A COMPARISON OF THE PHARMACOKINETICS OF ^{14}C TRANS CYPERMETHRIN IN A RESISTANT AND A SUSCEPTIBLE STRAIN OF HELIOTHIS VIRESCENS

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ABSTRACT

Penetration studies revealed that trans (1-^{14}C cyclopropyl) cypermethrin was absorbed at a faster rate in a susceptible (BRC) strain of Heliothis virescens as compared to a resistant one (PEG87). The rapid elimination of radiolabelled metabolites of cypermethrin from PEG87 suggests a higher metabolic turnover of the insecticide in the resistant as compared to the susceptible strain. The major metabolic product in the faeces of both strains was polar conjugated material, with PEG87 larvae excreting more conjugate than larvae of the BRC strain. Analysis of the primary metabolites of trans cypermethrin present in the faeces, larval bodies and haemolymph of each strain indicates that the major route of detoxification is via a monooxygenase which leads to the formation of hydroxylated cypermethrin. The appearance of the products of hydrolysis suggests additional esterase activity although this may be a secondary effect.

INTRODUCTION

The tobacco budworm, Heliothis virescens, is a major pest of cotton in the United States, Central and South America. Control of H.virescens, especially in the late season, is primarily achieved by use of the synthetic pyrethroids, representing approximately 51% of the total U.S. insecticides market.

Metabolism of pyrethroids in H.virescens has been reported to occur by both oxidative (Nicholson and Miller,1985) and hydrolytic attack (Dowd et al,1987), although the relative importance of these two pathways is unclear. In order to identify the critical pharmacokinetic and metabolic difference between resistant and susceptible larvae the in vivo fate of radiolabelled trans cypermethrin in H.virescens has been investigated. This study forms part of a wider programme investigating the major mechanisms of pyrethr oid resistance in field strains of the insect, leading to the development of a comprehensive management strategy.

MATERIALS AND METHODS

Insects

Resistant (PEG87) and susceptible (BRC) strains of Heliothis virescens supplied by ICI Americas Incorporated, Goldsboro, N. Carolina, USA and ICI Agrochemicals, Jealotts Hill, UK., respectively were reared in the laboratory in a similar manner to that detailed by Ahmad and McCaffery (1988). The PEG87 strain displayed a 520-fold resistance to trans cypermethrin as compared to the BRC strain after treatment by topical application.

Treatment of larvae with ^{14}C trans cypermethrin

Third instar larvae (19-25mg) of the two Heliothis strains were topically dosed with a 1µl solution of 1(RS) trans (α-RS)(1-^{14}C cyclopropyl) cypermethrin (Sp.Act.2.0 GBq/mmol, >99% radiochemical purity) in acetone, applied to the thoracic dorsum using a microsyringe (Hamilton). Resistant PEG87 larvae were treated at a sublethal rate of 0.008µg/µl, but this dose was found to cause unacceptable losses in the susceptible BRC strain which were subsequently dosed with 0.002µg/µl (LD_{10} for this strain). Larvae were held individually in glass petri-dishes, with a small quantity of food, for periods up to 48h. For each time interval studied three replicates of ten insects, or three replicates of twenty insects were used for the PEG87 and BRC strains respectively.

Thin layer chromatography (TLC)

TLC was carried out on Merck plates of silica gel 60 F254 (BDH). Plates were developed in one of two solvent systems (Table 1). Unlabelled reference compounds (Table 1) were detected by viewing in ultra-violet light and the Rf of each was calculated. Areas of radioactivity were located and quantified using a Berthold automatic TLC linear analyser (LB2820-1). ^{14}C trans cypermethrin was run as a standard on all plates and metabolites were identified by their Rf values.

TABLE 1 Reference compounds and their R_f values by TLC in two solvent systems

	R_f value in solvent [a] 1	2
trans cypermethrin	0.73	0.70
trans(1-^{14}C cyclopropyl) cyper.	0.75	0.74
4'-methoxy cis,trans- cyper.	0.67	0.65
trans-3-2-(2,2-Dichlorovinyl) -2-2-dimethyl-cyclopropane carboxylic acid (Cl_2CA)	0.37	0.53
trans-hydroxy-Cl_2CA (t-HO-Cl_2CA)	0.11	0.13

[a] solvents (proportions by volume), Crawford et al, 1981.
1 toluene; ethyl acetate; acetic acid (75; 25; 1)
2 benzene(saturated with formic acid); diethyl ether (10; 3)

Pharmacokinetics and metabolism

At appropriate times after dosing the larvae were surface washed in acetone (3x1ml/larva; 60secs/wash) and the washes for each replicate pooled. Aliquots (500µl) were removed for liquid scintillation counting (LSC), using a Packard Tricarb scintillation counter employing Ecoscint scintillation fluid. The petri-dishes in which the larvae were held were also washed in acetone (1ml) and washes for each replicate pooled with 500µl removed for LSC. Penetration of trans cypermethrin was calculated from results obtained by LSC. (Little et al, unpublished)

Haemolymph was removed from each larva and extracted in methanol, centrifuged, aliquots taken for counting and the samples reduced in volume and analysed by TLC (Little et al, unpublished). Excreta collected in petri-dishes were extracted in methanol, centrifuged, aliquots taken for counting, and the remainder analysed as above. After rinsing with acetone, batches of whole larvae were stored at -20°C prior to extraction. Replicate groups were homogenised in methanol, using an

electric tissue homogeniser (Jencons). The homogenate was extracted as above and aliquots removed for LSC, with the remaining volume reduced prior to application on TLC plates.

RESULTS

Penetration of ^{14}C trans cypermethrin into resistant and susceptible larvae

Trans cypermethrin was absorbed significantly faster ($P<0.001$) into the BRC susceptible larvae than in to the resistant PEG87 larvae over a 48h period (Fig 1).

Levels of radioactivity in the haemolymph of BRC and PEG87 larvae

Radioactivity was detected in the haemolymph of both strains reaching a maximum at 2-4h after dosing, representing 65.5% of the penetrated dose (equivalent to 4% of the penetrated dose per µl of haemolymph) in PEG87 larvae and 14.2% of the penetrated dose (equivalent to 0.6% of the penetrated dose per µl of haemolymph) in the BRC larvae. At 4-48h after dosing the level of radioactivity in both strains did not exceed 0.2% of the penetrated dose per µl of haemolymph.

TLC analysis of 2h haemolymph extractions from PEG87 larvae identified two main metabolites that were in the correct position to be one of the hydroxy trans cypermethrin metabolites, hydroxylated on the 2' or 4' position of the benzyl ring (2'/4'-HO-t-cypermethrin) and trans cyclopropane carboxylic acid (Cl_2CA) (Table 1), representing 43% and 10.4% of the penetrated dose respectively. A peak remaining at the baseline was identified as 'conjugate' and represented 11.4% of the penetrated dose. At the equivalent time point for BRC larvae, two peaks in radioactivity were detected one of which by remaining at the baseline was identified as conjugate, representing 11% of the penetrated dose; the other was in the correct position for trans cypermethrin, constituting 3.5% of the penetrated dose. No peaks of radioactivity were detected by TLC in either strain later than 2h after treatment.

Excretion of metabolites of trans cypermethrin from resistant and susceptible H.virescens

A considerably higher rate of excretion of radioactivity was found from PEG87 as compared to BRC larvae over the 48h sampling period (Fig. 2).

TLC analysis identified the major metabolic product in both strains to be relatively polar conjugated materials; Cl_2CA appeared at later time points, while trans-hydroxy-Cl_2CA was identified in those samples where benzene was the developing solvent. Fig.3 shows the increase in conjugate in the faeces of each strain over the 48h sampling period. Conjugates represented the highest percentage of the penetrated dose in both strains, but excretion of this metabolic product by the PEG87 larvae far exceeded that of BRC larvae, such that at 12h PEG87 larvae excreted 20 times more conjugate than the larvae of the BRC strain. In faecal samples taken from PEG87 larvae 1h after dosing, a metabolite was detected and identified as 2'/4'-HO- t cypermethrin, but this metabolite could not be detected at later time points.

Levels of trans cypermethrin and its metabolites within the larvae of the PEG87 and BRC strains

Two to four hours after dosing the internal concentration of ^{14}C activity was 2-4 fold greater in BRC larvae as compared to PEG87. Beyond this time point differences in internal levels of radioactivity between the two strains were negligible.

TLC analysis of larval extracts from both strains identified the major differences to be the percentage of the penetrated dose attributed to parent trans cypermethrin and conjugate. In PEG87 larvae, conjugates represented the highest percentage peaking at 38% 12h after dosing, while in BRC larvae it rarely exceeded 20% of the penetrated dose. The level of trans cypermethrin in PEG87 larvae fell below 5% of the penetrated dose within 4h, but accounted for 20-30% in BRC larvae 2-4h after treatment, falling gradually to approximately 8% by 24h. Two further components were also identified in the larval extracts with Rf values in both solvent systems equivalent to 2'/4'-HO-t cypermethrin and Cl_2CA (Table 1). Similar levels of 2'/4'-HO-t cypermethrin were identified in each strain except at 2h when the metabolite was absent from BRC larvae, but accounted for just over 20% of the penetrated dose in PEG87 larvae. Cl_2CA accounted for 10% of the penetrated dose in BRC larvae at 2h, but fell to around 2% for the remaining period. This metabolite was only identified after 12 and 24h in PEG87 larvae, representing 3-6% of the penetrated dose.

DISCUSSION

The approach in this study has been to examine the relative roles of penetration, metabolism and excretion of trans cypermethrin in a resistant (PEG87) and susceptible (BRC) strain of H.virescens. This isomer of cypermethrin was chosen for an initial study as it is metabolised at a higher rate than the more efficacious cis isomer. The results reported here are consistent with the PEG87 strain possessing a metabolic resistance based primarily on the oxidative enzyme system and to a lesser degree a penetration resistance. A comparison of the rates of penetration between the two H.virescens strains indicates that penetration into PEG87 larvae is delayed by the order of 1.5-2 fold, but this would appear to be of secondary importance compared with increased detoxication in the PEG87 strain.

Results from the metabolism studies indicate that at the doses used PEG87 larvae can metabolise and excrete cypermethrin at a considerably faster rate than the larvae of the BRC strain. Evidence for an enhanced metabolism of trans cypermethrin in PEG87 is seen by the rapid appearance of metabolites both in the haemolymph and faeces within 2-4h after dosing as well as considerably higher internal levels of parent material in the BRC larvae as compared to PEG87 larvae at the same time period. The major product of metabolism identified from the faecal and larval extracts of each strain was polar conjugated material and with time this became an increasingly important excretory product.

Evidence that both the monooxygenase and esterase enzyme systems may be involved in metabolism, with the formation of the 2'/4'HO t cypermethrin and Cl_2CA, is given by TLC analysis of the haemolymph, faecal and larval extracts. The major metabolite identified in the haemolymph of the PEG87 strain of H.virescens was 2'/4'-HO-t-cypermethrin; a product of hydroxylation, suggesting monooxygenase

activity. 2'/4'-HO-t-cypermethrin was also detected in the faeces of PEG87 larvae 1h after dosing and was present internally in the larvae of both strains throughout the time course of the study. The involvement of the oxidase system and evidence of hydroxylation at the 2' or 4'-position of the α-cyano 3 phenoxybenzyl compounds has been seen in other insects (Shono et al, 1978; Ishaaya et al,1983).

The involvement of the cytochrome P450 monooxygenase system in pyrethroid resistance in H.virescens is suggested by other workers (Nicholson and Miller,1985) although ester cleavage has been implicated as the major resistance mechanism in an H.virescens population from California (Dowd et al, 1987). Oxidase activity has also been identified in pyrethroid resistant H.armigera from Thailand (Ahmad,1988) and has been found to be the major mechanism contributing to pyrethroid resistance in field strains of H.armigera from Australia (N.Forrester and J.Daly, 1988, personal communications). From this study it is difficult to ascertain the relative importance of the two enzyme systems and it may be possible that the monooxygenase activity enhances ester cleavage, or the hydroxylated metabolites (2'/4'-HO-t-cypermethrin) may in fact be better esterase substrates than the original t-cypermethrin.

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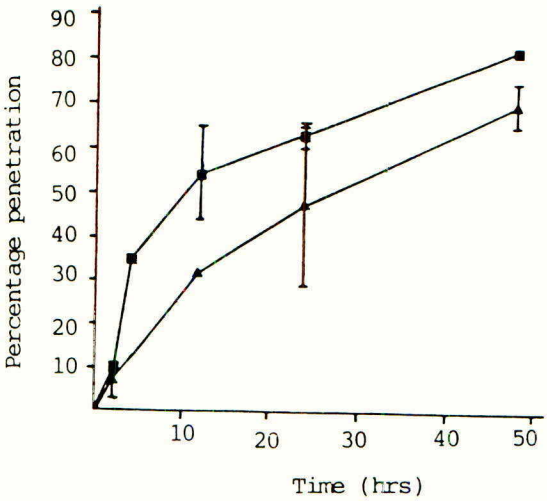
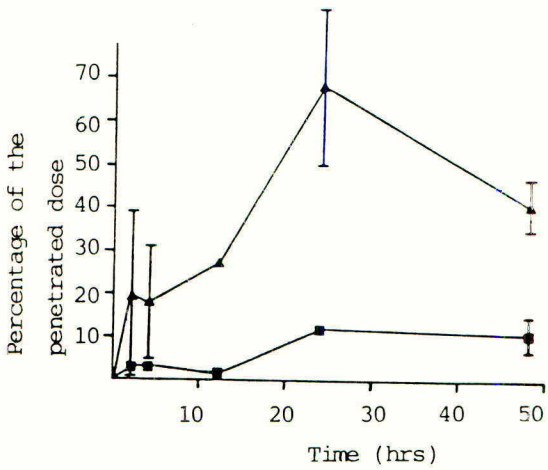


Fig.1 The penetration of 14C trans cypermethrin in PEG87 (▲) and BRC (■) larvae of Heliothis virescens

Fig.2. Radioactvity in the faeces of PEG87 (▲) and BRC (■) larvae topically dosed with 14C trans cypermethrin



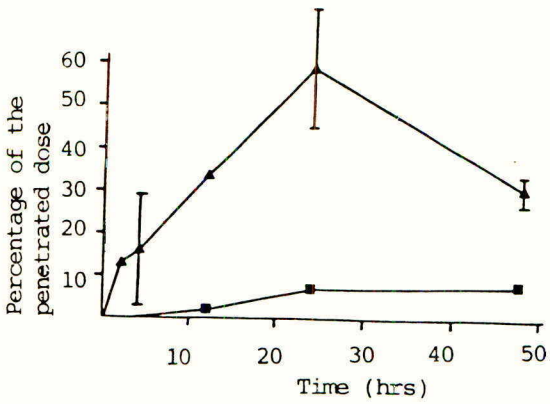


Fig.3. Radioactivity attributed to conjugates in the faeces of PEG87 (▲) and BRC (■) larvae topically dosed with 14C trans cypermethrin

RESISTANCE TO PYRETHROIDS IN HELIOTHIS SPP.: BIOASSAY METHODS AND INCIDENCE IN POPULATIONS FROM INDIA AND ASIA

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ABSTRACT

Using Heliothis virescens a range of insecticide bioassays was compared. The first instar foliar residue test was the most effective to discriminate between resistant and susceptible strains. The dip test was comparatively ineffective. The topical test produced similar, moderate levels of tolerance throughout development. The adult vial test was rather ineffective and male insects were less tolerant than females. In field strains of H. armigera the topical test was more efficient than the foliar residue test. Resistance to pyrethroids was detected in strains from India and Indonesia.

INTRODUCTION

The development of resistance to the synthetic pyrethroids has led to recent failures to control Heliothis armigera in cotton in various locations (reviewed by Ahmad & McCaffery, 1988) and H. virescens in cotton and soybeans in the USA (Luttrell et al., 1987). We report here on two further instances of resistance to pyrethroids in H. armigera which resulted in field failures in India and Indonesia in 1987.

A variety of bioassays are used to detect resistance in these species. Because of differences in the rate of pyrethroid uptake in these tests the pharmacodynamics of intoxication will differ. The tests will therefore vary in their discrimination between susceptible and resistant strains, and variations in the presence and potency of resistance mechanisms will influence this. Using H. virescens we have compared the efficiency of a range of bioassays. Comparisons have been made with H. armigera and two tests used for examination of material shipped to the UK.

MATERIALS AND METHODS

Insects

Susceptible (BRC/A) and pyrethroid resistant (PEG87/A) strains of H. virescens were obtained from ICI Agrochemicals, Jealott's Hill, UK and ICI Americas Incorporated, Goldsboro, N.C., USA respectively. Samples of H. armigera larvae were collected from cotton growing areas of Thailand (1987), and Indonesia (1988) and both cotton and pulse growing areas of India (1986-1988). The larvae were fed, allowed to pupate and transported to the UK. The first laboratory generations of larvae were used for the bioassays. Insects were reared in a similar manner to that described by Ahmad & McCaffery (1988).

Insecticides

An 11% e.c. formulation of cypermethrin ('Cymbush'), technical grade cis-cypermethrin (98.4%) and cypermethrin (90.5%, cis:trans ratio = 49:51) were obtained from ICI Agrochemicals, Jealott's Hill, UK. Fenvalerate (97%) was obtained from Shell Research Limited, Sittingbourne, UK.

Foliar residue bioassay

Leaves from 5 week old cotton plants were dipped in aqueous dilutions of formulated cypermethrin and allowed to dry. First instar larvae were taken within 6h of hatching and groups of 5 placed in 30ml plastic pots. A treated leaf was placed over the top of each pot and a snap-on lid closed over the leaf and pot. For the control and each treatment group 40 insects were tested and mortality was assessed after 48h. For 3rd instar larvae the base of a 90mm glass petri dish was lined with a treated leaf and plastic webbing placed on the leaf. A single larva was placed in each cell and the top closed with a second dish.

Vial residue bioassay

In a bioassay based on that described by Plapp (1979) 20 ml glass scintillation vials were evenly coated inside with acetone solutions of technical cypermethrin. Newly emerged moths were fed for 6h and placed individually in treated vials closed with cotton wool. Mortality was assessed 24h later. In similar tests with 3rd instar larvae mortality was assessed after 72h.

Topical bioassay

1 ul drops of acetone solutions of technical grade insecticides were applied to the thoracic dorsum of each 3rd instar (H. virescens 19-25mg, H. armigera 30-40mg) (Anon 1970) or 5th instar (H. virescens 100-150mg) and to the eye of adults. Control insects were treated with acetone alone. For each control and experimental group at least 40 insects were treated and mortality assessed after 72h.

Dip test

Aqueous dilutions of formulated cypermethrin were prepared and groups of 1st or 3rd instar larvae submerged for 30 sec in a way similar to that described by Watkinson et al.(1984). For each control and test group at least 40 insects were treated. Mortality of 1st and 3rd instar insects was assessed at 48h and 72h respectively.

Resistance factors at LD_{50} and LD_{90} were obtained by dividing the values for the R strain by those for the S strain.

RESULTS

In each test in a series of bioassays with H. virescens the slope values for the probit lines were lower for the resistant PEG87 strain than the susceptible BRC strain, and the resistance factors at LD_{90} were therefore higher than those at LD_{50} (Table 1). At the LD_{50} response level the foliar residue tests gave much greater discrimination than any other tests. With 3rd instar insects relatively huge quantities of residue were required to obtain a dose response. In contrast, the dip tests were relatively inefficient (Table 1).

