdroplet applicator.



N = Needle S = Hypodermic Syringe

instar nymphs (both females and males sex ratio 1:1) aged 5-6 days old were treated topically with doses of each insecticide that had been found to give 50 - 70% (LC_{50} - LC_{70}) of the test insects in the experiment reported in section 2.1.3. The insects were subjected to these doses over 5 generations. During the 6th generation, the insects were then exposed to the full range of the insecticidal doses as follows: cypermethrin, 1.25 to 0.028 mg/ml, fenvalerate, 3.66 to 0.201 mg/ml and permethrin, 3.1 to 0.262 mg/ml. It was then possible to determine the LC_{50} and LC_{95} values of the insects after their selection with each of the test insecticides over 5 generations. Depending on the availability of the test insects, 4-6 concentrations in geometric series of each insecticide were used.

In this experiment, the procedure of treatment of the test insects was similar to that in the experiment reported in section 2.1.3. Treated insects were kept in batches of ten replicated five times. The experiment was repeated three times.

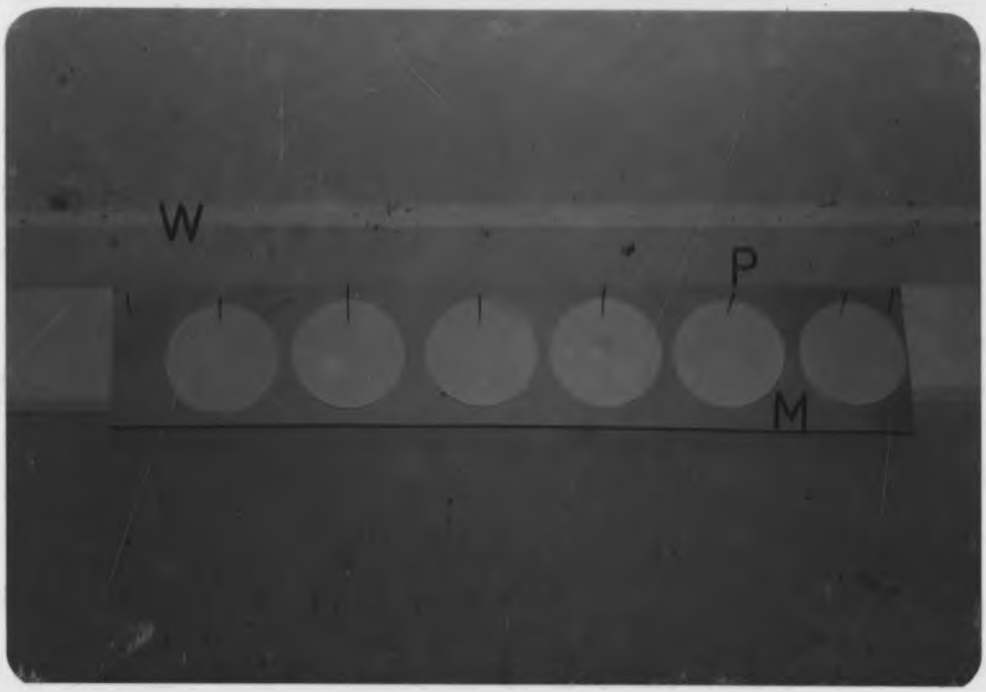
The data collected were the number of insects killed at various insecticidal concentrations used. The data was subjected to probit analyses to ascertain the lethal concentrations which killed 50 and 95 per cent of the individuals in the test populations of the insects. The resistance factors for the selected strains were obtained by dividing the LC_{50} and LC_{95} of the test insects in this experiment by those of the susceptible strains in the experiment reported in section 2.1.2. In this experiment Kibos strains of D. cardinalis and D. nigrofasciatus were used.

2.1.5 Experiment 4. Determination of the residual persistence of cypermethrin, fenvalerate and permethrin against *D. fasciatus* and *D. nigrofasciatus* at Kibos, Kenya

The objective of these experiments was to determine the residual persistence of cypermethrin, fenvalerate and permethrin under field conditions at Kibos. Laboratory experiments for the residual persistence of these chemicals were also conducted to serve as controls. In these experiments filter papers (Whatman No. 1; diameter 9.0 cm) were impregnated as uniformly as possible with 1ml of a mixture of insecticide-oil solution and Diethyl ether. These had been previously mixed in the ratio of 1:2. The filter papers were pinned on a porous pith wall (Plate 6) in the laboratory under conditions of temperature, light and RH described in the general procedure.

Prior to the actual studies, preliminary investigations had been conducted to identify doses killing 100 per cent of the test insects using the three test insecticides. It was important to determine this dose (concentration) because it would then be possible to monitor the rate of breakdown (decrease in the residual persistence) of each of the three insecticides with time after bioassays with each of the test insects. This was done by subjecting test insects to filter papers which had been impregnated with insecticides diluted in geometric series. The following concentrations were used: cypermethrin, 20, 10, and 5 mg/ml; fenvalerate, 100, 40 and 16 mg/ml; and, permethrin, 100, 40 and 16 mg/ml. In this investigation 50 insects were used at each concentration. Each treatment (concentration) was replicated three times. Three trials were conducted for this

Plate 6: Insecticide-treated filter papers pinned on a porous pith wall in the laboratory



P = Pins; W = Porous pith wall; M = Manila paper

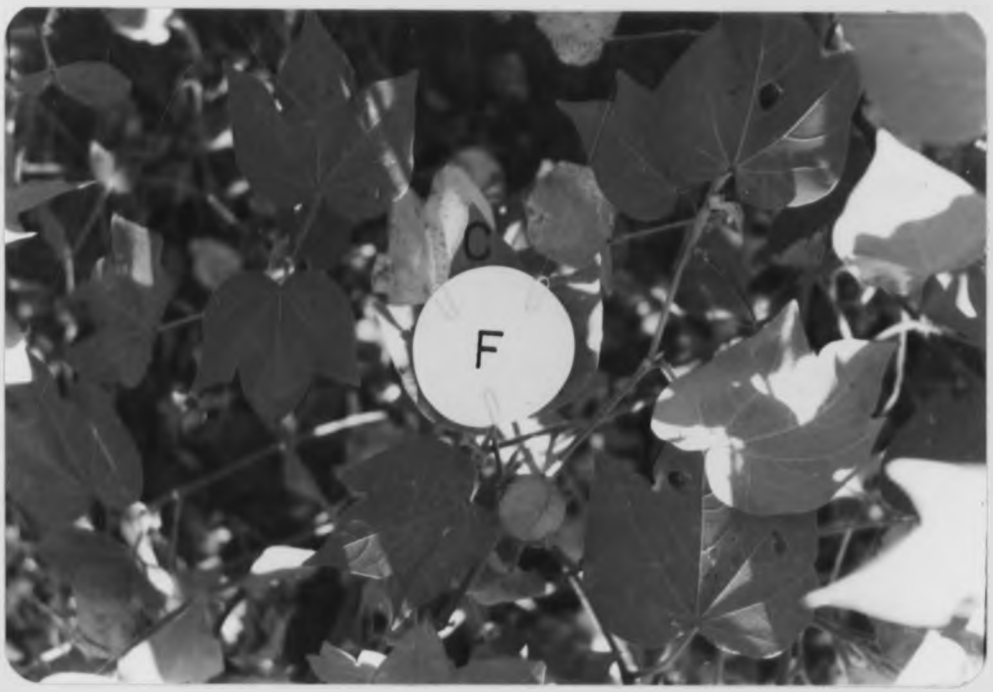
experiment. The dose(s) killing 100% of the test insects were subsequently used in experiments to determine the residual persistence of cypermethrin, fenvalerate and permethrin under field conditions at Kibos.

Filter papers were subsequently treated using the concentration of each of the three insecticides that killed 100% of the test insects. For each test insecticide, 24 filter papers were impregnated. Half of the filter papers were placed in the field while the remaining half were placed in the laboratory to serve as controls. The treated filter papers that were placed in the field were installed on leaves held on the upper surface by paper clips about 1m above the ground (Plate 7).

The leaves and cotton plants on which the filter papers were fixed were randomly chosen from a plot size of 50 x 50m with 49 rows of cotton plants. The spacing was 90 x 30 cm between and within the rows respectively with 2 plants per stand. The filter papers were placed on every 10th plant in a row of cotton plants starting from the 3rd cotton plant. Cotton plants comprising three rows on either side of the plot were not used. The experimental design used was the completely randomised block design. The number of insects used at every interval and for each insecticide was 120. Two trials were conducted for this experiment.

The chemical impregnated on the filter papers was tested for residual action (persistence) at intervals of 7, 14, 21 and 28 days after they had been treated. At each interval 3 of the filter papers placed in the field and 3 in the laboratory were removed and placed in petri-dishes. The insecticides were bioassayed by the tarsal contact method recommended by Busvine and Nash (1954) and

Plate 7: Insecticide-treated filter paper fixed on a cotton leaf, photographed from sideview



C = Cotton leaf F = Filter paper

Spielberger et. al. (1979). Test insects were confined in petri-dishes in which filter papers had been placed at the bottom to permit the insects to walk on them for five minutes. At the end of five minutes, the insects were transferred into fresh uncontaminated petri-dishes. Records on mortality were then taken after 24 hours. The data collected was used to calculate per cent mortality of the treated insects. The data was further subjected to Arcsine transformation as explained earlier in section 2.1.2. The transformed data was then statistically analysed for significant F-values. Duncan's New Multiple Range Test was applied to all the means that were found to be significantly ($P \leq 0.05$) different from each other. The mortality values were regressed with time (days) after chemical application to ascertain if there was any relationship between the residual persistence of each of the insecticides tested.

CHAPTER THREE

3.1 RESULTS

3.1.1 Experiment 1. Results of tests conducted to identify a suitable solvent for the synthetic pyrethroids used in bioassays against Dysdercus species.

The results of tests conducted using several solvents to determine which of them was the most suitable for dissolving and diluting the synthetic pyrethroid insecticides namely cypermethrin, fenvalerate, and permethrin, are presented in Table 2.

When 5-6 day old 5th nymphal instars of D. fasciatus were treated with 1µl of each of Ordinary motor engine oil, acetone and Isobutyl methyl ketone, it was observed that acetone caused a higher mortality (63.52%) than either Isobutyl methyl ketone (59.72%) or ordinary motor engine oil (13.49%) (Table 2). The differences in the mortalities resulting from the use of these solvents against D. fasciatus were significantly ($P \leq 0.05$) different with ordinary motor engine oil giving the least mortality (Table 2). Table 2 also shows that when D. nigrofasciatus were dosed with 1µl of each of the test solvents, Isobutyl methyl ketone caused the highest mortality (58.74%) than either acetone (50.45%) or ordinary motor engine oil (9.64%). As for D. fasciatus the differences in the mortalities of D. nigrofasciatus resulting from the application of the three solvents were significantly ($P \leq 0.05$) different from each other. Ordinary motor engine oil as in the previous case gave the least mortality (9.64%).

It was also evident from the results presented in Table 2 that all the three solvents tested were more harmful to D. fasciatus than

Table 2: Means of mortalities in percentages (transformed values) of four species of cotton stainers when dosed with 1 µl of each test solvent.

