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1. SUMMARY

Research under this grant has yielded data in the following areas:

1. Establishment of baselines of the general esterase that is associated with organophosphate resistance in many species of mosquitoes. Four Anopheles and two Culex species were used.

2. Malathion resistance levels were investigated in 14 strains of An. stephensi and 4 strains of An. culicifacies from Pakistan.

3. Anopheles albimanus resistance due to reduced sensitivity of acetylcholinesterase was investigated with emphasis on kinetic properties and spectrum of cross-resistance.

4. The use of synergists as suppressors of resistance caused by insecticide metabolizing enzymes was investigated in Culex.

This research was hindered by the unavailability of colonized strains of Anopheles aconitus and the recalcitrance of relatively small, new collections of Anopheles stephensi to be propagated in adequately large numbers for selection experiments in the laboratory. Because of these difficulties, research emphasis was placed on Anopheles albimanus as well as on Culex spp. A limited number of tests were also made on a collection of An. aconitus obtained by the P.I. from Indonesia. Susceptible and malathion-resistant colonies of An. stephensi were obtained from Pakistan and were used in studies of the carboxylesterase resistance mechanism. However, the lack of a newly colonized strain of this species has made impossible the initiation of selection experiments with fenitrothion.

The P.I. will be travelling to Pakistan during October 3-25, 1985 as AID consultant to the malaria control program. He has been in contact with the AID malaria advisor in Pakistan (Dr. W. Chin) and was promised adequately large collections of both An. stephensi and An. culicifacies. We, therefore, request reactivation of the project to December 31, 1987 without increased funding, in order to further pursue the research objectives which were outlined in the original proposal. The only modification that becomes essential in the substitution of An. albimanus from Central America for An. aconitus. Dr. Erickson (AID) had indicated his agreement for including An. albimanus in the project.

2. RESEARCH PROGRESS

2.1. Establishment of baseline esterase levels in adult mosquitoes.

Increased levels of insecticide detoxifying enzymes have often been shown to be responsible for insecticide resistance. Elevated levels of one or more esterases have been strongly associated with organophosphorus (OP) resistance in several insect species, including three mosquitoes: Culex pipiens L. complex (Stone and Brown 1969, Pasteur and Sinegre 1975, Georghiou and Pasteur 1978), Culex tarsalis Coquillet (Matsumura and Brown 1961, Georghiou and Pasteur 1978) and Culex tritaeniorhynchus Giles (Yasutomi 1971).

The present study was undertaken to determine the "baseline" general esterase activity for a number of susceptible mosquito strains of four Anopheles and two Culex species for future comparison with resistant strains.

Esterase levels can be readily determined with nonspecific substrates such as naphth-1-yl acetate (α-NA) and naphth-2-yl acetate (β-NA) (Van Asperen 1962). Individual one-day-old adults were homogenized in phosphate buffer, incubated with α- or β-NA for 30 min and color formation was measured spectrophotometrically (Van Asperen 1962).

Table 1 gives the mean O.D. values for the general esterase activity (GEA) toward α- and β-NA in the populations tested. All populations showed a normal distribution of absorbance values for both substrates. The esterase activity in the susceptible strains covered a similar range and were not significantly different. However, the susceptible strain of Cx. tarsalis gave higher values than the other species due, in part, to the larger size of the individuals of this species. Correcting the O.D. values for the weight of the insects tested shows similar activity in Cx. tarsalis and the anopheline species.

GEA activity in the organophosphate-resistant colonies of Cx. quinquefasciatus and Cx. tarsalis was significantly higher than in the susceptible colonies of all species when measured with either α- or β-NA. Comparison of the two resistant colonies showed that Cx. tarsalis had significantly higher activity with α-NA than did the Cx. quinquefasciatus colony, however there was no difference in the β-NA values.

All the susceptible strains of Culex and Anopheles tested showed lower GEA than the OP-resistant populations of Cx. quinquefasciatus and Cx. tarsalis tested. In the field, changes in the baseline levels of GEA of these susceptible populations may indicate the development of OP-resistance. However, the lack of an increase in GEA does not necessarily mean that resistance is not developing. In contrast to the situation in Culex species, there are cases of malathion resistance in which specific esterase based mechanisms have not resulted in an increase in esterase activity against α- and β-NA. For example, Anopheles arabiensis Patton from Sudan and An. stephensi from Pakistan both show 24-fold resistance to malathion, which is completely syner-

gized by the carboxylesterase inhibitor triphenyl phosphate, but these resistant strains had slightly lower levels of GEA with α- and β-NA (Hemingway 1982, Hemingway 1983).

