ques, chemosterilants, negatively correlated compounds, biological control, genetic methods, hormone-like materials, photochemical substances, detergents, botanicals or synthetic botanicals, and oils. Each of these has factors limiting its general use—incomplete development, adverse effects on nontarget organisms, or extreme cost. Further research in these areas, however, may alter some of these conditions and make some of the above control methods more promising.

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RESISTANCE IN FLORIDA AND COUNTERMEASURES INVOLVING CHEMICALS

JAMES B. GAHAN, CARROLL N. SMITH AND B. MICHAEL GLANCEY

Entomology Research Division, Agr. Res. Serv., U.S.D.A., Gainesville, Florida

During the past 20 years a vast majority of the mosquito control projects in Florida have been directed against the two species most prevalent in salt marshes, *Aedes taeniorhynchus* (Wiedemann) and *Aedes sollicitans* (Walker). Although permanent control measures have been emphasized wherever practicable, large quantities of insecticides have been used to give inhabitants of heavily infested areas temporary relief while more permanent measures were being instituted.

DDT was used extensively between 1945 and 1949, with excellent results in most places. However, as early as 1947 and 1948, Bertholf (1950) and Cain (1950) were having difficulty obtaining good results in Broward and Brevard counties, and by the summer of 1949 other districts were reporting poor control with DDT. When Deonier and Gilbert (1950) compared the susceptibility of adults and larvae from treated and untreated areas, they found that resistance had developed both to DDT and TDE. Unfortunately the districts that had been the most conscientious in their efforts to kill mosquitoes were the ones with the worst problems. A direct relationship obviously existed between the percentage of mosquitoes killed and the rapidity with which resistance developed.

Good control was temporarily re-established by changing from DDT to benzene hexachloride. However, Keller and McDuffie (1952) soon found that larvae from the Cocoa Beach area were developing a tolerance for benzene hexachloride, and Keller and Chapman (1953) found that mosquitoes in the same area were becoming resistant to dieldrin. Nonetheless, benzene hexachloride continued to be the most widely used insecticide, and it produced satisfactory control in most places between 1950 and 1953–1954. Whenever resistance to benzene hexachloride became acute, dieldrin was substituted. By the summer of 1955, however, resistance had developed to such a degree in some of the intensely treated areas of Florida that satisfactory control could not be obtained with DDT, benzene hexachloride, or dieldrin (Beidler 1956, Bertholf 1956, Stutz 1956.)

By the spring of 1955, considerable uncertainty existed over a future course to follow in controlling salt-marsh mosquitoes with insecticides. Malathion was commercially available, and Gjullin and Peters (1956) had obtained satisfactory control of adults of *Aedes nigromaculis* Ludlow in California by applying aerial sprays of this insecticide at the rate of 0.5 pound per acre. However, there was some fear that the compound might be too toxic to use over inhabited areas. Because the salt-marsh mosquito problem was so acute in Broward County, J. H. Bertholf, director of the mosquito control district in that county, requested our laboratory to evaluate malathion in his area. In October 1955, Gahan *et al.* (1956) applied solutions of malathion plus cyclohexanone in fuel oil by airplane to areas heavily infested with adults of *Aedes taeniorhynchus* and *Aedes sollicitans* that were highly resistant to chlorinated hydrocarbon insecticides. Treatments at 0.5, 0.25, and 0.1 pounds of malathion per acre gave control of 95 to more than 99 percent. In 1956 Rogers *et al.* (1957) found that thermal aerosol formulations of malathion were highly effective against these two species when they were applied by ground equipment.

During the latter half of the 1950's and the early part of the 1960's malathion was the principal insecticide used to control salt-marsh mosquitoes in Florida. Rogers and Rathburn (1964) reported that in 1957 the Florida State Board of Health strongly recommended to the various mosquito control agencies in the State that they use organaphosphates only to control the adult mosquitoes. These control agencies were advised to conduct no large-scale campaigns with malathion in uninhabited areas. This restriction was suggested in order to maintain a large population of mosquitoes that had received no treatment which would breed with strains that had been sprayed with malathion. The only larvicides recommended were diesel oil and granular formulations containing paris green.

In 1956, Rogers and Rathburn (1964) obtained an average kill of 93 percent when they exposed DDT-resistant *Aedes taeniorhynchus* to a thermal aerosol containing 8 ounces of malathion per gallon of #2 fuel oil, applied at the rate of 40 gallons an hour from a vehicle moving at 5 mph. The same type of test was repeated in 1957, 1958, 1961, and 1963 with no appreciable reduction in kill or indication of resistance.