Species	solvent		
	Isobutyl methyl ketone	acetone	oil
	(mean % insect mortalities)		
D. fasciatus	59.72[a]	63.52[b]	13.49[c]
D. nigrofasciatus	58.74[a]	50.45[b]	9.64[c]
S.E. of a treatment mean	2.97		
F value (treatments)	67.60***		
F value (blocks)	0.5 NS		

Transformed means followed by different letters (a,b,c) across the table are significantly different from each other ($P \leq 0.05$) (Duncan's New Multiple Range Test). Transformation of % mortalities was done using Arcsine proportion.
Significant: *** $P \leq 0.001$; NS = not significant

to D. nigrofasciatus. For example, when D. fasciatus and D. nigrofasciatus were treated with Isobutyl methyl ketone, the mortalitie recorded were 59.72% and 58.74% respectively. It was therefore concluded that D. fasciatus was more susceptible to each of the three solvents tested than D. nigrofasciatus.

From the data obtained (Table 2) it was clear that Ordinary motor engine oil was the least toxic solvent to both insect species studied. Based on this observation, Ordinary motor engine oil was selected for use as a solvent for dilution of cypermethrin, fenvalerate and permethrin in subsequent experiments reported here.

3.1.2 Experiment 2. The results of studies conducted to determine the susceptibility of various Dysdercus species to cypermethrin, fenvalerate and permethrin.

The concentration-probit regression equations representing various Dysdercus species tested are shown in Tables 3a-c. The LC_{50} and LC_{95} values for the three insecticides when bioassayed with these species are also shown in Tables 3a-c. The chi-square values in Tables 3a-c are a measure of the variation in the test material (insects). Non-significant ($P \leq 0.05$) chi square indicates that the standardisation of the test insects to remove variations resulting from differences in age, sex etc. was achieved. Where this value was significant ($P \leq 0.05$) appropriate transformations were carried out to make points used in plotting the probit line lie on a straight line. Susceptibility tests performed with cypermethrin showed that there were differences in the response of the species of cotton stainers studied to the compound (Table 3a). Table 3a also shows that of the four species studied, D. fasciatus was the most susceptible to

Table 3a: Susceptibilities of 5-6 day old fifth instar Dysdercus female nymphs to cypermethrin.

Species	Strain	Regression equation of probit line	S.E. of b	Chi-square (D.F.)	LC_{50} (mg/ml)	Confidence limits (95%) of LC_{50}	LC_{95}
D. fasciatus	Mwea-Tebere	y=2.783+2.558x	0.312	6.503(4)NS	0.0736	0.0728-0.0743	0.2455
D. fasciatus	Masongoleni	y=3.023+2.420x	0.316	4.932(4)NS	0.0656	0.0650-0.0662	0.2317
D. fasciatus	Kibos	y=2.625+2.679x	0.330	3.805(4)NS	0.0769	0.0764-0.0776	0.2427
D. fasciatus	Kitui	y=2.521+2.820x	0.368	3.827(3)NS	0.0757	0.0748-0.0767	0.2188
D. cardinalis	Kibos	y=4.744x-0.589	0.765	7.380(4)NS	0.1507	0.1500-0.1514	0.2884
D. superstitiosus	Kibos	y=2.485+2.525x	0.533	11.577(4) S	0.0991	0.0968-0.1014	0.3388
D. nigrofasciatus	Kibos	y=0.699+3.501x	0.486	2.948(4)NS	0.1690	0.1683-0.1702	0.4121
D. nigrofasciatus	Busia	y=0.199+3.417x	0.509	5.995(3)NS	0.2541	0.2530-0.2559	0.6237
D. nigrofasciatus	Homabay	y=2.744+1.642x	0.226	1.497(4)NS	0.2366	0.2317-0.2415	1.5136

NS - Non significant; significant ($P \leq 0.05$); b = slope of the probit line;

D.F. = degrees of freedom (n-2), where n is the number of concentration levels of insecticide used.

cypermethrin followed in order of susceptibility by D. superstitiosus, D. cardinalis and D. nigrofasciatus. The LC_{50s} of cypermethrin for D. fasciatus ranged between 0.0736 and 0.0769 mg/ml while that for D. superstitiosus was 0.0991 mg/ml. The LC_{50s} of the same insecticide for D. nigrofasciatus ranged between 0.1690 and 0.2541 mg/ml while that for D. cardinalis was 0.1507 mg/ml.

The LC_{95s} of cypermethrin for D. fasciatus ranged between 0.2188 and 0.2455 mg/ml. Those for D. superstitiosus, D. nigrofasciatus and D. cardinalis were 0.3388, 0.4121 to 1.5136 and 0.2884 mg/ml respectively (Table 3a).

It was considered from the results presented in Table 3a that the response of D. superstitiosus (LC_{50} and LC_{95} of 0.0991 and 0.3388 mg/ml, respectively) to cypermethrin was intermediate between the response of D. fasciatus (LC_{50}, 0.0736 - 0.0769 mg/ml and LC_{95}, 0.2188 - 0.2455 mg/ml) and that of D. nigrofasciatus (LC_{50}, 0.1690 - 0.2541 mg/ml and LC_{95}, 0.4121 - 1.5136 mg/ml). It was also evident in Table 3a that the least susceptible species to cypermethrin were D. cardinalis and D. nigrofascitus.

Tests performed with Fenvalerate revealed that D. fasciatus was the most susceptible species to this insecticide judging from the LC_{50s} and LC_{95s} which ranged between 0.335 - 0.469 mg/ml and 1.023 - 1.096 mg/ml respectively (Table 3b). The LC_{50s} obtained for D. nigrofasciatus ranged between 1.349 - 1.698 mg/ml. The least susceptible species to fenvalerate was D. cardinalis in which a concentration of 0.714 mg/ml killed 50% of the individuals in the test population. A slightly higher concentration of 1.862 mg/ml led to 95% mortality of the treated insects (Table 3b). From the results presented in Table 3b for fenvalerate, it was concluded that D. nigrofasciatus was intermediate in susceptibility to Fenvalarate as compared to the rest of the species tested.

Table 3b: Susceptibilities of 5-6 day old fifth instar Dysdercus female nymphs to fenvalerate.

Species	Strain	Regression equation of probit line	S.E. of b	Chi-square (D.F.)	LC_{50} (mg/ml)	Confidence limits (95%) of LC_{50}	LC_{95}
D. fasciatus	Kibos	y=3.565+2.663x	0.365	3.350(3)NS	0.346	0.342-0.350	1.096
D. fasciatus	Mwea-Tebere	y=2.976+3.142x	0.394	3.064(4)NS	0.441	0.438-0.444	1.202
D. fasciatus	Kitui	y=3.041+2.919x	0.390	2.440(3)NS	0.469	0.466-0.472	1.349
D. fasciatus	Masongoleni	y=3.536+2.786x	0.419	3.159(3)NS	0.335	0.332-0.339	1.023
D. nigrofasciatus	Busia	y=2.835+3.083x	0.357	4.032(4)NS	0.504	0.500-0.508	1.349
D. nigrofasciatus	Homabay	y=2.964+2.984x	0.350	4.184(4)NS	0.481	0.478-0.508	1.380
D. nigrofasciatus	Kibos	y=3.318+2.457x	0.314	3.814(4)NS	0.484	0.479-0.489	1.698
D. cardinalis	Kibos	y=2.430+3.088x	0.326	2.445(5)NS	0.714	0.711-0.719	1.862

NS - Non-significant. ($P \leq 0.05$); b = slope of the probit line;

D.F. = degrees of freedom (n-2), where n is the number of concentration levels of insecticide used.

When D. fasciatus, D. cardinalis, D. superstitiosus and D. nigrofasciatus were treated with permethrin, the most susceptible species was D. fasciatus (Table 3c). Table 3c shows that the doses of the toxicant lethal to 50 and 95% of individuals in the population of insects studied ranged between 0.442 - 0.505 mg/ml and 1.047 - 1.161 mg/ml, respectively. The rest of the species namely D. cardinalis, D. superstitiosus and D. nigrofasciatus were the least susceptible judging from their LC_{50} and LC_{95} values presented in Table 3c.

The LC_{50s} presented in Tables 3a-c were plotted on a logarithmic scale to give a visual impression of the differences (relative susceptibilities) in the susceptibilities of the pests to each of the insecticides tested. The data as shown in Fig. 5 indicate that D. fasciatus was the most susceptible species to the three insecticides while D. nigrofasciatus was the least susceptible species. On the other hand, it was possible to compare the potencies of the three insecticides to the cotton stainers studied using Fig. 5. According to this figure, the potencies of the three insecticides were in descending order (ypermethrin, Fenvalerate and permethrin. For example, cypermethrin was found to be x6.7 and x5.1 more potent than permethrin and fenvalerate respectively when D. fasciatus (Masongoleni) was used (since potency is the inverse of the ratio of an equitoxic dose). These potency values were obtained by dividing the LC_{50s} of ermethrin and Fenval rate by that of cypermethrin for the stated species.

The overall impression gained from data presented in Fig. 5 is that D. fasciatus was the most susceptible species of the four species treated with cypermethrin, fenvalerate and permethrin. The susceptibility of the other three Dysdercus species varied from insecticide to insecticide.

Table 3c: Susceptibilities of 5-6 day old fifth instar Dysdercus female nymphs to permethrin.

Species	Strain	Regression equation of probit line	S.E. of b	Chi-square (D.F.)	LC_{50} (mg/ml)	Confidence limits (95%) of LC_{50}	LC_{95}
D. fasciatus	Kitui	y=2.174+4.252x	0.543	4.281(4)NS	0.462	0.460-0.463	0.933
D. fasciatus	Kibos	y=1.928+4.363x	0.531	3.547(5)NS	0.505	0.504-0.506	1.161
D. fasciatus	Mwea-Tebere	y=1.806+4.710x	0.647	3.870(3)NS	0.476	0.475-0.479	0.920
D. fasciatus	Masongaleni	y=2.660+3.626x	0.518	2.346(2)NS	0.442	0.439-0.446	1.047
D. nigrofasciatus	Kibos	y=0.576+4.862x	0.654	4.966(5)NS	0.813	0.811-0.815	1.531
D. nigrofasciatus	Homabay	y=6.268x-0.840	1.004	2.429(3)NS	0.851	0.849-0.855	1.109
D. nigrofasciatus	Busia	y=5.945x-0.102	0.830	0.659(3)NS	0.721	0.719-0.724	1.202
D. superstitiosus	Kibos	y=0.851+4.530x	0.555	5.346(4)NS	0.824	0.820-0.826	1.288
D. cardinalis	Kibos	y=1.028+4.403x	0.550	4.682(4)NS	0.798	0.794-0.802	1.06

NS - Non-significant; ($P \leq 0.05$); b= slope of the probit line;

