SUSCEPTIBILITY TO ORGANOPHOSPHATE INSECTICIDES IN LARVAL *AEDES ALBOPICTUS*

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ABSTRACT. Larvae of 26 strains of *Aedes albopictus* were tested for susceptibility to 5 organophosphate insecticides. The recently collected geographic strains included 13 from the USA, 5 from Brazil, 3 from Southeast Asia and 5 from Japan. The greatest amount of variability was seen among U.S. populations, ranging from quite susceptible to potentially resistant. Brazilian samples were generally quite susceptible to all insecticides tested. Three Japanese strains showed tolerance or low level resistance to fenitrothion (2×, 3× and 8×), as did 2 U.S. strains (3× and 5×). Two U.S. strains showed tolerance to malathion (3× and 4×), while one strain from Chicago, Illinois, showed low level resistance (22×). Selection for resistance to malathion in a laboratory strain composed of multiple U.S. geographic strains resulted in a resistance ratio of 21× after 6 generations of selection.

INTRODUCTION

Continued expansion of the geographic range of *Aedes albopictus* (Skuse) in the United States (Moore et al. 1988) and recent findings concerning the vector potential of the mosquito (Grimstad et al. 1989, Mitchell et al. 1987) have led to an increase not only in public awareness of the mosquito, but also in attempts to control it. In southern regions of the country, this mosquito is currently a significant pest species tying up relatively large amounts of money and labor[1] (Peacock et al. 1988). In the Midwest, populations are generally smaller and control efforts vary greatly (Wesson et al. 1989), depending on local vector control agencies and resources. Some areas have attempted no control while others have achieved local eradication.[2]

Those populations of *Ae. albopictus* which are being exposed to control efforts are prone to develop insecticide resistance. Organophosphate insecticides are commonly used in mosquito control, either alone or in combination with other types of insecticides; in the U.S. malathion, temephos and chlorpyrifos are most often used, either as adulticides or larvicides.

Information on *Ae. albopictus* larval susceptibility to organophosphate insecticides is lacking for most areas in the U.S. where the mosquito occurs, although exceptions exist[3] (Khoo et al. 1988, Robert and Olson 1989, Cilek et al. 1989). In addition, there is little information on organophosphate susceptibility from Japanese and Northern Asian populations of *Ae. albopictus* (Hawley 1988). Similarly, there are no reports on the organophosphate susceptibility of Brazilian populations.

This study examined the susceptibility of a variety of geographic strains of *Ae. albopictus* to 5 commonly used organophosphate insecticides to provide baseline insecticide susceptibility information, and to allow for comparison among various populations. In addition, laboratory selection of *Ae. albopictus* larvae for insecticide resistance to malathion was conducted.

MATERIALS AND METHODS

Mosquito strains: Mosquito strains had been field-collected and colonized within the past 2 years before testing (Table 1). Exceptions were 2 long-established laboratory strains, OAHU of *Ae. albopictus* and ROCK of *Ae. aegypti* (Linn). The latter strains served as standard susceptible strains for comparison. After initial tests, the SABAH strain of *Ae. albopictus* became the standard because it was more susceptible than OAHU to all insecticides tested.

Larval bioassay: Log dosage-probit mortality tests were performed with chlorpyrifos, fenitrothion, fenthion, malathion and temephos. Alcoholic dilutions of analytical or technical grade insecticides were used. Test protocol followed that outlined by the World Health Organization (1981). Special attention was given to production of uniform, uncrowded larvae (fed on a liver powder suspension of 4 g/liter). Lots of 20–25 fourth instar larvae were tested for 24 hours in 250 ml of water with 1 ml of the appropriate insecticide concentration. Tests were conducted in 16-oz (400-ml) polyethylene-coated paper cups (Nestyle); they consisted of at least 2 replicates per concentration and 4 concentrations per insecticide series, excluding untreated controls. Most tests were replicated at least 4 times. Resulting survival data were subjected to probit analysis (Finney 1971), using a BASIC microcomputer program (Raymond 1985).

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[1] Monthly report of the New Orleans Mosquito Control Board, December, 1987, cover story.

[2] Berry, R. L. 1989. Reported at the 24th Annual Meeting of the Ohio Mosquito Control Association—Mansfield, OH.

[3] Annual report of the New Orleans Mosquito Control Board, 1986, p. 20–24.