In 1956 and 1957 Davis *et al.* (1959) compared salt-marsh mosquito larvae collected in Florida with others collected as eggs from an area in Georgia where very little control work had been done. In the larvicide tests the Georgia strain was 8-10 times more susceptible to DDT, benzene hexachloride, and dieldrin than larvae collected in Florida, but there was no more than a 2-fold difference in susceptibility of the two strains to five organophosphorus insecticides. The Florida larvae were at least equally as susceptible to malathion as the Georgia larvae. In wind tunnel tests, adult mosquitoes from Florida were almost 8 times as resistant to DDT as those from Georgia but there was little difference in the susceptibility of the two strains to benzene hexachloride, and the adults from Florida were 2.4 times more susceptible to malathion than adults from Georgia.

It seems probable that the judicious use of malathion greatly prolonged the period of effectiveness of this insecticide in Florida, since Gjullin and Isaac (1957) reported that *Culex tarsalis* Coquillett developed resistance to malathion in California within 2½ years after it was first used on a large scale.

The first indications that salt-marsh mosquitoes in Florida might be developing resistance to malathion appeared during the summer of 1965. The Lee County mosquito abatement district had been using airplane applications of malathion successfully for several years. They found their treatments were not as effective in 1964 as they had been previously, and in 1965 the control obtained with this insecticide was poor. During the fall of 1965, laboratory tests were made by Glancey *et al.* (in press) to compare *Aedes taeniorhynchus* collected from this area with a strain of the same species that has been reared in the laboratory since 1958 without intentional exposure to insecticides. The results of these tests indicated that the strain from Lee County was definitely developing resistance to malathion: the quantity of insecticide required to produce similar kills in the wild strain was about 6 times higher at the LC_{50} and 14 times higher at the LC_{90} with larvae and 10 times higher at the LC_{50} and 13 times higher at the LC_{90} with adults than that required for the laboratory strains.

Our laboratory has begun a survey to determine the extent of resistance to malathion in Florida. Only six counties have been sampled to date so the present report is incomplete. Larvae from eggs of *Aedes taeniorhynchus* have been collected in the field from Brevard, Indian River, Dade, Pinellas, Broward, and St. Johns counties and brought to the laboratory. Some of these mosquitoes were used in larvicide tests, while others were reared to the adult stage and tested in a wind tunnel. Adults and larvae from the same laboratory colony of *Aedes taeniorhynchus* employed by Glancey *et al.* (in press) were used for comparison. Other similar studies were made on the same strains to determine whether cross resistance had also developed to some of the newer insecticides. Because of a shortage of mosquitoes, no larvicide tests were made with the strains collected in Broward County, and no adulticide tests were made on the strain from St. Johns County.

The larvicide tests were conducted by dissolving the chemicals in acetone and adding them to distilled water. The concentrations varied with the toxicity of the compounds, but with each larvicide at least four concentrations were used to produce a range in mortalities between 20 and 100 percent. The acetone solution of the insecticide was first introduced into 225 ml. of water in a 250-ml. beaker, then 25 larvae were counted into another 25 ml. of water, and the water containing the larvae was poured into the beakers containing the insecticide. Each concentration was tested against two batches of larvae, produced or collected on different days, and duplicate beakers of 25 larvae each were used in the tests with each batch, a total of 100 larvae. The mortality that occurred in 24 hours was recorded. The LC_{50} and LC_{90} were calculated from the mortalities obtained.

The wind-tunnel (adulticide) tests were conducted with insecticides dissolved in odorless kerosene. As in the larvicide studies, the concentrations varied with the insecticide. The wind tunnel consisted essentially of a cylindrical tube 4 inches in diameter through which a column of air was moved at 4 mph by a suction fan. A cage containing 25 female mosquitoes was placed in the center of the tube. One-fourth ml. of insecticide solution was atomized at a pressure of 1 psi into the mouth of the tunnel, and the mosquitoes were exposed to the spray momentarily as it was drawn through the cage. Duplicate tests were run at each concentration. After treatment, the mosquitoes were anesthetized with carbon dioxide, transferred to untreated screen holding cages, and held at a temperature of 29.5° C. and a relative humidity of about 70 percent. A cotton pad saturated with a 20 percent honey-water solution was placed on the top of each cage, and the mortality was recorded after 24 hours.

