ARTICLES

CURRENT RESEARCH BY THE USDA OF POTENTIAL SIGNIFICANCE TO WORLD-WIDE MOSQUITO CONTROL [1]

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Our Insects Affecting Man and Animals Research Branch which is, of course, part of the United States Department of Agriculture, is, at the time of this presentation (Spring of 1967) involved in a program of research on mosquito biology and control that includes: (1) the development and evaluation of insecticides for adult and larval control, (2) the search for and evaluation of mosquito pathogens as control agents, (3) the development and evaluation of repellents and attractants, and (4) the development and evaluation of new approaches to mosquito control. At present, our research program is being conducted in laboratories at Gainesville, Florida; Corvallis, Oregon; and Lake Charles, Louisiana. At Gainesville, insecticidal control, repellents and attractants, biological control, and new approaches to control are being emphasized. At Corvallis, Oregon, effort is concentrated on insecticidal control and attractants. At Lake Charles, the emphasis is on biological and ecological studies and research on mosquito pathogens. The purpose of this paper is to highlight the current progress of the whole research program and to present specific examples of results that are of potential significance to world-wide mosquito control. The discussion is divided to reflect the four areas of research. Compounds tested in the research described are listed in Table 1; if no common name has been approved, the chemical name is given.

[1] Mention of a proprietary product does not necessarily imply endorsement by the U. S. Department of Agriculture.

DEVELOPMENT AND EVALUATION OF INSECTICIDES. New and potentially effective adulticides or larvicides are evaluated in laboratory screening tests and by small- and large-scale field testing.

LARVICIDES. At our Gainesville, Florida, laboratory an average of 100–150 new compounds are screened annually for toxicity to larvae of *Anopheles quadrimaculatus* Say. From one-third to one-half of these materials are rated Class IV (more than 50 percent mortality at 1.0 p.p.m.). However, only a small number are effective at lower concentrations and are evaluated against other species of mosquitoes. Data for seven such compounds and two standards are given in Table 2.

No new compounds were as toxic as Dursban or Abate; however, most of them gave 100 percent kill at 0.025 p.p.m. and will therefore have further evaluation.

Some examples of the comparative effectiveness of earlier better known experimental materials against *Aedes taeniorhynchus* (Wiedemann) are presented in Table 3.

The larvae used in these tests were taken from a laboratory colony that has not been exposed to insecticides for nine years but was originally highly resistant to DDT. A review of data obtained with this colony in 1950 shows that concentrations of 0.01 to 0.05 p.p.m. of DDT caused 100 percent kill of larval *Aedes taeniorhynchus* collected in the field. From the current LC_{50} and LC_{90} shown in Table 3 the colony larvae are still extremely resistant to DDT but not to the other insecticides. Thus, data such as those in Table 3 clearly demonstrate what materials could be used to

TABLE 1.—Common or chemical names of test compounds.