Table 1. Geographic strains of *Aedes albopictus* tested for organophosphate susceptibility.

Strain—Origin (year collected)	Strain—Origin (year collected)
USA strains	*Brazilian strains*
1. ALSACE—New Alsace, IN, USA (1987)	1. ANCH—Anchieta, ESS, Brazil (1988)
2. DOM—Chicago, IL, USA (1987)	2. ANCH 2—Anchieta, ESS, Brazil (1988)
3. DUNN—Evansville, IN, USA (1987)	3. CARI—Cariaciaca, ESS, Brazil (1986)
4. ESL—East St. Louis, IL, USA (1988)	4. PAULO—Sao Paulo, SPS, Brazil (1988)
5. FAUST—Findlay, OH, USA (1987)	5. TEREZA 2—St. Tereza, ESS (1988)
6. HCMCD—Harris Co., TX, USA (1986)	*Southeast Asian strains*
7. HOUSTON—Houston, TX, USA (1986)	1. SERIAN—Sarawak, E. Malaysia (1987)
8. INDY—Indianapolis, IN, USA (1986)	2. SABAH—Sabah, E. Malaysia (1986)
9. JAX-2—Jacksonville, FL, USA (1987)	*(Hawaiian)*
10. LEX—Lexington, KY, USA (1987)	3. OAHU—Honolulu, HI, USA (1971)
11. NO86—New Orleans, LA, USA (1986)	*Japanese strains*
12. OAK—Oak Hill, OH, USA (1987)	1. KABE—Kabeshima, Japan (1988)
13. OAK-2—Oak Hill, OH, USA (1988)	2. KUBO—Kuboizumi, Japan (1988)
ROCK—Rockefeller Institute (prior to 1959)	3. NAGA—Nagasaki, Japan (1988)
Aedes aegypti	4. OKIN—Okinawa, Japan (1988)
	5. SEBURI—Seburi, Japan (1988)

Baseline susceptibility: Using the SABAH strain (2-SE ASIA, Figs. 1–5), the following concentrations were established as baseline susceptibilities for *Ae. albopictus:* 0.01 mg/liter—chlorpyrifos, 0.02 mg/liter—fenthion, 0.20 mg/liter—malathion, 0.02 mg/liter—temephos, and 0.02 mg/liter—fenitrothion. Doubling the baseline concentration for each insecticide gives a concentration diagnostic for potential resistance, i.e., individuals surviving this insecticide dose may be considered resistant. The World Health Organization (1980) has proposed similar diagnostic standards for *Ae. aegypti.*[4] The greater of these 2 diagnostic values, either for *Ae. albopictus* or for *Ae. aegypti,* is listed as the diagnostic concentration. This gives a more conservative estimate of resistance potential in *Ae. albopictus.*

Resistance selection: Georghiou et al. (1987) were successful in selecting for resistance in *Ae. aegypti* after first combining a number of geographic strains to form a "synthetic" laboratory colony. That technique has also been used in this study.

Geographic strains of *Ae. albopictus* were combined from the 4 major regions shown in Table 1. Fifty males and 50 females from each strain were used as stock material: 18 U.S., 4 Brazilian, 15 Southeast Asian and 9 Japanese strains were used to make a composite strain from the U.S., Brazil, Southeast Asia and Japan, respectively. Each geographic composite was then tested and selected with all 5 insecticides.

RESULTS

Insecticide susceptibility: Figures 1–5 compare LC_{95} values for all geographic strains. The ROCK strain of *Ae. aegypti* was highly susceptible to all 5 insecticides.

Temephos: (Fig. 1.) Three U.S. strains (nos. 2, 5 and 13) and a Japanese strain (no. 1) had LC_{95} values at or above the diagnostic concentration of 0.04 mg/liter. The resistance ratio of the Oak Hill strain (no. 13) was 3×. All Brazilian and Southeast Asian strains had LC_{95} values below the diagnostic concentration.

Chlorpyrifos: (Fig. 2.) The LC_{95} values obtained with chlorpyrifos for 3 U.S. strains (nos. 2, 4 and 13), were equal to or just above the diagnostic concentration of 0.02 mg/liter. A Brazilian strain (no. 2) and a Southeast Asian strain (no. 1) also displayed tolerance to this insecticide.

