

with the Dustbuster Plus cordless vacuum. Since all of the modifications involve only the crevice tool, the vacuum can still be used in the laboratory for general cleanup as originally designed.

The cost per aspirator, regardless of model type discussed, is approximately \$35.00. This cost includes the total unit with the appropriate charger base, batteries, accessory attachments, etc.

In summary, we believe that the units described, and the detailed instructions for converting them into aspirators for small flying insects, offer several distinct advantages to ones previously described in the literature. Besides being portable, lightweight, battery-powered and durable, the most important advantage for field operations is the quick and easy disconnection and reattachment of sample containers (i.e., less "down time").

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### ISOLATION OF AN ORGANOPHOSPHATE SUSCEPTIBLE STRAIN OF *CULEX QUINQUEFASCIATUS* FROM A RESISTANT FIELD POPULATION BY DISCRIMINATION AGAINST ESTERASE-2 PHENOTYPES<sup>1</sup>

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Investigations of insect resistance to insecticides are often hindered by the absence of genetically related susceptible strains to which tolerances can be compared. These strains are also essential in the elucidation of the biochemical and genetic characteristics of resistance.

The southern house mosquito, *Culex quinquefasciatus* Say, is a common pest problem in California, with multi-resistance often hindering effective control (Womeldorf et al. 1968, Apperson and Georghiou 1974, Al-Khatib 1983<sup>2</sup>). Many authors have found this species a

convenient tool for studies of the evolution of resistance and in biochemical or genetic research (Georghiou et al. 1975, Ranasinghe 1976<sup>3</sup>, Lagunes 1980<sup>4</sup>, Ferrari and Georghiou 1981, Pasteur et al. 1981, Vazquez-Garcia 1983<sup>5</sup>, Al-Khatib 1983<sup>2</sup>, Hemingway and Georghiou 1984).

<sup>2</sup> Al-Khatib, Z. I. 1983. Compatibility and biotic potential of different genotypes of OP-resistant *Culex quinquefasciatus* Say (Diptera: Culicidae) with reference to strategies for disrupting the development of resistance. Ph.D. dissertation, University of California, Riverside. 242 pp.

<sup>3</sup> Ranasinghe, L. B. E. 1976. Role of synergists in the selection of specific organophosphorus resistance mechanisms in *Culex quinquefasciatus* Say (Diptera: Culicidae). Ph.D. dissertation, University of California, Riverside. 122 pp.

<sup>4</sup> Lagunes, A. L. 1980. Impact of the use of mixtures and sequences of insecticides in the evolution of resistance in *Culex quinquefasciatus* Say (Diptera: Culicidae). Ph.D. dissertation, University of California, Riverside. 209 pp.

<sup>5</sup> Vazquez-Garcia, M. 1983. Investigations of the potentiality of resistance to *Bacillus thuringiensis* ser. H-14 in *Culex quinquefasciatus* through accelerated selection pressure in the laboratory. Ph.D. dissertation, University of California, Riverside. 201 pp.

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Starch gel electrophoresis of the esterase patterns of insecticide-resistant and susceptible strains revealed that at least one highly active esterase is present in this species in California. This esterase (*Est-2<sup>A</sup>*) catalyzes the hydrolysis of  $\alpha$ -naphthyl acetate, and is suppressible by the synergist DEF (*S,S,S*-tributyl phosphorothioate) (Georghiou and Pasteur 1978). *Est-2<sup>A</sup>* was also found to be identical to or dissociable from resistance to temephos, chlorpyrifos, parathion, methyl parathion, malathion and fenthion (Georghiou and Pasteur 1978, Georghiou et al. 1980). By conducting single pair matings, Pasteur et al. (1980) demonstrated the presence of multiple alleles of *Est-2<sup>A</sup>* with variable levels of resistance to the selecting agent (temephos). Pasteur and Georghiou (1981) also developed a filter paper test which permits fast detection of *Est-2<sup>A</sup>* and hence OP-resistance in this species.

This study demonstrates the possibility of obtaining an OP-susceptible strain of *Cx. quinquefasciatus* from a mixed population by the removal of *Est-2<sup>A</sup>* phenotypes.

The following strains were used in this study:

1. LA, collected as larvae and pupae in large numbers (ca. 3,000) from a drainage ditch within the Southeast Mosquito Abatement District, Los Angeles, California, in June 1980. The strain was chosen for the purpose of this study because it exhibited moderate OP-resistance. At the time of collection the frequency of resistant individuals (RR + RS) in LA was 0.44 with and *Est-2<sup>A</sup>* frequency of 0.25. A plateau between the 20–50% levels of mortality, demarcating the approximate proportion of the susceptible component, was evident in the dosage-mortality regression line (1d-p) established with temephos.