Chemical	Chemical Definition
Abate®	*o,o*-dimethyl phosphorothioate, *o,o*-diester with 4,4′-thiodiphenol
Bay 38799	*o*-cyclopentylphenyl methylcarbamate
Bay 39007	*o*-isopropoxyphenyl methylcarbamate
Bay 41831	*o,o*-dimethyl *o*-4-nitro-*m*-tolyl phosphorothioate
Bay 62863	2,3-dihydro-2-methyl-7-benzofuranyl methylcarbamate
Bay 77488	*o,o*-diethyl phosphorothioate, *o*-ester with phenylglyoxylonitrile oxime
Bay 78182	*o,o*-diethyl phosphorothioate, *o*-ester with (*o*-chlorophenyl)glyoxylonitrile oxime
Bromophos	
Chevron RE-5353	*m*-(1-methylbutyl)phenyl methylcarbamate
CIBA C-8874	*o*-(2,5-dichloro-4-iodophenyl) *o,o*-diethyl phosphorothioate
CIBA C-9491	*o*-(2,5-dichloro-4-iodophenyl) *o,o*-dimethyl phosphorothioate
DDT	
Dursban®	*o,o*-diethyl *o*-3,5,6-trichloro-2-pyridyl phosphorothioate
Dupont 1179	methyl N-[(methylcarbamoyl)oxy]thioacetimidate
Fenthion	
Geigy GS-13005	*o,o*-dimethyl phosphorodithioate *S*-ester with 4-(mercaptomethyl)-2-methoxy-Δ^2-1,3,4-thiadiazolin-5-one
General Chemical GC-9879	4-hydroxy-2-mercapto-butyrateσ-lactone, *S*-ester with *o,o*-diethyl phosphorodithioate
Hercules 9326	5-*tert*-butyl-2-chlorophenyl methylcarbamate
Hercules 9485	*o*-(allyloxy)phenyl methylcarbamate
Malathion	
Mobil MC-181	7-methylbenzo[*b*]thien-4-yl methylcarbamate
Mobil MC-A-600	benzo[b]thien-4-yl methylcarbamate
Montecatini L-561	ethyl mercaptophenylacetate *S*-ester with *o,o*-dimethyl phosphorodithioate
Naled	
Neopynamin®	2,2-dimethyl-3-(2-methylpropenyl)cyclopropanecarboxylic acid ester with N-(hydroxymethyl)-1-cyclohexene-1,2-dicarboximide
Parathion	
Schering 34615	*m*-cm-5-yl methylcarbamate
Shell SD-8211	2-chloro-1-(2,5-dichlorophenyl)vinyl dimethyl phosphate
Shell SD-7438	*S*-benzylidene *o,o*-dimethyl phosphorodithioate
Shell SD-8447	2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate
Shell SD-8967	1-(4-bromo-2-chlorophenyl)-2-chlorovinyl dimethyl phosphate
Stauffer R-7240	*o,o*-dimethyl phosphorodithioate, S-ester with 3-(mercaptomethyl)-2,4-thiazolidinedione
Union Carbide UC-21149	2-methyl-2-(methylthio)propionaldehyde *o*-(methylcarbomoyl)oxime
Union Carbide UC-8454	5,6,7,8-tetrahydro-1-naphthyl methylcarbamate

TABLE 2.—Toxicity of nine compounds to fourth instar of *Anopheles quadrimaculatus.*

Chemical	% kill in 24 hours at indicated concentrations (p.p.m.)					
	0.1	0.05	0.025	0.01	0.005	0.0025
Montecatini L-561	..	..	100	100	56	..
Bay 77488	..	100	100	84	38	..
Bay 78182	..	100	100	58	36	..
CIBA C-8874	..	100	100	2	..	..
CIBA C-9491	..	100	100	8	..	..
Abate *	..	..	100	100	74	..
Dursban *	..	..	100	100	100	92

* Typical results. Tests not run concurrently.

TABLE 3.—Toxicity of selected compounds to fourth instar *Aedes taeniorhynchus* from a laboratory colony (average of 2 replications of duplicate jars, 25 larvae per jar).

Chemical	LC-50 (p.p.m.)	LC-90 (p.p.m.)
Dursban	0.0011	0.0017
Abate	.0015	.0025
Parathion	.0032	.005
Shell SD-7438	.0039	.007
Fenthion (Standard)	.0051	.0084
Bay 41831	.018	.03
Shell SD-8211	.019	.031
Bromophos	.028	.078
Shell SD-8447	.036	.07
Malathion (Standard)	.058	.092
Naled (Standard)	.11	.2
DDT	.8	7.5

control mosquito larvae that are resistant to DDT and other chlorinated hydrocarbons. As a result, such materials as Abate, parathion, and fenthion are already in use, and unregistered materials such as Dursban are potential substitutes. This kind of information is of world-wide interest because it may be of help in maintaining effective control of mosquitoes in problem areas.