It has still to be determined whether selection with organophosphorus compounds other than malathion will produce resistance associated with elevated levels of GEA in Anopheles. If they do not, then the questions of why such mechanisms seem to readily appear in Culex, but not in Anopheles, and what effect this may have on future developments of resistance and the strategy for control needs to be asked.

2.2 Screening for resistance to malathion in Anopheles mosquitoes.

Resistance to malathion in Anopheles mosquitoes is an important obstacle for malaria control in certain countries today. In order to rationally choose alternative insecticides we must first understand the nature of the resistance mechanism. In an attempt to colonize a resistant strain of Anopheles, we obtained four strains of An. culicifacies and 14 strains of An. stephensi from Pakistan in the fall of 1983. Twelve of these strains were effectively colonized in our lab. These 12 strains, plus one from India and two other previously colonized strains (see Table 2 for the history of each strain), were analyzed for larval malathion resistance by exposing early 4th instars to 1.0 ppm malathion. The results of these assays are shown in Table 3. The choice of 1 ppm for these tests produced 100% mortality in the susceptible An. C. strain and, therefore, any survivorship at this dose would indicate the presence of resistant individuals. All the An. culicifacies strains showed high susceptibility to malathion. The only culicifacies strain showing any resistance was the Baghica strain, in which only 0.59% of the larvae survived (Table 3). The Guatemala (An. albimanus) strain showed no resistance to malathion. This, however, was an older strain that was maintained at the SNEM Laboratory, Guatemala, for several years. Of the eight strains of An. stephensi tested, two strains, Khano Harni and Mal-R, had appreciable levels of malathion resistance.

We further investigated the level of resistance in the Mal-R and Khano Harni strains at the WHO recommended diagnostic dose of 3.125 ppm by our test methods and those of the WHO. Our results, shown in Table 4, indicate a similar kill in the Khano Harni strain, but a greater kill by the WHO method for the Mal-R strain.

The Mal-R strain is currently being reared under malathion selection pressure in an attempt to maximize the level of resistance. With this strain we shall investigate the mechanisms responsible for the resistance and the cross resistance spectra caused by selection with malathion. It is hoped that these studies will add to our knowledge of malathion resistance mechanisms in anopheline species and aid in the intelligent selection of a pesticide to replace malathion where malathion resistance prevents effective control.

2.3. Comparison of the acetylcholinesterase in susceptiole and resistant Anopheles albimanus.

OP and carbamate insecticides act by inhibiting acetylcholinesterase (AChE). Some resistant strains of arthropods have developed a modified AChE which is insensitive to some of these insecticides. Such a modified enzyme does not confer resistance to all OP's and carbamates however. The present study looks at the inhibitory effect of various insecticides on the altered AChE of An. Albimanus (EP-R strain) from El Salvador. The relationship between the degree of enzyme inhibition and the resistance levels was also investigated.

In vitro enzyme activity (and inhibition) was measured by the technique of Ellman et al. (1961) using acetylthiocholine iodide (ATCI) as the substrate. The hydrolysis of ATCI was monitored for five minutes in the presence and absence of an inhibitor. The bimolecular rate constant (k_i) was calculated by the method of Aldridge (1950).

Table 5 indicates the resistance ratios (RR) of the EP-R strain to a number of OP and carbamates compared to the susceptible (Gorgas) strain. The highest levels of resistance (>1000- and 73-fold) were to the carbamates propoxur and carbaryl. Resistance to the organophosphorus compounds tested ranged from 37.5-fold for fenitrothion to 1.2-fold for temephos.

The k_i's for various OPs and carbamates are given in Table 6. In the case of fenitrothion, k_i values were calculated for both the P=O and P=S analogs for the susceptible strain. This demonstrated, as expected, that the P=O compound had higher activity than the P=S analog (approximately 1000-fold).