In the topical test very similar tolerance values of between 194 and 228 were obtained when 3rd or 5th instar larvae or adults were bioassayed. The discrimination at LD_{90} was 6.5- and 5.8-fold greater respectively than at LD_{50}. The results with male moths were almost identical to those obtained with third instar larvae. In contrast, female moths of both strains were about twice as tolerant of cypermethrin as male moths although this was less marked at the LD_{90} response (Table 1).

TABLE 1

A comparison of the resistance factors obtained using cypermethrin in various types of bioassay on susceptible (BRC) and resistant (PEG87) strains of H. virescens.

Instar	Bioassay	Strain	LD_{50}[1]	RF	LD_{90}[1]	RF
I	Foliar residue	S	0.33	-	2.13	-
		R	340	1030	3100	1455
I	Dip test	S	0.19	-	0.92	-
		R	7.2	38	164	178
III	Foliar residue	S	9.5	-	51	-
		R	18100	1905	175000	3431
III	Dip test	S	1.2	-	8.9	-
		R	20	17	1070	120
III	Topical test	S	0.02	-	0.08	-
		R	4.5	225	118	1472
III	Vial test	S	3.7	-	25	-
		R	77	21	640	26
V	Topical test	S	0.20	-	1.4	-
		R	39	194	1580	1125
Adult Male	Topical test	S	0.02	-	0.08	-
		R	4.5	225	118	1475
Adult Female	Topical test	S	0.04	-	0.27	-
		R	8.3	208	195	722
Adult Male	Vial test	S	16	-	58	-
		R	65	4	1200	21
Adult Female	Vial test	S	37	-	156	-
		R	186	5	3200	21

[1] LD values expressed as ppm (foliar and dip tests), ug/larva (topical test) or ug/vial (vial test).

The vial residue tests with both 3rd instar larvae and adults gave poor discrimination between the two strains (Table 1). As with the topical test female moths were two- to three-fold more tolerant than male moths.

With field strains of H. armigera the first instar foliar residue and topical bioassays were used. With this species the foliar residue bioassay (Table 2) was less discriminating than the topical test (Table 3). In the topical bioassay discrimination at the LD_{90} level was around 1.2- to 2.6-fold greater than at LD_{50} except in the case of highly resistant insects (India 1987/88) where up to 17-fold better discrimination was obtained.

Resistance to pyrethroids was found in populations from cotton in Thailand, India and Indonesia (Tables 2, 3). In Hyderabad in India H. armigera was found to be suscptible to pyrethroids in 1986 but later, lowing migration of insects from areas of field failure in cotton in Juzzuru, resistance to pyrethroids was found to be high.

TABLE 2

Mortality of first instar larvae of various strains of H. armigera after 48h exposure to dry residues of cypermethrin on cotton leaves. RF=resistance factor.

Strain	LC_{50} (ppm)	RF	LC_{90} (ppm)	RF	Slope	(S.E.)
Reading (S)	0.17	-	1.8	-	1.25	(0.25)
Nakansowan, Thailand 1987	3.3	19	47	26	1.11	(0.12)
Lopburi, Thailand 1987	58	341	1276	709	0.96	(0.15)
Juzurru, India 1987	9.5	56	581	323	0.96	(0.15)
Hyderabad, India 1987	1.0	5.9	19	11	0.35	(0.13)
Hyderabad, India 1988	38	224	845	469	0.95	(0.11)

TABLE 3

Mortality of third instar larvae of various strains of H. armigera 72 h after topical treatment with pyrethroids. RF=resistance factor.

Strain and Insecticide	LD_{50} (ug/larva)	RF	LD_{90} (ug/larva)	RF	Slope	(S.E.)
Reading (S)						
cis-cypermethrin	0.009	-	0.025	-	1.24	(0.13)
cypermethrin	0.020	-	0.050	-	1.31	(0.14)
fenvalerate	0.015	-	0.030	-	1.81	(0.22)
Nakansowan, Thailand 1987						
cis-cypermethrin	0.55	61	3.3	132	1.06	(0.25)
Lopburi, Thailand 1987						
cypermethrin	6.8	340	45	900	1.57	(0.19)
Hyderabad, India 1986						
cis-cypermethrin	0.005	0.6	0.020	0.8	2.21	(0.32)
Juzurru, India 1987						
cypermethrin	6.5	325	57	1140	1.35	(0.18)
Hyderabad, India 1988						
cypermethrin	15	750	627	1.3×10^4	0.70	(0.17)
fenvalerate	4.3	287	112	3.7×10^3	0.91	(0.14)
Sulawesi, Indonesia 1988						
cypermethrin	1.3	65	7.5	150	1.70	(0.25)
fenvalerate	0.3	20	1.5	50	1.81	(0.21)

DISCUSSION

There are large differences in the efficiency with which bioassays of different types distinguish between resistant and susceptible strains of H. virescens. This is because physical and behavioural differences between the life history stages tested and differences in the method of application of the insecticide will affect the uptake and intoxication of the insect. Self-dosing tests in which the insects walk over or ingest residues are likely to lead to a steady uptake of toxicant whereas single dose tests such as the topical and dip tests may lead to a relatively massive but short-lived exposure.

With H. virescens the first instar foliar residue test provides the most effective discrimination between resistant and susceptible strains. The use of this test with third instar insects is clearly unrealistic since such large quantities of the insecticide are needed. This may be due to avoidance of the residue by the larvae and it could reflect the presence of a behavioural mechanism of resistance. Only the self dosing bioassays using residues will take account of such mechanisms.

The topical test gives consistent results irrespective of the life-history stage used. Adults of H. virescens appear as resistant as larvae by this test and the doses required to achieve similar mortalities are of the same order. On a dose per unit weight basis adults are around 10- to 20-fold less tolerant than larvae and 5th instar larvae are 1.5- to 2-fold more tolerant than 3rd instar larvae. This presumably reflects the generally greater metabolic capacity of larger larvae.

The adult vial test devised by Plapp (1979) is used extensively in the USA to detect resistance in H. virescens. In our hands it is not the most sensitive test available and a discriminating dose test based on the topical bioassay would, we believe, be more sensitive as has been shown in Australia with H. armigera (Forrester & Cahill, 1987; Daly, 1988). Interestingly, male moths are less tolerant than female moths (of a similar weight) in the adult vial test and recent work in our laboratory suggests that one reason is because males are more active than females and hence acquire higher doses of the insecticide.

Effective management of insecticide resistance in Heliothis spp. in the field requires the routine screening of large numbers of insects. In practice, the use of a discriminating dose is more efficient than the conventional dose-response assay (Roush & Miller 1986). The choice of bioassay method should therefore depend on the most efficient separation of resistant and susceptible individuals. In the field, the most effective bioassays are those most sensitive to changes in tolerance as well as being easy to perform on large numbers of individuals in a discriminating manner.

The use of these tests has detected resistant strains of H. armigera from Thailand, India and Indonesia. In the latter two countries resistance to pyrethroids is reported for the first time bringing the total number of countries with documented resistance to the pyrethroids in this species to five. Others presumably remain as yet undetected or unconfirmed. In all these cases programmes of resistance management will depend on the reliable use of sensitive bioassays.

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MONITORING FOR INSECTICIDE RESISTANCE IN MIGRANT POPULATIONS OF *MYZUS PERSICAE*

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ABSTRACT

Myzus persicae shows varying degrees of resistance to insecticides due to increased production of a carboxylesterase; the enzyme can be measured in individual insects, so enabling the proportions of resistant variants to be determined in field populations. However, aphids sampled in suction traps could not be analysed as they are usually collected in alcohol which inactivates the enzyme. A storage solution has now been developed, based on glycerol and antibiotics in water, which has been used in both suction traps and in laboratory tests to preserve enzyme activity so allowing detection of insecticide resistance by immunoassay in samples maintained even at 30°C for up to 15 days.

INTRODUCTION

The peach-potato aphid (*Myzus persicae*) is a world-wide pest causing both direct damage and transmitting many plant viruses on a range of crops, including sugar beet, potatoes and oilseed rape (Blackman & Eastop, 1984). Resistance to organophosphorus, carbamate and pyrethroid insecticides is conferred by increased production of a carboxylesterase, esterase-4 (E4) (Devonshire & Moores, 1982).

Individual *M. persicae* have been categorised as susceptible (S), moderately resistant (R_1), very resistant (R_2) and extremely resistant (R_3) to insecticides, according to their E4 content. This is a simplification of a greater spectrum of resistance (Devonshire & Sawicki, 1979), but the terms are in common use and will be used here. Recent field surveys have shown that R_1 aphids predominate in the U.K., R_2 individuals are common, especially after crops have been treated with insecticides, and R_3 individuals are now found occasionally in the field as well as being common in glasshouses (Furk, 1986; Brookes & Loxdale, 1987; ffrench-Constant & Devonshire, 1988). These surveys have used electrophoresis (Loxdale *et al.*, 1983) or an immunoassay (Devonshire *et al.*, 1986) of either fresh or deep frozen insect samples to give a measure of E4 activity and hence the resistance type of individual aphids. Crop samples provide valuable information on the levels of insecticide resistance of *M. persicae* on the host plants sampled, but further information is required to study the temporal and spatial dynamics of insecticide resistance.

The Rothamsted Insect Survey (RIS), which has operated a network of suction traps throughout Britain since 1968, monitors continuously the numbers of aphids flying at a height of 12.2 m (Taylor, 1986). The daily records give a standard random sample of the numbers of aphids, including *M. persicae*, flying between host plants (Taylor, 1974). Such samples provide an ideal opportunity to study the temporal and spatial dynamics of aphid populations, including those of insecticide resistance. However, samples from suction traps are collected in 50% methanol and after a number of days the aphids are macerated in 75% lactic acid at 100°C to aid identification

(Taylor, 1984). Such procedures destroy enzyme activity in the aphid so that the samples are no longer suitable for the assay of E4. Less-extreme storage conditions (30% ethanol at room temperature) have been shown to preserve E4 activity for at least six days so that an immunoassay method involving protein detection can be used (Devonshire *et al.*, 1986). However, to preserve E4 activity, as required by the presently used immunoassay procedure, it was necessary to develop an improved storage solution in which insects could be collected in trap samples at remote sites and sent to Rothamsted for assay. The present routine of handling trap samples means that insects arriving at Rothamsted were collected up to eight days previously (Woiwod *et al.*, 1984).

This paper reports the development of a storage solution in which aphids can be collected and kept for 7-10 days, even at temperatures as high as 30°C, and an assessment still made of E4 activity. This covers the extremes of operating conditions for Rothamsted Insect Survey suction traps in Great Britain.

MATERIALS AND METHODS

Aphids

The clones of *M. persicae* used in this study were susceptible (S; clone US1L), moderately resistant (R_1; clone 405D), very resistant (R_2; clone T1V) and extremely resistant (R_3; clone 794J). The origins and rearing of these clones on excised potato leaves has been described previously (Sawicki *et al.*, 1980). When large numbers of aphids were required, they were reared on nicotine-fumigated Chinese cabbage started from 'nucleus' aphid stocks on excised potato leaves.

Laboratory studies

Esterases are known to be fairly stable, and 50% (v/v) glycerol is used commercially for storing many enzymes; it has also been used to preserve muscle activity, as it breaks down membranes allowing antibiotic compounds to penetrate (Loxdale & Tregear, 1983). For these reasons a number of solutions based on aqueous glycerol were tested. Fungal and bacterial growth were found to be associated with loss of E4 activity, so a range of antibiotics was also tested.

Initial testing was carried out by placing alate *M. persicae* from laboratory clones of known insecticide resistance into the solutions at 4, 10, 18 and 30°C. Three individuals were taken from each treatment after three, six and ten days, together with three from the reference clone to act as controls and the E4 assessed by electrophoresis using 6% vertical slab polyacrylamide gels (Loxdale *et al.*, 1983). By iteration an aqueous solution (No. 21) was found that maintained most of the E4 activity at 30°C for 15 days. This solution contained:

25% glycerol
0.5% Triton X-100 - to reduce surface tension to ease insect capture
100 mM KCl - to maintain ionic strength
20 mM Tris(hydroxymethyl)aminomethane - to maintain pH at 9.0
1 mM oxytetracycline - antibacterial, antimycotic agent
10 µM $CuSO_4$ - antimycotic agent

All were purified grades and solutions were made up in distilled water.

Other solutions tested that proved less successful included varying concentrations of ethylene glycol, the antibiotics neomycin sulphate, Benelate, and a Sigma (cat. no. A 9909) antibiotic/antimycotic solution. The omission of individual components from solution 21 was unsuccessful. 25% glycerol was the minimum concentration found to be effective in E4 activity preservation.

Electrophoresis only provides a subjective measure of E4 activity, and in subsequent work E4 activity was quantified after different treatments by immunoassay, using homogenates equivalent to 0.08 aphid per assay, and diluted to 0.02 aphid to distinguish between the R_2 and R_3 variants (Devonshire *et al.*, 1986). Apterous S, R_1, R_2 and R_3 aphids were placed in the storage solution at 4, 18 and 30°C and E4 measured after 0, 1, 4, 7, 10 or 15 days. Twenty four individuals were tested at each treatment. The same number of standards was taken from the aphid cultures at the same time as test aphids, deep frozen at -25°C and assayed alongside the test aphids.

Field testing

To test the storage solution under operating conditions, it was used as the collecting medium in a 12.2 m high suction trap at Rothamsted instead of the 50% methanol used normally. Each collecting bottle was seeded with apterous R_2 *M. persicae* so they could be separated readily from any trapped alate *M. persicae*. Samples were collected daily, transferred to fresh storage solution and then the aphids removed from the other insects at intervals ranging from 1-7 days to simulate the RIS weekly routine. At all stages samples were retained in the solution at room temperature for the duration of the test period and then stored deep frozen for later testing. E4 activity in both the test apterous aphids and the migrant alate *M. persicae* was assessed by immunoassay. The numbers of apterae tested ranged from 24-48 per time interval.

RESULTS

Laboratory samples

No differences were observed between temperatures; data were therefore combined at each time interval for each strain of *M. persicae*. No loss of E4 activity occurred in any resistant strain of *M. persicae*, even when maintained in the storage solution for 15 days (Fig. 1). In all treatments resistant aphids could be separated from susceptible aphids with treatment means within the distributions fitted previously (Devonshire *et al.*, 1986).

Trap samples

In apterous R_2 aphids seeded into trap samples, E4 activity was maintained well for the experimental period (seven days) at room temperature (Fig. 2).

Insecticide resistance in migrant populations

Of the 75 *M. persicae* found in the suction trap samples at Rothamsted while the storage solution was being used as a collecting medium (mainly from 6 June to 3 July), the majority were R_1, with 19 S and only two R_2 individuals (Fig. 3).

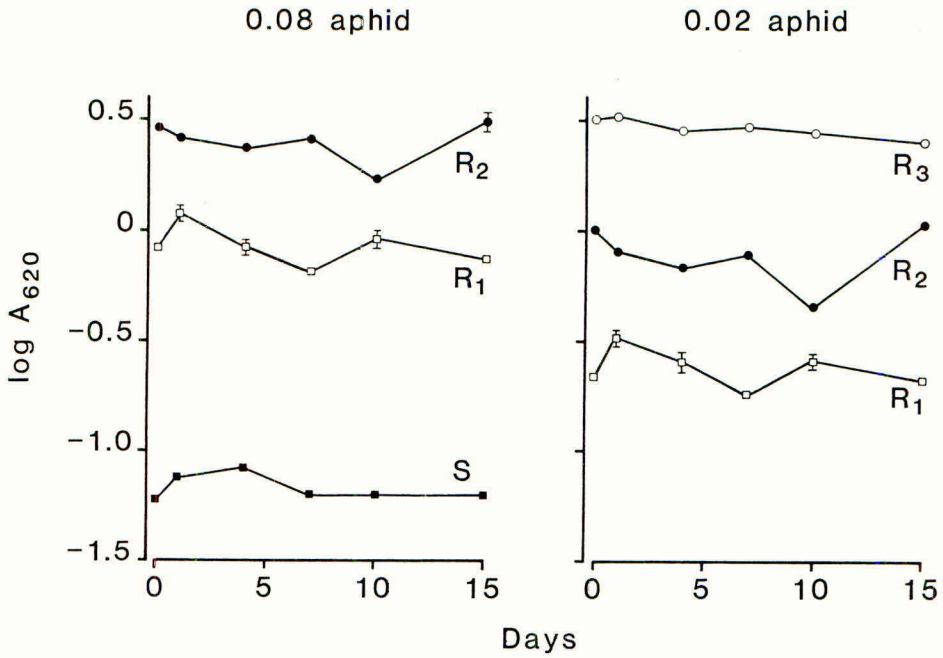

Fig. 1 E4 activity, at two aphid fractions (0.08 and 0.02), in susceptible (S), moderately resistant (R_1), very resistant (R_2) and extremely resistant (R_3) apterous *M. persicae* after maintaining in the storage solution for different periods. Bars indicate S.E. (those less than 0.03 are not shown) n = 72.

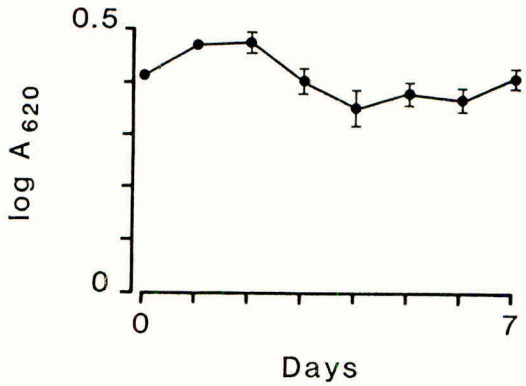

Fig. 2 E4 activity in very resistant (R_2) *M. persicae* after storing in suction trap sample in the storage solution at room temperature for up to 7 days. Bars indicate S.E. (those less than 0.02 are not shown) n = 24-48.

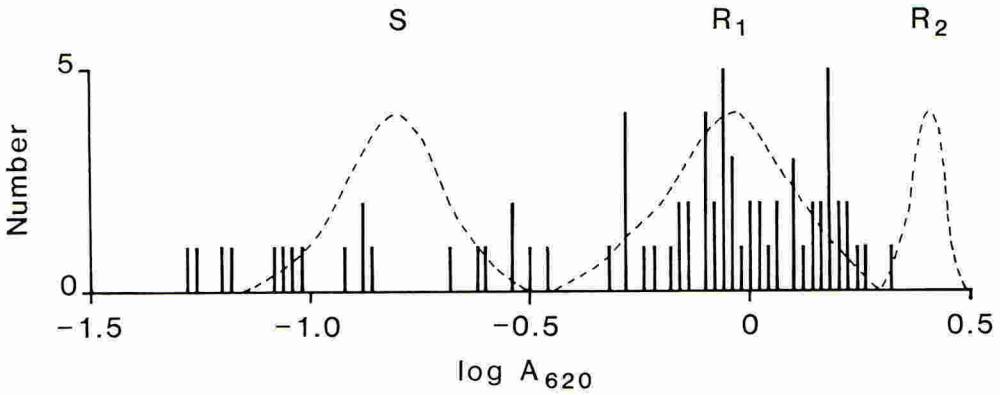

Fig. 3 Observed distribution of E4 activity in 0.08 alate *M. persicae* trapped in the 12.2 m suction trap at Rothamsted. Broken lines show the fitted distributions for fresh susceptible (S), moderately resistant (R_1) and very resistant (R_2) laboratory standards with known quantities of E4.