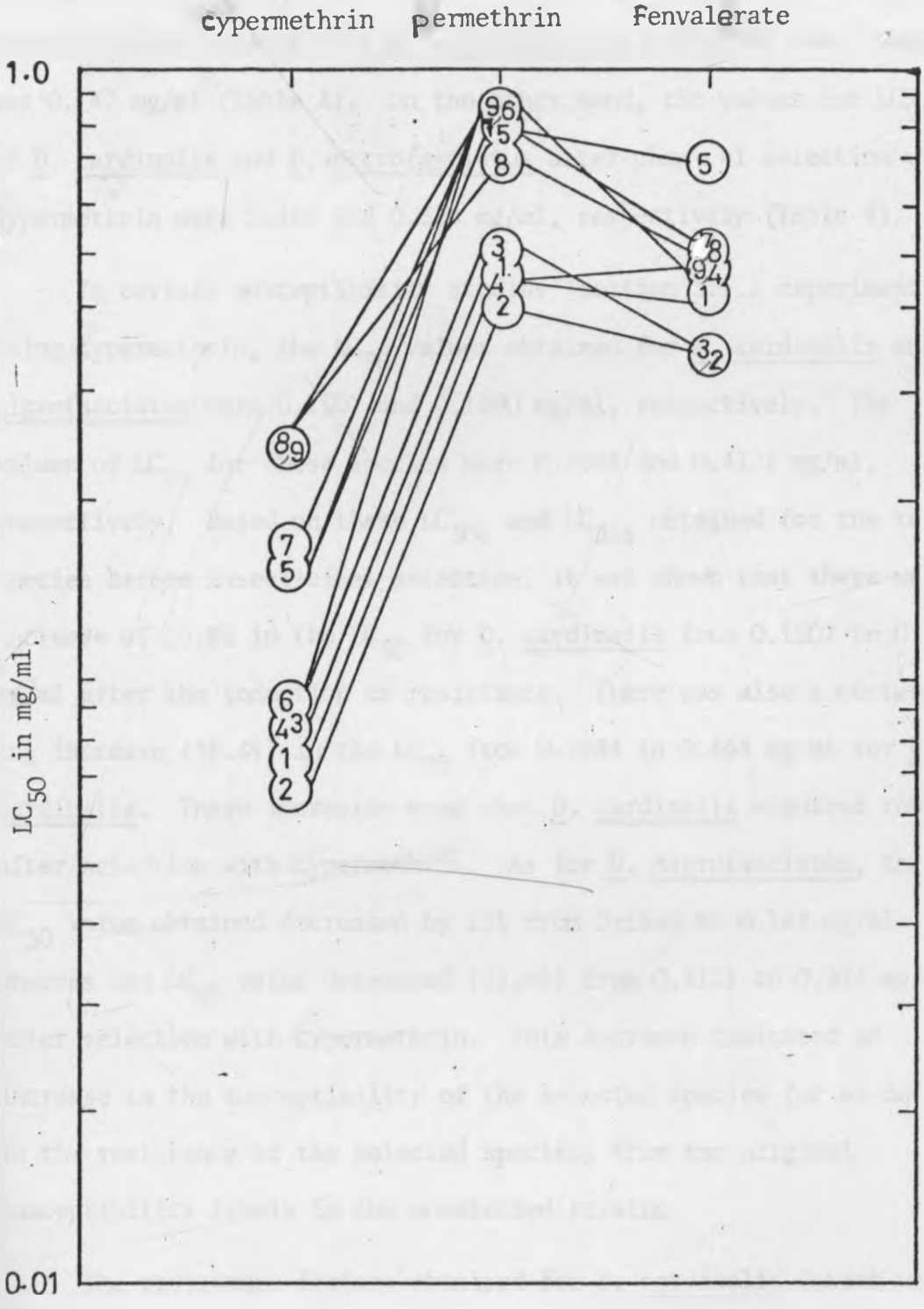
D.F. = degrees of freedom (n-2), where n is the number of concentration levels of insecticide used.

It is also evident in Tables 3a-c and Fig. 5 that there were differences in the susceptibilities of different strains of Dysdercus species to cypermethrin, fenvalerate and permethrin. For example, when treated with cypermethrin, the LC_{50s} of all strains of D. fasciatus tested were as follows: Mwea-Tebere strain, 0.0736 mg/ml; Kibos strain, 0.0769 mg/ml; Kitui strain, 0.0757 mg/ml and the Masongoleni strain, 0.0656 mg/ml (Table 3a). The strains of D. nigrofasciatus tested with cypermethrin reacted as follows: Kibos strain, 0.1690 mg/ml; Busia strain, 0.2541 mg/ml and Homabay strain, 0.2366 mg/ml. The results show that the susceptibilities of strains of D. fasciatus were higher than those of strains of D. cardinalis, D.nigrofasciatus and D. superstitiosus to the test insecticides. It was therefore concluded from these observations that the magnitude of susceptibility of the stainers varied according to the species and strain of the pest tested.

3.1.3 Experiment 3. Results of studies conducted to induce resistance in Dysdercus species by subjecting them to insecticide selection pressure with cypermethrin, fenvalerate and permethrin

The probit regression equations representing the responses (slope) of D. cardinalis and D. nigrofasciatus which were the species that were used in resistance studies reported here are shown in Table 4. These regression equations were used to plot the log concentration (dose) - probit regression curves presented in Fig. 6a-f. Table 4 also shows the median lethal concentrations (LC_{50}) and LC_{95} values of the two test Dysdercus species after selection with each of the three test insecticides.

Fig. 5: Median lethal concentrations (mg/ml) of the indicated insecticides to 5-6 day old fifth instar female Dysdercus nymphs.



Concentrations are indicated on a vertical logarithmic scale and the various species are indicated by numbers as follows:

1. D. fasciatus, Mwea-Tebere
2. D. fasciatus, Masongoleni
3. D. fasciatus, Kibos
4. D. fasciatus, Kitui
5. D. cardinalis, Kibos
6. D. superstitiosus, Kibos
7. D. nigrofasciatus, Kibos
8. D. nigrofasciatus, Busia
9. D. nigrofasciatus, Homabay

When D. cardinalis was subjected to selection pressure using cypermethrin the LC_{50} value obtained was 0.2060 mg/ml. The LC_{50} value obtained after selection of D. nigrofasciatus using the same chemical was 0.147 mg/ml (Table 4). On the other hand, the values for LC_{95} of D. cardinalis and D. nigrofasciatus after chemical selection using cypermethrin were 0.468 and 0.324 mg/ml, respectively (Table 4).

In earlier susceptibility studies (section 3.1.2 experiment 2) using cypermethrin, the LC_{50} values obtained for D. cardinalis and D. nigrofasciatus were 0.1507 and 0.1690 mg/ml, respectively. The values of LC_{95} for these species were 0.2884 and 0.4121 mg/ml, respectively. Based on these LC_{50s} and LC_{95s} obtained for the two species before insecticidal selection, it was shown that there was an increase of 26.8% in the LC_{50} for D. cardinalis from 0.1507 to 0.2060 mg/ml after the induction of resistance. There was also a corresponding increase (38.4%) in the LC_{95} from 0.2884 to 0.468 mg/ml for D. cardinalis. These increases mean that D. cardinalis acquired resistance after selection with cypermethrin. As for D. nigrofasciatus, the LC_{50} value obtained decreased by 13% from 0.1690 to 0.147 mg/ml whereas its LC_{95} value decreased (21.4%) from 0.4121 to 0.324 mg/ml after selection with cypermethrin. This decrease indicated an increase in the susceptibility of the selected species (or as decrease in the resistance of the selected species) from the original susceptibility levels in the unselected strains.

The resistance factors obtained for D. cardinalis (which were based on LC_{50} and LC_{95}, respectively) were x1.4 and x1.6 (Table 4). As for D. nigrofasciatus, these were x0.9 and x0.8. Judging from these resistance factors, it was concluded that D. cardinalis had a higher response to chemical selection than D. nigrofasciatus when cypermethrin was used. This indicated that D. cardinalis would

Table 4: Response of Dysdercus species (Kibos strains) to insecticides after insecticidal selection over five generations.

Selecting insecticide	Species	Regression equation of probit line	LC_{50} (mg/ml) before selection	LC_{95} (mg/ml) before selection	LC_{50} (mg/ml) after selection	LC_{95} (mg/ml) after selection	Resistance factors based on LC_{50}	Resistance factors based on LC_{95}
cypermethrin	D. cardinalis	y=0.150+3.690x	0.1507	0.2884	0.206	0.468	1.4	1.6
cypermethrin	D. nigrofasciatus	y=0.290+4.024x	0.1690	0.4121	0.147	0.324	0.9	0.8
fenvalerate	D. cardinalis	y=5.634x-0.474	0.714	1.862	0.937	1.622	1.3	0.9
fenvalerate	D. nigrofasciatus	y=3.156+2.388x	0.484	1.698	0.592	2.138	1.2	1.3
permethrin	D. cardinalis	y=2.245+2.847x	0.798	1.06	0.928	2.754	1.2	2.7
permethrin	D. nigrofasciatus	y=0.160+5.019x	0.813	1.531	0.921	1.698	1.1	1.1

develop resistance much faster than D. nigrofasciatus in fields treated with this pyrethroid.

The log concentration-probit regression curves (Fig.6a-f) indicate that there was a linear relationship between the insecticidal doses applied and insect mortality obtained. This meant that as the chemical dose was increased, there was a corresponding increase in the number (or percentage) of insects killed. For example, it is shown in Fig.6a that there was both a decrease in slope and a shift to the right of the curve representing strains of D. cardinalis used in resistance studies with cypermethrin. This suggests that a higher dose (Fig. 6a, point x) was required to kill as many insects as those killed in the unselected population at point u (Fig. 6a). As shown in Table 4, the LC_{50} for the unselected strain of D. cardinalis increased from 0.1507 to 0.206 mg/ml in the selected strain. On the other hand, there was a shift to the left of the curve for D. nigrofasciatus when cypermethrin was used. This suggested that there was a decrease in the dose (Fig. 6b, point x) of cypermethrin used on D. nigrofasciatus after selection.

