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DETECTION of INSECTICIDE RESISTANCE in *AEDES AEGYPTI* (DIPTERA: CULICIDAE) from CUBA and VENEZUELA.

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ABSTRACT

Four strains of *Aedes aegypti (*L.), one from Cuba and three from Venezuela, were bioassayed for susceptibility to 8 pesticides, including the organophosphorus insecticides, temephos, malathion, fenthion, pirimiphos methyl, and chlorpyrifos, and the pyrethroids deltamethrin, lambda cyhalothrin and cypermethrin. S, S, S, -tributyl phosphorotrithioate and piperonyl butoxide were used as synergists to assess the involvement of esterases and monooxygenases in organophosphate resistance. Venezuelan strains had low levels of resistance to fenthion and malathion, and moderate to high resistance to temephos, pyrimphos methyl and chlorpiriphos. All strains were susceptible to the pyrethroids, except the Cuban strain, which had moderate levels of resistance to cypermethrin. The organophosphate resistance in *Ae. aegypti* is a serious threat to control operations. Integrated strategies for *Ae. aegypti* control to prevent or delay pyrethroid resistance in Venezuela and Cuba are suggested.

KEY WORDS: *Aedes aegypti*, insecticide resistance, mechanisms, Cuba, Venezuela.

Control of *Aedes aegypti* (L.), in the Caribbean has become a public health priority, because this species is the vector of dengue (Nathan 1993). Epidemic dengue hemorrhagic fever (DHF) occurred for the first time in Cuba in 1981 (Kourí *et al.* 1983) and a DHF epidemic occurred in Venezuela in 1989-90 (PAHO 1990).

Vector control is the primary means of preventing or reducing dengue transmission, and the development of resistance to organochloride, organophosphorus (OP), carbamate and pyrethroid insecticides in *Ae. Aegypti* from subtropical and tropical regions of the world (WHO 1986) hinders these control efforts. Georghiou *et al.* (1987) reported larval resistance to temephos, malathion, fenthion, and propoxur in *Ae. aegypti* collected from 28 sites in the Caribbean. Other authors have reported 10-fold levels of resistance to OP, which resulted in control problems (Rawlins and Ragoonansigh 1990; Rawlins and Ou Hing Wan 1995; Rawlins 1998).

The role of metabolism in OP resistance was demonstrated in *Ae. aegypti* larvae which had higher carboxylesterase activity (Chen & Sudderuddin 1978). Mazarri and Georghiou (1995) found that non-specific esterase and monooxygenases played a significant role in organophosphate and carbamate resistance, respectively, in *Ae. aegypti* from Venezuela. Recently, elevated esterase activity was associated with temephos resistance in *Ae. aegypti* (Wirth and Georghiou 1999).

An outbreak of dengue occurred in Santiago de Cuba municipality in 1997, and the Venezuelan states of Aragua, Miranda and Apure, reported a high incidence of dengue and vector prevalence between 1997 and 1998 (Mazarri et al. 1998). In the current study, for the *Ae. aegypti* collected from each of these localities, the insecticide resistance status was determined for compounds which are in current, or planned for future use.

MATERIALS AND METHODS

Strains

The SANTIAGO DE CUBA was collected from Cuba in 1997 as were 3 strains from Venezuela (APURE, MIRANDA and ARAGUA). All strains were maintained in the laboratory without selection pressure.

The ROCKEFELLER susceptible laboratory strain, of Caribbean origin, was colonized in the early 1930's. This strain was provided by the Centers for Disease Control laboratory in San Juan, Puerto Rico.

Bioassay procedure

Standard WHO larval bioassays were used to establish complete dosage-mortality lines (WHO, 1981). Twenty early 4^{th} instar larvae of uniform size were placed in plastic cups containing 99 ml of tap water and 1 ml of insecticide solution. Five or more concentrations of each insecticide, prepared in standard (weight/volume) acetone solution, were used in at least 5 replicates on different days. Controls were treated with 1 ml of acetone. Mortality was determined after 24 h of insecticide exposure. Each bioassay was replicated at least twice; i.e. 500 larvae were assayed for each insecticide. Results were subjected to probit analysis by the method of Finney (1971) using a basic program (Raymond 1985). Resistance ratios were calculated from the LC_{50} for the tested population divided by the LC_{50} of the reference ROCKEFELLER strain.