New larvicides that show promise in laboratory tests are usually evaluated against natural populations of several species of mosquito larvae. Unfortunately, sufficient quantities of only a few materials can be obtained for this purpose; indeed, at the moment, only Dursban is available in the quantity necessary for comparisons with Abate, the best of the registered larvicides. In field tests in Florida, the two materials were equally effective against species of *Aedes, Psorophora,* and *Culex*. Typical percentage kills with these compounds at various doses per acre in small-scale field tests were as follows:

	At rates of application of—		
	0.1 lb./acre	0.01 lb./acre	0.005 lb./acre
Abate	100%	100%	97%
Dursban	100%	99+%	96%

In Oregon, one of the most important sources of mosquitoes, primarily *Culex* sp., is log ponds. These ponds are normally so polluted that they provide an unusually severe test of larvicidal efficacy. Promising new larvicides are therefore thoroughly evaluated in them, especially with respect to residual effects. In initial comparative tests, Dursban at 0.025 and Abate at 0.035 and 0.05 pound per acre gave complete initial control of larvae, but the effect lasted only a short time. However, in subsequent tests Dursban at 0.035 pound per acre and Abate at 0.06 pound per acre prevented breeding for 15–22 days (average 17.5) and 9–15 days (average 13.2), respectively. Fenthion at 0.1 pound per acre was slightly less effective than Abate. The results obtained with these and other materials in the polluted log ponds suggest that these materials would be effective in such environments as sewage lagoons where *Culex* vectors of encephalitis, filariasis, and other diseases breed prolifically. This kind of information is of special interest in countries where efforts are underway to eradicate mosquito-borne diseases.

ADULTICIDES. New compounds (50–100 per year) that are highly effective in screening tests against human lice and mosquito larvae are evaluated as sprays for effectiveness against adult *Aedes taeniorhynchus* in a modified wind tunnel by the procedure described by Davis (1959) and Davis and Gahan (1961); malathion is the standard. Results obtained with a number of materials are shown in Table 4.

The most effective compounds were Bay 62863 and Union Carbide UC-21149 which had LC-90's of 0.008 and 0.009 percent, respectively. However, five other compounds were also effective and equal or superior in toxicity to malathion.

Materials that show promise as adulticides in laboratory tests are often field tested as thermal or nonthermal aerosols against caged adults of *Aedes taeniorhynchus.* Sometimes adults of *Aedes aegypti* (L.), *Culex pipiens quinquefasciatus* Say, and *Anopheles quadrimaculatus* are included. The materials are usually tested as emulsions at four concentrations

TABLE 4.—Toxicity of seven compounds against adult *Aedes taeniorhynchus* in contact spray tests (average of duplicate cages; 25 adults per cage).

Chemical	Estimated LC-50 (% conc.)	Estimated LC-90 (% conc.)
Bay 62863	0.004	0.008
Union Carbide UC-21149	0.004	0.009
Shell SD-8967	0.006	0.013
Schering 34615	0.0062	0.018
Montecatini L-561	0.01	0.027
Stauffer R-7240	0.02	0.03
Mobil MC-181	0.017	0.035
Malathion *	0.008	0.037

* Results represent means and extremes of LC-50's and LC-90's in seven tests.

by the procedures of Mount *et al.* (1966). Partial results of recent tests with nine materials are given in Table 5.

Four new materials—Bay 41831, Dursban, Schering 34615, and Shell SD-8211—were about equally effective and gave kills that were comparable to fenthion and naled, two of the three standards. All these materials were slightly superior to malathion, the third standard, and considerably more effective than Abate or Shell SD-8447.

Usually the final step in the evaluation of a new insecticide is aerial application against natural populations of mosquitoes. Also for the past several years, special attention has been given to the application of low volume sprays, i.e., 0.5 to 8.0 ounces per acre. Results of some of these tests have been published by Glancey *et al.* (1965) and Glancey *et al.* (1966). Table 6 reproduces the results reported in 1966.

Application of only 0.5 ounce per acre of naled alone gave 98.5 percent control after 6 hours. Combinations of 0.5 ounce per acre each of naled and malathion and of even 1.0 ounce per acre of naled and 2.0 ounces per acre of malathion were no more effective than 0.5 ounce per acre of naled alone at 6 hours but all were more effective after 24 hours. Fenthion at 0.5 ounce per acre and the combination of fenthion at 0.8 and Bay 39007 at 1.6 ounces per acre were somewhat less effective than naled alone and in combination with malathion.

Low volume aerial spraying is of world-

TABLE 5.—Effectiveness of nine insecticides applied as nonthermal aerosols in field tests against caged adults of three species of mosquitoes (average mortality at 150 and 300 feet; 2 to 6 replications of 25 adults each).