In addition to 39.5x resistance to temephos at the LC<sub>95</sub> this population was also resistant to chlorpyrifos methyl (66x) and moderately resistant to malathion (20x) (Table 1).

2. S-Lab, a susceptible reference strain that originated from the San Joaquin Valley in 1950 and has been reared in this laboratory since then free of insecticide pressure.

Technical grades of chlorphoxim, chlorpyrifos methyl, chlorpyrifos, fenitrothion, fenthion, malathion and temephos were dissolved in acetone and used in larval bioassays. For these tests, 100 ml of water were placed in waxed paper cups and 20 late-3rd- or early-4th-instar larvae were added. Each cup was dosed with 1 ml of the required insecticide concentration. For each insecticide 5 or more dosages were tested on at least 4 different days. The 1d-p lines were calculated from the 24-hr mortality data according to the method of Finney (1971).

The presence of *Est-2<sup>A</sup>* in individual mosquitoes was detected by the following technique after Pasteur and Georghiou (1981). Individual adults were crushed on a glass plate in 50  $\mu$ l drops of distilled water and the homogenates were transferred to filter paper strips. These were then immersed for 90 sec in a solution of 10 ml 1%  $\alpha$ -naphthyl acetate and 90 ml of phosphate buffer (9.2 gm KH<sub>2</sub>PO<sub>4</sub> + 4.8 gm Na<sub>2</sub>HPO<sub>4</sub> per 1000 ml water). The strips were then dipped in a solution of 0.5% GBC salt for another 90 sec and fixed in 10% acetic acid. The presence of the active esterase was indicated by a blue color.

Single pair matings were established with virgin individuals from the LA population. Since *Est-2<sup>A</sup>* is known to be dominant (Georghiou et al. 1980), the F1 and F2 progeny of 23 pairs of parents that recorded no esterases were tested to confirm the absence of *Est-2<sup>A</sup>* before these families were mixed to form the susceptible strain, LA-S. The absence of any *Est-2<sup>A</sup>* phenotypes indicated the total elimination of this gene in the derived population. The susceptibility of the LA-S strain towards the tested

Table 1. Comparative susceptibility to organophosphates of a susceptible reference strain of *Culex quinquefasciatus*, Lab-S; a field population, LA; and a derivative susceptible strain, LA-S from Los Angeles, California.

Insecticide	Lab-S <sup>a</sup>		LA <sup>b</sup>				LA-S			
	LC <sub>50</sub>	(LC <sub>95</sub> )	LC <sub>50</sub>	(LC <sub>95</sub> )	RR <sub>50</sub>	(RR <sub>95</sub> )	LC <sub>50</sub>	(LC <sub>95</sub> )	RR <sub>50</sub>	(RR <sub>95</sub> )
Fenitrothion	0.028	(0.083)					0.014	(0.03)	0.5	(0.36)
Chlorphoxim	0.0059	(0.0084)					0.003	(0.008)	0.51	(0.95)
Chlorpyrifos	0.0041	(0.0062)					0.0024	(0.0051)	0.58	(0.82)
Temephos	0.0026	(0.0038)	0.05	(0.15)	19.23	(39.5)	0.002	(0.003)	0.77	(0.79)
Malathion	0.076	(0.135)	0.5	(2.65)	6.58	(19.6)	0.076	(0.224)	1.0	(1.66)
Fenthion	0.025	(0.037)					0.035	(0.075)	1.4	(2.03)
Chlorpyrifos methyl	0.0028	(0.0047)	0.02	(0.31)	7.14	(65.9)	0.004	(0.009)	1.43	(1.91)

<sup>a</sup> All LC values in mg/liter.

<sup>b</sup> RR (Resistance Ratio) calculated from  $\frac{LA}{Lab-S}$  values.

organophosphates proved to be of the same order as those of the Lab-S strain with resistance levels ranging from 2.03 for fenthion to 0.36 for fenitrothion (Table 1).

Since intensive selection by organophosphates leads to prevalence of resistance genes, this study demonstrated that the opposite could be achieved by discriminating against the mutant gene (*Est-2<sup>A</sup>*) in successive generations of the mosquito *Cx. quinquefasciatus*. The elimination of this gene from a colonized field population resulted in a population homozygous for the wild type susceptible gene. The slightly higher tolerance in the derived line to malathion, fenthion and chlorpyrifos methyl could be due to ancillary genes inherited from the parental wild population or caused by other minor mechanisms of resistance. Since the removal of gene *Est-2<sup>A</sup>* from a field population of *Cx. quinquefasciatus* led also to the elimination of resistance to several organophosphates this study confirms earlier reports that *Est-2<sup>A</sup>* gene is associated with resistance to a large variety of organophosphates.

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