Resistance mechanisms

The action of two synergists, S,S,S, tributyl phosphorotrithioate (DEF) and piperonyl butoxide (PBO), were investigated by exposing 4^{th} instar larvae to 0.008 mg/l DEFor 5.0 mg/l PBO for 4 h prior to the addition of insecticide solution (Ranasinghe and Georghiou 1979). There was no mortality due to the synergist alone.

Biochemical tests

Esterase and glutathione S-transferase (GST) activities were determined using a modification of the techniques described for *Culex quinquefasciatus* Say (Peiris and Hemingway 1990). The tests were modified according to the methodology in: Measurement and Magnitude of enzymatic rate constants chapter, Enzyme Structure and mechanism (Fersht 1985).

Measurement of esterase activity

The saturate concentration of β-naphthyl acetate and the optimum time of the reaction for esterase activity in *Ae. aegypti* was determined using the ROCKEFELLER and SANTIAGO DE CUBA strains. The optical density (O.D) of the reaction product for each concentration of β-naphthyl acetate (1.0mM, 0.66 mM, 0.44 mM, 0.29 mM, 0.195 mM and 0.13 mM) was analysed at one minute intervals (from 1 to 25 min) to determine the optimum reaction time. The values of Vo (slope of the plot of O.D. vs. reaction time) were plotted against the concentrations of β-naphthyl acetate mentioned above, to obtain a saturate concentration for the substrate of 70 mM and an optimum reaction time of 10 min.

Esterase activity was determined for 4^{th} instar larvae using a modification of the method of Peiris and Hemingway, (1990). 20 µl of each larval homogenate was added to a 96 well microtitre plate and mixed with 200 µl of 0.7 mM β-naphthyl acetate. After 10 min, 40 µl of Fast-blue stain was added and the optical density read at 570 nm in a Labsystems iMS Reader MF, manufactured in Finland by Labsystems company.

The mean ± standard deviation baseline value for esterase activity (measured as optical density) for 1,108 larvae of the ROCKEFELLER strain was 0.528 ± 0.233 - values >1.227 (mean + 3SD) were considered as elevated esterase activity.

Modification of GST activity test

The saturate concentration of 1-chloro-2,4-dinitrobenzene (CDNB) was determined using different concentrations of substrate (15 mM, 20 mM, 30 mM, 40 mM, 50 mM & 60 mM) with a constant concentration of 30 mM reduced glutathione. The saturate concentration of reduced glutathione (GSH) was found subsequently by changing its concentration (30 mM, 25 mM, 20 mM, 15 mM, 10 mM and 5mM) and maintaining CDNB concentrations established previously. The saturate concentrations of GSH and CDNB were 20 mM and 50 mM. The optimum time for reading the reaction was 3 min**.**

Glutathione S-transferase activity was determined using a modification of the method of Booth *et al*. (1961). In a 96 well microtitre plate, 20 µl of larval homogenate was added to a reaction mixture containing 250 µl of 50 mM CDNB and 5ml of 20 mM GSH. After 3 min, the O. D. was read at 340 nm in Labsystems iMS Reader MF.

The range of GST activity was established for 288 larvae of the ROCKEFELLER strain. The specific GST activity was expressed in µmol/ mg.min. The mean ± standard deviation value for specific GST activity was 0.37±0.0998. Values above 0.6694 µmol/ mg.min (mean + 3SD) were considered as elevated GST activity.

An estimate of resistance gene frequencies for esterase and GST mechanisms were calculated from the number of homozygous susceptible individuals for each assay, assuming that the population was in Hardy- Weinberg equilibrium.

RESULTS

The LC_{50} values for 5 organophosphate insecticides tested against the susceptible ROCKEFELLER strain of *Ae. aegypti* are shown in Table 1. Malathion was the least toxic insecticide with an LC_{50} of 0.445 mg/l, whereas chlorpyrifos was the most toxic with an LC_{50} of 0.00687 mg/l. There was > 11-fold resistance to pyrimiphos methyl in all Venezuelan strains; ARAGUA had the highest resistance levels to pyrimiphos methyl (27.6-fold) and chlorpyrifos (22.6-fold). In contrast, the SANTIAGO DE CUBA strain had 5 - 10-fold resistance to fenthion and pirimiphos methyl and 16.1-fold resistance to chlorpyrifos. The remaining strains, with the exception of MIRANDA had the lowest level of resistance to fenthion. APURE was the only strain susceptible to chlorpiryfos.