DISCUSSION

These preliminary findings indicate that it is now possible to use the Rothamsted Insect Survey network of suction traps, which provide a random sample of migrant aphids (Taylor, 1974), to monitor the temporal and spatial dynamics of insecticide resistance in migrant *M. persicae* populations with only minor modifications to operating procedures (Woiwod *et al.*, 1984). It will be necessary to collect all samples and sort the aphids from other insects in the storage solution. In order to retain E4 activity in *M. persicae* the whole aphid sample will then either have to be identified without clearing, which may reduce the accuracy of identification in the first instance, or *M. persicae* (forming about 1% of the total aphid sample) will have to be identified and removed from the sample prior to the remaining aphids being cleared for identification. This latter option adds an extra stage in an already tight weekly schedule (Woiwod *et al.*, 1984).

The unequivocal classification of the resistance levels of individuals as S, R_1, R_2 or R_3, when trapped in suction traps and collected in the storage solution, will be difficult because of both the widespread presence of intermediate levels of resistance in the field (ffrench-Constant & Devonshire, 1988), and the small loss in E4 activity that may take place in samples kept in unfavourable conditions due to postal delays, very large insect samples or excessive rain. The development of the storage solution (21) therefore only provides half the answer (Adams, 1977); it is now necessary to determine distribution curves of E4 activity for samples collected and stored in the solution for different periods. Despite its limitations, the technique indicates the proportion of migrant *M. persicae* that are resistant to insecticide and should enable the regional variation in resistant variants (Furk, 1986) to be investigated further.

The development of this storage solution, or minor variations of it, should also make possible the use of a wide range of biochemical diagnostic techniques such as the identification of viruses in vector aphids. This would enable suction traps to be used for giving a qualitative measure as well as a simple quantitative measure of aerial population density.

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CHANGES IN SUSCEPTIBILITY TO INSECTICIDES OF *SPODOPTERA LITTORALIS* LARVAE SELECTED WITH DIFLUBENZURON AND THREE CONVENTIONAL INSECTICIDES.

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ABSTRACT

The development of tolerance and cross-tolerance in *Spodoptera littoralis* larvae to diflubenzuron, methomyl, profenofos and fenvalerate has been investigated. An appreciable level of tolerance to and cross-tolerance between methomyl, profenofos and fenvalerate developed in *S.littoralis* larvae during 4 generations of breeding under selection pressure (at LC25 level) with any of these compounds. In contrast: the level of tolerance to diflubenzuron and cross-tolerance between diflubenzuron and any of the above-mentioned conventional insecticides was relatively low. Further selection of methomyl-, profenofos- and fenvalerate tolerant strains with diflubenzuron during the 5th generation reduced the earlier developed tolerance levels to these conventional insecticides. These results give rise to the supposition that the use of diflubenzuron in the official cotton pest control programme of the Egyptian Ministry of Agriculture contributes to the suppression of resistance to conventional insecticides.

INTRODUCTION

In Egypt the use of chemicals is still the only reliable method of controlling this pest. However, as a result of continuous applications of synthetic insecticides, resistance, cross-resistance and multi-resistance-phenomena have developed (El Guindy *et al.*, 1975, 1979, 1982; El-Dahan *et al.*, 1982). To avoid the rapid appearance of resistance, the Ministry of Agriculture has recommended since 1978 a chemical control programme consisting of 3 or 4 different sprays/season starting on July 1st. The first spray (primarily aimed against *Spodoptera*) consists of an organophosphorous or a carbamate compound mixed with a benzoylphenylurea (moulting inhibitor).

The aim of the present study was to investigate the rate of development of tolerance to and cross-tolerance between four commonly used insecticides in cotton (diflubenzuron, methonyl, profefonos and fenvalerate) in *S. littoralis* larvae under selection pressure from any of these compounds. Extra attention was given to diflubenzuron because of its special role in the cotton spraying programme in Egypt.

MATERIALS AND METHODS

Egg masses of *S.littoralis* were collected from cotton plants at the Sakha Agricultural Research Station in early June 1987 before the start of the chemical control programme. This field strain was then

reared for two generations in the laboratory without exposure to insecticides before being subjected to the toxicological tests. Rearing techniques were chosen of El-defrawi *et al.* (1964).

The 4 insecticides used for this work were the moulting inhibitor 'Dimilin' 25% WP (diflubenzuron) and 3 conventional insecticides: 'Lannate' 90% SP (methomyl), 'Curacron' 72% EC (profenofos) and 'Sumicidin' 20% EC (fenvalerate).

A leaf dip technique was used to determine the toxicity of the test compounds. Castor bean leaves were dipped for 5 seconds in an aqueous dilution of the formulated toxicant. The treated leaves were left to dry and then offered to one day old 4th instar larvae. The LC-P lines were established from the mortality figures of 5 replicates of 10 larvae for each of 5 concentrations per toxicant. LC50 and LC90 figures in the tables are expressed in ppm.

Due to the differences in mode of action between the 3 fast acting conventional insecticides and diflubenzuron, mortality counts were established after different periods of feeding. Mortality due to the conventional compounds was determined after exposure to treated leaves. Mortality due to diflubenzuron was recorded after a 72 h period during which larvae were first fed with treated leaves for 48 h and thereafter with untreated leaves for 24 h. This method is in agreement with the official methods for testing moulting inhibitors in Egypt. Mortality was calculated using Abbott's formula (Abbott, 1925).

To test the effect of repeated selection with the 4 toxicants on susceptibility, the field colony was divided into 5 subcolonies, of which the first was reared without exposure to insecticides. The other subcolonies were subjected to repeated selection for 4 generations with diflubenzuron, methomyl, profenofos and fenvalerate, respectively at LC25 level. Changes in susceptibility to each selecting agent were estimated every generation. The cross-tolerance of the 4 selected strains was assessed after the 4th generation of selection. The effect of diflubenzuron selection for 1 generation on the susceptibility of the 4 selected strains was examined by selecting the larvae of F4 of each selected strain to selection pressure with diflubenzuron at the LC25 level. The susceptibility of each strain in F5 to the 4 test toxicants was estimated.

RESULTS AND DISCUSSION

Changes in susceptibility of *S.littoralis* larvae subjected to selection pressure by 4 selected insecticides during 4 generations

The least toxic of the 4 compounds tested against the parental larvae was profenofos, followed by methomyl and fenvalerate. Most toxic was diflubenzuron (Table 1). The same table shows that the susceptibility of subsequent generations of this strain to the test toxicants increases when reared free of any insecticidal contamination. After 4 generations larvae were only 0.6-0.7 x more sensitive to the insecticides tested.

Selection pressure of field strain larvae to the test insecticides (at LC_{25} level) resulted in a gradual increase of resistance (Table 2). The eventual tolerance ratios (T.R) at LC50 for F4 4th instar larvae were 2.3, 4.1, 4.4 and 4.2 for strains selected with diflubenzuron, methomyl, profenofos and fenvalerate, respectively. In the same sequence the LC90

T.R.'s were 2.1, 4.0, 2.5 and 4.4. These results were similar to data obtained by Radwan *et al.* (1985) who recorded T.R.'s of 1.55 for diflubenzuron and 3.4 for methomyl (at LC50 level) after 5 generations of selection pressure. The relatively high T.R.'s in strains subjected to selection pressure with fenvalerate, profenofos and methomyl as compared to that of diflubenzuron indicates that resistance of *S.littoralis* to these conventional insecticides may develop faster than resistance to diflubenzuron.

TABLE 1

Susceptibility of 4th instar larvae of *S.littoralis* (field strain) reared during 4 generations under insecticide-free conditions in the laboratory.

Insecticide	Generation tested							
	P			F4			T.R.*	
	LC50	LC90	Slope	LC50	LC90	Slope	LC50	LC90
Diflubenzuron	168	420	2.17	100	280	1.96	0.6	0.7
Methomyl	860	2200	3.51	600	1600	3.11	0.7	0.7
Profenofos	1100	4800	3.98	760	3400	3.11	0.7	0.7
Fenvalerate	180	620	3.37	100	440	2.78	0.6	0.7

* Tolerance Ratio= $\frac{\text{LC50 (LC90) of F4-generation}}{\text{LC50 (LC90) of P-generation}}$

TABLE 2

Changes in susceptibility of *S.littoralis* larvae (L4) to 4 insecticides during 4 generations of selection pressure from these insecticides

Generation tested	Insecticidal selection pressure during F1-F4 generations									
	Diflubenzuron					Methomyl				
	Susceptibility			T.R.*		Susceptibility			T.R.*	
	LC50	LC90	Slope	LC50	LC90	LC50	LC90	Slope	LC50	LC90
P	168	420	2.17	-	-	860	2200	3.51	-	-
F1	180	480	2.32	1.1	1.2	1400	3100	3.92	1.6	1.4
F2	270	590	1.98	1.6	1.4	1800	4200	3.64	2.1	1.9
F3	410	720	2.19	2.4	1.7	2900	5900	3.28	3.4	2.7
F4	380	880	2.18	2.3	2.1	3600	8800	3.01	4.1	4.0

Generation tested	Insecticidal selection pressure during F1-F4 generations									
	Profenofos					Fenvalerate				
	Susceptibility			T.R.*		Susceptibility			T.R.*	
	LC50	LC90	Slope	LC50	LC90	LC50	LC90	Slope	LC50	LC90
P	1100	4800	3.98	-	-	180	620	3.37	-	-
F1	1800	5500	3.86	1.6	1.1	310	770	2.96	1.7	1.2
F2	2500	6700	3.19	2.3	1.4	450	1400	2.81	2.5	2.3
F3	4100	9300	2.98	3.7	1.9	710	2300	2.52	3.9	3.7
F4	4800	12100	2.69	4.4	2.5	750	2700	2.44	4.2	4.4

* Tolerance Ratio= $\frac{\text{LC50 (LC90) of Fx-generation}}{\text{LC50 (LC90) of P-generation}}$

Cross tolerance of *S.littoralis* selected strains to insecticides

Data presented in Table 3 suggest a very slight cross-tolerance of the diflubenzuron-selected strain to methomyl, profenofos and fenvalerate. T.R.'s at LC50 level are 1.6, 1.8 and 1.9 respectively, whereas at LC90 level the T.R.'s were as low as 1.5, 1.3 and 1.5 respectively.

The mutual cross-tolerance levels of the methomyl, profenofos and fenvalerate selected strains to these selecting agents were variable but relatively higher with T.R.'s ranging from 2.5-4.7 at LC50 levels and 1.5-2.7 at LC90 levels. Cross-tolerance levels of these strains to diflubenzuron were always lower, namely T.R.'s of 1.7 (LC50) and 1.4 (LC90) for the methomyl-selected strain, 1.9 (LC50) and 1.5 (LC90) for the profenofos-selected strain and of 2.1 (LC50) and 1.3 (LC90) for the fenvalerate-selected strain. Hence the susceptibility of *S.littoralis* larvae to diflubenzuron seems to be little affected by a preceding period of selection pressure from the conventional insecticides under consideration.

TABLE 3

Cross-tolerance of *S.littoralis* larvae (L4) after 4 generations of selection pressure with four insecticides

Selection agent during F1-F4 generations	Insecticide tested	Susceptibility F4-generation				
		Susceptibility			T.R.*	
		LC50	LC90	Slope	LC50	LC90
Diflubenzuron	**Diflubenzuron**	**380**	**880**	**2.18**	**2.3**	**2.1**
	Methomyl	1400	3400	3.11	1.6	1.5
	Profenofos	2000	6400	2.96	1.8	1.3
	Fenvalerate	350	940	3.09	1.9	1.5
Methomyl	Diflubenzuron	280	600	2.69	1.7	1.4
	Methomyl	**3600**	**8800**	**3.01**	**4.1**	**4.0**
	Profenofos	5200	12800	4.15	4.7	2.7
	Fenvalerate	450	940	3.25	2.5	1.5
Profenfos	Diflubenzuron	320	620	2.91	1.9	1.5
	Methomyl	2400	4900	4.01	2.8	2.2
	Profenofos	**4800**	**12100**	**2.69**	**4.4**	**2.5**
	Fenvalerate	460	1100	3.18	2.6	1.8
Fenvalerate	Diflubenzuron	350	560	2.94	2.1	1.3
	Methomyl	2400	5400	4.11	2.8	2.5
	Profenofos	4200	9400	4.31	3.8	2.0
	Fenvalerate	**750**	**2700**	**2.44**	**4.2**	**4.4**

* Tolerance Ratio= $\frac{\text{LC50 (LC90) of F4-generation}}{\text{LC50 (LC90) of P-generation}}$

The results were confirmed by El-Guindy *et al.* (1982) who found that lower levels of resistance were exhibited to several insecticides in a diflubenzuron-tolerant strain of the cotton leafworm. Also, Radwan *et al.* (1985) found that slight cross-tolerance ranging between a T.R. of 1.2-2 was detected in a diflubenzuron-selected strain of *S.littoralis* when the susceptibility was tested using profenofos, fenvalerate and methomyl. They also added that a methomyl-selected strain exhibited negative cross-tolerance to diflubenzuron (TR=0.6) and clear cross-tolerance to fenvalerate (2.6 fold) and profenofos (4.3 fold).

Susceptibility of 5th generation *S.littoralis* larvae subjected to selection pressure by conventional insecticides during 4 generations and to diflubenzuron during the 5th generation

The tolerance of the diflubenzuron-selected strain after a further generation of selection with this compound slightly increased (Table 4). However, exposure of the methomyl, profenofos or fenvalerate-selected strain to diflubenzuron during the 5th generation resulted in a consistent decrease in tolerance levels of all 4 insecticides as compared to the levels of the previous generation. The only exception found was the slightly increased T.R. (1.5) to fenvalerate of the methomyl and profenofos-selected strain. The tolerance level of the fenvalerate selected strain to fenvalerate was reduced (T.R.=0.2 at LC90 level).

TABLE 4

Cross-tolerance of *S.littoralis* larvae (L4) of the 4 selected strains after a further generation of selection with diflubenzuron

Selection regime F1-F4 generation	Insecticide tested	Susceptibility F5-generation Susceptibility			T.R.*	
		LC50	LC90	Slope	LC50	LC90
Diflubenzuron	**Diflubenzuron**	**590**	**900**	**2.34**	**1.6**	**1.0**
	Methomyl	1600	4600	2.81	1.1	1.4
	Profenofos	2300	6800	3.15	1.2	1.1
	Fenvalerate	370	980	2.76	1.1	1.0
Methomyl	Diflubenzuron	220	510	3.32	0.8	0.9
	Methomyl	**1800**	**4200**	**3.19**	**0.5**	**0.5**
	Profenofos	2800	7800	3.28	0.5	0.6
	Fenvalerate	340	1400	3.17	0.8	1.5
Profenofos	Diflubenzuron	260	560	3.12	0.8	0.9
	Methomyl	1400	3200	3.86	0.6	0.7
	Profenofos	**3000**	**8000**	**3.69**	**0.6**	**0.7**
	Fenvalerate	360	1600	3.25	0.8	1.5
Fenvalerate	Diflubenzuron	200	380	2.84	0.6	0.7
	Methomyl	1200	2800	3.82	0.5	0.5
	Profenofos	2200	6200	3.88	3.5	0.7
	Fenvalerate	**480**	**600**	**3.42**	**0.6**	**0.2**

* Tolerance Ratio= $\frac{\text{LC50 (LC90) of F5-generation}}{\text{LC50 (LC90) of F4-generation}}$

These results indicate that the insect growth regulator, diflubenzuron, may be effective in reducing the tolerance of *S.littoralis* to conventional insecticides. Also El-Guindy *et al.* (1980) showed that the cross-resistance levels could be remarkably reduced by intermittent breeding of this strain under selection pressure of diflubenzuron for one generation only.

CONCLUSION

The presence of diflubenzuron in the official cotton pest control program in Egypt seems to reduce the chances for resistance of *S.littoralis* to conventional insecticides and therefore prolongs their usage period. Further research will be required to support the preliminary data described in this paper.

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CHARACTERISING ACETYLCHOLINESTERASE GENOTYPES IN RESISTANT INSECT POPULATIONS

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ABSTRACT

A rapid technique for characterising and monitoring the insensitivity of acetylcholinesterase (AChE) to organophosphorous and carbamate insecticides in single insects has been applied to study AChE variation in insecticide-resistant populations of the housefly (*Musca domestica*), tobacco whitefly (*Bemisia tabaci*), and two species of aphids (*Myzus persicae* and *Aphis gossypii*).

INTRODUCTION

Biochemical tests for specific insecticide resistance mechanisms provide a powerful means of monitoring the selectivity of insecticide treatments, and appraising putative tactics for delaying or overcoming resistance. To be fully effective, however, such techniques must be applicable on a large scale to distinguish between individual heterozygotes and homozygotes for resistance genes. This is now possible for insecticide-insensitive acetylcholinesterase(s) (AChE), using a rapid microtitre plate assay to characterise enzyme inhibition in the presence of organophosphorus (OP) and carbamate insecticides (Moores *et al.*, 1988).

We report here on the use of this technique to study AChE variation in insecticide-resistant populations of the housefly (*Musca domestica*), tobaocco whitefly (*Bemisia tabaci*), and two species of aphid (*Myzus persicae* and *Aphis gossypii*).

ASSAY PROCEDURE

AChE activity is measured using acetylthiocholine as substrate, detecting the released thiol colorimetrically by reaction with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB; Ellman *et al.*, 1961). 96 insects in separate wells of a microtitre plate (NUNC) are homogenised simultaneously in phosphate/Triton (0.1M phosphate buffer, pH 7.5 containing up to 1% Triton X-100) using a multihomogeniser designed for microtitre plates, and left at 4°C for at least 1h to ensure adequate tissue solubilization. After dilution, replicate aliquots (equivalent to 0.05 of a housefly, 0.2 of an aphid or 0.4 of a whitefly) are assayed in another microtitre plate with or without the appropriate insecticide as inhibitor.

Full details of the housefly assay are given by Moores *et al.* (1988). Whitefly adults (*ca.* 20 µg) and aphids (100-400 µg) are homogenised in 5 µl buffer containing 0.1% Triton X-100, diluted to 250 µl (with the same mixture), and aliquots (100 µl for whiteflies, 50 µl for aphids) assayed. For whiteflies, substrate and DTNB concentrations are 0.5 mM, whereas for aphids, the final DTNB concentration is reduced to 15 µM to avoid AChE inhibition caused by the thiol reagent at higher concentrations (Smissaert, 1976). This reduced concentration does not become limiting provided that final absorbance readings (at 405 nm) are below 0.2.

Assays are performed on a V_{max} kinetic microplate reader (Molecular Devices), utilizing the SOFTmax software package run on a Packard-Bell (IBM-type) microcomputer. Repeated absorbance readings, taken simultaneously for each well at increasing time intervals, are displayed as graphs in a 12 x 8 microtitre plate format on the computer screen for continuous assessment during the assay. On completion of a kinetic run, linear regressions are fitted to the absorbance-time data for each well.

APPLICATION TO HOUSEFLIES

Houseflies possess several allelic AChE variants with different levels and patterns of insensitivity to OP and carbamate insecticides (e.g. Moores *et al.*, 1988). These variants often coexist with each other and with numerous detoxication mechanisms, rendering the genetic and biochemical basis of resistance to these compounds very complex. The present technique can be used to investigate the role of insensitive AChE in resistance by monitoring temporal changes in genotype frequencies, and by analysing the extent of AChE polymorphisms in fly samples collected from the field.