In all cases, the k_i values for the resistant strain were greater than those for the susceptible, i.e., the AChE of the resistant strain was less sensitive to inhibition than that of the susceptible. However, the differences between the resistant and susceptible strains were low (1.2- to 5.6-fold) for all compounds with the exception of propoxur and carbaryl. Table 7 shows that there is a good correlation between the RR and the k_i ratios of the EP-R and susceptible Gorgas strains. The greater the levels of resistance, the larger the difference in k^i values. The only exception to this is chlorpyrifos methyl, which has the same k_i ratio as malaoxon but a lower RR. The correlation between RR and k_i ratios may indicate that the altered AChE mechanism is the only resistance mechanism present in this population, and this is in agreement with the monofactorial inheritance of resistance in this population (Georghiou et al. 1974).

It would be desirable to develop a rapid, simple method for detection of this altered AChE in field populations. In an attempt to achieve this goal, we compared the EP-R and Gorgas strains by the filter paper technique described by Miyata et al. (1980) for N. cincticeps. This method was too insensitive for use with either adults or larvae of An. albimanus as no differences were detected between our two strains.

Recently we have developed a simple filter paper test (nitrocellulose fiber test) for detection of AChE insensitivity to carbamate insecticides in Culex quinquefasciatus (Omar, Georghiou and Wedding, in

prep.). We are currently investigating the application of this method to An. albimanus.

The present study indicates that the altered AChE mechanism extends to a wide range of organophosphorus and carbamate compounds, although the resistance levels it confers toward organophosphorus compounds are low.

2.4. The use of synergists to suppress organophosphate resistance.

As was previously mentioned in Section 3.1, resistance to organophosphates (OPs) can be due to quantitative or qualitative changes in esterases which effectively detoxify the OPs. Synergists are compounds which selectively block detoxification enzymes. Three synergists that inhibit esterases (IBP, DEF and TPP) were examined against a strain of Culex quinquefasciatus (Tem-R) resistant to OPs due to an elevated esterase (Est-2), in an attempt to suppress the resistance.

A backcross population (BC) was initiated by crossing Tem-R females to C-Lab males. The resulting heterozygote females were backcrossed to C-Lab males and the progeny selected wtih temephos at the LC_{50}. This procedure was repeated for seven generations. Progeny from the seventh backcross were left unselected and allowed to breed panmictically for one generation to establish BC_{7-1}. This stock with a known frequency of resistant and susceptible genotypes, was used to determine the effect of selection with various synergist/insecticide combinations. The BC_{7-1} was treated in five ways: unselected (BNS); selected with malathion (BM); temephos (BT); IPB + malathion (BKM); or IBP + temephos (BKT). Esterase measurements were conducted as explained in Section 2.1.

At the start of this investigation, the Tem-R strain was 1133X more resistant to temephos and 155X more resistant to malathion than the C-Lab strain at the LC_{50}. DEF, IBP, and TPP all reduced the level of malathion and temephos resistance in the Tem-R strain when any combination of synergist and insecticide were applied together. The LC_{50} values given in Table 8 show that DEF was a more effective synergist of temephos than IBP, which was more effective than TPP, the LC_{50} values for each of the synergists + temephos being significantly different; however, all synergists were almost equally effective with malathion. IBP and DEF had a small but significant effect on the toxicity of malathion to the susceptible line, the fiducial limits of the log dosage probit mortality lines for TPP + malathion and the malathion alone overlapped at the LD_{50} value (see Table 8). None of the synergists had any significant effect on the toxicity of temephos to the susceptible strain.

The Tem-R strain had elevated esterase activity against both α- and β-NA when compared to the susceptible (Table 9). However, 24 hours exposure of Tem-R larvae to 20 mg/liter IBP, TPP, or 10 mg/liter DEF, followed by washing and assaying of esterase activity demonstrated that all three synergists decreased the esterase activity against both α- and β-NA to or below the level found in the unsynergized susceptible strain. Table 9 shows that the synergists had a greater effect on the α- than

the β-NA activity in the susceptible strain. These in vitro results agree well with the large reductions in resistance seen in Table 8.

BC_{7-1} was selected with various insecticide/synergist combinations. Figure 1 shows the effect of these selections over four successive generations. The parental generation (BC_{7-1}) displayed a classical heterogenous response to both malathion and temephos with a single plateau around 70% mortality. Bioassays of BC_{7-1} with IBP + temephos produced a much narrower plateau at around the same level, whereas BC_{7-1} behaved as a homogeneous population with a steep LD-P line and no plateau after exposure to IBP + malathion. This indicates that the resistance (R) gene has a major effect on the strain's response to malathion and temephos, but a marginal effect on the response to IBP + temephos and little or no effect on the IBP + malathion response.