The LC_{50} values for 3 pyrethroid insecticides tested against the ROCKEFELLER strain are shown in Table 2. In general, there was < 5-fold pyrethroid resistance in the Venezuelan strains, and 7.2-fold resistance to cypermethrin in SANTIAGO DE CUBA.

Synergism with DEF indicate that esterases play a significant role in chlorpyrifos resistance in ARAGUA, SANTIAGO DE CUBA and MIRANDA, (synergism ratios (S.R.) 306.1, 16.2 and 10.2 respectively), (Table 3). Similarly, the LC_{50} of temephos in the APURE strain was reduced from 0.149 mg/l to 0.00764 mg/l (S.R. 19.7) in the presence of DEF, confirming the importance of esterases in temephos resistance.

PBO synergised pyrimiphos methyl < 5 fold in APURE, ARAGUA and MIRANDA, (Table 4), indicating that resistance did not depend on MFO activity. Absence of substantial synergism with DEF was also evident. These results indicated that pyrimiphos methyl resistance in all strains was not metabolic based. In general the results of the test with PBO indicated the lack of any significant role by MFO in resistance toward any of the evaluated organophosphate insecticides.

Biochemical tests

Esterase gene frequencies in *Ae. aegypti* colonies ranged from 0.42 for ARAGUA to 1.0 for APURE and SANTIAGO DE CUBA (Table 5). Genes for GST resistance were less frequently observed, ranging from 0.041 for APURE to 0.8 for SANTIAGO DE CUBA. The ROCKEFELLER strain frequencies were 0 for both, indicating this colony remained homozygous susceptible.

The comparison of the distribution patterns of esterase and GST activity between ROCKEFELLER and SANTIAGO DE CUBA strain are showed in fig 1 and fig 2, respectively.

DISCUSSION

Ae. aegypti populations from Venezuela and Santiago de Cuba had variable levels of resistance to organophosphate and pyrethroid insecticides. The Venezuelan strains generally had low resistance to fenthion and malathion. APURE and SANTIAGO DE CUBA, treated in the field with 1 ppm temephos, had high levels of resistance to temephos. However, selection of an Indian strain of *Ae. aegypti* for 20 generations with temephos, increased temephos tolerance by only 2-3-fold (Madhukar and Pillai 1970).

The primary insecticides used in Venezuela are organophosphates, including temephos for larval control and malathion in thermal foggers and ultra-low volume (ULV) sprays for adult control (Mazarry and Georghiou 1995). As malathion has been used in the control of *Ae. aegypti* for more than 25 years, the low level of resistance observed in the strains from Venezuela may indicate that ULV adult treatments result in lower selection pressure than larval treatments. This is a result of a larger proportion of the population remaining untreated, providing a pool of susceptible individuals for the repopulation of the treated areas (Georghiou and Taylor 1977).

Additionally, pyrethroids (lambda cyhalothrin and deltamethrin) are often incorporated into control programs mostly in an emergency when an outbreak of dengue or DHF is declared. Among the pyrethroid insecticides, lambda cyhalothrin was the most toxic to the strains tested.

During the last outbreak of dengue in Santiago de Cuba, *Ae. aegypti* populations were controlled with temephos as larvicide and the pyrethroids lambda cyhalothrin and cyfluthrin as ULV adulticides.

The high synergism ratios with DEF and chlorpyrifos indicated that resistance was mediated by an esterase mechanism. Other mechanisms well documented in *Ae. aegypti* like the kdr-like target insensitivity or GST metabolic resistance may be involved in DDT resistance (Malcolm and Wood 1982; *et al*. 1989; Grant and Matsumura 1989; Grant *et al* 1991). Elevated GST's were seen in almost all the strains tested.