Tests with D. cardinalis after selection with Fenvalerate gave an LC_{50} value of 0.937 mg/ml (Table 4). The LC_{50} value before the insects were subjected to fenvalerate selection pressure was 0.714 mg/ml. The LC_{95} values as determined for this species before and after selection with fenvalerate were 1.862 and 1.622 mg/ml respectively (Table 4). The resistance factor obtained for this species based on LC_{50} was x1.3 while that based on LC_{95} was x0.9. The log concentration-probit regression curves for the unselected and selected strains of D. cardinalis for fenvalerate cross each other at point z (Fig. 6c). This indicates that after insecticidal selection, there was a mixture of both strains of D. cardinalis which had acquired resistance and those that had not acquired resistance. Thus the insects treated at

Fig. 6a: Log concentration-probit lines of D. cardinalis before and after selection

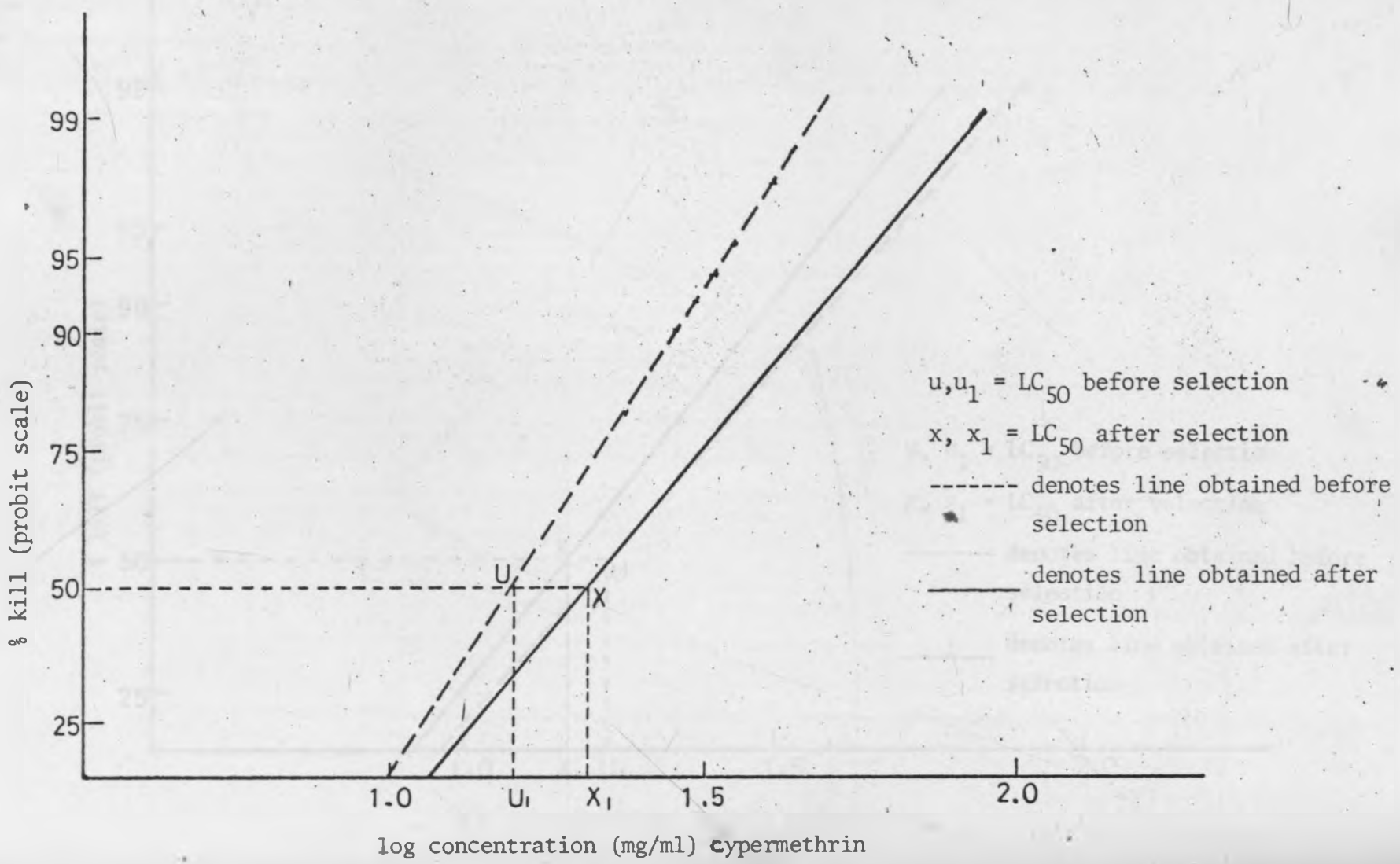


Fig. 6b: Log concentration-probit lines of D. nigrofasciatus

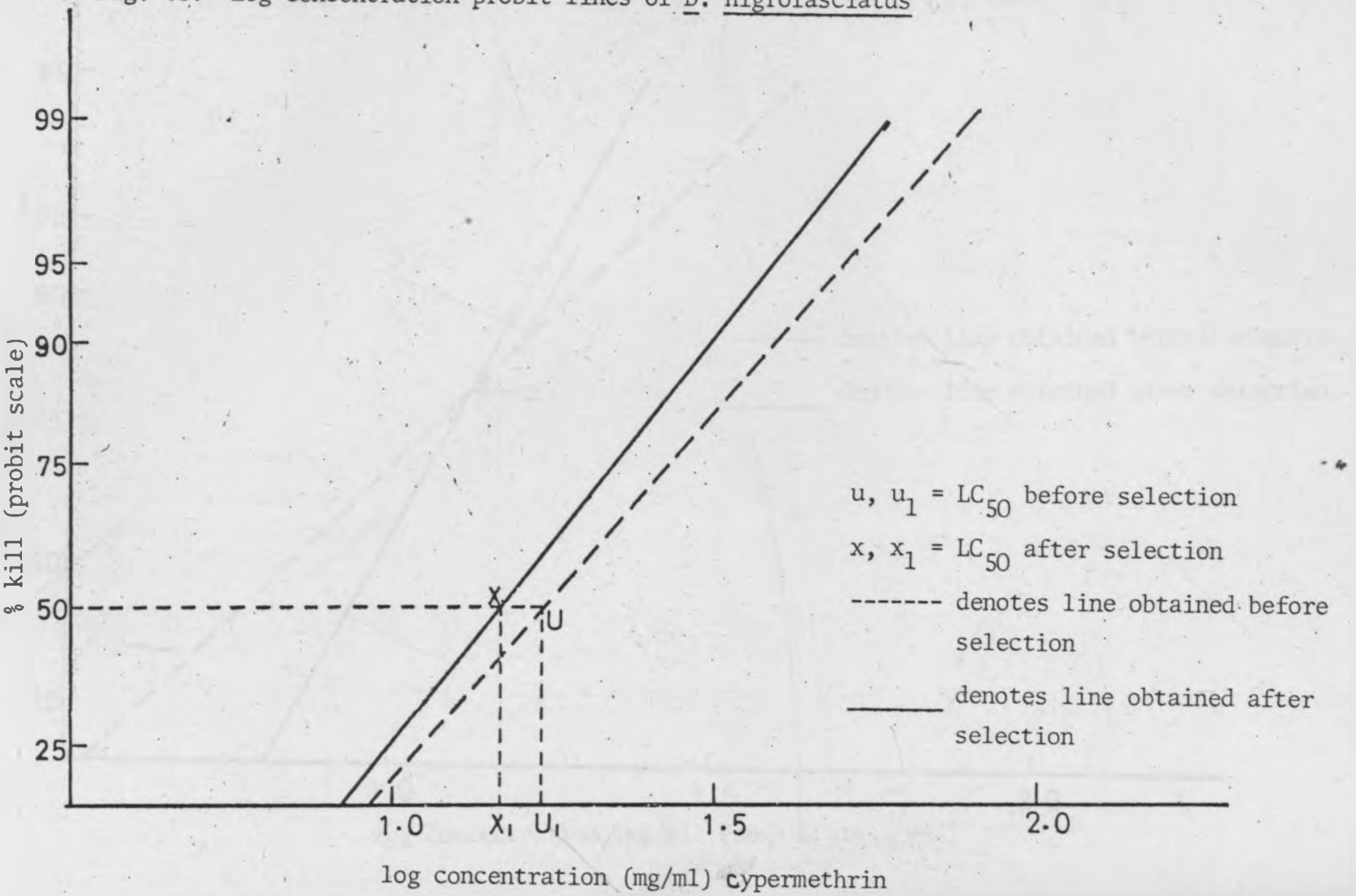
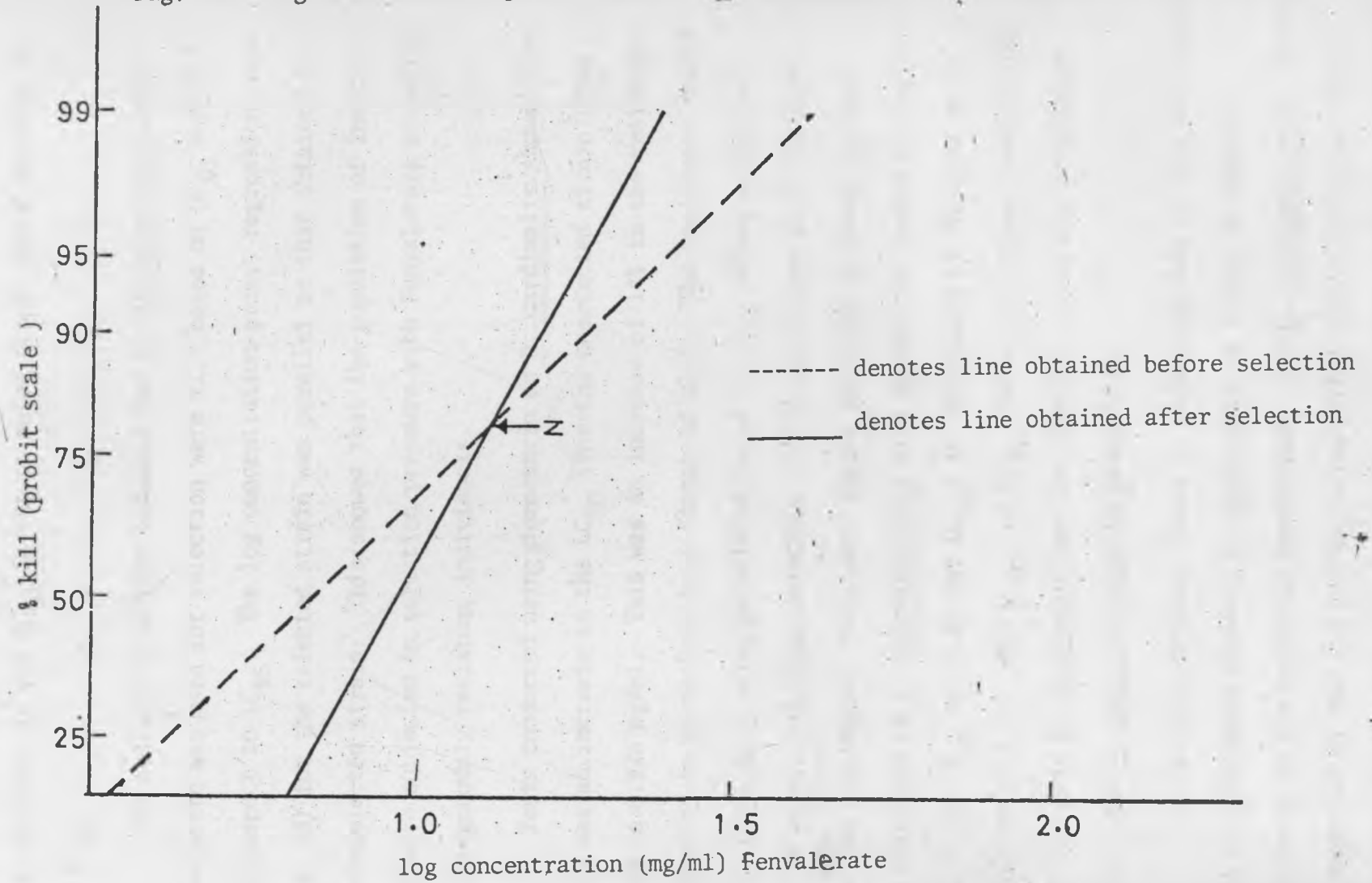


Fig. 6c: Log concentration-probit lines of D. cardinalis before and after selection



the lower doses of the insecticide were more resistant at these doses than were those subjected to treatment at the higher doses. This is shown by an increase in the LC_{50} in the selected strain and a decrease in its LC_{95}.