Fenitrothion: (Fig. 3.) While many of the strains were quite susceptible to fenitrothion, 3 had LC_{95} values in excess of the diagnostic concentration of 0.06 mg/liter. The 1988 Oak Hill strain from the U.S. (no. 13) had a resistance ratio of 5×, while the Japanese strains from Kabeshima (no. 1) and Kuboizumi (no. 2) had resistance ratios of 3× and 8×, respectively.

Malathion: (Fig. 4.) The majority of strains tested were susceptible to malathion. Three exceptions were the U.S. strains from Chicago (no. 2) and Houston (nos. 6 and 7). The 2 strains from the Houston area had LC_{95} values just above the diagnostic dose (1.00 mg/liter), while

[4] Diagnostic concentrations for *Ae. aegypti:* 0.01 mg/liter—chlorpyrifos, 0.05 mg/liter—fenthion, 1.00 mg/liter—malathion, 0.02 mg/liter—temephos, and 0.06 mg/liter—fenitrothion.

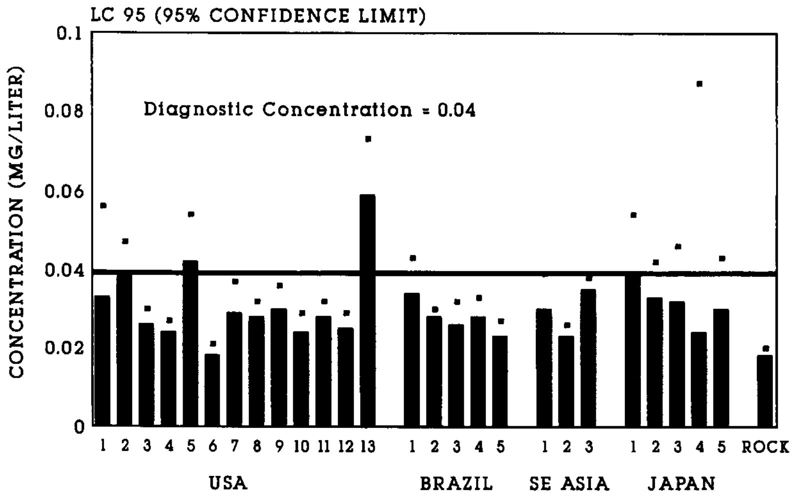

Fig. 1. Temephos susceptibility in 26 strains of *Aedes albopictus* larvae. Bar height denotes LC_{95} value; point denotes 95% confidence limit. Horizontal line is at the diagnostic concentration. Strain numbers correspond to numbers in Table 1. All strains were tested in 4 replicate trials except SE ASIA nos. 2 and 3, which were replicated 6 and 8 times, respectively.

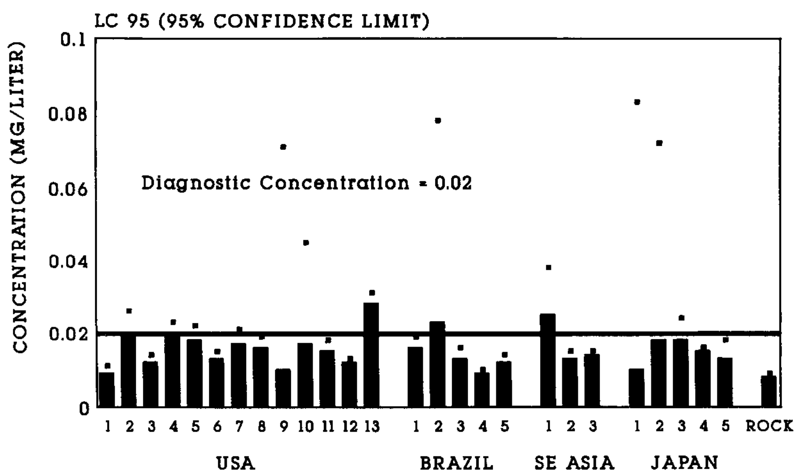

Fig. 2. Chlorpyrifos susceptibility in 26 strains of *Aedes albopictus* larvae. Bar height denotes LC_{95} value; point denotes 95% confidence limit. Horizontal line is at the diagnostic concentration. Strain numbers correspond to numbers in Table 1. All strains were tested in 4 replicate trials except USA no. 13 and SE ASIA nos. 2 and 3; these were replicated 5, 6 and 8 times, respectively.

the Chicago strain more than tripled that value with a resistance ratio of 22× at the LC_{95} level. While not obvious from the graph, the Chicago population was highly heterogeneous, resulting in a very shallow Ld-pm line slope (1.32).