Monitoring genotype frequencies in age-structured populations

Research presently underway at Rothamsted aims to quantify how several insecticide treatment regimes select for resistance genes in age-structured fly populations resembling those in the field (Denholm, 1988). As part of this work, two AChE variants with contrasting patterns of sensitivity have been isolated from genetically-heterogeneous stocks and inbred into the genetic background of the insecticide-susceptible Cooper strain. Strain 6401 is derived from strain CH_2 of American origin, with AChE indistinguishable from that of the Weymann strain (Moores *et al.*, 1988); compared to the baseline sensitivity of the S enzyme in the Cooper strain, it is less sensitive to dichlorvos but slightly hypersensitive to azamethiphos. Variant 49R, of Danish origin (Moores *et al.*, 1988), is slightly insensitive to azamethiphos and dichlorvos. Differences between the CH_2, 49R and S enzymes are such that a bivariate plot of inhibition rates (expressed as a percentage of uninhibited AChE activity) with 10 µM dichlorvos and 0.2 µM azamethiphos for the same insect clearly resolves all six possible genotypic combinations of these three alleles (Fig. 1a).

Age-structured laboratory populations polymorphic for these alleles were established by releasing similar numbers of unmated male and female adults of strains homozygous for each variant into population cages (80 x 40 x 40 cm) containing water, sugar and fresh pasteurised milk. The populations were maintained by innoculating eggs collected thrice-weekly onto a bran-based larval medium and transferring, one week later, the resulting pupae back into the cage (Denholm *et al.*, 1986). The cages were left untreated for 12 weeks to examine the persistence of each variant in the absence of insecticide. One population was then exposed continuously to a residue of trichlorphon (a non-volatile precursor of dichlorvos) by attaching aluminium panels sprayed with an aqueous dilution (500 mg a.i./m^2) of formulated insecticide ('Dipterex 80', Bayer UK) to the rear wall of the cage. AChE genotype frequencies were monitored as above after rearing weekly samples of eggs to adults under uncrowded conditions.

Throughout the experiment there was no difficulty in assigning adults unambiguously to one of the six genotype classes. After some initial fluctuations, allele frequencies remained markedly stable in the absence of insecticide, indicating little or no difference in the competitive fitness

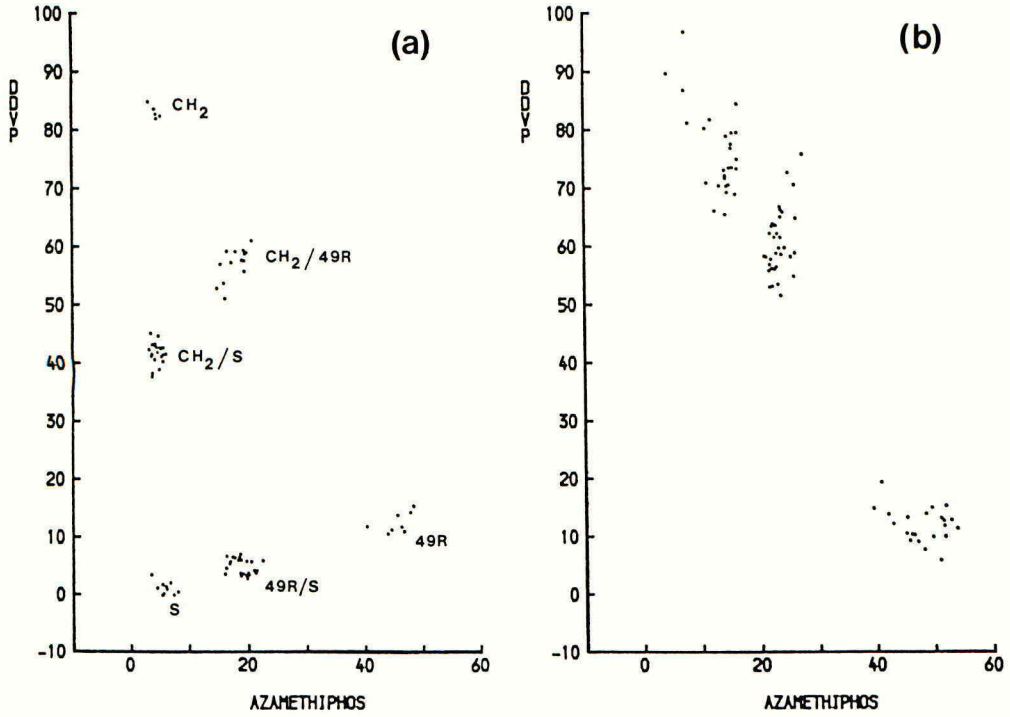

Fig. 1. Bivariate plots of mean % activity remaining during inhibition of AChE by 10 µM dichlorvos and 0.2 µM azamethiphos: (a) - the six possible AChE genotypes derived from strains CH_2,49R and S; (b) - individuals of the multiresistant French strain. 88 individuals were assayed for each plot.

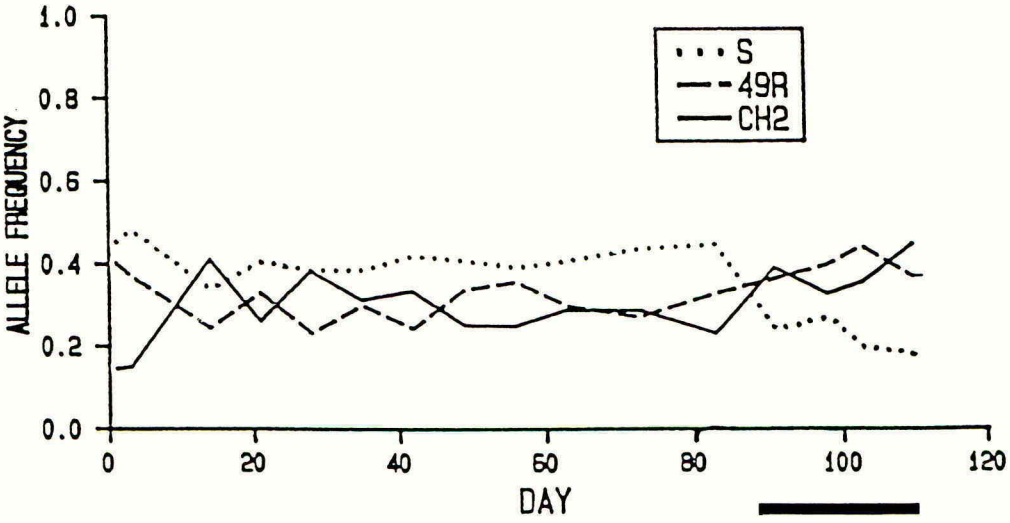

Fig. 2. AChE allele frequencies in a housefly population unexposed to insecticide, and during exposure to a residue of trichlorphon (solid bar denotes the period of trichlorphon treatment).

conferred by each AChE variant (Fig. 2). Under trichlorphon selection there was a perceptible but slow increase in frequency of the CH_2 and 49R alleles at the expense of the S variant. This result supports toxicological findings that the level of resistance conferred by these insensitive alleles alone is relatively weak (Denholm, unpublished data).

Analysis of allelic variation in a field strain

In addition to resolving well-characterised variants the assay can, by enabling several inhibitors to be tested against the same insect, be used to investigate the number and type of AChE alleles in strains of unknown genetic composition. To illustrate this, AChE from flies of a multi-resistant field strain collected from Ain, France in 1987 was assayed with five OP and carbamate inhibitors: dichlorvos (10 µM), malaoxon (10 µM), paraoxon (40 µM), azamethiphos (0.2 µM) and methomyl (100 µM). These diagnostic concentrations were determined from preliminary experiments with the S enzyme. Results were analysed by calculating correlation coefficients for each pair of percentage inhibition data, and by plotting the data in pairwise combinations to assess visually the clustering of individual points.

Results for dichlorvos and azamethiphos (Fig. 1b) resolved individuals into three distinct groups, which on this graph are similar to the previously-characterised genotypes CH_2/CH_2, CH_2/49R, and 49R/49R (*cf.* Fig. 1a). However, insensitivity to dichlorvos, unlike that of the CH_2 enzyme, showed a strong positive correlation with insensitivity to methomyl (r_{86}=0.96), indicating that this dichlorvos-insensitive enzyme is distinct biochemically from the CH_2 variant. The azamethiphos-insensitive enzyme appears from this analysis to be identical to the 49R variant. The fully sensitive S enzyme was not detected in this strain.

Single-pair crosses established to test the genetic interpretation indicate that there are at least two alleles contributing to the top left-hand cluster of points in Fig. 1b. Further work to resolve them is presently underway.

APPLICATION TO WHITEFLIES

The development of insecticide resistance in *B. tabaci* has enabled this species to rise from a secondary pest to become the most important cotton pest in some areas. Insensitive AChE has been implicated in this resistance (Dittrich *et al.*, 1985), and its role is being further examined using two whitefly strains - one susceptible to presently-used insecticides, and the other a Sudanese field strain resistant to a wide range of pyrethroids and OPs.

The insensitive AChE can be detected readily in individual whitefly adults using the microtitre plate assay, since the S and R enzymes differ over 500-fold in response to paraoxon. Frequency distributions of inhibition rates (expressed as for houseflies) distinguish homogenous enzymes in haploid males, but do not separate female heterozygotes (SR) from susceptible homozygotes (SS) since the S variant dominates the assay; this is because it has approximately 10-fold more uninhibited activity than the R variant. However, by comparing visually the progress of paraoxon-inhibited and uninhibited reactions, individual females are readily classified as SS, SR or RR, and males as S or R (Fig. 3).

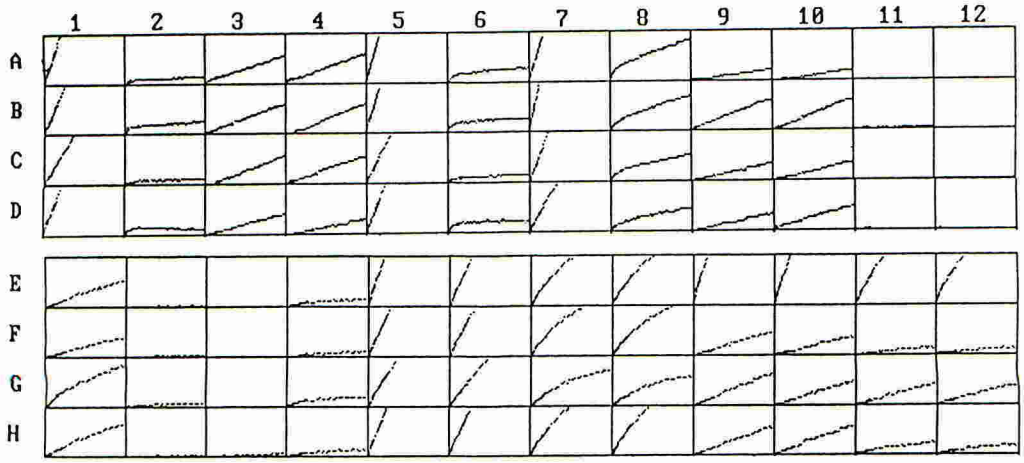

Fig. 3. Kinetic plots of individual reactions in microtitre plates.
Rows A-D: *B. tabaci* assayed for 30 min in the absence (odd columns) and presence (even columns) of 30 µM paraoxon. Columns 1-2, S♂ ; 3-4, R♂ ; 5-6, SS♀; 7-8, SR♀; 9-10, RR♀; 11-12, buffer blanks.
Rows E-H: *A. gossypii* assayed for 20 min in the absence of inhibitor, or presence of 10 µM pirimicarb, 400 µM omethoate, and 40 µM demeton-S-methyl.

Assays have shown that this insensitive AChE variant predominates in the resistant Sudanese population, but is absent from the susceptible strain. The technique is now being used to monitor the role of this mechanism in the build-up of resistance in age-structured *B. tabaci* populations simulating field conditions.

APPLICATION TO APHIDS

Insensitive AChE has previously been shown to cause strong and specific pirimicarb resistance in cotton-melon aphids *A. gossypii* (Silver, 1984; reviewed by Devonshire, 1988). For the present work, susceptible (strain 81-171, from cucumbers) and pirimicarb-resistant (strains 80-005 and 85-052 from chrysanthemums in UK glasshouses) *A. gossypii* individuals were examined with the microtitre plate assay. Visual assessment of inhibition curves (Fig. 3) shows clear pirimicarb-insensitivity of enzymes from the resistant aphids, with smaller differences in response to omethoate and demeton-S-methyl. In contrast to whiteflies, the insensitive enzyme is 2-6 fold more active than the sensitive form. Even so, expressing mean inhibited slope as a percentage of uninhibited rate clearly discriminated between the susceptible and two resistant strains:

Strain	n	pirimicarb	omethoate	demeton-S-methyl
81-171	6	8 ± 3	3 ± 5	27 ± 3
80-005/85-052	13	87 ± 12	42 ± 9	38 ± 9

In peach-potato aphids, *M. persicae*, the only resistance mechanism so far identified is increased detoxication of insecticides by a carboxylesterase (Devonshire, 1988). Several standard reference clones, US1L, 405D, T1V, 794J, FrenchR and Ferrara (Devonshire *et al.*, 1986) together with clones established from field samples (916, taken from

potatoes at Rothamsted, and 922 from peaches at Ferrara, Italy), were examined for AChE insensitivity. Mass homogenates of each clone were prepared, and replicate aliquots, equivalent to 0.2 aphid, tested with malaoxon, heptenophos, paraoxon and pirimicarb, each chemical at 1,3,10,30 and 100 μM. All eight clones responded identically, confirming earlier preliminary observations in 1976 (Devonshire & Moores, unpublished data) that AChE insensitivity does not contribute to resistance in this species.

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COLORADO POTATO BEETLE RESISTANCE TO INSECTICIDES IN ONTARIO, CANADA

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ABSTRACT

Colorado potato beetle (CPB), Leptinotarsa decemlineata, from 7 potato-growing areas across Ontario were monitored for resistance to representative insecticides. High resistance to carbofuran was identified in 6 of 7 populations; endosulfan resistance was >30x in 4 of 6 collections. Resistance to deltamethrin and fenvalerate varied, reaching a high of 30x in one collection. All populations showed moderate resistance to azinphosmethyl but remained susceptible to aldicarb. In laboratory tests, abamectin, cyhalothrin, and cyfluthrin were highly toxic to insecticide-susceptible CPB; the 2 pyrethroids were ca. 1/10 as toxic to a resistant strain. In field microplot tests, foliar applications of RH-5849 and cyhalothrin provided excellent control of insecticide-susceptible CPB. Bacillus thuringiensis var san diego and B. thuringiensis var tenebrionis gave fair to good control of small larvae early in the season.

INTRODUCTION

The Colorado potato beetle (CPB), Leptinotarsa decemlineata, was first reported as a pest of potatoes in midwest North America about 1860 and by 1874 had spread to the eastern United States (Casagrande 1987); the first CPB were reported in Ontario in 1870 (Reed 1872). After its introduction into France in 1922, it spread rapidly through Europe, except in the British Isles where rigid quarantine restrictions have prevented its establishment. Intensive use of insecticides to control CPB has led to rapid and widespread development of insecticide resistance, particularly in the northeastern United States (Casagrande 1987). In Canada, CPB first developed resistance to organochlorine (OC) insecticides, then, in 1979, in an isolated area of Quebec to carbofuran and most other recommended organophosphorus (OP) and carbamate insecticides, other than aldicarb, and soon after to pyrethroids (Harris & Svec 1976, 1981; Harris & Turnbull 1986). CPB resistance to carbofuran has also been demonstrated in New Brunswick (Boiteau et al. 1987). In Ontario, in an effort to slow resistance development, growers were encouraged to alternate insecticides, choosing from a different chemical group for each application. Concurrently, resistance levels were monitored in "problem" areas, and several experimental insecticides were evaluated in laboratory and/or field experiments in an attempt to expand grower options for CPB control. Results of these studies are summarized in this report.

MATERIALS AND METHODS

Beginning in 1984, CPB were collected annually from the Thedford Marsh near Lake Huron in southwestern Ontario; following widespread control failures in 1987 and 1988, collections were expanded to most major potato-growing areas of Ontario. The insects were reared on potatoes as described by Harris & Svec (1976). Adults were tested the day after collection; larvae were reared to adults, which were tested when 24-48 h old. Carbofuran and fenvalerate were used as test insecticides in the initial stages of the monitoring program. Subsequently, endosulfan, azinphosmethyl, aldicarb, and deltamethrin were included to provide a representative cross-section of insecticides currently recommended for CPB control. Seven potential CPB insecticides - cyhalothrin, tefluthrin, cyfluthrin, flucythrinate, abamectin, chlorethoxyfos, and thianitril - were evaluated against both an insecticide-susceptible (S) strain (Harris & Svec 1976) and an insecticide-resistant (R) strain (Harris & Turnbull 1986) maintained in our laboratory. The R strain, originally collected in 1982 near Sherbrooke, Quebec contained the highest levels of resistance seen in Canada to date against all recommended insecticides (carbofuran >1600x, azinphosmethyl = 17x, pyrethroids = 23x-38x) except aldicarb. The R strain has since been maintained in the laboratory with regular selection with permethrin. In direct contact toxicity tests, technical (>95% purity) grade insecticides were dissolved in 19:1 acetone: olive oil and were applied using a Potter spray tower as described by Harris et al. (1962). A range of insecticide concentrations was used which was suitable for differentiating between S and R strains. Two replicates of 10 beetles were treated at each concentration, with the test being repeated the following day if enough beetles were available. Ten beetles treated with the solvent mixture served as a control for each test. Treated beetles were placed in clean, waxed cups covered with glass petri dishes, and were provided with fresh potato foliage and held at 27°C and 65% RH, under continuous light for 18 h, when mortality counts were made. Corrections for natural mortality were made by Abbott's formula.

Insecticides were evaluated in field tests as follows: on 12 May 1988, a single row of potatoes, cv. Chieftain, was planted in organic soil contained in field microplots (Harris et al. 1971). On 10 June, due to low and uneven natural CPB infestations, a total of 35 adults, collected from adjacent field plantings, was infested into each microplot. On 17 June, 4 plants, selected at random in each microplot were flagged. Insecticides were applied at 275 kPa in 900 L of water/ha using a single nozzled (D-4 orifice disc, #25 swirl plate) Oxford precision sprayer. Treatments, including controls were replicated 3x in a randomized complete block design. Rates and timing of application are shown in Table 1. CPB life stages were counted on all flagged plants on 20, 27 June, and 4, 12, 20 July. Feeding damage on foliage was assessed visually on 6, 15, 26 July.

RESULTS AND DISCUSSION

After developing resistance to most OC insecticides in the early 1970's (Harris & Svec 1976), Ontario CPB remained susceptible to recommended OP, carbamate, and pyrethroid insecticides for several years. Beetles collected from the Thedford Marsh in 1985 were as susceptible to carbofuran as the S

TABLE 1

Experiment design for field microplot studies of insecticides for CPB control

Treatment	Active Ingredient	Formulation Applied	Rate of Application /ha	Date of Application June 20	June 28	July 5	July 12
1	RH-5849*	240 EC[1]	200 g a.i.	+	+		+
2	RH-5849	"	300 g a.i.	+	+		+
3	B.t.**var san diego	'M-ONE' FW[2]	7.0 l	+	+	+	+
4	B.t. var san diego	"	14.0 l	+	+	+	+
5	B.t. var tenebrionis	'SAN 418' I[3]	10.0 l	+	+	+	+
6	B.t. var tenebrionis	"	14.0 l	+	+	+	+
7	cyhalothrin	'KARATE' 50 EC[4]	7.5 g a.i.	+			+
8	cyhalothrin	"	10.0 g a.i.	+			+
9	deltamethrin	'DECIS' 2.5 EC[5]	7.5 g a.i.	+			+
10	CONTROL	-	-				

* 1,2-dibenzoyl-1-tert-butylhydrazine; ** Bacillus thuringiensis
1) Rohm and Haas Canada Inc; 2) ELANCO, division of Eli Lilly Canada, Inc.;
3) SANDOZ Agro Canada, Inc.; 4) Chipman, a business unit of C.I.L. Inc.;
5) Hoechst Canada Inc.