Rawlins and Ragoonansingh (1990) reported high levels of chlorpyrifos resistance in populations of *Ae. aegypti* from Puerto Rico, St Lucia and Trinidad. As chlorpyrifos has not been used for control of *Ae. aegypti* in Venezuela nor in Cuba, it is probable that chlorpyrifos resistance is a result of cross-resistance from temephos or other organophosphates (Mazarri and Georghiou 1995).

In the current study, elevated esterase were linked to resistance to temephos in synergist and biochemical assays of the APURE strain. Recently, Georghiou (1999) reported the significant role of the elevated esterase activity in a temephos resistant *Ae. Aegypti* strain after 13 generations of selection.

Elevated esterase activity associated with chlorpyrifos and temephos resistance, respectively, has been reported in *Ae. aegypti* from Venezuela (Mazarri and Georghiou 1995), and Trinidad (Vaugan *et al*. 1998).

The frequencies of elevated GST activity in the MIRANDA and APURE strains were low and this detoxification mechanism is unlikely to play a significant role on the OP's resistance detected in these strains. The ARAGUA and SANTIAGO DE CUBA strains had moderate and high frequencies of elevated GST's respectively.

All the pyrethroids were suitable replacements for the control of *Ae. aegypti,* because resistance was not found (RR < 5-fold). However, Mazarri and Georghiou (1995) reported moderate resistance to lambda cyhalothrin in two states of Venezuela. Hemingway *et al*. (1989) found that pyrethroid resistance was attributed to a kdr (nerve insensitivity) gene in Puerto Rican *Ae. Aegypti*. DDT resistance mechanisms are well documented in *Ae. aegypti* strains, (McDonald and Wood,1979).

It is evident that this important vector species has developed OP resistance, which may result in potential control problems. Biological control agents, such as *Bacillus thuringiensis* var. and *Israelensis* could be seriously considered for incorporation into larval *Ae. Aegypti* control programs. Continued monitoring of insecticide susceptibility in *Ae. aegypti* populations is necessary for informed decisions on insecticide use, however the emphasis on source reduction and environmental sanitation must continue to decrease reliance on insecticides and reduce selection pressure on resistant population.

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Table 1. Toxicity of 5 organophosphates to *Aedes aegypti* strains from Cuba and Venezuela compared to the susceptible ROCKEFELLER strain.

	Temephos	Malathion	Fenthion	Pirimiphos methyl	Chlorpyrifos
S. DE CUBA LC_{50} [a]	0.0713 (0.0601-0.0763)	0.788 (0.729-0.855)	0.0521 (0.0477-0.0565)	0.0641 (0.060-0.0683)	0.114 (0.103-0.140)
Slope [b]	7.2 (± 0.71)	5.1 (±0.66)	4.6 (±0.44)	6.5 (±0.59)	6.7 (±1.42)
$\chi 2$	0.97	1.36	1.90	0.14	0.65
RR [c]	5.9	1.8	0.25	8.1	16.2
APURE LC_{50} [a]	0.149 (0.0116-0.0180)	0.367 (0.331-0.402)	0.0330 (0.0311-0.0432)	0.140 (0.127-0.157)	0.0212 (0.0184-0.0239
Slope [b]	2.2 (±0.22)	4.5 (±0.41)	12.6 (± 0.51)	5.1 (±0.51)	2.9 (±0.23)
c**2**	4.10	4.1	3.81	0.59	5.21
RR [c]	11.1	3.5	0.2	17.8	3.1
ARAGUA LC_{50} [a]	0.0468 (0.0424-0.0514)	0.529 (0.471-0.597)	0.0379 (0.0358-0.0401)	0.224 (0.193-0.256)	0.150 (0.121-0.201)
Slope [b]	5.2 (±0.69)	4.3 (±0.52)	10.7 (±1.14)	3.9 (±0.40)	1.8 (±0.25)
c**2**	2.11	2.53	2.13	5.93	3.75
RR [c]	3.9	4.9	0.18	27.6	22.1
MIRANDA LC_{50} [a]	0.0559 (0.0510-0.0619)	0.104 (0.0770-0.130)	0.0308 (0.0276-0.0335)	0.170 (0.0716-0.229)	0.0387 (0.0321-0.0468
Slope [b]	4.5 (±.0.56)	2.9 (±0.45)	5.8 (±0.69)	3.6 (±0.91)	2.3 (±0.29)
c^2	2.23	2.13	0.50	1.24	1.65
RR	4.7	0.97	3.1	21.5	5.7
ROCKEFELLER LC_{50} [a]	0.0127 (0.0116-0.0144)	0.445 (0.381-0.532)	0.00981 (0.00911.0.0109)	0.00701 (0.00698-0.00912)	0.00687 (0.00619-0.0075
c**2**	4.49	3.42	0.36	1.32	0.43
Slope [b]	6.3 (± 0.73)	2.2 (± 0.27)	6.0 (± 1.28)	3.6 (± 0.52)	5.1 (± 0.82)