Chemical	% Conc.	Percentage kill of indicated species after 18 hours: *Aedes taeniorhynchus*	*Culex pipiens quinquefasciatus*	*Anopheles quadrimaculatus*
		New Materials		
Bay 41831	1	86	87	87
	4	98	100	100
Schering 34615	1	73	86	86
	4	100	93	100
Dursban	1	54	70	65
	4	96	100	100
Shell SD-8211	1	45	53	34
	4	96	98	84
Abate	4	74	88	49
Shell SD-8447	4	76	38	8
		Standards		
Fenthion	1	93	83	84
	4	100	99	99
Naled	1	80	54	68
	4	100	99	96
Malathion	1	47	32	51
	4	95	84	90

TABLE 6.—Control of adult salt-marsh mosquitoes with various insecticides applied as low volume sprays from a Stearman airplane equipped with Mini-Spin nozzles. (*A. taeniorhynchus* predominating; plane speed 80 m.p.h., 100-foot swaths; 50–75 feet altitude; average of three tests.)

Insecticide	Amount/acre		Pretreatment count (avg/man/min)	Percent reduction after—	
	Pounds:	Fl. oz.		6 hr.	24 hr.
Naled	0.05	0.5	47	98.5	32
Naled	.05	.5	26	95	55
Naled +	.05	.5	45	98.5	59
Malathion	.04	.5	..	..	..
Naled +	.05	.5	113	72	84
Malathion	.16	2.0	..	..	..
Naled +	.11	1.0	118	97	97
Malathion	.32	4.0	..	..	..
Fenthion	.016	.25	49	17	42
Fenthion	.03	.5	89	49	64
Fenthion +	.05	.8	139	83	93
Bay 39007	.02	1.6	..	..	..

wide interest because of its effectiveness and the economy of application. Also, many researchers have reported that low volume sprays of technical insecticides are effective longer than comparable doses applied in diluted sprays at 2–4 quarts per acre.

Perhaps the most important aspect of our research on mosquito control is the search for new residual insecticides for use in world-wide campaigns to eradicate malaria and other mosquito-borne diseases. Our Gainesville laboratory screens chemicals provided by chemical companies all over the world. The World Health Organization also provides selected compounds and provides financial support for and evaluation of their residual effectiveness.

Between 100–150 compounds a year are usually evaluated on plywood panels at 100 mg. per square foot for residual effectiveness against adults of *Anopheles quadrimaculatus.* The most effective materials tested the past year were Schering 34615, Dursban, Union Carbide UC-8454, DuPont 1179, Neopynamin, and Bay 62863; all lasted 24 or more weeks and all of which were equal to or better than the standards, malathion and DDT. A few of these materials may remain effective as long as a year. Since long-lasting materials are of special interest, all that could be obtained in sufficient amounts were evaluated against *Anopheles quadrimaculatus* in farm buildings in the rice-growing areas of Arkansas and Louisiana. The buildings consisted mostly of wooden animal barns and sheds. The results of recent field tests with nine promising materials in Louisiana are shown in Table 7.

All the new materials gave 95–100 percent control of adult mosquitoes for 16–19 weeks and were as effective as the standard, malathion. Some treatments probably would have continued to kill adults

TABLE 7.—Control of adult *Anopheles quadrimaculatus* in farm buildings treated with nine insecticides at a rate of 200 mg. per square foot (applied as emulsions (E) or wettable powders (WP).

Chemical and formulation	No. of weeks of 95–100 percent control in indicated buildings		
	Bldg. #1	Bldg. #2	Bldg. #3
Mobil MC-A-600 WP	17	19	..
Hercules 9326 EC	19	16	16
Hercules 9485 EC	19	17	16
Bay 38799 WP	..	16	16
Geigy GS-13005 EC	17	16	16
Chevron RE-5353 EC	17	17	16
General Chemical GC-9879 EC	17	17	..
Schering 34615 EC	19	16	16
Malathion EC (standard)	19	16	16

for additional weeks if populations had not become so small after harvest of the rice fields and the advent of cool fall weather that further evaluations were not possible. However, observations are usually resumed in the spring when adult populations reappear to determine what materials are still effective. Several years ago, treatments of Bay 39007 were effective over a year (through two seasons).