If we assume that the strain of *Aedes taeniorhynchus* reared in the laboratory was nonresistant, it is obvious from the results shown in Tables 1 and 2 that all the field strains showed considerable resistance to malathion. The tests with

TABLE 1.—Results of larvicide tests with malathion against *Aedes taeniorhynchus* from various sections of Florida and a laboratory colony.

Mosquito strain	Lethal concentration (in ppm.)	
	LC_{50}	LC_{90}
Brevard	0.634	17.4
Laboratory	.047	.102
Indian River	.17	.57
Laboratory	.053	.11
Pinellas	.308	1.845
Laboratory	.05	.109
Dade	.096	.2
Laboratory	.02	.04
Lee	.326	1.144
Laboratory	.055	.087
St. Johns	.30	1.3
Laboratory	.01	.03

TABLE 2.—Results of wind-tunnel tests with malathion against adults of *Aedes taeniorhynchus* from various sections of Florida and a laboratory colony.

Mosquito strain	Lethal concentration (in percent)	
	LC_{50}	LC_{90}
Brevard	0.23	1.32
Laboratory	.019	.036
Indian River	.059	.32
Laboratory	.013	.021
Pinellas	.082	.557
Laboratory	.03	.05
Dade	.096	.204
Laboratory	.02	.033
Broward	.06	.2
Laboratory	.02	.033
Lee	.09	.329
Laboratory	.009	.025

larvae show that the strains from Brevard and St. Johns counties have developed a high degree of resistance; these insects were more than 13 and 30 times as difficult to kill at the LC_{50} and about 170 and 40 times as difficult to kill at the LC_{90} as the laboratory-reared strain. The Lee and Pinellas strains were 13 to almost 17 times as resistant as the laboratory strain at the LC_{90}. The resistance of the Indian River and Dade strains were only 3-5 times higher than the laboratory strain at both the LC_{50} and LC_{90}.

The Brevard strain of adults was also one of the most resistant in the wind-tunnel tests. It was about 12 times as resistant at the LC_{50} and over 36 times as resistant at the LC_{90} as the laboratory strain. The Lee strain, collected from the area that first reported difficulty in obtaining control with malathion, was less resistent than the Brevard strain and probably no more resistant than the Indian River or Pinellas strains at the LC_{90}. The Dade and Broward strains were 3-6 times more difficult to kill than the laboratory strain and were the least resistant of the group.

The Florida State Board of Health (unpublished data obtained during the same period) also reported that resistance to malathion is developing in *Aedes taeniorhynchus*.

It can be argued that these field collected insects may have received some exposure to insecticides before being used, because they were tested without being reared to the F_1 generation. However, no chemicals were applied to these breeding areas during the month before the collections were made. Since none of the larvicides in current use remain effective more than a few days in the presence of organic matter, it seems impossible that any effective residual deposits were present.

Field tests will be needed to determine whether other insecticides must be immediately substituted for malathion in the areas in question. However, the results obtained should forewarn those engaged in mosquito control operations in Florida that a change is occurring in the mosquito population and that control procedures may soon have to be revised again.

Fortunately, in 1966, more good insecticides with low toxicity to warm-blooded animals are available than there were in 1955. As early as 1959 Davis *et al.* (1960) and Davis and Gahan (1961) found that fenthion and naled were highly effective when they were applied by airplane to areas that were naturally infested with adults of *Aedes taeniorhynchus.* Fuel oil solutions applied from a Stearman airplane at the rate of 0.025 pound of naled or 0.05 pound of fenthion per acre caused reductions of 99 percent in the adult population. Fenthion gave 87 percent control at 0.025 pound per acre. Fenthion also was much more toxic than naled and malathion against larvae of this species in laboratory tests. Shortly thereafter Rathburn and Rogers (1961; 1963) found that naled and fenthion were effective against *Aedes taeniorhynchus* when they were used as thermal aerosols.

Naled was first sold to mosquito control districts in Florida during the summer of 1962. It has been used extensively since that time. Some fenthion has been applied during the past year, but so far this compound has not received general acceptance.

To determine whether or not the strains resistant to malathion showed cross-resistance to fenthion and naled, larvicide tests were run against the Brevard, Indian River, Pinellas, and St. Johns strains, and wind-tunnel tests were conducted against adults of the Pinellas strain to compare their susceptibility with that of the laboratory colony. As shown in Tables 3 and 4 there was no cross-resistance to either of these compounds. There seems little doubt that fenthion and naled could substitute for malathion in these areas.