The resistance factors obtained for D. nigrofasciatus when fenvalerate was used for selection were x1.2 based on LC_{50} and x1.3 with respect to LC_{95}. The log concentration-probit regression curve (Fig. 6d) for the selected strain was parallel to that obtained for the unselected strain. This showed that the population of insects that were subjected to selection pressure with Fenvalarate consisted of homogenously resistant individuals.

Tests conducted using permethrin on D. cardinalis showed that there was an increase in the LC_{50} (for the unselected strain) from 0.798 to 0.928 mg/ml. This was an increase of 14% in the resistance of insects to permethrin by a factor of x1.2. The resistance factor of D. cardinalis using permethrin based on LC_{95} before selection (1.06 mg/ml) and after selection (2.754 mg/ml) was x2.7 (Table 4). This was the highest resistance factor recorded in these studies. The selection of D. nigrofasciatus with permethrin caused an increase (11.7% for LC_{50} and 9.8% for LC_{95}) in resistance of the pest by a factor of x1.1 for both LC_{50} and LC_{95} (Table 4). These results also indicate that D. cardinalis has the capacity to develop resistance faster than D. nigrofasciatus to permethrin.

The results obtained (Table 4) indicated that of the two species used in resistance studies, D. cardinalis is likely to develop resistance to the synthetic pyrethroids than D. nigrofasciatus. This is supported by the log concentration-probit regression lines which show the large shifts for D. cardinalis as compared to those obtained for D. nigrofasciatus. It was concluded for these studies that

Fig. 6d: Log concentration - probil lines of D. nigrofasciatus before and

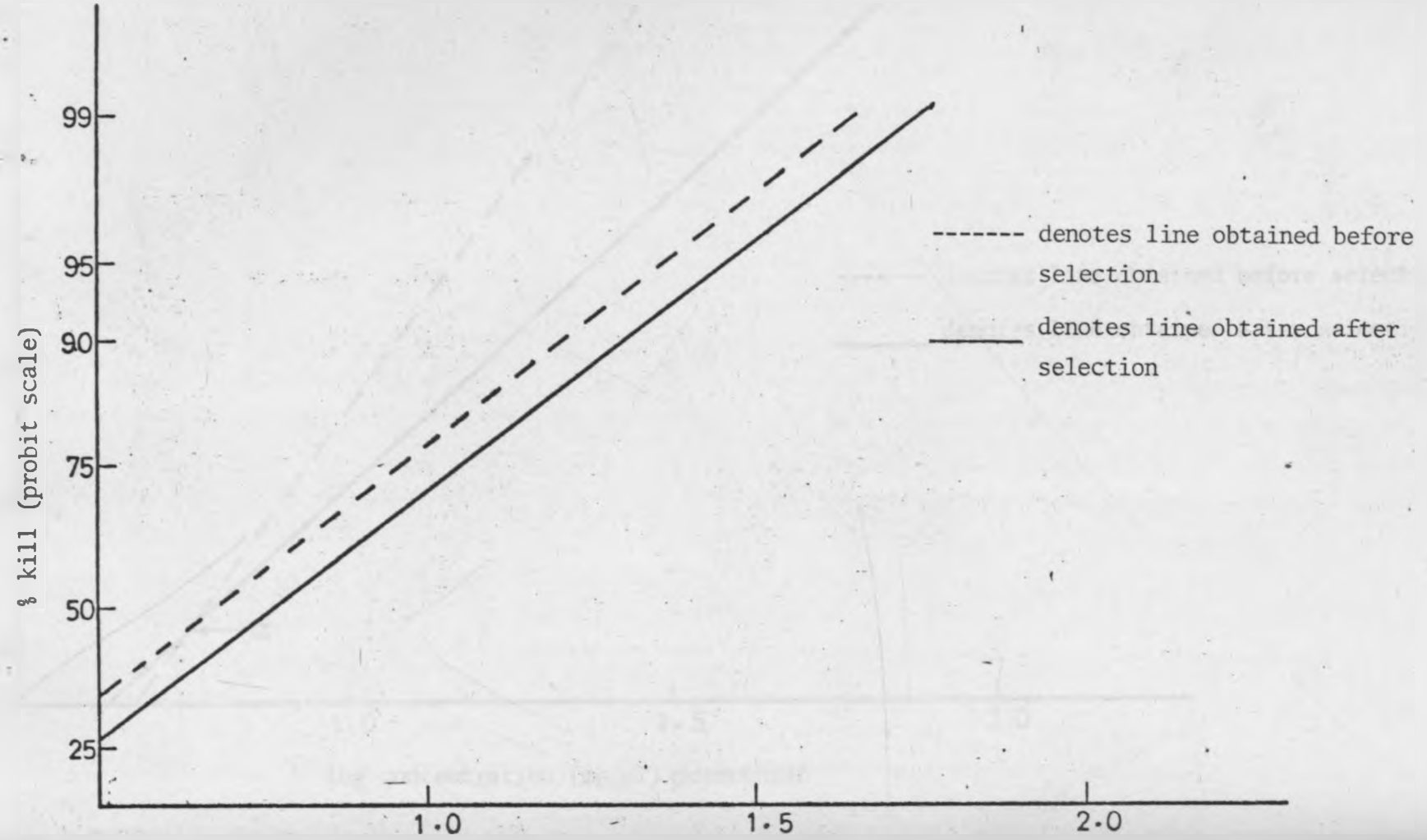


Fig. 6e: Log concentration-probit lines of D. cardinalis before and after selection

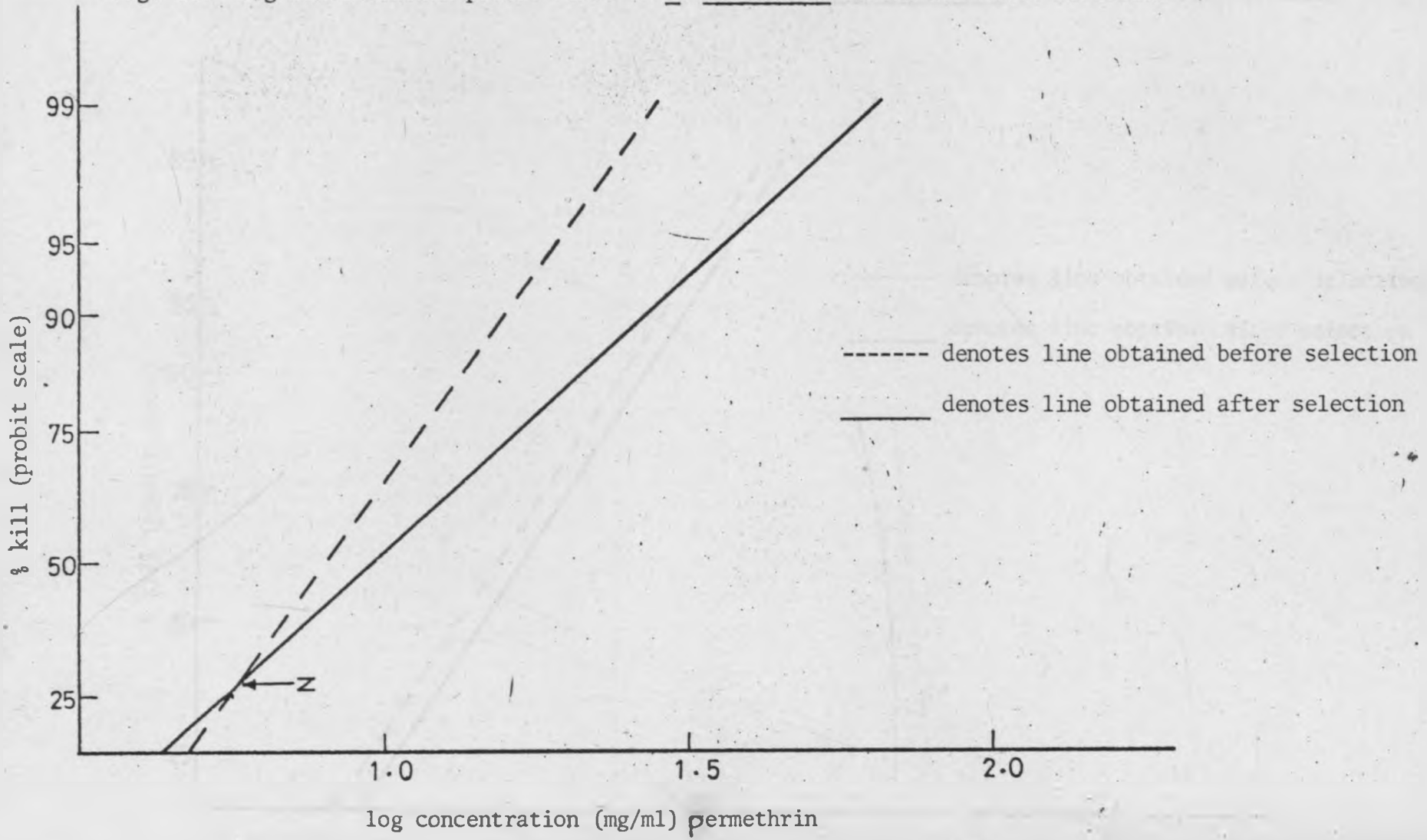
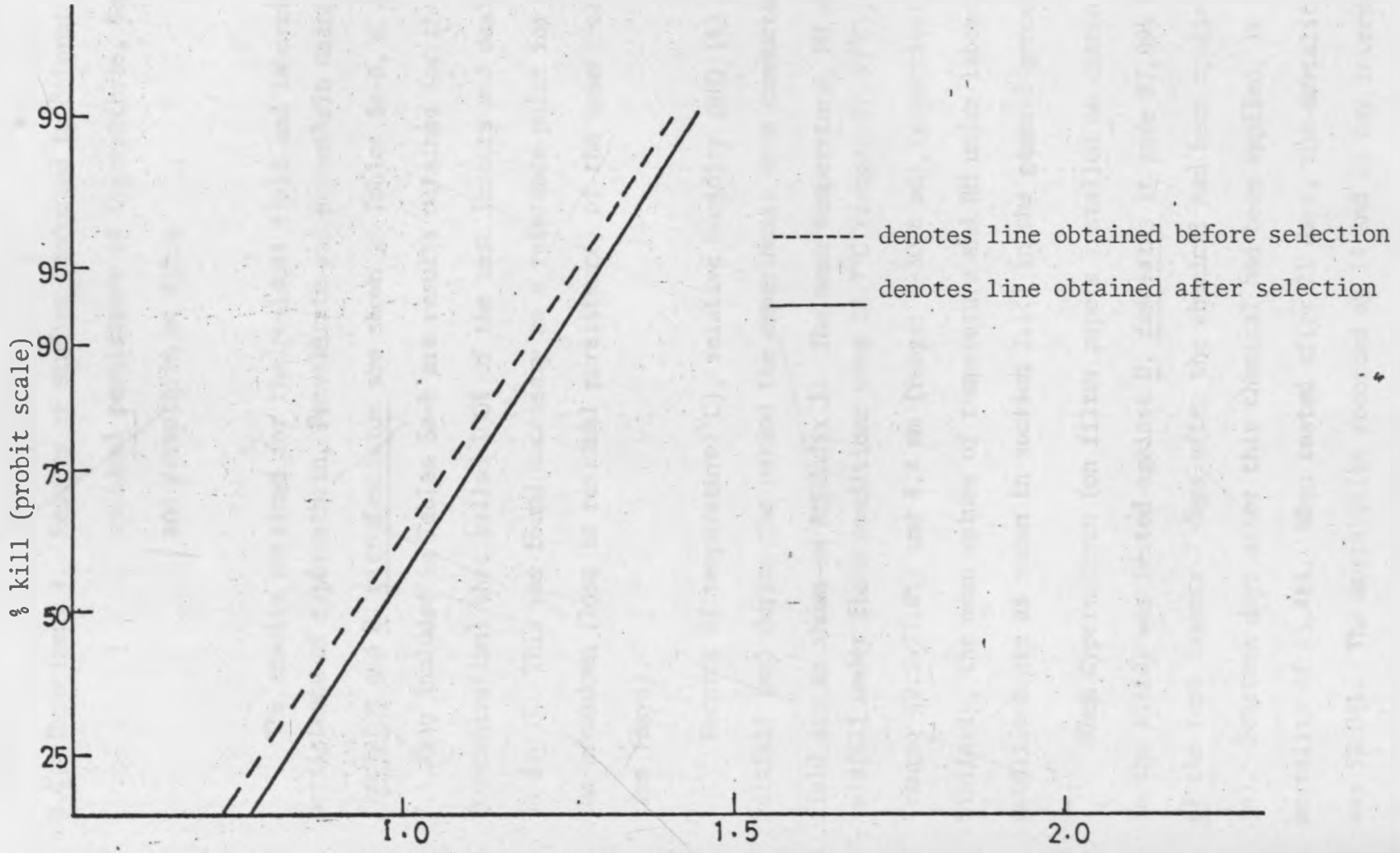