TABLE 2

Direct contact toxicity of carbofuran and fenvalerate to adult CPB

Strain	Year	Mean % mortality at % solution indicated 0.0033	0.010	0.033	0.10	0.33	1.0	Approximate level of resistance*
				Carbofuran				
S		0	93	100	100	100		-
Thedford	1985	10	100	100	100	100		1
	1986	0	50	95	100	100	100	3
	1987		0	5	15	10	5	>100
	1988	5	25	30	20	25	25	>100
				Fenvalerate				
S		0	50	70	93	99	100	-
Thedford	1984	5	0	58	63	85	95	10
	1985	0	0	25	60			10
	1986	0	20	23	73	98	100	10
	1987		60	70	100	100		1
	1988	0	35	25	52	100		10

* relative to insecticide-susceptible strain

strain (Table 2) even though it had been widely used for more than a decade. However, in 1986 the Thedford strain showed first indications of resistance development and numerous control failures were reported in 1987, when resistance increased dramatically to >100x, a level that persisted in 1988. Resistance development to fenvalerate, first recommended for use in 1980, progressed in a different fashion. By 1984, when monitoring began at Thedford following field reports of reduced CPB control by fenvalerate, ca. 10x resistance was already present (Table 2). The resistance level has not increased since then, probably due to reduced use of pyrethroids.

To date, resistance monitoring in 1987 and 1988 has indicated moderate to high levels of resistance to all tested insecticides, except aldicarb, in 7 different populations of Ontario CPB (Table 3). Carbofuran resistance was lower in the Ridgetown strain, collected from a research farm where many different insecticides are tested annually; yet, despite this extreme case of "alternation of insecticides", resistance to the pyrethroids was 13x.

Aldicarb was first recommended in Ontario for CPB control in 1978. Although initially very effective, reported efficacy declined after several years. However, as the survey data indicate, reduced efficacy is not due to development by CPB of resistance to aldicarb. A more likely explanation is the development by soil microbes of the capacity for rapid aldicarb degradation (Suett & Jukes 1988, R.A. Chapman, pers. comm. 1988).

TABLE 3

Relative resistance of selected populations of adult CPB to recommended insecticides in Ontario potato-growing areas, 1987-1988

Location	Estimated resistance level* at LD_{50}					
	carbo-furan	fenval-erate	delta-methrin	aldi-carb	azinphos-methyl	endo-sulfan
Thedford	>100	10	3	1.8	3	10
Sylvan	>100	5	9	1.8	5	30
Union	>100	5	8	1.0	4	>100
Ridgetown	4	13	13	1.7	2	het.**
Alliston	>100	30	30	1.0	3	80
Greenville	>100	3	4	1.8	5	>100
Sarnia	>100	10	6	-	3	-

* relative to insecticide-susceptible strain
** heterogeneous population

In other laboratory studies, several potential CPB insecticides were highly toxic to S CPB (Table 4). Cyhalothrin was most effective, appearing as toxic as the currently recommended deltamethrin (Harris & Turnbull 1986). All tested pyrethroids were, however, less toxic to the R as compared to the S strain. The levels of resistance ranged from ca. 5x with flucythrinate to 30x with tefluthrin; cyhalothrin and cyfluthrin were intermediate with resistance levels of 10x and 7x, respectively. Thianitril and chlorethoxyfos were equitoxic to the S and R strains. Abamectin was highly toxic to both S and R strains, but was relatively slow acting; toxicity was 10x higher 3 DAT (days after treatment) as compared to 1 DAT.

TABLE 4

Direct contact toxicity of 7 insecticides to an insecticide-susceptible (S) and an insecticide-resistant (R) strain of CPB in laboratory bioassays

Insecticide	Strain	Mean % mortality at % solution indicated							
		0.00033	0.001	0.0033	0.01	0.033	0.1	0.33	1.0
Cyhalothrin	S	10	90	100	100	100			
	R		0	12	83	100	100		
Abamectin	S	5	40	90	100				
	R	0	45	100	100				
Cyfluthrin	S	0	0	63	98	100			
	R	0	0	0	18	87	100		
Flucythrinate	S			10	70	100	100	100	
	R			0	0	50	100	100	
Tefluthrin	S		0	0	30	95	100		
	R		0	0	0	0	0	40	100
Thianitril	S		0	0	20	85	100	100	
	R		0	0	5	90	100	100	
Chlorethoxyfos	S		0	0	0	0	18	54	100
	R		0	0	0	0	19	58	84

In field studies, there were no significant differences among CPB populations in microplots prior to the 1st insecticide application on 20 June (Table 5), when most CPB were present as 1st and 2nd instar larvae. By 27 June, CPB populations in plots treated with cyhalothrin (treatments #7,8) and deltamethrin (#9) had fallen by as much as 93%. In remaining treatments, CPB numbers, while lower than in CONTROL (#10) plots, had actually risen. These treatments (Table 1) were thus applied again on 28 June. By 5 July, CPB in plots treated with RH-5849 (#1,2) had fallen to low levels; CPB remained low in these plots for the remainder of the experiment. RH-5849 appears to be a slow-acting but persistent insecticide. On 5 July, CPB populations in plots treated with both formulations of B. thuringiensis, while much lower than in CONTROL plots, were still fairly high and both

treatments were applied for the third time. B.t. var tenebrionis (#5,6) appeared more effective than B.t. var san diego (#3,4). Foliar feeding damage on 6 July was minimal in all treated plots except those treated with B.t. var san diego; CONTROL plots were nearly defoliated by that date.

TABLE 5

Control of CPB in field microplots

Treatment[1]	Number of CPB/plant					Mean feeding damage rating[2]		
	20/6	27/6	5/7	12/7	20/7	6/7	15/7	26/7
1	29.1 a[3]	31.4 c	3.7 d	0.6 c	2.5 bc	0.2 c	0.1 d	0.8 d
2	20.7 a	31.8 c	1.9 d	1.1 c	2.3 bc	0.1 c	0.1 d	0.5 d
3	36.8 a	41.9 bc	50.3 b	33.0 a	7.5 a	1.0 b	3.5 b	3.5 b
4	26.1 a	36.0 c	28.8 bc	16.8 b	3.9 abc	0.3 c	1.7 c	2.2 c
5	36.9 a	56.5 ab	15.8 cd	11.8 bc	4.7 ab	0.2 c	0.7 d	2.2 c
6	26.2 a	36.5 c	14.0 cd	4.4 c	1.2 c	0.1 c	0.1 d	0.6 d
7	27.1 a	10.9 d	6.4 cd	6.5 bc	3.9 abc	0.1 c	0.6 d	0.4 d
8	34.2 a	2.4 d	3.0 d	6.2 bc	4.8 abc	0.0 c	0.1 d	0.2 d
9	32.4 a	3.4 d	3.8 d	8.7 bc	4.1 abc	0.1 c	0.4 d	0.5 d
10	27.2 a	69.3 a	91.0 a	*	*	5.0 a	5.0 a	5.0 a

[1]refer to Table 1 for description
[2]rating scale: 0=no feeding; 1=10% defoliation; 2=25% defoliation; 3=50% defoliation; 4=75% defoliation; 5=100% defoliation
[3]figures in the same column followed by the same letter do not differ significantly at P=0.05 (Duncan's Multiple Range Test)
*plots defoliated

CPB counts taken on 12 July, prior to reapplication of all treatments showed generally low numbers in all plots except those treated with B.t. var san diego (#3,4) and the low rate of B.t. var tenebrionis (#5) (Table 5). On 20 July, there was little difference among CPB populations; highest numbers were recorded in the presence of the low rate of B.t. var san diego (#3), lowest in plots treated with the higher rate of B.t. var tenebrionis (#6). A final damage rating on 26 July showed defoliated CONTROL plots (#10), more than 50% defoliation in plots treated with the low rate of B.t. var san diego, ca. 30% defoliation in plots treated with either the high rate of B.t. var san diego or the low rate of B.t. var tenebrionis and less than 10% defoliation in remaining treatments.

In summary, Ontario potato growers are faced with a significant and increasing insecticide resistance problem in CPB. Several insecticides, however, appear promising. Some appear equally effective against resistant CPB. Both strains of B. thuringiensis tested gave fair to good CPB control. Although the strains are reportedly genetically identical (Krieg et al. 1987), the Sandoz formulation proved more effective. 'M-One' received a full registration in the United States on 13 May (Anon. 1988a). As this biopesticide is effective only against early instar larvae (Anon. 1988b) field scouting, already practiced by progressive growers, assumes even greater importance.

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REACTIVE AND PREVENTATIVE STRATEGIES FOR THE MANAGEMENT OF INSECTICIDE RESISTANCE

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ABSTRACT

Many pest insects have developed resistance to the synthetic compounds applied to control them. To manage the development of resistance, a variety of management strategies have been implemented, such as rotation or mixtures or insecticides. Models are presented here that simulate the rate of development of resistance under rotation and mixture strategies. The models assume that resistance is due to the inheritance of a single semi-dominant allele (R) and that there is no cross-resistance. The results indicate that a mixture strategy may delay the development of resistance most effectively when the initial R allele frequency is low. However, when the frequency of R exceeds about 0.02, rotation becomes the better strategy. The results are discussed in relation to the incentive to implement alternative strategies. A critical assessment of the value of such models is also made with reference to the mode of inheritance of insecticide resistance.

INTRODUCTION

A rapidly increasing number of pest insects and mites are becoming resistant to the chemical insecticides and acaricides used to control them (Georghiou & Mellon, 1983). This is especially true of a wide range of phytophagous, pre-harvest Lepidopteran tests such as those belonging to the genera Heliothis (Luttrell et al., 1987, Ahmed & McCaffery, 1988), Spodoptera (El-Guindy et al., 1982), Plutella (Liu, 1982) and Pectinophora (Haynes et al., 1987). A significant proportion of the global use of insecticides is directed against Heliothis spp. and reports of resistance are becoming more frequent.

Difficulties in the control of insects occurring as a result of resistance are frequently found where the host plant acts as a trap crop and/or where a high proportion of the pest population is continually exposed to the selecting insecticide. Such difficulties have lead to the concept of using mixtures, rotations or mosaics of insecticides in order to combat resistance (Georghiou, 1983, Knipling & Klassen, 1984, Curtis, 1985, Mani 1985).

A number of management strategies of this sort have been devised and successful examples include the control of mites in Zimbabwe (Sawicki & Denholm, 1987), the cotton bollworm Heliothis armigera in Australia (Forrester & Cahill, 1987) and the Egyptian cotton leafworm Spodoptera littoralis and the pink bollworm Pectinophora gossypiella in Egypt (Sawicki & Denholm, 1987). In all cases these strategies are based on an alternation or rotation of pesticides between sprays or seasons and all depend on either voluntary or enforced compliance from farmers and agrochemical suppliers.

Since the number of novel compounds being discovered for the control of

certain pest insects is dwindling (Ware, 1983), it is of paramount importance to develop models from which strategies can be devised to maximize the life of existing insecticides. The modelling carried out here considers two strategies, mixture and rotation of insecticides. The aim of the modelling was to identify the best strategy to maximise the useful life of a favoured insecticide, perhaps because it is very effective and relatively inexpensive. The rotation model is based on the strategy to control H. armigera in Australia, in which pyrethroid use is restricted in theory to one generation in four (or five).

THE MODELS

Both models assume that resistance is due to the inheritance of a single allele and that different alleles confer resistance to each insecticide. The resistance alleles are located at unlinked loci and produce no cross-resistance. Only two insecticides are considered, either in rotation or mixture. For both models, coverage following application of insecticide is set at 0.95. The favoured insecticide (insecticide A) is assumed to kill 0.99 of susceptible insects under field conditions, whilst only 0.9 of susceptible insects succumb to insecticide B in the field. The initial frequency (q_o) of the resistance allele to insecticide A (R) is varied and the initial frequency of the resistance allele to insecticide B is fixed at 0.001. The level of effective (H) dominance of R with respect to the susceptible wild type allele is varied from 0 (fully recessive) to 0.2. The level of dominance of the second resistance allele is fixed at 0. The rotation strategy consists of three generations when insecticide A is not applied followed by one generation of application and so on. When insecticide A is not being applied, a selection coefficient of 0.3 against this effect on fitness is assumed and R is recessive in the absence of insecticide. The insecticides are applied at full rate in mixture. The strategies are considered to have failed when the frequency of R (q) exceeds 0.9.

RESULTS

Figure 1 summarizes the results of the modelling. When the q is less than 0.01, the level of dominance has a marked effect on the time to failure. Time to q>0.9 is maximized when H=0 for both the rotation and mixture strategies and under the above conditions mixture is the better strategy. If the resistance allele is semi-dominant in the field, H=0 can be effectively achieved by using a sufficiently high dose to kill susceptible homozygotes and heterozygotes. However, if a dose is used that allows just a small level of dominance in R, the time to failure is reduced by an order of magnitude and rotation becomes the better strategy. As q increases towards 0.1, the time to failure decreases dramatically and as q increases H has less and less effect.

When q_i=0.02-0.04 rotation takes over as the better strategy, irrespective of the level of dominance. The rotation scenario modelled in Figure 1 assumes a selection coefficient of 0.3 against R in the absence of insecticide. Selection against R in the absence of insecticide probably occurs in most cases (Curtis et al., 1978, Muggleton, 1983), but a value of 0.3 may have been an overestimate. Therefore, the rotation model was run again setting R as neutral in the absence of insecticide. When R is neutral, rotation becomes the better strategy when q=0.03-0.07, depending on the level of dominance in the presence of insecticide.

DISCUSSION

In accordance with other studies (Curtis, 1985, Mani, 1985), the models confirmed the value of mixture strategies, providing q is low (<0.02) and that sufficiently high doses can be used to ensure that R is effectively recessive. Therefore, for a population of pest insects that has never been exposed to the two proposed insecticides and in which there is no cross-resistance, mixture is the better of the two strategies modelled to prevent the development of resistance.

Methods for the detection of resistance in many of these species depend, at the present time, on bioassays that are highly limited in their capacities to detect low levels of resistance. Therefore it is usual for resistance to remain undetected until the frequency of the gene(s) responsible reaches about 0.1 (Roush & Miller, 1987). From the present models, q=0.02-0.04 appears to the critical point above which rotation is better than mixture. When q=0.02, only 4 insects in every 1000 (q^2) would be resistant homozygotes, well below the practicable level of detection.

The management of pest insects and strategies to counteract the development of resistance are mediated in practice by financial considerations. If a mixture strategy is used at a time when one of the resistance alleles is at a relatively high frequency, the number of insects in the pest population resistant to one of the compounds in the mixture will increase rapidly. However, the strategy may continue to appear to work if the pest population remains susceptible to the second insecticide. To avoid the costly waste of applying an ineffective insecticide in mixture, continual monitoring of the level of resistance to both compounds is necessary.

Strategies to control resistance have rarely been put into operation without the impetus of widespread field failure. Despite this, many models devised to predict the progress of resistance in insect populations have been based on scenarios in which the resistance genes are present in the population at selection mutation balance frequencies found before insecticide application (Holloway, 1986). Clearly, practical use of a strategy for the control of many pest species begins at a time when the gene(s) for resistance to one or more of the compounds involved is relatively common in the population. Where management strategies are implemented in reaction to the detection of resistance, Figure 1 shows that rotation is likely to be the better strategy.

The models are sensitive to several variables, including the level of dominance. Laboratory work suggests that resistance alleles are semi-dominant with respect to the wild type allele (Roush & McKenzie, 1987), but it is not known whether the level of dominance seen in the laboratory reflects the situation in the field. Deleterious alleles are generally recessive (Sheppard, 1967), hence it is likely that resistance alleles are generally recessive, hence it is likely that resistance alleles are recessive (initially at least) in the absence of insecticide. Are they also recessive in the presence of insecticide? Laboratory studies of the inheritance of resistance usually involve the crossing of laboratory susceptible and field resistant populations. This procedure may induce dominance breakdown (Kettlewell, 1970) in which case the semi-dominance often observed with respect to resistance could be an artefact.

The models also assume that resistance is controlled by a single allele. This may not be realistic for many pest populations. For example, several

mechanisms of resistance are known in Heliothis spp. (Nicholson & Miller, 1985) and it is unlikely that they are controlled by one and the same allele. Hence management strategies devised to protect economically important crops (e.g. cotton) may be based on inappropriate models. Since there is a deficit of novel insecticides to use against resistant pest insects it is critical that more effort is made to study the genetics of individual mechanisms of resistance, how the genes involved interact and the contribution of each mechanism to total resistance, with a view to refining models and hence management strategies.

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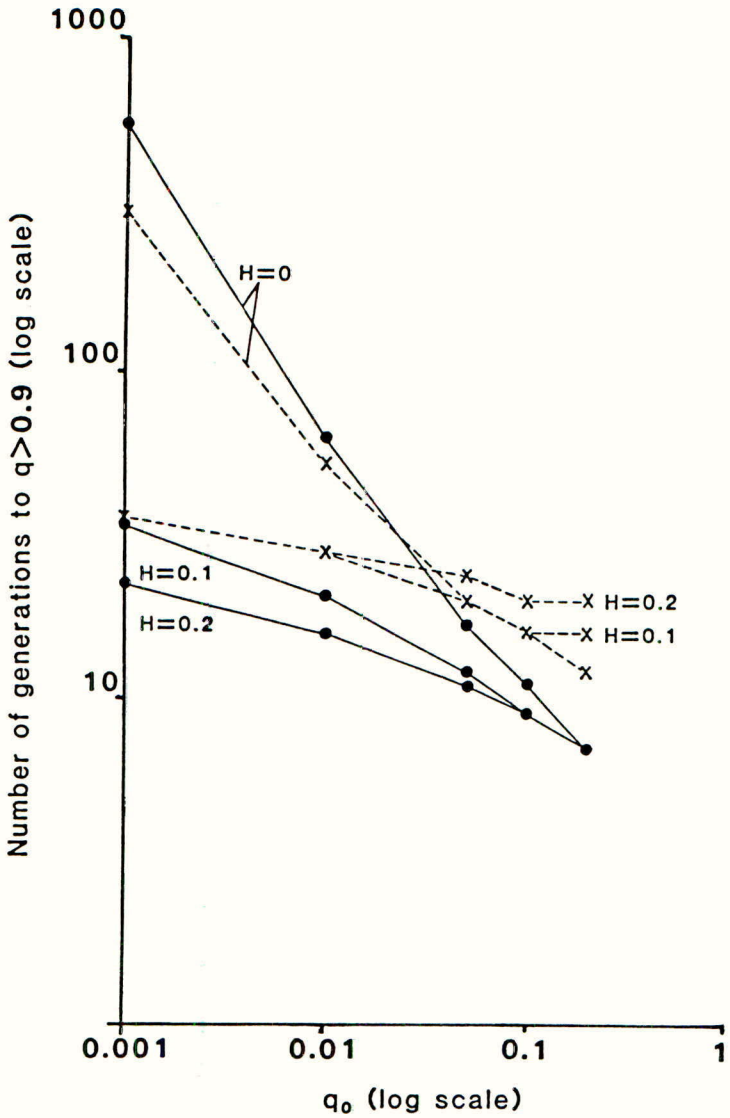

Figure 1. Number of generations until loss of efficacy of favoured insecticide (i.e. when frequency (q) of resistance allele >0.9) under rotation (x---x) and mixture (●——●) strategies from varying initial resistance allele frequency (q_o).