Application by thermal aerosol generators is one of the preferred methods of dispersing insecticides in Florida to control adult mosquitoes. Mount *et al.* (in press) recently compared one of the popular thermal aerosol generators with a nonthermal aerosol machine designed and developed by the U. S. Army Engineer Research and Development Laboratories, Fort Belvoir, Virginia (Morrill and Wesley, 1955 and Edmunds *et al.,* 1958), commonly called a "cold fogger." They found that both types of machines were about equally effective when malathion, fenthion, and naled were used as toxicants against caged adults of *Aedes taeniorhynchus.* They also found water equal to fuel oil as a diluent for insecticides dispersed as nonthermal aerosols. In other tests conducted against *Aedes taeniorhynchus* with the cold fogger by Mount *et al.* (in press), emulsions of fenthion, Bayer 41831 (*O,O*-dimethyl *O*-4-nitro-*m*-tolyl phosphorothioate), Baygon® (*o*-isopropoxyphenyl methyl carbamate), Dursban® (*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate) and Geigy GS-13005 (*O,O*-dimethyl phosphorodithio-

TABLE 3.—Results of larvicide tests with fenthion and naled against *Aedes taeniorhynchus* from various sections of Florida and a laboratory colony.

Mosquito strain	Lethal concentration (in ppm.)			
	Fenthion		Naled	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}
Brevard	0.012	0.02	0.078	0.153
Laboratory	.011	.015	.059	.123
Indian River	.005	.008	.066	.133
Laboratory	.005	.008	.075	.148
Pinellas	.006	.014	.098	.17
Laboratory	.005	.008	.097	.173
St. Johns	.006	.01	.10	.18
Laboratory	.005	.009	.09	.17

TABLE 4.—Results of wind-tunnel tests with fenthion and naled against adults of *Aedes taeniorhynchus* from Pinellas County, Florida and a laboratory colony.

Mosquito strain	Lethal concentration (in percent)			
	Fenthion		Naled	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}
Pinellas	0.007	0.014	0.004	0.008
Laboratory	.009	.013	.013	.023

ate *S*-ester with 4-(mercaptomethyl)-2-methoxy-Δ^2-1,3,4-thiadiazolin-5-one) were about equally effective.

Wind-tunnel tests conducted by Gahan and Davis (1964) showed that Baygon was slightly inferior to fenthion, about equal to naled, and superior to malathion against adults of the laboratory colony of *Aedes taeniorhynchus.* They also found that Bayer 41831 was slightly more toxic than malathion at the LC_{50} but less toxic at the LC_{90}. In another unpublished study, our laboratory found Shell SD–8211 (2-chloro-1-(2,5-dichlorophenyl) vinyl dimethyl phosphate) considerably more toxic than malathion and about equal to fenthion.

Tests have also been conducted in our laboratory with other materials that might be used as larvicides against *Aedes taeniorhynchus.* Fenthion, naled, malathion, DDT, and parathion were used as standards (Table 5). Dursban and Abate[(R)]

TABLE 5.—Results of larvicide tests with new compounds against *Aedes taeniorhynchus* reared in the laboratory.

Insecticide name or company designation	Lethal concentration (in ppm.) LC_{50}	LC_{90}
New Insecticides		
Dursban	0.00092	0.0012
Abate	.0015	.0025
Shell SD–7438	.004	.007
Bayer 41831	.018	.03
Shell SD–8211	.019	.031
Shell SD–8447	.036	.071
Standard Insecticides		
Parathion	.0032	.005
Fenthion	.0051	.0084
Malathion	.058	.092
Naled	.11	.2
DDT	.8	7.5

(*O,O*-dimethyl phosphorothioate *O,O*-diester with 4,4′-thiodiphenyl) were the outstanding materials; they show promise of being two of the most effective, if not the most effective, compounds ever tested by our laboratory against larvae of *Aedes taeniorhynchus.* Shell SD–7438 (*O,O*-dimethylphosphorodithioate *S,S*-diester with toluene-*alpha,alpha*-dithiol) was slightly inferior to parathion, the best of the standards, but was superior to all the other standards. Bayer 41831, Shell SD–8211, and Shell SD–8447 (2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate) were the least effective of the new materials, but they also should be considered promising larvicides because they were more toxic than malathion, naled, and DDT.