Fig. 6f: Log concentration - probit lines of D. nigrofasciatus before and after selection



Dysdercus species are capable of developing resistance to the synthetic pyrethroids cypermethrin, fenvalerate and permethrin.

3.1.4 Experiment 4. Results of studies conducted to determine the residual persistence of cypermethrin, fenvalerate and permethrin at Kibos

The results obtained for the residual field and laboratory persistence of cypermethrin, fenvalerate and permethrin using D. fasciatus and D. nigrofasciatus are shown in Tables 5a-b, 6 and Fig. 7. Also included in Tables 5a-b are results obtained for the dose (concentration) that killed 100% of the test insects and designated as day 0. This was found necessary as a reference point for monitoring the breakdown (loss in residual persistence) of the insecticides with time (days).

Records of temperature(^{o}C), relative humidity (RH) (%) and rainfall (mm) during the period the experiments were conducted in the field are as shown in Appendix I. The mean temperature, RH and rainfall under field conditions were 28.7^{o}C (range: 27-30^{o}C), 68.4% (range: 49.5-77.8%) and 4.4 mm (range: 0-30.8 mm) respectively. Similarly, the mean values of temperature and RH under laboratory conditions are as shown in section 2.1.1 in the general procedure.

When cypermethrin (on filter papers installed on cotton plants in the field) was tested against D. fasciatus it gave 87.99% mortality of the test insects 7 days after the chemical had been applied (Table 5a). Fourteen days after this chemical had been applied, it caused a mortality of 65.48%. When tested after 21 days, the mortality recorded was 59.04%. The mortalities recorded at 14 and 21 day intervals were not significantly ($P \leq 0.05$) different from each other. However, there

was significant ($P \leq 0.05$) difference between these mortalities and that recorded after 7 days. When tested after 28 days, 48.09% of the insects were recorded dead. This mortality was significantly ($P \leq 0.05$) different from those recorded at all the other previous intervals (Table 5a).

Filter papers treated with cypermethrin and placed in the laboratory caused the following mortalities after tests: at 7 days, 90%; at 14 days, 84.50%; at 21 days, 75.41% and 63.02% at 28 days (Table 5a). These mortalities were significantly ($P \leq 0.05$) different from each other. It is shown by the results presented in Table 5a that cypermethrin applied on filter papers which were kept in the laboratory caused higher mortalities than was the case with the same chemical applied on filter papers which were placed on cotton plants in the field.

It was also observed that when D. fasciatus was used to test the residual action of fenvalerate in the field the mortalities it incurred were: 72.12, 65.96, 55.06 and 29.14% at intervals of 7, 14, 21 and 28 days respectively (Table 5a). There were significant ($P \leq 0.05$) differences in the mortalities obtained (Table 5a) except those obtained at intervals of 7 and 14 days. For filter papers which were placed in the laboratory the mortalities realised for D. fasciatus with fenvalerate were significantly ($P \leq 0.05$) different at all the intervals (Table 5a). It is also shown by data presented in Table 5a that fenvalerate applied on filter papers which were kept in the laboratory caused higher mortalities than was the case with the same chemical applied on filter papers which were placed in the field.

Tests in which permethrin was used gave 83.96% kill of D. fasciatus 7 days later (Table 5a) under field conditions. When tested 14 days after exposure in the field the chemical killed 61.21% of the

Table 5a: Mean mortalities (transformed % values) of D. fasciatus when exposed to insecticide treated filter papers with cypermethrin, fenvalerate and permethrin.

Interval (days)	Insecticide					
	cypermethrin		fenvalerate		permethrin	
	(percentage mortality of cotton stainers)					
	F_{90}[a]	L_{90}[a]	F_{90}[a]	L_{90}[a]	F_{90}[a]	L_{90}[a]
0	90[a]	90[a]	90[a]	90[a]	90[a]	90[a]
7	87.99[a]	90[a]	72.12[b]	82.47[b]	83.96[a]	90[a]
14	65.48[b]	84.50[b]	65.96[b]	68.64[c]	61.21[b]	73.37[b]
21	59.04[b]	75.41[c]	55.06[c]	51.54[d]	46.41[c]	67.03[b]
28	48.09[c]	63.02[d]	29.14[d]	34.52[e]	42.24[c]	32.44[c]
Overall means	70.12	8059	62.46	65.43	64.76	7057
S.E. (treatment means)	2.83	1.48	2.77	2.06	2.13	3.27
C.V. %	7.00	3.19	7.67	5.46	5.69	8.04
F. values (treatments)	41.88***	59.98***	66.31***	120.99***	102.99***	51.99***
F values (blocks)	0.33NS	3.59NS	0.26NS	0.93NS	3.90NS+	0.09NS

Transformed means in each column with different letters are significantly different from each other ($P<0.05$) (Duncan's New Multiple Range Test). Transformation of % mortalities was done using Arcsine proportion. NS: non-significant; significant ***$P<0.001$. F = mortalities recorded using filter papers put in the field; L = mortalities recorded using filter papers put in the laboratory. Concentrations of insecticides on filter papers were 0.3 mg per sq. cm for cypermethrin and 1.6 mg per sq. cm for each of fenvalerate and permethrin.

test insects. The magnitude of individuals killed decreased with longer exposure of the chemical in the field. This was evidenced by the fact that longer exposures of 21 and 28 days caused 46.41 and 42.24% mortality respectively. There was significant ($P<0.05$) difference in the mortalities obtained for this chemical at 7 and 14 day intervals (Table 5a).

The overall impression gained is that cypermethrin gave higher mortalities of D. fasciatus than either fenvalerate or permethrin. For example the residual toxicity of cypermethrin after 21 days exposure in the field resulted in 59.04% kill of the test insects. The corresponding mortality values for fenvalerate and permethrin after the same period of exposure were 55.06 and 46.41% respectively. Under laboratory conditions, these were 75.41% for cypermethrin, 51.54% for fenvalerate and 67.03% for permethrin (Table 5a).

When D. nigrofasciatus was used to determine the residual persistence of cypermethrin in the field, the mortalities obtained were 81.01, 62.22, 51.89 and 41.31% at 7, 14, 21 and 28 days respectively (Table 5b). These mortalities were significantly ($P\leq0.05$) different for the intervals studied.

On the other hand, results of insect mortality for filter papers treated with cypermethrin and kept in the laboratory were 87.99, 68.21, 65.37 and 56.5% after exposure periods of 7, 14, 21 and 28 days respectively. The mortalities obtained at 14 and 21 day intervals were not significantly ($P<0.05$) different (Table 5b). For filter papers impregnated with fenvalerate and exposed to field conditions, the mortalities recorded after 7 and 14 days were not significantly ($P<0.05$) different. However, these mortalities were significantly ($P\leq0.05$) different from those obtained at 21 and 28 day intervals as shown in Table 5b. The mortalities caused by this

Table 5b: Mean mortabilities (transformed % values) of D. nigrofasciatus when exposed to insecticide treated filter papers with cypermethrin, fenvalerate and permethrin

Interval (days)	Insecticide					
	Cypermethrin		Fenvalerate		permethrin	
	(Percentage mortality of cotton stainers)					
	F	L	F	L	F	L
0	90^{a}	90^{a}	85.15^{a}	90^{a}	90^{a}	90^{a}
7	81.01^{b}	87.99^{a}	61.53^{ab}	73.17^{b}	72.57^{b}	87.99^{a}
14	62.22^{c}	68.22^{b}	55.72^{ab}	63.88^{c}	55.22^{c}	64.99^{b}
21	51.89^{d}	65.37^{b}	45.27^{bc}	42.64^{d}	42.13^{d}	59.83^{b}
28	44.31^{d}	56.50^{c}	33.44^{d}	32.27^{e}	32.23^{d}	41.45^{c}
Overall mean	65.89	73.62	56.22	60.39	58.43	68.85
S.E. (treatment means)	2.48	2.13	3.34	2.45	3.74	3.27
C.V.%	6.52	5.00	10.17	7.03	11.10	8.23
F values (treatments)	60.40***	47.57***	32.80***	89.77***	38.53***	38.81***
F values (blocks)	0.49NS	2.70NS	0.45NS	2.36NS	0.57NS	0.06NS

Transformed means in each column with different letters are significantly different from each other ($P<0.05$) (Duncan's New Multiple Range Test). Transformation of % mortalities was done using Arcsine proportion. NS: non-significant; significant ***$P<0.001$. F = mortalities recorded using filter papers put in the field; L = mortalities recorded using filter papers put in the laboratory. Concentrations of insecticides on filter papers were 0.3 mg per sq. cm for cypermethrin and 1.6 mg per sq. cm for each of fenvalerate and permethrin.

chemical on filter papers which were placed in the laboratory were all significantly ($P \leq 0.05$) different (Table 5b).