THE RELATIVE EFFICACY OF RH-7988 AGAINST STRAINS OF MYZUS PERSICAE (SULZER) (HOMOPTERA: APHIDIDAE) IN LABORATORY TESTS

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ABSTRACT

Three UK strains of Myzus persicae showing varying degrees of insecticide resistance (susceptible-S, moderately resistant-R1 and strongly resistant-R2) were tested by the cage dip test to determine the relative contact efficacies of RH-7988 and pirimicarb. The resistance factors (RF) for the R1 and R2 strains compared with the S strain against RH-7988 were 1.4 and 1.9 respectively. The RF's against pirimicarb were 1.6 and 5.5. Further tests using cage dip, immunoassay and electrophoresis were done on two Italian M.persicae strains to determine their resistance status. Results showed that the strain from the Novara province was similar in its esterase and its resistance to RH-7988 and pirimicarb to the UK R2 strain. The other strain, from the Ravenna province, had higher levels of esterase, a faster migrating esterase E4 (the resistance-conferring enzyme), and had higher resistance to RH-7988 and pirimicarb than the UK R2 strain.

INTRODUCTION

Myzus persicae (Sulzer) is a cosmopolitan pest species which has been shown to be resistant to insecticides in many countries throughout the world, for example, Denmark (Pedersen, 1981), Turkey (Zumreoglu, 1978), Australia (Attia, et al., 1979), Holland (Oppenoorth and Voerman, 1975), Korea (Choi and Kim, 1986) and America (Koziol, et al., 1984) as well as the UK (Furk, 1986). Electrophoresis and a modified immunoassay test distinguishes between the susceptible and resistant variants found in the UK (Devonshire, 1975 and Devonshire et al., 1986).

The insecticide RH-7988 is an aphicide the action of which may circumvent the resistance mechanism found in M.persicae. Field trials by Rohm and Haas in Italy since 1985 against M.persicae on peach suggested that different rates of pirimicarb were necessary to achieve economically acceptable control at 2 trial sites 300 km apart. These were Sozzago, Piedmonte in Novara province and Villa Grappa, Emilia in Ravenna province. The normal rate of pirimicarb for M.persicae control on peaches is higher in the Villa Grappa region than in the Sozzago region. This suggests an increased level of M.persicae insecticide resistance in the former area. The rate for RH-7988 necessary to give economically acceptable control was the same at each site.

The aims of this study were two-fold. Firstly to investigate and compare with pirimicarb the contact activity of RH-7988 against three M.persicae strains presently

found in UK field populations using a cage-dip bioassay. Secondly to compare the relative performance of RH-7988 and pirimicarb against two Italian strains of M.persicae from Villa Grappa and Sozzago with the compound's performance against the UK M.persicae strains. It was hoped that the levels of resistance present in the two Italian field samples could then be calculated.

MATERIALS AND METHODS

Insects

Stock cultures representing the three common variants of M.persicae in the UK, susceptible (S), moderately resistant (R1) and strongly resistant (R2), were reared under glass at the Harpenden Laboratory at approximately 20°C and an 18 h daylength within large aphid-proof cages (457 x 457 x 610 mm). Host plants used were Chinese cabbage (Brassica pekinensis). The Italian strains were brought into the UK as eggs on peach (Prunus persica) twigs whose buds had begun to break. First instar fundatrices were transferred from the twigs onto container-grown peach trees. Spring migrants developing from colonies on the peach trees were transferred to Chinese cabbage and cultured under similar conditions to the stock cultures.

Esterase E4 Determinations

Tests were done on the Italian strains to characterize their resistance levels using an electrophoretic method (Furk, 1986) and an immunoassay method (Devonshire et al., 1986).

Bioassays

The three laboratory populations and the two Italian strains of M.persicae were tested using the FAO-recommended dip-test method for the detection of resistance of adult aphids to pesticides (Anon, 1979). A range of concentrations of each formulated pesticide (RH-7988 and pirimicarb) was made up in distilled water (Table 1). At each concentration of the insecticide, at least 50 aphids (in replicate batches of 10) were dipped for 10 seconds in the insecticide. As a control, batches of aphids were similarly dipped in distilled water. Mortality was assessed after one hour by putting the aphids onto filter paper and looking for movement of appendages after stimulation with a needle.

For all insecticide tests, log concentration-probit (lc-p) lines were fitted and LC_{50} and LC_{90} values calculated by probit analysis (Finney, 1971).

TABLE 1

Concentrations of RH-7988 and pirimicarb used against five strains of M.persicae.

Insecticide	Aphid strain	Concentrations (mg a.i./l)				
Pirimicarb	Susceptible, R1	10	17	30	50	90
	R2, Sozzago, Villa Grappa	150	190	240	310	400
RH-7988 (46% e.c.)	Susceptible	3	9	25	70	200
	R1	25	42	71	119	200
	R2, Sozzago, Villa Grappa	50	80	130	220	350

RESULTS

E4 Determinations of the Italian Strains

The electrophoretic tests showed that the E4 type esterase from the Villa Grappa strain was faster moving (FE4) than the E4 from the Sozzago strain. The degree of staining showed all aphids tested to be at least of an R2 level of resistance. The tests also showed that each of the two strains was homogenous. The immunoassay test showed that the Villa Grappa strain had esterase E4 quantities above R2 level and the Sozzago strain had esterase E4 quantities at the R2 level.

Dip Tests

a) Pirimicarb

The LC_{50} and LC_{90} values for the one hour assessment of the S, R1 and R2, Sozzago and Villa Grappa strains are shown in Table 2. Table 3 shows the chi squared and probability values obtained when fitting data from 5 pairs of aphid strains to common pirimicarb dose response lines. None of the pairs fitted a common line at the 5% probability level but the R2 and Sozzago strains were not significantly different at the 1% level.

b) RH-7988

The LC50 and LC90 values for the one hour assessment of the S, R1, R2 Sozzago and Villa Grappa strains are shown in Table 2. When the dose responses of pairs of strains were fitted to common lines (Table 3), the R2 and Sozzago strains were not significantly different at the 5% probability levels.

Analysis of parallelism showed that for pirimicarb and RH-7988 there was no evidence of different slopes for tests on the different strains. Resistance factors were therefore calculated from the LD_{50} values using the results of the susceptible strain as the baseline (Table 2).

DISCUSSION

The cage-dip test method evaluated the contact activity of RH-7988 and pirimicarb. RH-7988 is a systemic cholinesterase inhibitor being primarily absorbed through the gut wall (Murray et al., 1988). It is less active by contact action. Consequently, using this test method it might be expected that RH-7988 would be

less effective than pirimicarb (whose modes of action include contact poisoning) at similar concentrations against M.persicae strains. However, the RF values were lower for all strains tested against RH-7988 than pirimicarb. In addition, results from field trials on peach show that RH-7988 at 30 gai/hl + oil can control M.persicae populations as well as or better than pirimicarb at 37.5/50 gai/hl (Murray et al., 1988).

The cage-dip test results show that pirimicarb required increasing concentrations to kill both UK R1 and R2 strains as shown by their LC_{50} and LC_{90} figures. This supports the evidence that at commercial use rates pirimicarb does not effectively control R2 aphids in field situations (ffrench-Constant et al., 1988).

RH-7988 also required increased concentrations to kill R1 and R2 strains. However, the RF values were lower than those for pirimicarb. Indeed, recent studies using sprays against R1 and R2 strains on sugar beet (Dewar et al., 1988) have indicated that RH-7988 can effectively control the R2 strain.

The laboratory techniques employed were successful in determining the resistance status of the two Italian M.persicae samples. The Sozzago sample corresponded to the UK R2 strain in the cage dip, electrophoretic and immunoassay tests. The aphids from Villa Grappa had higher RF values for both pirimicarb and RH-7988 in the cage-dip tests. In addition, they had a faster electrophoretically migrating E4 than the UK R2 strain. This shows conclusively that the aphids are of a type already documented from the same Ravenna province (Devonshire et al., 1983). In this case, it had been shown that the electrophoretic assay overestimated the resistance to carbamates in aphids with FE4. However, in the present work the cage-dip test still showed that the Villa Grappa strain was more resistant to both pirimicarb and RH-7988 than the UK R2 strain. These differences in esterase and resistance levels explain why the commercial use rate of pirimicarb necessary for M.persicae control in peach orchards is higher in the Ravenna province than in the Novara province.

TABLE 2

LC_{50} and LC_{90} values of two insecticides against five strains of M.persicae (RF values calculated using susceptible results as a baseline).

Strain	LC_{50} (mg a.i./l)	Fiducial Limits (P = 0.95)	LC_{90} (mg a.i./l)	Fiducial Limits (P = 0.95)	RF
Pirimicarb after 1 hour					
Susceptible	26.5	(23-31)	67.0	(55-89)	1
R1 resistance	42.7	(37-51)	127.2	(97-193)	1.6
R2 resistance	144.8	(118-164)	278.1	(249-331)	5.5
Sozzago	125.9	(90-147)	226.3	(202-270)	4.8
Villa Grappa	200.1	(171-225)	428.8	(356-605)	7.6
RH-7988 after 1 hour					
Susceptible	62.1	(50-75)	147.5	(116-213)	1
R1 resistance	87.9	(69-107)	252.3	(191-411)	1.4
R2 resistance	115.9	(96-133)	229.4	(196-290)	1.9
Sozzago	112.3	(91-133)	246.2	(203-331)	1.8
Villa Grappa	168.9	(79-221)	392.1	(288-1245)	2.7

TABLE 3

Chi squared and probability (1 degree of freedom) of 5 pairs of aphid strains having the same intercept against two pesticides.

Strains	Pirimicarb		RH-7988	
	chi squared	p	chi squared	p
S v R1	22.0	<0.001	7.6	0.001-0.01
R1 v R2	28.2	<0.001	5.9	0.01-0.02
R2 v Sozzago	5.9	0.01-0.02	0.02	0.80-0.90
R2 v Villa Grappa	24.4	<0.001	15.1	<0.001
Sozzago v Villa Grappa	47.8	<0.001	13.1	<0.001

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THE EFFICACY OF NOVEL AND EXISTING APHICIDES AGAINST RESISTANT MYZUS PERSICAE ON SUGAR BEET IN THE LABORATORY

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ABSTRACT

A laboratory bioassay which involved clip-caging R_1 and R_2 resistant Myzus persicae on young potted sugar beet plants at various times after spraying was developed to examine the efficacy and persistence of some novel aphicides compared to existing standards. Results from two trials showed that of the currently approved aphicides for sugar beet in the UK, pirimicarb gave the best initial control of R_1 aphids, followed by demeton-S-methyl and a formulated mixture of deltamethrin plus heptenophos. All three aphicides gave poor control of R_2 aphids. Ethiofencarb gave variable but intermediate control of R_1's and better control of R_2 aphids than any of the first three. Cyhalothrin gave reasonable control of R_1 aphids, but virtually no control of R_2's. A new compound, RH 7988, controlled both R_1's and R_2's at the high rate tested and exhibited excellent repellent effects on survivors throughout the 2-week trial period. Further testing of this compound at several rates revealed that rates as low as 70 g a.i./ha gave excellent control.

INTRODUCTION

In recent years the proportion of Myzus persicae in the field that are resistant to insecticides has increased (ffrench-Constant & Devonshire, 1988; Dewar, ffrench-Constant & Devonshire, 1988). The nature of this resistance, i.e. the production within the aphid of an esterase, E4, which can hydrolyse ester bonds in insecticide molecules, means that all currently available aphicides for use in sugar beet in the UK are liable to degradation, although some are more prone to this than others (Devonshire & Moores, 1982). Therefore, there is a need to develop novel classes of insecticide which are not degraded by this enzyme.

This paper describes a bioassay technique which has been developed as a first step in assessing the potential of novel insecticides on sugar beet. The results of two trials are presented which compare the efficacy of representatives from the currently available classes of aphicides, namely organophosphates, carbamates and pyrethroids, with that of a novel insecticide, RH 7988.

MATERIALS AND METHODS

Aphid Clones

To avoid any effect of the host plant on aphid behaviour, clones of moderately resistant (R_1) and highly resistant (R_2) aphids (Sawicki & Rice,

1978) originating from IACR, Rothamsted were reared on young sugar beet plants (circa 8-10 leaf stage). The cultures were maintained in aphid proof boxes and kept as far apart as possible, in separate rooms, to avoid cross-contamination. Samples of aphids from each clone were periodically checked at Rothamsted using the immunoassay technique described by Devonshire et al (1986) to ensure that the clones were still pure R_1 or R_2.

Insecticides

In trial 1, two carbamates, pirimicarb ('Aphox', ICI Agrochemicals), and ethiofencarb ('Croneton', Bayer AG), an organophosphate, demeton-S-methyl ('Metasystox', Bayer UK) and a formulated mixture of a pyrethroid and an organophosphate, deltamethrin plus heptenophos ('Decisquick', Hoechst) were compared with a new compound, RH 7988,ethyl (3-tert-butyl-1-dimethyl carbamoyl-1H-1,2,4-triazol-5-ylthio)acetate (Rohm & Haas; Murray, et al, 1988), which was mixed with Sunoil at 0.25%. In trial 2, these same five compounds were tested again in comparison with another pyrethroid, cyhalothrin ('Karate', ICI Agrochemicals).

Application of treatments

The insecticides were applied to young beet plants (4-6 leaves) which were grown in a glasshouse at an average temperature of 18°C in 10 cm pots containing SHL potting compost. In Trial 1, begun in November 1987, the five insecticides were applied at recommended rates (Table 1) using a 3 m, hand-held, gas powered, Oxford precision sprayer fitted with 8002 T jets giving an output of 200 l/ha at a walking pace of 1 m/sec. Plants, 50 per treatment, were placed in 3 rows on a concrete road at spacings consistent with field grown beet, namely, 50 cm between rows, 17 cm within rows. In Trial 2 the six insecticides were applied at recommended rates (Table 2), to plants placed in a pot sprayer which delivered the chemicals at 238 l/ha through a sprayer, fitted with T-jet 8002 nozzles, travelling on a gantry. This gave a more accurate application of insecticides because (a) the speed of the machine was more constant than walking, (b) chemicals were applied at temperatures more comparable to those likely to be encountered in the field in early summer, and (c) there was no possible effect of wind on deposition rates. After application, the insecticides were allowed to dry before the plants were returned to the glasshouse until needed for the trials.

Bioassay technique

Apterous adult or 4th instar aphids from each clone were clip-caged separately, 5 per cage, on the under side of one of the first pair of true leaves on ten test plants per treatment. Experiments began when the plants were at the 4-6 leaf stage and were completed two weeks later when plants were at the 6-8 leaf stage. On such plants the first two true leaves were always fully expanded and assumed to be physiologically identical. Infestation of the plants with aphids was always done with one clone at a time to avoid contamination of the cultures. In Trial 1, aphids were caged on test plants 4 hours, 2, 7, 10 and 14 days after application of treatments; in Trial 2, this was done 1, 3, 7, 10 and 15 days after treatment. Aphids were exposed to treatments for 24 hours, after which they were recorded as dead, feeding or walking round the cage. The latter record was regarded as a measure of an anti-feedant effect.

Analyses

Because some of the aphids originally exposed to the treatments disappeared during the experiments by escaping under the cage, results were calculated as percent mortality of those remaining and analysed using a Generalised Linear Model incorporating data from all test occasions.

RESULTS

Initial knockdown

In both trials pirimicarb and RH 7988 gave the best control of R_1 aphids, killing over 80% of the aphids; demeton-S-methyl (DSM), ethiofencarb, and deltamethrin plus heptenophos (D+H) killed less than 60% of R_1's, while, in Trial 2, cyhalothrin killed 68% (Tables 1 & 2).

The efficacy of these treatments against R_2 aphids was more variable. In Trial 1, RH 7988 gave best control followed by ethiofencarb. Pirimicarb and DSM killed less than half the test aphids, while D+H killed only 21% (Table 1). In Trial 2, RH 7988 was again the best treatment killing 67% of R_2's, but none of the other treatments killed more than 30% of this clone. D+H and cyhalothrin treatments were particularly poor (Table 2). The differences between the two trials may have been due to the 24 hour difference in the time of first exposure after treatment.

Persistence

Efficacy of all insecticides declined over the trial period, but, after 7 days, only RH 7988 and ethiofencarb gave some measure of control. In both trials, RH 7988 killed more than 60% of both R_1 and R_2 aphids, while ethiofencarb only killed 40% of both types in Trial 1 and 60% of R_1's and 40% of R_2's in Trial 2. Less than 40% of R_1's and 10% of R_2's were killed by any of the other treatments after 7 days (Tables 1 & 2).

TABLE 1

Percent mortality of moderately resistant (R_1) and highly resistant (R_2) Myzus persicae after exposure to insecticides on sugar beet : Trial 1.

Treatment	Rate of application (g a.i./ha)	Aphid Clone R_1 Days after treatment (4 hrs)	2	7	10	14	R_2 (4 hrs)	2	7	10	14
Untreated	-	0	8	0	2	0	0	8	15	0	0
pirimicarb	140	91	84	28	4	9	50	30	10	4	0
demeton-S-methyl	244	54	88	36	14	5	46	20	0	12	0
ethiofencarb	500	52	76	50	42	17	71	41	43	10	2
RH 7988+sunoil	280	80	94	93	38	29	83	90	66	12	18
deltamethrin + heptenophos	7.5 120	42	60	13	9	19	21	20	5	10	0

S.E.'s vary with % mortality and number of aphids recovered. Between 10% and 90% mortality S.E.'s lie between 4 and 8%. Outside this range the S.E. decreases towards zero.

TABLE 2

Percent mortality of moderately resistant (R_1) and highly resistant (R_2) Myzus persicae after exposure to insecticides on sugar beet : Trial 2.

Treatment	Rate of application (g a.i./ha)	Aphid Clone R_1 Date of Treatment					Aphid Clone R_2 Date of Treatment				
		1	3	7	10	15	1	3	7	10	15
Untreated	-	3	3	3	2	2	2	2	0	0	4
pirimicarb	140	87	35	47	23	14	14	11	0	8	9
demeton-S-methyl	244	47	31	20	16	2	27	3	12	4	5
ethiofencarb	500	52	59	63	13	0	30	26	40	2	4
RH 7988+sunoil	280	93	83	68	28	31	67	6	90	64	22
deltamethrin + heptenophos	7.5 120	33	43	19	11	9	6	15	2	6	2
Cyhalothrin	5	68	59	20	17	7	3	13	3	2	2

See note on Table 1 for comments on statistics.

Anti-feeding

Most of the surviving aphids from any of the treatments were feeding during the time of assessments, except on plants treated with ethiofencarb and RH 7988, on which proportionally more aphids were walking round the cage. This suggests anti-feedant effects of these treatments.

Rates of application

A further trial to ascertain whether the excellent control achieved with RH 7988 could be achieved with lower rates was carried out using the pot sprayer. RH 7988 plus 0.25% sunoil was applied at 70, 140 and 280 g a.i./ha and compared to pirimicarb applied at 70, 140 and 280 g a.i./ha. Aphids were exposed to the insecticides 4 hours, 2, 7 and 15 days after application.

RH 7988 applied at all three rates gave good control of both R_1 and R_2 aphids for 7 days after application, and, even after 15 days, the two highest rates controlled R_1's very well (Table 3). Pirimicarb gave good control of R_1's after 4 days at the 140 and 280 g a.i. rates, but, only at double the recommended rate (280 g a.i./ha), was control of R_2's as good. The efficacy of all rates of pirimicarb declined rapidly and after 7 days, control of both R_1's and R_2's was poor.

TABLE 3

Percent mortality of moderately resistant (R_1) and highly resistant (R_2) Myzus persicae exposed to pirimicarb and RH 7988 applied at different rates to sugar beet.