[a] LC_{50} in mg/liter; 95% CI in parenthesis, [b] Standard deviation (± SD) are in parenthesis; The linearity of the log dose-probit mortality response are well represented by a line (P> 0.05, and D. F=4). [c] Resistance ratio (RR): LC_{50} resistant strain/ LC_{50} ROCKEFELLER strain. Number of larvae tested were 500 per insecticide

Table 2. Toxicity of 3 pyrethroids insecticides to the *Ae.aegypti* strains from Cuba and Venezuela.

	Deltamethrin	Lambda cyhalothrin	Cypermethrin
S. DE CUBA LC_{50} [a]	0.00038 (0.00011-0.00063)	0.00824 (0.00759-0.00908)	0.00941 (0.00877-0.0104)
Slope [b]	2.1 (± 0.497)	4.6 (±0.54)	6.1 (±0.93)
$\chi 2$	1.68	2.37	3.027
RR [c]	4.7	0.77	7.2
APURE LC_{50} [a]	0.00374 (0.00270-0.00627)	0.0128 (0.0116-0.0145)	0.00343 (0.00301-0.00386)
Slope [b]	1.8(±0.25)	6.6 (± 0.81)	2.9 (±0.259)
c**2**	2.43	3.15	3.88
RR [c]	3.5	1.2	2.6
ARAGUA LC_{50} [a]	0.00138 (0.00124-0.00152)	0.00853 (0.00797-0.00924)	0.00354 (0.00285-0.00440)
Slope [b]	4.1 (±0.38)	5.2 (±0.56)	1.9 (±0.28)
c**2**	4.89	3.46	4.90
RR [c]	1.6	0.82	3.1
MIRANDA LC_{50} [a]	0.00322 (0.00286-0.00357)	0.00461 (0.00422-0.00499)	0.00629 (0.00578-0.00684)
Slope [b]	3.6 (±0.33)	4.3 (±0.39)	4.3 (±0.418)
c^2	3.47	3.53	1.56
RR	3.7	0.44	4.8
ROCK LC_{50} [a]	0.00008 (0.00007-0.00008)	0.00103 (0.00084-0.0012)	0.00129 (0.00076-0.0018)
c**2**	5.063	6.45	1.68
Slope [b]	2.9 (±0.33)	2.3 (±0.23)	1.5 (±0.24)

[a] LC_{50} in mg/liter; 95% CI in parenthesis, [b] Standard deviation (± SD) are in parenthesis; [c.] Resistance ratio (RR): LC_{50} resistant strain/ LC_{50} susceptible strain. The linearity of the log dose-probit mortality response are well represented by a line (P> 0.05, and D. F=4). Number of larvae tested =500 per insecticide

Table 3. Toxicity of five organophosphate insecticides with the synergist DEF to four strains of *Aedes aegypti.*