In tests involving residual persistence of permethrin in the field, the following mortalities were obtained: 72.57, 55.22, 43.13 and 32.23% respectively (Table 5b). The mortalities realised for this chemical at 21 and 28 days were not significantly ($P \leq 0.05$) different. On the other hand, there was significant ($P < 0.05$) difference between these mortalities and those obtained at 7 and 14 days. Filter papers containing permethrin kept in the laboratory caused 87.99% of the test insects at 7 days. At the other intervals, the mortalities recorded were: 64.99% at 14 days, 59.83% at 21 days and 41.45% at 28 days (Table 5b). There was no significant ($P \leq 0.05$) difference between the mortalities obtained for this chemical at 14 and 21 days. However, there were significant ($P \leq 0.05$) differences between these mortalities and those obtained at the rest of the intervals (Table 5b).

Based on the results shown in Table 5b it is concluded that cypermethrin caused higher mortalities of D. nigrofasciatus than either fenvalerate or permethrin.

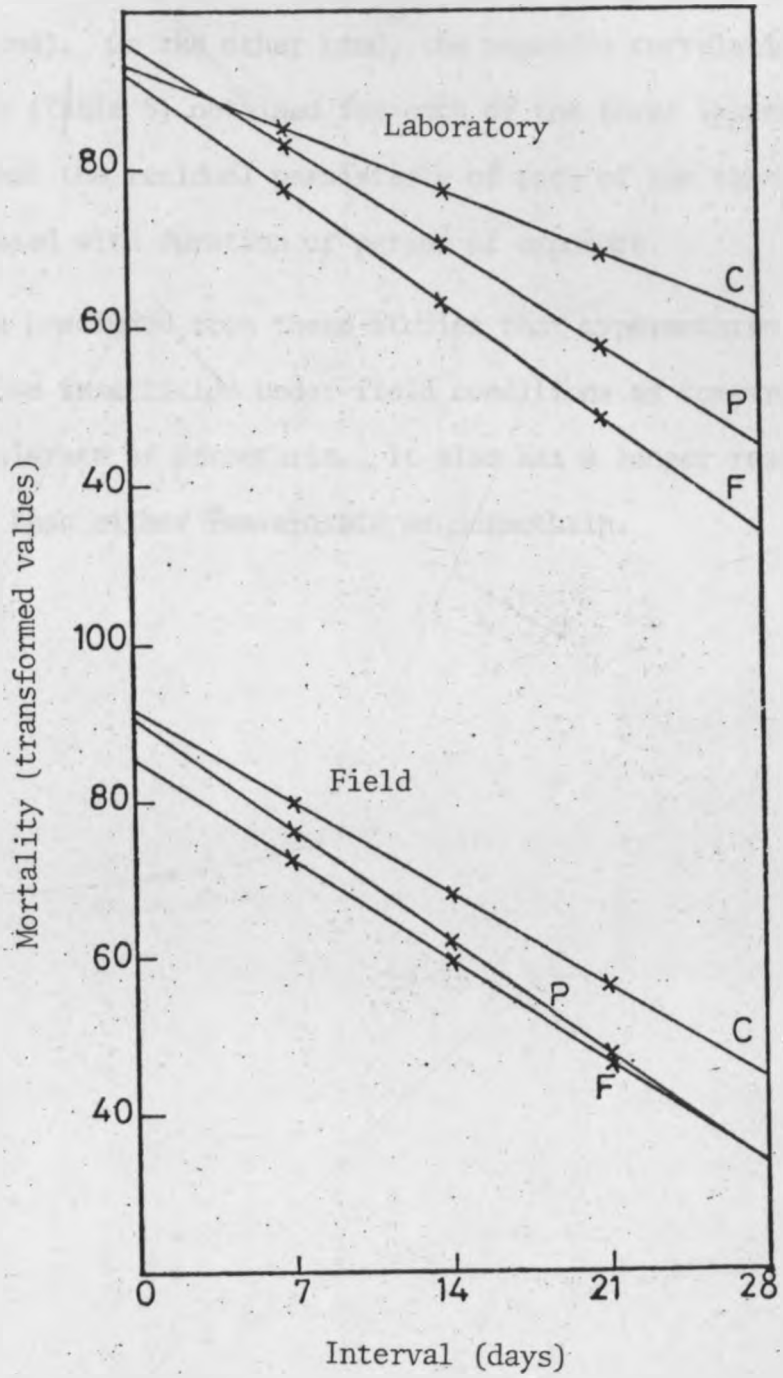
Data for mortalities of both D. fasciatus and D. nigrofasciatus were regressed on intervals (days) Table 6. This operation revealed that there was significant ($P < 0.05$) difference in the regression coefficents of cypermethrin ($r = -1.691$) as compared to those of fenvalerate ($r = -2.028$) and permethrin ($r = -1.871$). The regression coefficients for fenvalerate and permethrin were not significantly ($P \leq 0.05$) different from each other. This indicated that the residual persistence of cypermethrin differed significantly ($P \leq 0.05$) from those of fenvalerate and permethrin. It also indicated that the residual persistence of both fenvalerate and permethrin were similar.

Table 6. Regression equations of Dysdercus spp. mortalities on interval (days) for cypermethrin, fenvalerate and permethrin.

Insecticide	Field	*r	laboratory	*r
cypermethrin	y=91.74x-1.691b	- 0.9836	y=93.22x-1.131[b]	- 0.9846
permethrin	y=90.49x-2.028[a]	- 0.9862	y=96.51x-1.860[a]	- 0.9695
fenvalerate	y=85.64x-1.871[a]	- 0.9862	y=92.46x-2.080[a]	- 0.9933

Regression coefficients with different letters indicate significant difference ($P \leq 0.05$). *r signifies correlation coefficient.

Fig. 7: Regression of Dysdercus spp. mortalities on interval (days).



The insecticides used are designated by letters as follows:

C - cypermethrin, P - permethrin,
F - fenvalerate

The highly significant ($P \leq 0.05$) regression F-ratios (Appendix II) obtained for each of the three chemicals imply that there was a strong linear relationship between the rate of decrease of the residual persistence of the three insecticides with the length of period of exposure (time). On the other hand, the negative correlation coefficients (Table 6) obtained for each of the three insecticides indicated that the residual persistence of each of the three insecticides decreased with duration of period of exposure.

It was concluded from these studies that cypermethrin was the most effective insecticide under field conditions as compared to either fenvalerate or permethrin. It also has a longer residual persistence than either fenvalerate or permethrin.

CHAPTTER FOUR

4. DISCUSSION AND CONCLUSION

Studies reported in this thesis were based on the need to quantify the response of Dysdercus species occurring in Kenya to three synthetic pyrethroid insecticides namely cypermethrin, fenvalerate and permethrin. These are the insecticides recommended by the Ministry of Agriculture for use by farmers on cotton (Anon., 1981b, 1982). The emphasis of these studies was mainly on three most important attributes of insecticides: initial toxicity (expressed as susceptibility levels in the text), residual persistence and the possibility of development of resistance by insects against which the insecticides are aimed at controlling. The quantitative data obtained based on these parameters forms a useful basis upon which reference can be made should any species develop insecticide resistance in future.

In experiments on the susceptibility of Dysdercus species reported in this thesis, the most important parameter measured was the LC_{50} which is the concentration of insecticide that resulted in 50% kill of the test insects (also referred to as the median lethal concentration in the text). According to Busvine (1971), this value is statistically and more reliably determined than other LC values. For this reason, it was felt that the LC_{50} values determined for the Dysdercus species found in this country could be used as a reference point in future investigations involving the synthetic pyrethroids.

The accuracies of these determinations depended on the procedure adopted for the standardisation of the test insects as recommended by Busvine (1971) and Finney (1971). The conditions of temperature and RH under which the test insects were reared as reported in section

2.1.1 of the general procedure were considered to be ideal in promoting the development of cotton stainers (Geering, 1956; Pearson and Maxwell, 1958). The cages used in rearing these insects were thought to be ideal for their development. Similar cages were successfully used by Nyamasyo (1978) in rearing cotton stainers for his studies.

Fifth instar nymphs aged 5-6 days were found to be the most convenient stage for use in studies reported here. The rest of the nymphal stages were found difficult to handle owing to their small size and delicate bodies which also made them unsuitable for bioassays using the topical application technique. The other problem related to their use was that it was not possible to sex these instars. On the other hand, the adults spend most of their time mating and laying eggs and so could not be used in these studies.

In the determination of the LC_{50s} and LC_{95s} for these insects, ordinary motor engine oil, a high boiling paraffinic mineral oil was used as solvent for test insecticides. This oil was found to have the least lethal effect on the test insects. It was hoped that a fairly high level of accuracy was achieved in the assessment of the lethal effect (toxicity) of the insecticides without interference from this solvent. However, it was not known what effect the ordinary motor engine oil used in this study had on each of the three insecticides. Several workers, notably Busvine and Nash (1953) have used mineral oils in the dilution of insecticides in their studies of contact insecticides. The finding that ordinary motor engine oil had the least lethal effect on Dysdercus species and was a good solvent for the synthetic pyrethroids could be utilised by other workers in similar studies. It is cheaper and readily available at most petrol filling stations in the country. It is also safer to handle than most organic solvents which are likely to be harmful to the user.

Some interesting observations were made on the results of the susceptibilities obtained for the four (nine strains) Dysdercus species used in this study. The most susceptible species was D. fasciatus. Strains of this species were also the most susceptible. The least susceptible species was D. nigrofasciatus including its strains collected from different parts of Kenya.

The variation in the susceptibilities of the different species used in this study could be explained partly in terms of the "intrinsic contact toxicities" of the insecticides. Porter, et. al. (1947) defined "intrinsic contact toxicity" as the toxicity at the site of action of the poison (insecticide) in the insect. The three synthetic pyrethroid insecticides used in the bioassay are closely allied since they are all synthetic pyrethroids. It appears that the differences in the susceptibilities shown by the different insect species could be explained by differences in the structures and functional groups (Appendix III) of these insecticides. These two factors could have been responsible for influencing the speed of action and effect produced by each insecticide. Yamamoto (1970) suggested a structure-activity relationship in the action of synthetic pyrethroids when applied to insects.

On the other hand the phenomenon of species specificity could explain the differences in the response of the four different Dysdercus species to insecticides used. According to Busvine (1971), there are two major causes of species specificity to contact insecticides namely differences in contamination and penetration of the cuticle. The insects used in this study were applied with uniform droplets (1µl) of each of the test insecticides. This factor being constant, the rate of penetration seems to the most decisive factor in determining the different toxicities exhibited by these

insecticides. Further evidence for species specificity based on cuticular penetration was provided by Menusen (1948) when he bio-assayed a wide range of insecticides by contact, by injection or as stomach poisons.

Similar explanations could be advanced for the observed differences in the susceptibilities of the different strains of the same species and also the different strains of dissimilar species. It is also possible that the different strains of Dysdercus species were likely to have varying levels of susceptibilities (resistances) in view of their places of origin in the field. Based on this, it is logical to assume that genetic differences in resistance (susceptibilities) were already present in the nine different strains even before exposure to the three insecticides because they were collected from different localities where they had formed distinct and peculiar genetic pools. It is these genetic differences which in turn dictate the different biological and biochemical differences in populations that result in different responses (susceptibilities) when exposed to an insecticide (Sawicki, 1979).