Selection of BT, BKT, BM and BKM for four generations increased the levels of resistance to the particular selecting agents in all cases, but the greatest increases were in BT, BKT and BM (see Fig. 1).

The frequency of the R gene in each generation was determined by assaying α- and β-NA activity. The numbers of RR and RS were calculated by assuming a Hardy-Weinberg equilibrium. Figure 2 shows the R gene frequencies in three generations of the selected lines. Selection with malathion, temephos, or IBP + temephos produced a significant increase in the R gene frequency, whereas there was a significant decrease in the IBP + malathion and unselected lines. Malathion selection produced the largest increase in R gene frequency. There was no significant difference between the R gene frequencies in BT and BKT selected lines in any generation.

A subpopulation of Tem-R was selected with either IBP + malathion (TKM) or IBP + temephos (TKT) for five generations. There was a small increase in resistance to the selecting chemicals in both TKM and TKT. The LC_{50} of TKT changed from 0.023 to 0.052 mg/liter and TKM from 0.044 to 0.23 mg/liter after five generations. Assays of esterase activity with α- and β-NA in each generation showed that there was no significant increase in esterase activity in any of the selected generations compared to the parental Tem-R population, although the mean activity in each selected generation was slightly higher than that of the parental.

As it is possible that the increase in resistance is also due to selection of an IBP detoxification mechanism, the dosage-mortality lines for IBP alone against the Tem-R and the fifth selected generations of TKM and TKT were compared. There was a small significant increase in the LC_{50} of the selected lines compared to the parental. The LC_{50} increased from 95 ± 10.2 mg/liter for Tem-R to 150 ± 12.6 and 162 ± 14.8 for TKT_4 and TKM_4, respectively. There was no significant difference between the LC_{50}'s of these populations with regard to temephos or malathion.

Temephos selection of Cx. quinquefasciatus in the laboratory over a period of ten years has produced a high level of resistance to temephos (1133-fold) and a moderate level of resistance to malathion

(155-fold) both being inherited monofactorially. This indicates that the resistances are either dependent on the same gene, or on genes that are closely linked on the same chromosome. Synergist studies indicate that esteratic degradation of both malathion and temephos is the major detoxification pathway in the resistant strain. Malathion resistance is suppressed almost completely by DEF, IBP, and TPP, whereas temephos resistance is strongly suppressed only by DEF (i.e., to a 6X level), while IBP and TPP reduce it to 7.4-fold and 118.2-fold, respectively. This is not inconsistent with a common single gene involvement in the two resistances. The very high level of temephos resistance, compared to that of malathion, may indicate an elevated level of phosphotriesterase, with a greater affinity for temephos than for malathion. Thus, one may expect TPP, generally considered to be a specific inhibitor of carboxylesterase, to be a poor "competitor" for this enzyme when compared to temephos, but not when compared to malathion.

IBP has also recently been shown to inhibit malathion degrading carboxylesterases. The synergism of temephos by IBP reported here, seems to indicate that the range of esterases inhibited by IBP extends beyond the carboxylesterase group, as temephos does not contain a carboxylester bond. However, IBP is not as strong a synergist for this esterease as DEF.

Since DEF is a defoliant, mixtures of this synergist and insecticide cannot be used in the field; however, mixtures of IBP and insecticide may be of practical use. As IBP suppresses malathion resistance almost completely but has only a partial effect on temephos resistance, it may be expected that selection of a heterogeneous population (with regard to resistance) with temephos or malathion alone would concentrate the esterase resistance mechanism, whereas IBP + temephos would select it at a slower rate and IBP + malathion would not select for this elevated esterase mechanism.

The decrease in the R gene frequency in the IBP + malathion (BKM) and unselected population (BNS) may indicate that there is a selective disadvantage associated with this resistance gene. The possibility that the disadvantage is associated wtih the genetic background of the resistance gene rather than the gene itself cannot be entirely discounted. However, seven generations of backcrosses to the susceptible were carried out to establish BC_{7-1} from which BKM and BNS were selected. This reduced any background effect, but the R gene was still virtually lost after three generations in both BKM and BNS. The effects of random genetic drift should also have been weak due to the large number of indivuduals selected at every generation (1000-2000) and careful sampling of progeny from a large number of egg rafts when seeding larval bowls to establish future generations. A similar instability of the temephos resistance gene has also been shown by Georghiou et al. (1983). Very recent work (Mouches et al. 1985) indicates that the high levels of esterase in the Tem-R strain are due to specific amplified DNA sequences. It is very likely that the observed instability of Tem-R resistance is caused by rapid reduction in the extent of gene amplification in the absence of the selecting chemical.