	Temephos	Malathion	Fenthion	Pirimiphos. methyl	Chlorpyrifos
S. DE CUBA LC_{50} [a]	0.0112 (0.0103-0.0130)	0.778 (0.710-0.859)	0.0267 (0.0230-0.0313)	0.0981 (0.0914-0.109)	0.00678 (0.00638-0.00718)
Slope [b]	7.7 (± 1.40)	3.8 (± 0.43)	9.4 (±1.85)	6.6 (±1.68)	7.4 (±0.74)
c**2**	3.40	1.28	1.15	0.10	5.97
SR [c]	6.45	1.45	0.37	0.65	16.2
APURE LC_{50} [a]	0.00764 (0.00708-0.00824)	0.621 (0.541-0.754)	0.011 (0.0102-0.0136)	0.052 (0.0490-0.0560)	0.00538 (0.00496-0.00571)
Slope [b]	5.43(± 0.70)	2.7 (± 0.36)	8.6 (±0.23)	9.9 (± 1.53)	7.5 (± 0.92)
c**2**	6.95	4.17	8.86	0.411	0.53
SR [c]	19.7	0.59	3.0	2.7	3.9
ARAGUA LC_{50} [a]	0.00918 (0.00808-0.0108)	0.0886 (0.0789-0.0978)	0.0171 (0.00912-0.0232)	0.0522 (0.0454-0.0591)	0.00049 (0.00038-0.00064)
Slope [b]	4.6 (± 0.68)	6.0 (± 1.81)	4.3 (± 1.91)	3.2 (± 0.45)	1.6 (± 0.25)
c**2**	2.23	6.75	2.31	3.33	4.44
SR [c]	5.1	6.0	2.2	4.3	306.1
MIRANDA LC_{50} [a]	0.00524 (0.00476-.00563)	0.348 (0.319-0.375)	0.0131 (0.0123-0.0152)	0.0268 (0.0227-0.0310)	0.00378 (0.00311-0.00490)
Slope b	7.1(± 0.91)	6.6 (± 0.82)	6.8 (± 0.85)	3.8 (±0.32)	2.02 (± 0.26)
c**2**	0.68	0.27	3.40	3.08	1.76
SR [c]	10.7	0.29	2.4	6.3	10.2
ROCK LC_{50} a	0.0121 (0.0102-0.0161)	0.246 (0.21-0.27)	0.0134 (0.012-0.015)	0.0121 (0.011-0.014)	0.00401 (0.004-32-00.565)
Slope [b]	4.6 (± 0.98)	6.7 (± 0.93)	4.3 (± 0.45)	6.4 (± 0. 0.64)	4.4 (± 0.44)
c**2**	2.18	3.42	4.78	3.22	0.40
SR [c]	1.0	1.8	0.75	0.64	1.5

[a] LC_{50} in mg/liter with DEF; 95% CI in parenthesis; [b] Standard deviation (SD) are in parenthesis; [c]Synergism ratio (SR)= LC_{50} insecticide alone/ LC_{50} insecticide + synergist DEF. The linearity of the log dose-probit mortality response are well represented by a line (P> 0.05, and D. F=4). Number of larvae tested = 500 per insecticide.

Table 4. Toxicity of five organophosphates insecticides with the synergist PB to four strains of *Aedes aegypti.*

	Temephos	Malathion	Fenthion	P. methyl	Chlorpyrifos
S.DE CUBA LC50 a	0.0885 (0.0838-0.0943)	1.115 (1.00714-1.251)	0.0503 (0.0471-0.0534)	0.181 (0.157-0.205)	0.0861 (0.0786-0.0949
Slope [b]	7.9 (± 1.11)	3.8 (± 0.38)	7.6 (± 0.67)	4.6 (± 0.42)	4.2 (± 0.49)
c**2**	6.63	5.73	4.99	2.98	5.29
SR [c]	0.8	1.0	1.0	0.35	1.3
APURE LC50 a	0.0377 (0.0337-0.0416)	0.481 (0.448-0.514)	0.0210 (0.0189-0.0232)	0.0758 (0.0606-0.0839)	0.0296 (0.0262-0.0329
Slope [b]	3.9 (± 0.34)	6.5 (± 0.57)	5.2 (± 0.40)	4.9 (± 1.26)	3.9 (± 0.38)
c**2**	2.16	4.30	3.81	0.59	5.29
SR [c]	3.9	0.8	1.6	2.7	0.7
ARAGUA LC50 a	0.0420 (0.0379-0.0461)	0.193 (0.160-0.232)	0.0281 (0.0275-0.0391)	0.151 (0.138-0.167)	0.0457 (0.0402-0.0508
Slope [b]	5.4 (± 0.71)	3.9 (± 0.42)	4.2 (± 0.40)	5.4 (± 0.46)	3.9 (± 0.48)
c**2**	0.81	0.69	4.22	3.80	1.63
SR [c]	1.1	2.7	1.3	1.5	3.3
MIRANDA LC50 a	0.0423 (0.0373-0.0478)	0.296 (0.263-0.331)	0.0241 (0.0211-0.0265)	0.0779 (0.0716-0.0849)	0.0358 (0.0311-0.0406
Slope [b]	3.2 (± 0.32)	3.9 (± 0.46)	6.7 (± 0.91)	4.7 (± 0.63)	2.6 (± 0.24)
c**2**	3.59	2.26	2.71	2.68	6.41
SR [c]	1.3	0.35	1.3	2.2	1.1
ROCK LC50 a	0.0223 (0.0191-0.0252)	0.300 (0.271-0.333)	0.0101 (0.00902-0.0111)	0.0269 (0.024-0.029)	0.0156 (0.013-0.018)
Slope [b]	3.1 (± 0.24)	4.8 (± 0.51)	9.9 (± 0.22)	4.2 (± 0.36)	3.2 (± 0.27)
c**2**	5.30	3.80	1.74	3.69	1.61
SR [c]	0.6	1.5	0.98	0.29	0.43