It is also important to examine the operational factors (Georghiou and Taylor, 1977a,b) prevailing at the collection sites of the cotton stainers. These factors include dosage, threshold below which no treatment is applied, frequency of application of insecticides and whether some portion of the population is left purposely untreated (refugia) and also the type of chemical used. These factors could be manipulated to accelerate or decrease the rate of build-up of resistance (Gonzalez, 1976; Sawicki, 1979). Depending on the degree of intensity of these factors, which vary from one area to another, there are bound to be differences in the responses of cotton stainers to an insecticide.

In Kenya, insecticide spraying on cotton in all the cotton growing areas is based on fixed spray schedules (Brown et. al., 1972; Anon., 1980, 1981b) resulting in 5 - 6 spray rounds throughout the crop spraying period. These insecticidal applications are done regardless of the degree of infestation by the insect pest species to be controlled. Although the number of insecticidal applications are limited (5-6 applications), they are applied to the crop so widely that selection pressure is exercised on a high proportion of the treated population. Additional pressure is exercised as subsequent generations which infest the crop are sprayed as has happened in other countries (Sawicki, 1979). This situation is likely to produce genotypes which have differing susceptibilities to an insecticide when applied to insects obtained from different areas.

Another important factor that could explain the differences in the relative susceptibilities of the strains used in the study is the presence of 'refugia' (Georghiou and Taylor, 1977a,b). These are plant tissues, distorted foliage, growth buds, soil and other shelters which enable some members of the target population to escape selection. This concept can be extended to apply to the situation in the cotton growing areas of Kenya where the degree of agricultural use of land differs from one area to another (Acland, 1971). The role of 'refugia' in these areas would accordingly differ from one area to another. Perhaps the most important 'refugia' in most of the cotton growing areas in Kenya are the alternate host plants of Dysdercus spp. For example in Eastern Province of Kenya, the alternate tree hosts of Dysdercus spp. such as Sterculia rhynchocarpa K. Schum and Ceiba pentandra Gaertn. (Kapok) provide natural wild refuges for these insects. Any immigration into already treated cotton from these wild hosts could lead to periodic dilution of resistance or its elimination through

competition for fitness hence confering differences in relative susceptibilities to the different strains from different areas. It is possible that the Masongoleni strain was the most susceptible strain because of such influence.

It can therefore be concluded that no one single factor can explain the differences in the responses of the four Dysdercus spp. or the nine Dysdercus strains used in this study. This is so because of the multitude of genetic and operational influences operating in area where these insects were collected. On the other hand, the relative potencies of the three insecticides can be assessed from where it can be seen that cypermethrin was the most potent (toxic) followed by fenvalerate and permethrin in that order.

The practical implications of the results obtained in this study are two-fold. First, the results show that the synthetic pyrethroid insecticides, at least the ones used in this study, are still very effective against the Dysdercus spp. occuring in Kenya. Secondly, in order to maintain this effectiveness, emphasis will have to be put on their prudent use in future, in order to delay the development of resistant strains to them. This would be for the benefit of cotton farmers in particular and the cotton industry in general in this country since modern agriculture is virtually impossible without insecticides.

In experiments on the induction of resistance in Dysdercus spp. reported here, it was observed that the development of resistance was higher in D. cardinalis after selection with permethrin. The response of D. cardinalis after selection with fenvalerate and cypermethrin was low. Similarly, the response of D. nigrofasciatus after selection with each of cypermethrin, fenvalerate and permethrin was low. However, even the highest resistance factor obtained in this study was considered

to be too low. This is in comparison to the FAO (1969) standards which consider a resistance factor of x4 or above to be significant. The low responses in the development of resistance obtained in this study can be attributed to the frequent failure of laboratory strains in selection response. This was probably due to the restriction in their gene pool as a result of inbreeding and lack of selection for general vigour (Brown and Pal, 1971a,b).

It is possible that if heterogeneity had been maintained during selection in the populations of insects used in this study for more than five generations, the responses of each or some of the test insects would have been higher. Nyamasyo (1978) in his studies on insecticide resistance selected strains of D. fasciatus which developed a response of x6.1 with Carbaryl after six generations.

The response obtained after selection of D. cardinalis with Permethrin suggested that Dysdercus spp. are likely to develop resistance to the synthetic pyrethroids as has happened elsewhere in other insect species (Brown and Pal, 1971a,b; Keiding, 1977; Sawicki, 1979). It is possible that this response was obtained because Permethrin was the first synthetic pyrethroid to be introduced in this country against the cotton pest complex, including Dysdercus spp. (Anon., 1978, 1979). The field strains of Dysdercus could possibly have developed a defence mechanism to this insecticide which has been used intensively since its introduction in this country. In practice, the type of chemical used may be important in accelerating the development of resistance. It has been shown that insect pest species can develop cross and multiple cross resistances depending on the history of pesticide usage in the area concerned (Gonzalez, 1976). The development of the same Kdr (knock down) resistance to DDT and the pyrethroids in the housefly has been documented elsewhere

(Sawicki, 1979). It is not known whether strains already resistant to carbaryl can develop an additional mechanism for resistance against pyrethroids.

It can be concluded that the resistance levels obtained are low and do not constitute any danger in the control of Dysdercus species.

The results obtained in this study confirmed that synthetic pyrethroids remained effective for as long as two weeks (14 days) in the field. The results of this study therefore agreed with the observations obtained in field trials with these insecticides (Anon., 1980, 1981b). This agrees with the present recommendations by the Ministry of Agriculture for farmers to spray their cotton crop at fortnightly intervals. However, cypermethrin which was found to have the longest residual persistence would be recommended for field application at 21 day intervals.

Appendix I: Data for July, 1984 of daily mean relative humidity* (%), rainfall (mm) and temperature (^{o}C) at Kibos

Date	Relative humidity (%)	Rainfall (mm)	Temperature (^{o}C) maximum	minimum
July				
1	66.3	NIL	29.5	16.5
2	68.5	NIL	28.9	16.0
3	56.8	NIL	29.5	16.5
4	56.5	NIL	29.6	16.0
5	65.0	4.8	28.5	14.5
6	49.5	30.8	28.5	14.0
7	72.3	NIL	28.0	10.5
8	65.0	NIL	29.0	14.0
9	69.0	NIL	30.0	16.0
10	74.5	1.1	29.0	16.0
11	68.5	NIL	28.8	16.0
12	66.8	9.6	28.9	16.0
13	65.8	NIL	29.5	16.5
14	68.5	6.5	29.0	15.5
15	73.4	2.9	27.0	16.0
16	76.8	NIL	28.8	14.5
17	67.5	3.9	29.0	14.0
18	76.5	4.0	28.0	15.8
19	76.0	TR**	28.5	14.0
20	77.8	1.1	28.5	15.0
21	68.0	NIL	28.0	15.0
22	70.0	54.9	27.0	16.8
23	73.5	8.0	27.0	14.0
24	68.5	NIL	27.5	15.4
25	74.4	9.0	27.5	15.0
26	69.5	0.7	28.5	16.6
27	73.0	NIL	29.0	14.0
28	67.0	NIL	29.0	16.0
29	66.8	NIL	29.5	16.0
30	67.0	NIL	29.5	16.0
31	61.3	NIL	29.5	15.0
Total	2120	137.3	888.5	473.1
Monthly mean	68.4	4.4	28.7	15.3

* records were taken at 9.00 hrs and 15.00 hrs on each day

** TR (trace)denotes less than.0.1 mm of rainfall

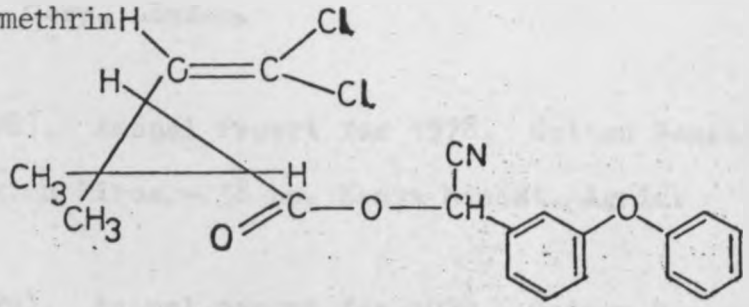
Appendix II Regression of Dysdercus spp. mortalities (transformed values) on intervals (days).

	Field			Laboratory		
	cypermethrin	permethrin	fenvalerate	cypermethrin	permethrin	fenvalerate
Total SS	4490.3974	6391.8735	5440.9341	1999.8514	5736.6104	6531.4261
Regression SS	4202.9636	6045.7925	5148.0380	1880.05	5084.0497	6358.6433
Error df	13	13	13	13	13	13
Error SS	287.4338	346.0810	292.8961	119.8014	652.5607	172.7828
Error MS	22.1103	26.6216	22.5305	9.2155	50.1970	13.2910
F	190.09***	227.10***	228.49***	204.01***	101.28***	478.42***
r	-0.9836	-0.9863	-0.9862	-0.9846	-0.9695	-0.9933
Parameter A	-1.691a	-2.028a	-1.871a	-1.131b	-1.860a	-2.080a
Parameter B	91.74	90.49	85.64	93.22	96.51	92.46
Mean x	14.00	14.00	14.00	14.00	14.00	14.00
Mean y	68.07	62.10	59.44	77.39	70.48	63.34

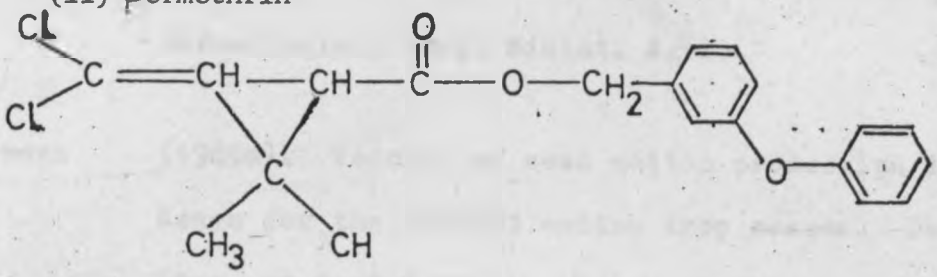
Regression coefficients (A) with different letters indicate significant difference ($P \leq 0.05$).
r signifies correlation coefficients. *** significant $P \leq 0.001$ respectively.

Appendix III: Structural configurations of cypermethrin, fenvalerate and permethrin (Source: MacCuaig, 1980)

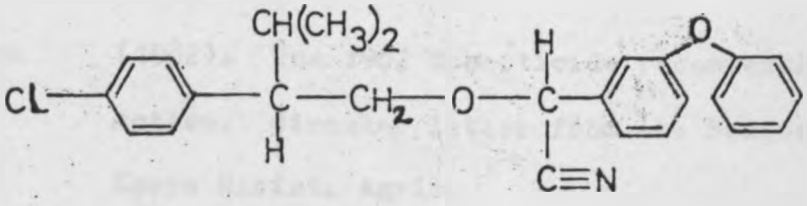
(i) cypermethrin



(ii) permethrin



(iii) fenvalerate



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