The synergistic effect of IBP with malathion may prove useful in reducing the rate of selection of resistance in the field or decreasing the frequency of resistance genes already present in field populations.

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Table 1

Mean O.D. values and standard deviations for general esterase activity in a number of insecticide susceptible and resistant laboratory colonies of *Anopheles* and *Culex*

Species	Substrate (O.D. x 10^{-3})	
	α-NA	β-NA
Susceptibles		
An. aconitus	4.7 ± 0.1	1.8 ± 0.3
An. culicifacies	2.4 ± 0.3	1.0 ± 0.2
An. stephensi	3.5 ± 1.6	1.5 ± 0.5
An. albimanus	3.6 ± 2.3	2.2 ± 0.8
Cx. quinquefasciatus	5.8 ± 1.8	2.3 ± 0.6
Cx. tarsalis	7.9 ± 2.4	2.5 ± 0.4
Resistants		
Cx. quinquefasciatus	31.8 ± 5.8	36.2 ± 4.0
Cx. tarsalis	59.2 ± 20	29.4 ± 3.1

Table 2

History of the *Anopheles* Strains Used in This Study

Species	Strain	Origin	Date First Colonized
An. culicifacies	An. C.	London School of Hygiene and Tropical Medicine	Unknown
	Vehari	Vehari, Pakistan	1980
	Multan	Multan, Pakistan	1975
	Rose	Pakistan	Unknown
	Sattoki	Sattoki, Pakistan	Unknown
	Baghica	Kot Baghica, Pakistan	1975
An. albimanus	Guatemala	Guatemala	Unknown
An. stephensi	Sattoki	Sattoki, Pakistan	Unknown
	Chirag	India	Summer 1983
	Peshwar	Peshwar, Pakistan	Unknown
	Black Scale	Khano Harni, Pakistan	Unknown
	Sheikhpura	Sheikhpura, Pakistan	Unknown
	Islamabad	Islamabad, Pakistan	Unknown
	Mal-R	Khano Harni, Pakistan	1978
	Khano Harni	Khano Harni, Pakistan	Unknown

Table 3

Susceptibility of 15 Strains of *Anopheles* Mosquitoes to Malathion

Species	Strain	Dose (PPM)	Number Tested	Percent Mortality (24 hr)
An. culicifacies	An. C.	1.0	96	100
	Vehari	1.0	536	100
	Multan	1.0	517	100
	Rose	1.0	405	100
	Sattoki	1.0	550	100
	Baghica	1.0	505	99.41
An. albimanus	Guatemala	1.0	29	100
An. stephensi	Khano Harni	1.0	336	15.18
	Sattoki	1.0	550	100
	Chirag	1.0	512	100
	Peshwar	1.0	549	100
	Black Scale	1.0	270	98.52
	Sheikhpura	1.0	552	100
	Islamabad	1.0	342	99.71
	Mal-R	1.0	341	8.50

Table 4

Susceptibility of 2 Strains of *Anopheles stephensi* to Malathion by 2 Methods

Method	Strain	Dose (PPM)	Number Tested	Percent Mortality (24 hr)
G.P.G. et al. (1966)	Mal-R	3.125	194	62.89
	Khano Harni	3.125	214	65.89
WHO (1976)	Mal-R	3.125	206	72.33
	Khano Harni	3.125	200	65.50

Table 5

LC_{50} Values for Resistant and Susceptible *Anopheles albimanus* for a Range of Organophosphate and Carbamate Insecticides

	LC_{50} (mg/l)		Resistance ratio
	Susceptible strain	Resistant strain	
Temephos	0.006	0.007	1.2
Fenthion	0.03	0.17	5.0
Methyl chlorpyrifos	0.02	0.03	1.3
Ethyl chlorpyrifos	0.008	0.22	26.8
Phenthoate	0.035	0.25	7.2
Fenitrothion	0.04	1.5	37.5
Malathion	0.35	6.2	17.7
Parathion	0.007	0.25	36.2
Chlorphoxim[a]	0.012	0.08	6.8
Carbaryl	0.89	65.3	73
Propoxur	0.39	>390	>1000

[a] 0,0-diethylthiophosphorylimino-(2-chlorphenyl) acetonitrile.