Fenthion: (Fig. 5.) With the exception of one U.S. strain (no. 13), all strains were susceptible to fenthion. The LC_{95} value for the 1988 Oak Hill strain equaled the diagnostic concentration (0.05 mg/liter) with a resistance ratio of 4×.

Selection experiment: Malathion selection of the U.S. composite proved most successful. Insecticide selection pressure was initiated at 40% in the first generation and increased to 50%, 70%, 70%, 75% and 80% after 6 generations. Selection in the U.S. composite strain resulted in a resistance ratio of 21× after 6 generations of selection (Fig. 6). As compared to the SABAH strain, the U.S. composite resistance ratio was 27× at the LC_{95} level.

DISCUSSION

Certain trends are evident from the *Ae. albopictus* organophosphate susceptibility results: 1)

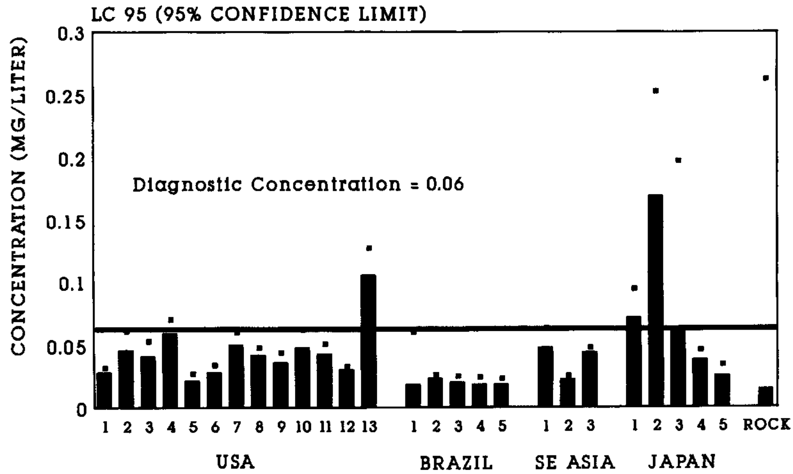

Fig. 3. Fenitrothion susceptibility in 26 strains of *Aedes albopictus* larvae. Bar height denotes LC_{95} value; point denotes 95% confidence limit. Horizontal line is at the diagnostic concentration. Strain numbers correspond to numbers in Table 1. All strains were tested in 4 replicate trials except SE ASIA nos. 2 and 3 which were replicated 6 and 8 times, respectively.

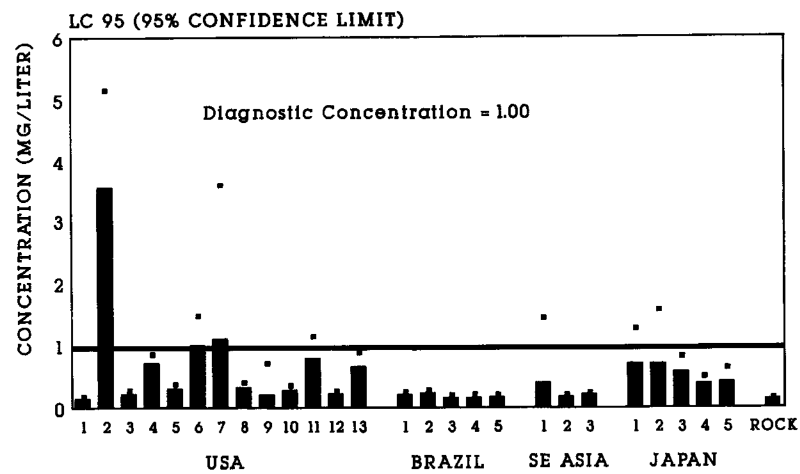

Fig. 4. Malathion susceptibility in 26 strains of *Aedes albopictus* larvae. Bar height denotes LC_{95} value; point denotes 95% confidence limit. Horizontal line is at the diagnostic concentration. Strain numbers correspond to numbers in the Table 1. All strains were tested in 4 replicate trials except USA no. 12 and SE ASIA nos. 2 and 3; these were replicated 3, 6 and 8 times, respectively.

when compared to the ROCK strain, *Ae. albopictus* strains were less susceptible than *Ae. aegypti* to all 5 insecticides; 2) Brazilian strains were, in general, highly susceptible to all of the insecticides, most notably to malathion, fenitrothion and fenthion; and 3) U.S. strains showed a wide range of variability in response to the insecticides, from quite susceptible to potentially resistant.