Of the new materials mentioned in this report, Abate, Baygon, Dursban, fenthion, and naled are commercially available. Based on the literature, on information received from manufacturers, or on the reports of the U. S. Food and Drug Administration, the oral LD_{50}s of these materials in mg./kg. of body weight in rats are at least 1,766 mg. for Abate, 95–104 mg. for Baygon, 135–163 mg. for Dursban, 215–245 mg. for fenthion and 430 mg. for naled. It appears that all should be safe enough to use. At the present time the three Shell compounds are not commercially available. However, Shell SD–8211 and Shell SD–8447 are extremely promising for field studies since they have oral LD_{50}s in rats of 3,680 and $>$2,500 mg./kg. of body weight, respectively, and appear to be both safe to warm-blooded animals and highly toxic to mosquitoes.

Little information is available on the resistance of other species of mosquitoes to insecticides. However, evidence collected by Evans *et al.* (1960) and Porter *et al.* (1961) showed that *Aedes aegypti* in the Miami area has developed some resistance to DDT. Abedi and Brown (1961) applied "selection pressure" with DDT to a strain of this species that was originally collected in Key West, Florida and found that the LC_{50} steadily increased. They concluded that larvae of *Aedes aegypti* from Key West, Florida are capable of developing high resistance to DDT. DDT has been used frequently to control larval *Aedes aegypti* in the southern part of Florida. Since a campaign to eradicate *Aedes aegypti* has recently been inaugurated in Florida, the problem of resistance to DDT assumes more importance than it has in the past.

Although resistance to insecticides continues to be a problem, we see no reason to be pessimistic about the future of chemicals in the control of mosquitoes. More than 50 years of research were required to find any toxicants that could be depended on to produce a high degree of control. Since statisticians tell us that scientific information is accumulating in a geometrical progression, it is not unreasonable to believe that a much shorter period of research will produce insecticides to which resistance cannot be developed.

SUMMARY. The two species of salt-marsh mosquitoes in Florida developed tolerance to the chlorinated hydrocarbons before 1955. At present, *Aedes taeniorhynchus* is becoming resistant to malathion also. However, the problem of finding suitable substitutes is not as acute as in 1955 because a larger number of effective materials are available that appear to be safe to use in inhabited areas. Commercially available insecticides that appear to be outstanding as both larvicides and adulticides are fenthion, naled, and Dursban® (*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate). Abate® (*O,O*-dimethyl phosphorothioate *O,O*-diester with 4,4′-thiodiphenyl) appears to be an excellent larvicide though not an outstanding adulticide, while Baygon® (*o*-isopropoxyphenyl methylcarbamate) is a highly effective adulticide but only a fair larvicide.

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SUMMARY OF SYMPOSIUM

A. W. A. BROWN

Department of Zoology, University of Western Ontario, London, Canada

It was rash to accept the chairman's invitation to summarize this symposium, for it has turned out to contain so many and diverse points. Fortunately, however, the order of the successive contributions makes sense, starting with the underlying biochemical and genetical causes of resistance, and then proceeding from the northeastern States where the problem is hesitantly developing, thence to Florida where organophosphorus-resistance is eventually being added to organochlorine resistance, and finally to California where every mosquito resistance problem that we can now think of has definitely developed.

Dr. Perry has shown us what research in the past 10 years has revealed about the biochemical mechanisms of resistance, and that a mosquito gets to be resistant essentially because it can break down the insecticide. With DDT, the detoxication mechanism removes hydrochloric acid from the molecule, and the enzyme that does it is thus a dehydrochlorinase. This dehydrochlorination has been found to occur in DDT-resistant culicines just as definitely as in resistant house flies. In anophelines the picture is not so clear, but probably Dr. Perry will agree that dehydrochlorination accounted for part of the DDT-resistance in the Turkish *Anopheles atroparvus* that he studied.

Attempts to counter this resistance in mosquitoes by adding to the DDT some dehydrochlorinase inhibitor, such as DMC or WARF-Antiresistant, proved effective at first but after a few generations of this treatment the mosquitoes developed a resistance to the DDT-synergist mixture. Substitution of DDT with compounds similar in molecular configuration but far less open to detoxication has proved more successful. Deutero-DDT, less detoxicable because the hydrogen in the center of the molecule is replaced by its isotope deuterium, is effective against DDT-resistant mosquitoes, and better than DDT against the susceptible ones. The compound CP-47412, containing cyclopropane instead of ethane as the central spine of the molecule, is perhaps even more successful. Nevertheless, the remedial insecticides for resistant strains are still usually