Table 6

Bimolecular Rate Constants (k_i)[a] for Inhibition of Acetylcholinesterase from Resistant and Susceptible *Anopheles albimanus*

	$10^{-4} \times k_i$ (M^{-1} min^{-1})		
	Resistant strain	Susceptible strain	k_i Ratio
Fenoxon	0.38	0.46	1.2
Chlorpyrifoxon methyl	0.21	0.28	1.3
Chlorpyrifoxon	1.8	3.5	2.0
Fenitrothion		0.39	
Fenitroxon	58.1	327	5.6
Malaoxon	71	94.9	1.3
Methyl paraoxon	4.3	6.8	1.6
Paraoxon	10.3	53.6	5.2
Chlorphoxon	4.6	5.5	1.2
Carbaryl	2.1	36.0	17.3
Propoxur	0.003	42.4	1570

[a]k_i values quoted are averages of not less than five replications.

Table 7

Ranking of resistance ratios (RR) and k_i ratios (k_i R) for multiple resistant *Anopheles albimanus* (EP-R) compared to a laboratory susceptible strain

Decreasing resistance	RR	Decreasing inhibition differential	k_i R
↓	Propoxur	↓	Propoxur
	Carbaryl		Carbaryl
	Fenitrothion		Fenitroxon
	Parathion		Paraoxon
	Chlorpyrifos		Chlorpyrifoxon
	Malathion		Malaoxon
	Chlorphoxim		Chlorpyrifoxon methyl
	Fenthion		Chlorhoxon
	Chlorpyrifos methyl		Fenoxon

Table 8

Resistance ratios of *Culex quinquefasciatus* (Tem-R and C-Lab) to synergist/insecticide combinations

Compound(s)	LC_{50} (mg/l) Susceptible (C-Lab)	LC_{50} (mg/l) Resistant (Tem-R)	Resistance ratio	Synergism ratio in Tem-R
Temephos	0.0015 ± 0.002	1.7 ± 0.25	1133.3X	
DEF + temephos	0.001 ± 0.0008	0.006 ± 0.0004	6X	283X
IBP + temephos	0.0027 ± 0.001	0.02 ± 0.004	7.4X	85X
TPP + temephos	0.0017 ± 0.001	0.2 ± 0.025	118.2X	8.5X
Malathion	0.065 ± 0.04	10.1 ± 1.5	155.3X	
DEF + malathion	0.012 ± 0.005	0.025 ± 0.025	2.1X	404X
IBP + malathion	0.015 ± 0.01	0.042 ± 0.01	2.8X	240.5X
TPP + malathion	0.024 ± 0.01	0.099 ± 0.02	4.1X	102X

Table 9

The effect of *in vivo* exposure of *Culex quinquefasciatus* larvae to DEF, IBP, and TPP on α- and β-naphthyl acetate activity

	Activity/min/mg protein			
	Control	IBP (20 mg/l)	TPP (20 mg/l)	DEF (10 mg/l)
α-NA				
Tem-R	2.4 ± 0.74	0.07 ± 0.03	0.02 ± 0.006	0.01 ± 0.004
C-Lab	0.09 ± 0.03	0.01 ± 0.006	0.009 ± 0.004	0.005 ± 0.002
% Inhibition				
Tem-R		97	99	99
C-Lab		89	90	95
β-NA				
Tem-R	0.4 ± 0.13	0.04 ± 0.02	0.02 ± 0.02	0.01 ± 0.009
C-Lab	0.04 ± 0.03	0.05 ± 0.04	0.03 ± 0.03	0.007 ± 0.005
% Inhibition				
Tem-R		89	95	97
C-Lab		0	25	83

Note. Values quoted are the means of at least 50 individuals ± standard deviation.