Treatment	Rate of application (a.i./ha)	Aphid Clone R_1 Days after treatment (4 hrs)	2	7	15	R_2 (4 hrs)	2	7	15
Untreated	-	8	2	4	6	0	0	0	0
pirimicarb	70	61	44	19	20	11	12	4	22
	140	81	73	16	6	37	27	19	5
	280	93	93	32	38	80	48	18	9
RH 7988 +	70	81	93	100	26	68	90	89	26
sunoil	140	84	93	100	74	89	100	86	36
	280	90	95	98	74	88	93	93	69

DISCUSSION

The results of the first two trials demonstrate the inability of currently available aphicides to control highly resistant aphids. The poor performance of DSM, the pyrethroid, cyhalothrin, and the pyrethroid-organophosphate mixture, D+H, against R_2 aphids confirms previous results on sugar beet (Dewar et al, 1988), and potatoes (ffrench-Constant et al, 1988) where little or no control of R_2 aphids was observed by these or similar chemicals. Even the carbamates, pirimicarb and ethiofencarb, while giving good initial control of R_1's, gave less than adequate control of R_2's. Only RH 7988 gave good control of both R_1 and R_2 clones, suggesting that this chemical is not degraded by the esterase E4 in the same way as the other insecticides. RH 7988 was also the most persistent of the insecticides tested giving good control of both clones for several days, even at quite low rates.

In view of the increase in proportion of highly resistant aphids in recent years, (ffrench-Constant & Devonshire 1988; Dewar et al. 1988), and the inadequacy of existing approved aphicides, it is important that new insecticides such as RH 7988 are developed to a marketable stage as quickly as possible.

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A NEW METHOD FOR THE EVALUATION OF RESIDUAL INSECTICIDES ON DIFFERENT SURFACES AGAINST MUSCA DOMESTICA L.

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ABSTRACT

A test method is described for exposing houseflies to commercial formulations of residual insecticides on different surfaces. The method can be used for long exposure periods of 1 day or more and for an assessment of recovery from knockdown to be made. The method enables efficacy data to be compared for different compounds, formulations or surfaces and strains of houseflies exhibiting different degrees of insecticide tolerance. To illustrate the technique, results are presented of dose-response tests for deltamethrin and permethrin formulations on different surfaces against a susceptible fly strain and for a deltamethrin formulation on 3 surfaces against a susceptible and 2 pyrethroid-resistant strains.

INTRODUCTION

The photostable pyrethroids, although no longer registered for use against Musca domestica in intensive animal units in the United Kingdom, are widely used in the public health and food processing sector. A number of workers such as Combs (1978), Scirocchi & Cesoroni (1979), Taylor (1981) and Hinkle et al. (1985), have employed different test methods to examine the efficacy of commercial insecticide formulations against houseflies. Most methods confined flies in unventilated or poorly ventilated systems for short periods of time and knocked-down (KD) flies usually fell onto the treated surface causing a greater area of the body to contact the insecticide. In practical situations where walls and other vertical surfaces are treated the KD insects could be expected to fall away from a treated surface. This is particularly significant in the case of pyrethroids and other compounds with a rapid action. If recovery from KD is to be assessed there is an additional complication of removing KD and unaffected insects from the test apparatus.

This paper describes a laboratory technique to examine the efficacy of commercial insecticide formulations applied to a variety of test surfaces to which resistant or susceptible flies can be exposed for a number of days, and any KD flies would fall away from the test surface. To illustrate the techniques, dose-response data are presented for commercial formulations of permethrin and deltamethrin on a variety of surfaces against a susceptible housefly strain. Data are also presented for a formulation of deltamethrin applied at a single-dosage rate to 3 different surfaces to which a susceptible and 2 pyrethroid-resistant strains were exposed at 0, 4 and 12 weeks after application of the insecticide.

MATERIALS AND METHODS

The Cooper strain is the Slough Laboratory standard insecticide-susceptible strain. The 2 resistant strains were tested for resistance to synergised pyrethrins by the topical application method (Chapman and Lloyd, 1981). The R_1 strain was 3.5x and R2 10.4x resistant at the KD50 level.

All flies were bred at 28°C and 50% RH with a 12h light/12h dark regime. Flies for test were fed on 10% sucrose solution until 2-4 days old when they were given a whole milk feed for 5h. They were then removed to test rooms where they were anaesthetised with CO_2 and batches of 20 female flies were put in 120ml plastic beakers covered with plastic mesh. These females were then left at 20°C, 50% RH and under constant light overnight to acclimatise to the test conditions. Ten percent sucrose solution was supplied during this time.

The commercial insecticide formulations used for the dose-response tests were permethrin EC ('Pynosect PLO', Mitchell Cotts) and WP ('Coopex', Wellcome) and deltamethrin SC ('Crackdown', Wellcome). For the aged formulation work, deltamethrin (0.04% ai) was supplied as a ready-to-use formulation by National Chemsearch. The test surfaces were 90mm diameter Whatman No 1 filter-papers, Whatman GF/A glass micro-fibre filters (GMFF) and 100mm x 100mm squares of glass, cork sheet, plywood or vinyl floor tile.

Insecticide solutions were applied to the test surfaces using a small scale laboratory sprayer (Morgan & Pinniger 1987). This sprayer used a motor and rack system to drive an arm mounted nozzle back and forth across a test surface. The sprayer was designed to apply commercial formulations at rates which simulate field applications.

The insecticide solutions for the dose-response tests were made up by serial dilution from the commercial formulation using tap water to give the required deposit using 'fixed' sprayer settings. However, where the dose-response tests required very low deposit rates, a reduction in the number of traverses per surface was used to give these reduced deposits. The required deposit rates of the deltamethrin formulation were achieved by varying the nozzle type and size, number of traverses and spraying pressure. For control treatments tap water was sprayed onto similar surfaces.

In order to determine the deposit rate achieved, 21cm diameter filter-papers were sprayed at each dose and analysed using high performance liquid chromatography (HPLC).

Following spraying, the test targets were allowed to dry overnight at 20°C and 50% RH in trays prior to use. For the aged surfaces, all targets were sprayed on the same day and kept for 4 and 12 weeks in a dust-free environment at 20°C and 50% RH.

The exposure apparatus consisted of a 75mm diameter x 20mm deep stainless-steel ring, one end of which was covered with plastic mesh, 10 apertures/cm, held in place with an elastic band. Twenty 3-5 day old female flies were lightly anaesthetised with CO_2 and placed onto the mesh inside a ring. A treated target was then placed over the ring holding the flies and a 150mm x 100mm supporting glass plate placed on top. An elastic band was used to secure this apparatus which was then placed in a wooden stand into which angled slots had been cut, 10mm deep, so that the treated target and ring faced downwards at an angle of 5° thus preventing the anaesthetised flies from contacting the treated surface until they recovered. Knocked-down flies also fell away from the treated surface. A source of 10% sucrose solution in a 4ml tube with a cotton wool wick was placed between the retaining elastic band and the plastic mesh so that the flies were able to feed through the mesh.

For the dose-response tests, 5 replicates of 20 3-5 day old female flies were used for each of 5 doses. The number of flies knocked down were assessed at 24 and 48 hours. The KD data were subjected to probit analysis. The tests with the deltamethrin EC used five replicates for each strain, surface and age period. Knockdown counts were taken at hourly intervals for the first 6h and also at 24h and 48h. Five tap-water sprayed control targets were used for each formulation, surface and strain for all experiments.

RESULTS AND DISCUSSION

Table 1 shows some examples of the accuracy of the deposit rates achieved as determined by HPLC. The achieved deposits were all within 10% of the intended deposits. The results of the dose response tests shown in Table 3 are based on intended deposit rates.

TABLE 1

Intended deposits and achieved (assessed by HPLC) deposits as sprayed on to 21cm filter papers.

Insecticide formulation	Intended Deposit (mg/m^2)	Achieved Deposit (mg/m^2)
Permethrin EC	100	93.6
	75	81.9
	3.4	3.2
	1.9	1.8
Permethrin WP	0.4	0.4
	0.16	0.16
	0.1	0.1
Deltamethrin SC	2.0	2.0
	1.0	1.0
	0.5	0.5
Deltamethrin EC	8.0	8.0

Table 2 shows the results as total KD responses at the hourly count periods for the aged deltamethrin deposits on 3 surfaces against the susceptible and resistant strains by M. domestica.

With the limited KD observations it is not known whether the apparent recovery shown by the susceptible strain on vinyl, the R1 strain on plywood and vinyl and the R2 strain on glass, is a single event with little further KD or whether it is a series of knockdown and recoveries resulting in the final 48h count. However, whatever the sequence of events these results show that where a residual pyrethroid formulation is applied to a vertical surface the resulting KD of houseflies may not necessarily result in death.

TABLE 2

Total numbers of M. domestica knocked down during exposure to a deltamethrin formulation (8.0 mg/m^2) sprayed onto 3 surfaces.

Surface	Strain	Weeks after spraying	Total KD of 5 replicates at count times (h) 1	2	3	4	5	6	24	48
Plywood	Cooper	0	100	100	100	100	100	100	100	100
		4	97	99	100	100	100	100	100	100
		12	100	100	100	100	100	100	100	99
	R_1	0	23	79	68	40	13	12	17	11
		4	15	64	51	41	33	13	15	15
		12	50	78	59	32	15	12	22	12
	R_2	0	1	4	5	3	1	3	3	3
		4	0	4	5	3	5	4	0	2
		12	0	4	4	0	1	4	0	0
Glass	Cooper	0	100	100	100	100	100	100	100	100
		4	100	100	100	100	100	100	100	100
		12	100	100	100	100	100	100	100	100
	R_1	0	100	100	100	100	100	100	99	99
		4	100	100	100	100	100	99	96	97
		12	100	100	100	99	100	99	94	98
	R_2	0	89	93	85	83	71	52	56	65
		4	99	100	100	98	97	95	73	90
		12	99	98	96	92	89	79	64	59
Vinyl tile	Cooper	0	42	100	100	100	97	97	95	93
		4	7	62	64	72	67	66	52	42
		12	2	27	51	55	53	44	15	9
	R_1	0	34	57	58	51	49	41	39	38
		4	7	18	10	8	3	5	3	3
		12	0	0	1	2	0	0	0	0
	R_2	0	21*	27*	26*	22*	21*	21*	20	20*
		4	0	0	0	0	0	0	0	0
		12	0	0	0	0	0	0	0	0

* Total KD occurred in 1 replicate (20).

The deltamethrin formulation performed best against the susceptible strain on glass with 100% KD in all tests; the performance on plywood was almost as good but on vinyl total KD was only obtained at 2, 3 and 4h count periods, followed by subsequent recovery, on the freshly treated surfaces. The formulation on plywood also showed poorly against the 2 resistant strains with a maximum KD of 79% in the R1 strain on the freshly sprayed surface 2h but with a high degree of recovery being observed after a further 3h.

On a number of occasions the maximum KD for all strains was not obtained until 2-3h after the flies were first exposed to the insecticide. There was little reduction in efficacy observed over the 12 week period on the plywood and glass surfaces. However on vinyl tile there was a definite loss of effect observed even against the susceptible strain with aged deposits.

There appear to be clear differences between the responses of the R1 and R2 strains on all 3 surfaces. In a survey of insecticide resistance in houseflies from Eastern England carried out in 1984-85 the resistance factors for synergised pyrethrins ranged from 0.9x to 6.8x at KD50 (P.A. Chapman, unpublished data). The R1 strain with a RF of 3.5x may be regarded as being on the higher side of the 'norm' for pyrethroid resistance in UK houseflies while the R2 strain with a RF of 10.4x could not be controlled using synergised pyrethrins in the field. The responses of the 2 resistant strains to the deltamethrin residues reflects their resistance levels to pyrethrins as determined by topical application.

TABLE 3

Dose-response data for Cooper susceptible strain of houseflies exposed to commercial formulations of pyrethroids on different surfaces.

	KD_{50} mg/m^2	95% Fiducial Limits of KD_{50}	Slope ± S.E.	χ^2	DF
Deltamethrin (SC) filter paper	0.0099	0.0089, 0.011	3.0 ± 0.2	14.4	18
Permethrin (EC) filter paper	38.8	35.2, 42.6	3.1 ± 0.3	28.4	23
Permethrin (WP) filter paper	0.18	0.15, 0.19	6.0 ± 0.9	37.6	18
Permethrin (EC) GMFF	9.3	8.5, 10.3	6.6 ± 0.9	47.3	18
Permethrin (WP) GMFF	0.02	0.018, 0.021	5.3 ± 0.4	25.2	18
Permethrin (EC) vinyl tile	72.8	70.0, 75.6	8.6 ± 0.6	22.9	18
Permethrin (EC) cork	13.9	13.0, 14.8	5.6 ± 0.5	20.6	18

From the dose-response data in Table 3, deltametrin SC was by far the most effective formulation on filter-paper against the susceptible strain. Permethrin WP was 215-fold and 465-fold more effective than the EC on filter-paper and on GMFF respectively at KD_{50}. The KD_{50} for permethrin EC was lowest on GMFF followed by cork, filter-paper and vinyl tiles.

CONCLUSIONS

These data suggest that many factors have to be taken into account when developing insecticide formulations for the control of houseflies. Chadwick (1985) also showed that the effectiveness of a formulation is directly influenced by the surface to which it is applied and therefore the surfaces tested should be relevant to the intended use of a formulation.

Tests should be carried out not only on susceptible strains but also strains showing levels of tolerance likely to be encountered in the field in order to arrive at a practical dosage.

The techniques described here show a potential for obtaining dose-response data for commercial formulations on a variety of surfaces. The data so generated could be used to indicate the field usage rates required for each compound or formulation. The efficacy of these rates could then be assessed and compared with other formulations against a number of housefly strains.

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USE OF AN IMMUNOASSAY TO DETERMINE THE OCCURRENCE OF INSECTICIDE RESISTANT STRAINS OF MYZUS PERSICAE IN NORTHERN ENGLAND BETWEEN 1985-87

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ABSTRACT

An enzyme immunoassay, designed to detect E4 activity in Myzus persicae, was used to determine the frequency of insecticide resistance in field populations of the aphid collected in Yorkshire and Lancashire between 1985-87. Aphids with an elevated level of E4 above that of the reference susceptible strain represented 86%, 87% and 70% of all aphids assayed from 1985-87 repectively. In 1985 the moderately resistant STR variant was dominant, but in 1986 a large increase in the percentage of individuals with an E4 content equivalent to the highly resistant STRR and >STRR variants was observed in both regions. This increase was not repeated in 1987 and suggests that considerable fluctuations in the proportion of resistant individuals present can occur between consecutive years. In each year a greater proportion of the highly resistant forms were collected in Lancashire than Yorkshire, but the major factors causing this and other observed variation are unresolved.

INTRODUCTION

Insecticide resistance in Myzus persicae is confered by the increased production of a carboxylesterase designated E4 (Devonshire & Moores, 1982). The relative resistance of a strain can be correlated with the amount of this enzyme present. Populations with moderate resistance occur frequently throughout the country (Furk, 1986), and the situation requires continual monitoring to ensure that the appropriate chemicals continue to be recommended for aphid control. The requirement for a technique allowing precise quantification of E4 in a portion of a single aphid has led to the development of an enzyme immunoassay that can readily distinguish between those variants commonly found in the field: susceptible, moderately resistant and very resistant, along with extreme forms that to date have mainly been associated with glasshouses. The immunoassay described has been used to monitor changes in the occurrence of susceptible and resistant strains of M.persicae in the north of England over a three year period from 1985-87.

MATERIALS AND METHODS

Aphids

Between June and September 1985-87 field populations of M.persicae were collected from various sites covering a wide area of Yorkshire and Lancashire. Potatoes, swede and cabbage crops were sampled and at most sites the latter crop proved to be the best source of aphids. Plants spaced evenly throughout a crop were sampled by removing infested leaves from plants that were at a minimum distance of 5-10m apart

depending on the size of the field. Only one leaf per plant was removed for swede and cabbage crops, but where aphids were obtained from potatoes, if possible, a bottom, middle and top leaf were collected. Both insecticide treated and untreated crops were sampled. Infested leaves were placed in polythene bags with tissue paper for transport to the laboratory where individuals were stored in 96 well microtitre plates at -70°C until required for assay.

Immunoassay

Ion-exchange high performance liquid chromatography was used to separate E4 from other esterases present in an homogenate of highly resistant aphids by modifying a column chromatography technique (Devonshire, 1977). Fractions containing E4 were pooled and used to raise an antiserum in rabbits following standard immunisation procedures. Their antiserum was used in an immunoassay to detect the presence of E4 in a portion of a single aphid. The immunoassay protocol was similar to that of Devonshire & Moores (1984), but eliminated the need to purify the IgG fraction from the crude serum.

In this procedure NUNC Immunoplate II, 96 well, flat bottom, microtitration plates are incubated overnight at 4°C with anti-rabbit antibody (Goat, Sigma), 200µl/well of a 10µg/ml dilution in coating buffer (0.02M sodium carbonate, pH9.6, containing 0.02% (w/v) NaN_3). Plates are subsequently washed with PBS-Tween 20 (0.05% v/v) (250µl/well), three washes of 3min each and excess liquid removed by shaking on a paper towel. E4 antiserum (1/1000 dilution in PBS-Tween 20 containing 0.02% (w/v) NaN_3) is incubated (200µl/well) for 2h at 25°C after which the plate is again washed with PBS-Tween 20. Preparations of aphid homogenate are incubated (0.25 aphid/well) for 3h at 30°C. Plates are again washed with PBS-Tween 20 and bound E4 assayed with 200µl/well anapthyl butyrate as described by Devonshire & Moores (1984). E4 activity is quantified using a Titretek R Multiskan set at 620nm.

Preparation of field collected aphids

Individual aphids were homogenised in distilled water (200µl) containing 0.05% (v/v) Tween 20, so that 50µl was equivalent to 0.25 aphid. For samples collected in 1985 homogenisation was carried out for individual aphids in a tissue homogeniser (Jencons). This procedure was eliminated in subsequent years by use of a multihomogeniser suitable for use in a 96 well microtitre plate and capable of consecutively homogenising 16 individual aphids. The homogeniser was made in the Department of Biophysics, Leeds University, based on a design described by Brookes & Loxdale (1985).

For each field collected aphid two replicates of 0.25 aphid were assayed, with two equivalent replicates of aphids from the reference susceptible (STS), moderately resistant (STR) and highly resistant (STRR) clones on each plate. These reference clones were originally obtained from Rothamsted Experimental Station and are equivalent to the S, R_1 and R_2 variants respectively.

RESULTS

A total of 1881 individuals of M.persicae were collected in Yorkshire and Lancashire between 1985-87 and assayed for E4 activity using the immunoassay. The results for each aphid are expressed as a

TABLE 1 The percentage of aphids in each resistance ratio category from 1985-87

RESISTANCE RATIO CATEGORY	1985 YORKS	1985 LANCS	1986 YORKS	1986 LANCS	1987 YORKS	1987 LANCS
STS 0.000 - 0.400	16.7	10.9	7.3	18.3	27.7	35.3
STR 0.401 - 0.800	63.5	62.2	38.7	24.6	34.9	12.3
STR/STRR 0.801 - 0.900	7.6	7.1	16.9	15.3	16.3	10.3
STRR 0.901 - 1.200	11.2	15.9	32.9	32.7	19.4	30.2
>STRR >1.200	0.6	3.8	4.2	9.1	3.6	12.3
TOTAL	334	181	450	332	332	252

resistance ratio relative to the value for a reference resistant clone (STRR). Resistance ratios are plotted to give frequency histograms for each region in each year (Fig 1). The distribution spread for each of the reference clones STS, STR and STRR (mean ratio ±2sd) is shown.