[a] LC_{50} in mg/liter with PBO; 95% CI in parenthesis; b Standard deviation (SD) are in parenthesis; c Synergism ratio (SR)= LC50 insecticide alone/ LC50 insecticide + synergist PBO. The linearity of the log dose-probit mortality response are well represented by a line (P> 0.05, and D. F=4). Number of larvae tested = 500 per insecticide

Table 5. Frequency values of the esterases and GST mechanisms observed in *Aedes aegypti* strains from three Venezuela states and Santiago de Cuba.

Mechanisms	ROCKEFELLER (n)	APURE (n)	ARAGUA (n)	MIRANDA (n)	STGO. DE CUBA (n)
Esterase[a]	0 (1108)	1.0 (285)	0.42 (300)	0.831 (350)	1.0 (350)
GST[b]	0 (288)	0.041 (285)	0.45 (300)	0.043 (150)	0.80 (285)

a Esterase activity was measured as optical density.
b Glutathion s transferase was measured as especific activity
(n) The total of individual tested are in breakers

Fig1. Distribution patterns of esterase activity in the susceptible ROCKEFELLER and laboratory strain of *Aedes aegypti* MIRANDA.

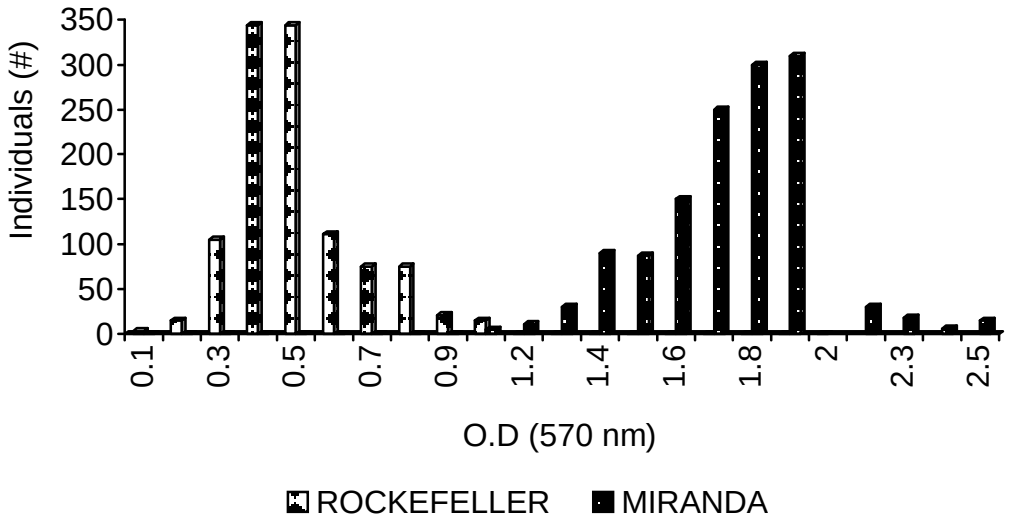

Fig. 2. Variation of the GST activity (D.O./min) of mosquito larval homogenates of Aedes aegypti in the presence of saturated concentrations of CDNB and GSH