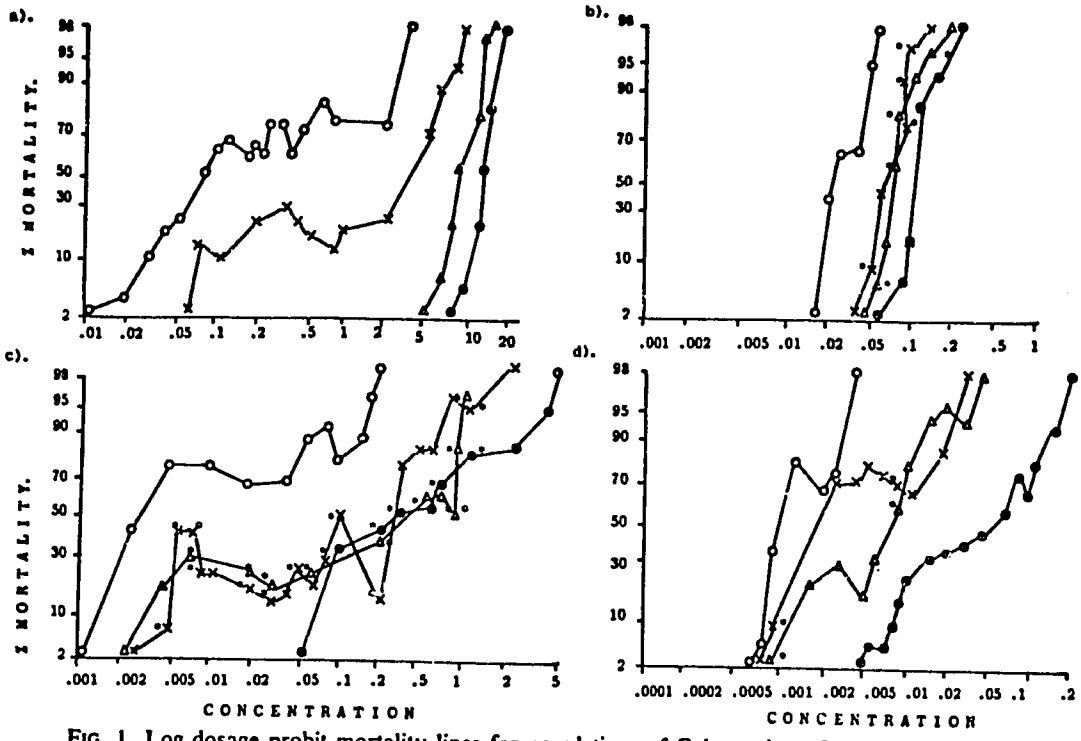

FIG. 1. Log dosage probit mortality lines for populations of Culex quinquefasciatus selected for four generations with (a) malathion, (b) IBP + malathion, (c) temephos, and (d) IBP + temephos. (* = the positions at which the 95% confidence limits of different lines overlap.)

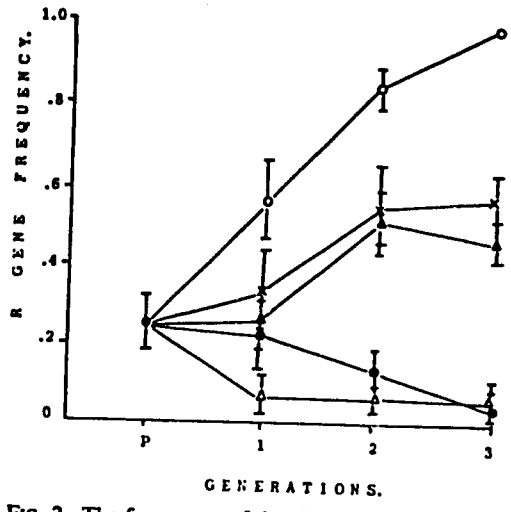

FIG. 2. The frequency of the elevated esterase gene estimated spectrophotometrically in populations of Culex quinquefasciatus selected with malathion (BM = O), IBP + malathion (BKM = Δ), temephos (BT = ×), and IBP + temephos (BKT = ▲), or left unselected (BNS = ●). 95% confidence limits are given at each point.

FISCAL DATA FOR PROJECT AID DPE1179-C-00-2008-00

August 1, 1982 to September 30, 1985

Budget Category	Appropriation	Actual Expenditures thru 9/30/85	Balance
Personnel	$27,122.00	$15,021.12	$12,100.88
Employee Benefits	6,755.00	46.01	6,708.99
Supplies	2,331.11	951.26	1,379.85
Travel	2,500.00	-0-	2,500.00
Total	$38,708.11	$16,018.39	$22,689.72
Indirect Costs	14,185.89	4,968.91	9,216.98
Balance	$52,894.00	$20,987.30	$31,906.70