The comparison of *Ae. albopictus* susceptibility to that of *Ae. aegypti* is useful since these mosquitoes occur in the same habitats in many areas in the southern U.S. If *Ae. albopictus* is less susceptible to organophosphate insecticides, this may be one factor in explaining why *Ae. aegypti* populations seem to be disappearing from areas where the 2 coexist (Ho et al. 1989), although other hypotheses to explain this phenomenon have been proposed (Nasci et al. 1989). However, in *Ae. aegypti*, as in *Ae. albopictus*, considerable variation exists between geographic strains in response to insecticide application. Samples of *Ae. aegypti* from the Caribbean have exhibited resistance to malathion (Fox 1980) and temephos (Georghiou et al. 1987). Hemingway et al. (1989), however, found no resistance to malathion in pyrethroid-resistant *Ae. aegypti* from Puerto Rico.

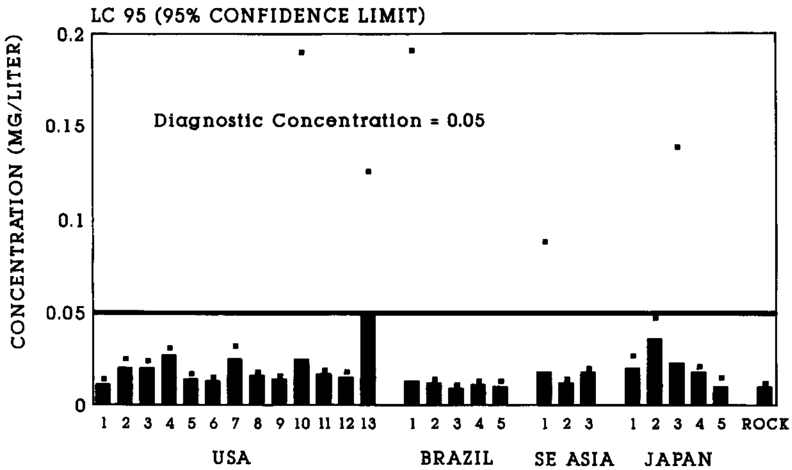

Fig. 5. Fenthion susceptibility in 26 strains of *Aedes albopictus* larvae. Bar height denotes LC_{95} value; point denotes 95% confidence limit. Horizontal line is at the diagnostic concentration. Strain numbers correspond to numbers in the Table 1. All strains were tested in 4 replicate trials except USA no. 13 and SE ASIA nos. 2 and 3; these were replicated 5, 6 and 7 times, respectively.

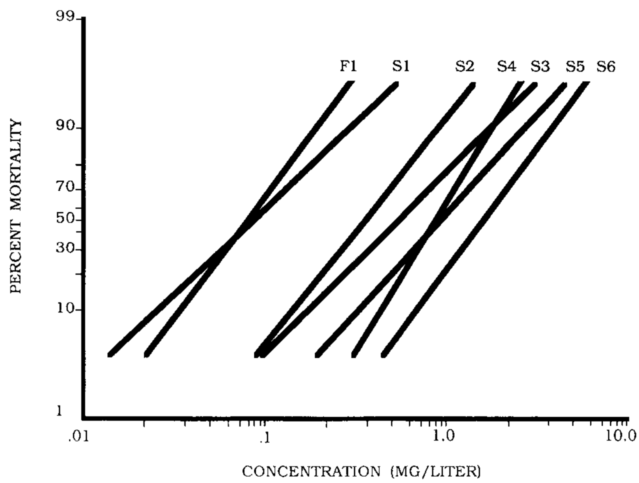

Fig. 6. Response to 6 generations of malathion selection in the U.S. composite strain of *Aedes albopictus*. S*n* refers to each consecutive generation of selection. In 6/7 Ld-pm lines, all but one or fewer data points fit the line according to chi-square analysis (Finney 1971).