The values (mean ratio ±2sd) obtained for the STS, STR and STRR clones were used to establish ratio categories relating to five of the seven known E4 variants recognised by Devonshire & Sawicki (1979) and characterised by successive doubling in esterase quantity (Table 1). The STS, STR and STRR categories given here are equivalent to V1, V4 and V16 variants (Devonshire & Sawicki, 1979) with the STR/STRR category equivalent to the V8 variant and intermediate between the STR and STRR types. It is not established whether those individuals identified in this study as possessing an E4 level higher than the STRR category are of the V32 or V64 variant of Devonshire & Sawicki (1979).

In 1985, the distribution of individuals between the five variant categories were similar for the two regions (Table 1), although a higher percentage of STRR and >STRR types were found in Lancashire while a greater number of susceptible individuals were collected in Yorkshire. In this year the majority of individuals in both regions, equivalent to 60% of each population, were in the STR category.

In 1986, the distribution of individuals between the five variant categories were again similar for the two regions, with the highest percentage of individuals of the STR and STRR type (Table 1). As in 1985 a higher proportion of highly resistant individuals (>STRR) were collected in Lancashire than Yorkshire, but Lancashire also possessed a greater percentage of susceptible aphids than Yorkshire. Comparison of the values for 1985 and 1986 (Table 1) indicates that between these two years an increase occurred in the percentage of individuals with an E4

content equivalent to the STR/STRR, STRR and >STRR variants and was accompanied by a decline in the percentage of individuals in the STR category and suggests a general trend toward the more resistant types.

The percentage of individuals in each ratio category did not show such close similarity between the two regions in 1987 as in 1985 and 1986 (Table 1). Although a large increase in the percentage of individuals in the STS category was recorded in each region the distribution of individuals among the four resistance categories differed. In Yorkshire the highest percentage of resistant aphids were of the STR type with a decline from 1986 in the number of aphids in the STRR category. However, aphids of the STRR type dominated in Lancashire with the percentage of aphids in this category remaining stable from the previous year. As in 1985 and 1986 a greater proportion of highly resistant forms (>STRR) were found in Lancashire, the percentage of which had increased gradually over the 3 year period from 3.8% in 1985 to 12.3% in 1987.

DISCUSSION

This study represents the most extensive examination of the incidence of insecticide resistant strains of M.persicae in northern England since the study by Sykes (1977), who found that all populations examined included some resistant aphids with almost one third of the populations possessing highly resistant forms.

The frequency histograms obtained for the three years show that susceptible and resistant aphids essentially form a bimodal distribution. Within both regions the resistant individuals formed a continuous distribution, representing approximately 86%, 87% and 70% of all aphids assayed in 1985, '86 and '87 respectively. This continuous pattern for resistant individuals was also identified by ffrench Constant & Devonshire (1988), after immunological estimation of E4 activity in M.persicae individuals collected in eastern and southern England.

The majority of aphids assayed from each region in 1985, displayed an E4 activity equivalent to the STR variant. In 1986 a dramatic increase in the percentage of aphids with E4 activities characteristic of the more resistant types was observed in both regions, with the majority of individuals within the STRR variant category in this year. However, this rise in the incidence of highly resistant forms was not repeated in 1987 and suggests that considerable fluctuations in the relative proportions of resistant individuals can occur between consecutive years. Data provided over the 3 year period indicates that a greater proportion of highly resistant forms of M.persicae are consistently found in Lancashire as compared to Yorkshire.

The data reported here confirms earlier reports (Furk, 1986), that highly resistant individuals of M.persicae occur more frequently in the north of England as compared to southern or eastern regions (ffrench Constant & Devonshire, 1988). All M.persicae populations examined within the two regions, between 1985-87, included some resistant forms, with 33-61% of all sites containing individuals of the >STRR variant. This indicates that there has been a considerable increase in the percentage of highly resistant individuals in the field in Yorkshire and Lancashire since the report by Sykes (1977). Susceptible individuals appeared at low

frequency at most sites, representing less than 15% of the total population in 1985-86, but their frequency increased in 1987, so that they represented more than 20% of the total population at the majority of sites, but in few cases exceeded 40%.

However factors governing the relative percentage of susceptible and resistant individuals of M.persicae at a particular site are not straightforward. Considerable local and regional variation was observed in the proportion of each variant present both between sites in one year and at an individual site between years. This variation could not be conclusively attributed to such factors as crop type, the frequency of insecticide usage or the type of insecticide used. Consequently in the open field other factors must be important in determining the relative proportions of susceptible and resistant variants of M.persicae.

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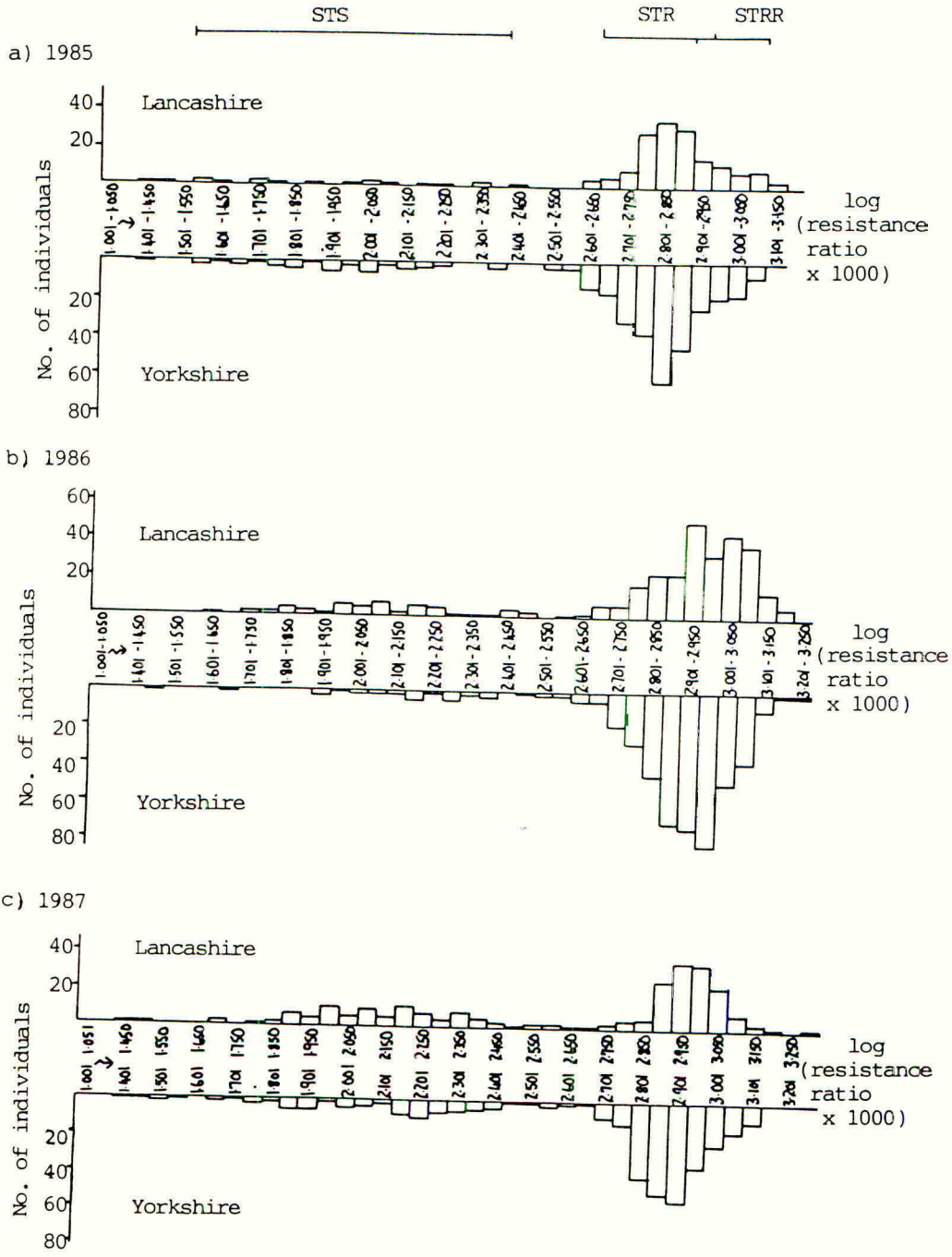
Fig.1. Frequency distributions for resistant and susceptible Myzus persicae



MANAGEMENT OF γHCH/DIELDRIN RESISTANCE IN MOSQUITOES - A STRATEGY FOR ALL INSECTS ?

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ABSTRACT

The effects of γHCH/dieldrin resistance genes on various fitness components in the absence of insecticide were investigated in backcrossed strains of *Anopheles gambiae*. The lifetime fecundity of RR females was only two-thirds that of SS or RS females despite similar longevities. Several reasons were identified: RR females were less responsive to oviposition-site stimuli; RR females produced fewer eggs per unit blood meal; their spontaneous activity, as measured in an acoustic actograph, was only half that of SS or RS females. Unlike SS and RS females, the activity pattern of RR females was the same in simulated moonlight as in darkness. In mate competition experiments, RR males were the least successful. These results indicate that RR mosquitoes have a hyper-inhibited nervous system and require greater stimulus intensity before making a response. This may be a feature of all species resistant to γHCH/dieldrin, and could also explain why this type of resistance often reverts in the absence of γHCH/dieldrin selection. An appropriate management strategy would be insecticide rotation.

INTRODUCTION

There are many examples of insect populations, polymorphic for insecticide resistance, becoming more susceptible when the selecting insecticide is withdrawn from use (Curtis *et al.* 1978). With cyclodiene resistance, for example, reversion has been observed in *Musca domestica* (Keiding 1967), *Lucilia cuprina* (Whitten *et al.* 1980), and anopheline mosquitoes (Hamon & Garrett Jones 1963) among others. If reversion goes far enough, it may be practical to re-introduce the selecting insecticide in an insecticide rotation strategy for several generations at a time.

Two factors can cause reversion: immigration of susceptible insects from outlying unsprayed areas and natural selection against the resistance genotypes if these are less fit in the absence of insecticide. Unless fitness is the cause, a strategy of insecticide rotation can only be a short-term control solution since migration is unlikely to be unidirectional and the outlying pool will gradually become resistant.

The aim of the present study was to find out if heterozygotes and homozygotes for cyclodiene resistance in *Anopheles gambiae* are less fit than the susceptibility genotype in the absence of insecticide. Several behavioural factors are identified which indicate that the resistance homozygote would be less fit.

METHODS

Insect strains

MU - homozygous for resistance, from Muheza, Tanzania in 1975.
KWA - homozygous for susceptibility, from Kwale, Tanzania in 1975.

MU was backcrossed to KWA eleven times to produce strains for experiments with homogeneous genetic backgrounds.

Life tables

Adult cohorts comprising 150 mosquitoes of each sex, age 0-1 days, were set up in population cages and bloodfed every 4 days. Oviposition bowls were provided 3 days after each feed and all eggs laid were counted. Deaths were monitored daily until all adults had died.

Investigations into genotypic differences in fecundity

The winglengths (a reliable indicator of adult size) of mosquitoes from the life table study were measured under a microscope.

Comparison was also made between the blood meal sizes and the number of eggs produced by SS and RR females. The procedure was to feed and then to split members into two subgroups, one for immediate calorimetric determination of meal size (Briegel *et al.* 1979) and the other kept for determining egg number when gravid.

In the test of responsiveness to oviposition-site stimuli, gravid mosquitoes were individually kept in confined, sub-optimal oviposition chambers: 8 x 2.5 cm diameter glass tubes partially filled with water.

Flight activity was recorded in an acoustic actograph, sensitive to mosquito wing-beat frequency (Jones *et al.* 1967). Activity was recorded over several days on 'Miniscript' event recorders and the scores averaged to produce histograms of mean hourly activity against time. Mosquitoes were recorded in two types of lighting regime: A. 12 h of 40 lux light alternating with 12 h of darkness (the dark regime), and B. 12 h of 40 lux light alternating with 12 h of 0.3 lux light (the 'moonlight' regime).

Mating competitiveness

Because mosquitoes mate at dusk in swarms, it is not possible to quantify mating success by direct observation. An indirect method of identifying the genotypes of successful males is to isolate mated females in oviposition chambers and to test the progeny of each using discriminating doses of insecticide.

Prior to mating, females were marked according to genotype using fluorescent powder. 90 males and 30 females of each genotype, age 5 days, were transferred to a 67-cm-cube mating cage, given 3 nights to mate, and then the females bloodfed. Two days later, females were isolated for oviposition.

RESULTS

Life tables and differences in fecundity

RR females laid only two-thirds as many eggs as SS or RS females, despite similar longevities (Table 1).

TABLE 1

Lifetime fecundity and calculation of female fitness.

	SS	RS	RR
Net fecundity ± s.e.	614 ± 40	597 ± 40	402 ± 40
Fitness w	1.00	0.97	0.65

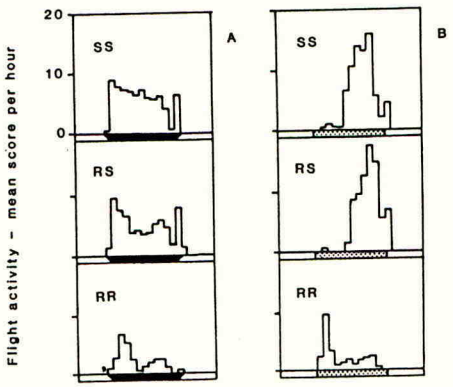
Several experiments were undertaken to find out why RR laid fewer eggs. Size was eliminated - the winglengths of the 3 genotypes did not differ significantly - nor did RR females take smaller meals (Table 2). However, RR females did produce 10% fewer eggs than SS from similar sized meals (Table 2). Two further, behavioural factors contributed to the lower fecundity of RR. In the test of responsiveness to oviposition-site stimuli, fewer RR females (12%, n = 26) oviposited in the glass tubes than SS (59%, n = 34) or RR (69%, n = 29).

TABLE 2

Comparison of mean blood meal sizes and mean egg production (± S.E) (N = 32).

	SS	RR
Meal size	3.8 ± 0.3	4.0 ± 0.3
Egg number	194 ± 6	174 ± 7

FIGURE 1. Mean flight activity per h of *A. gambiae* females: A. dark regime; B. 'moonlight' regime.



In the actograph, RR were less active than SS or RS (Fig. 1), indicating that this genotype might be less successful at finding oviposition sites. In the dark regime there were 3 peaks of activity: an evening peak (E), a night peak occurring several hours later (N), and a dawn peak (Fig. 1). When SS and RS were recorded in the 'moonlight' regime, activity increased and the E peak phase-shifted towards the N peak which became highly elevated. RR activity was not modified by 'moonlight' - the activity pattern was the same in both regimes.

Mating competitiveness

As might be expected from the actograph recordings, SS males were most, RS males were intermediately, and RR males were least successful at competing for females ($P < 0.001$) (Table 3). The genotype of females had no bearing on the outcome of matings.

TABLE 3

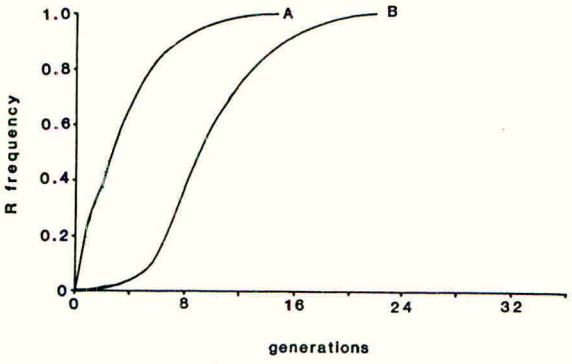
Results of mate competition experiment.

		Number of matings		
Females		SS	RS	RR
Males	SS	12	10	8
	RS	10	7	3
	RR	1	2	2

Population genetics

A population genetics model was developed which included female and male fitness parameters. Estimates of male fitness were based upon the differential mating competitiveness of the 3 genotypes. Estimates of female fitness in the absence of insecticide were based upon lifetime

FIGURE 2. Simulations showing the selection of the R allele by γHCH. A. Male genotypes equally competitive; B. Male genotypes compete according to mate competition results.

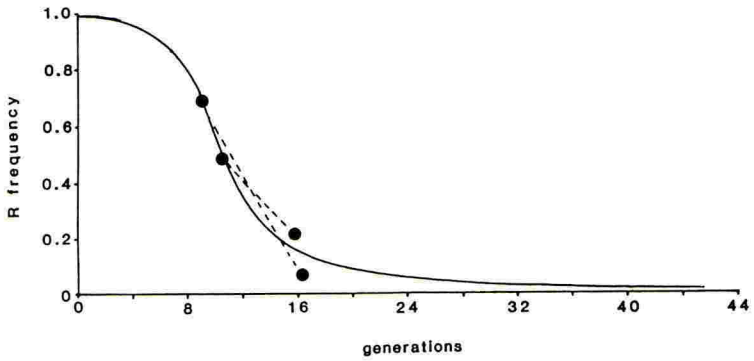


fecundity (Table 1) and, in the presence of insecticide, upon survivorship in γHCH-sprayed dwellings (Rawlings *et al.* 1981). Various computer simulations were run using these estimates.

Because RS and RR can tolerate γHCH better than SS, use of this insecticide selects the R allele (Fig. 2). The two simulations in Fig. 2 compare mating at random (Sim. A) with mating based upon the mate competition parameters (Sim. B). The inferior competitiveness of RR males (Sim. B) causes a slower rate of selection.

When insecticide is withdrawn from use, the frequency of the R allele decreases, due to the inferior fitness of RR males and females (Fig. 3). Comparison of this simulation with known field reversions (Rao *et al.* 1960, Bhatia & Deobhankar 1963) show a close correspondence.

FIGURE 3. Simulation showing reversion of resistance after withdrawal of γHCH. Field reversions (dashed lines) are superimposed.



DISCUSSION

The experimental evidence points to RR as being the least fit of the three genotypes. The life table study indicated that RR females are less fecund. Several reasons were identified: RR females produce fewer eggs per unit volume of blood, they are less responsive to oviposition-site stimuli, and they are less active. In the confines of a laboratory cage, activity would be unimportant, but in the field should considerably reduce the relative fitness of RR females seeking oviposition sites.

The physiology of dieldrin resistance provides insight into the behavioural characteristics of RR mosquitoes. The toxic action of dieldrin is to block the chloride channels of the nervous system and thereby antagonize the operation of the inhibitory neurotransmitter GABA (Kadous *et al.* 1983). Insects have become resistant to dieldrin by decreasing the sensitivity of the receptor at the chloride channels (Kadous *et al.* 1983). My results show that RR mosquitoes are relatively unresponsive to diverse types of stimuli. A possible mechanism is that chloride channels have become more permeable to chloride ions as a result of the change in the dieldrin receptor so that the level of inhibition in the 'resting' nervous system of RR is greater than in SS or RS, and greater stimulus intensity is required before a response is elicited.

The behavioural idiosyncrasies of RR mosquitoes puts this genotype at a considerable disadvantage when γHCH/dieldrin are not being applied.

Computer simulations produced rates of reversion comparable to those observed in the field, which suggests that γHCH might be effectively re-introduced when the frequency of resistance is low as part of an insecticide rotation control strategy.

In terms of cross-resistance pattern and dominance, dieldrin resistance in mosquitoes is similar to dieldrin resistance in many other species of insect (Oppernoorth 1985), and thus the mechanism is probably common to all species. It follows that the behavioural fitness disadvantages found in mosquitoes might also find expression in other species. This would explain why reversion is such a common phenomenon (Keiding 1967, Whitten *et al.* 1980) and suggests that rotation strategies might have widespread suitability.

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