Compared to U.S. and Asian populations of *Ae. albopictus*, Brazilian strains show less genetic variation, according to isozyme and DNA analysis (S. Kambhampati, personal communication). Brazilian strains are also highly uniform with regard to absence of photoperiodically induced diapause (G. Craig, personal communication) and laboratory transmission of La Crosse virus (T. Streit, personal communication). Similarly, Brazilian strains were uniformly insecticide-susceptible with the exception of ANCH-2 to chlorpyrifos. This lack of variability supports the concept that the Brazilian populations originated either from a low number of introductions from the same source or from a single introduction.

The variation in insecticide susceptibility evident in U.S. strains of *Ae. albopictus* has 2 possible explanations: 1) the populations in the U.S. stemmed from a number of different Asian sources with different insecticide exposure histories, or 2) insecticide selection has taken place since *Ae. albopictus* arrived in the U. S., giving rise to populations with different insecticide susceptibilities. Perhaps a combination of these 2 factors accounts for the observed variability.

As stated earlier, there is limited information regarding susceptibility of Asian strains of *Ae.*

albopictus to organophosphate insecticides. Brown (1986) reported populations from Madagascar, Malaysia, Singapore and Vietnam as resistant, but no resistance ratios were given. Japanese populations from Tokyo and Okinawa were tested for susceptibility to the organophosphates, fenitrothion, fenthion and temephos (Takahashi et al. 1985). The Tokyo strain showed a resistance ratio of 5× to fenthion, but was susceptible to the other insecticides tested; the Okinawa strain was susceptible to all 3 insecticides. Additional information is lacking regarding mosquito susceptibility in Japan, where U.S. populations of *Ae. albopictus* probably originated (Hawley et al. 1987). However, organophosphate insecticides are commonly used in Japan, not only for mosquito control, but also for control of agricultural pests affecting rice and barley, among other crops (M. Mogi, personal communication). Since the present study shows lowered susceptibility or resistance of Japanese strains to some insecticides, some selection for resistance in *Ae. albopictus* may have occurred in northern Asia, prior to U.S. introduction.

Some selection for insecticide tolerance has probably taken place since the arrival of *Ae. albopictus* in the U.S., especially to malathion. Khoo et al. (1988) found that 50% of adult mosquitoes of the HCMCD strain from Harris County, Texas, were resistant to malathion applied topically. Robert and Olson (1989) found adult female *Ae. albopictus* from Houston and Liberty County, Texas, resistant to malathion. Similarly, the present findings regarding the HCMCD, HOUSTON and especially the DOM (Chicago) strain point to the recent development of resistance in those populations. This seems likely since none of the Japanese strains tested showed unusually high tolerance to malathion. The selection of the U.S. composite strain resulting in a resistance ratio of 21× in only 6 generations demonstrates the potential for resistance development in this species.

Less easily explained is the apparent decrease in the susceptibility of the Oak Hill, Ohio, population over a one-year period (1987–88). One explanation is that heavy insecticide pressure on that population has resulted in decreased susceptibility to all 5 insecticides. However, since the 1988 colony originated from 3 field-collected inseminated females, genetic drift could have produced that result. Continued untreated tire introductions to the tire yard in Oak Hill may be importing less susceptible individuals from other localities—as evidenced by the finding of *Ae. aegypti* at Oak Hill in 1989 where it had not been collected in previous years.[2] These questions are being addressed through continued testing of the Oak Hill *Ae. albopictus* population on an annual basis; the results of 1989 susceptibility tests are not yet available.

Preliminary attempts have been made to detect nonspecific esterase amplification as a resistance mechanism in both the DOM (Chicago) and malathion-selected U. S. composite strains. Amplification of detoxifying esterase enzymes is often the basis for organophosphate resistance in *Culex* mosquitoes (Mouches et al. 1986, Raymond et al. 1987) and has been described in *Anopheles albimanus* Wied. (Brogdon et al. 1988). In *Ae. albopictus*, initial studies have shown no difference in intensity of esterase banding between the malathion-resistant and control (SABAH) strains, when using polyacrylamide gel electrophoresis. Selection for insensitive acetylcholinesterase might, alternatively, account for resistance development in these strains. Methods for detection of this resistance mechanism have recently been updated (ffrench-Constant and Bonning 1989).

Given the potential public health importance of *Ae. albopictus*, continued insecticide susceptibility surveillance is essential. Current information about the effectiveness of many commonly used insecticides against *Ae. albopictus* is spotty and often anecdotal. Given the wide range of response to insecticides seen in these populations, there is a particular need for testing on a local basis.

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