Outstanding materials such as Bay 39007 are of special interest to WHO and are usually subjected to additional practical tests in Africa and other countries where mosquito-borne diseases occur. For example, this year WHO and USDA cooperated in an extensive test of Bay 39007 on cheesecloth in Lagos and Kadana, Nigeria.

Early results in this test were exceptionally good, but final results are not yet available.

Also, in cooperative studies between military entomologists of the Department of Defense and our Gainesville laboratory, insecticides and repellents are evaluated in areas outside of the United States. During 1965, 13 new materials were evaluated in Thailand as larvicides and/or adulticides against *Aedes aegypti* and *Culex fatigans* Wiedemann in both laboratory and field tests (Lofgren *et al.* 1967) Abate, Dursban, fenthion, malathion, Shell SD-8211, and bromophos were effective as larvicides. Fenthion, Bay 41831 (Sumithion®), malathion, Bay 39007, Schering 34615, and naled were effective as thermal aerosols against adults.

Biological Control. Studies are underway at both our Lake Charles and Gainesville laboratories to identify pathogens of mosquitoes and to determine host-pathogen relationships. The goal is to develop methods of using these pathogens for mosquito control. Perhaps the clearest explanation of the status of our research on biological control of mosquitoes is given in a paper by Chapman *et al.* (1967). These researchers observed protozoa, bacteria, fungi, viruses, or nematodes in 27 of 43 species of mosquitoes collected in Louisiana. The most common pathogens were microsporidia (Protozoa: Nosematidae) and *Coelomomyces* (Blastocladiales: Coelomemycetaceae). The problem is now to elucidate these host-pathogens relationships to determine whether the pathogens can be used effectively in control. Progress in this area was reported at this meeting by Petersen *et al.* (1967) who studied a nematode that infects *Aedes sollicitans.*

Repellents and Attractants. Our program to develop repellents and attractants has been continued at Gainesville, Florida and Corvallis, Oregon. Of special interest is a report by Gouck *et al.* (1967) on preliminary studies designed to develop chemicals that are effective as space repellents. Of 242 materials tested, with netting having openings large enough to permit mosquitoes to pass through, five prevented mosquito penetration more than 100 days. Also, Gjullin *et al.* (1967) noted the presence of male pheromones in *Culex p. quinquefasciatus, C. tarsalis* Coquillett, and *C. pipiens pipiens* (L.), and Hazard *et al.* (1967) showed that bacteria isolated from hay infusions produced chemicals that stimulated gravid *Aedes aegypti* and *C. p. quinquefasciatus* to oviposit; attractions to these compounds were also demonstrated in an olfactometer.

New Approaches. Research has been continued on methods of sterilizing mosquitoes, either chemically or by gamma irradiation, and of determining whether the use of sterile insects is feasible for mosquito control. Also, the effects of chemosterilants on fertility, longevity, vigor, and mating competitiveness of *C. p. quinquefasciatus* have been emphasized in laboratory studies. Cycling populations of both *C. p. quinquefasciatus* and *Anopheles quadrimaculatus* have been established in large outdoor cages to study the release of sterile males as a method of control.

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SEASONAL ACTIVITY OF MOSQUITO PREDATORS IN WOODLAND POOLS IN ONTARIO

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INTRODUCTION. The present paper reports further on the insect predators of univoltine *Aedes* mosquitoes that breed in vernal, woodland pools near Belleville in Southern Ontario. The principal aim of this research was to obtain data on behavior and species succession of predaceous water beetles in both temporary and permanent waters. This information is basic to the development of effective biological and integrated control methods (Beirne, 1962).

METHODS. Three vernal (semi-permanent) pools were selected for study in 1964 in the Marsh Hill swamp at Chatterton. Pools 1 and 2 were situated 100 yards within the south side of the swamp; each was about 75 sq. yards in area in early April, soon after the spring thaw. The area of Pool 3, some 80 yds. to the west, was about 350 sq. yards. Water depths averaged 15 inches, with a maximum depth of 27 inches in Pool 3. Aquatic traps (James and Redner, 1965) were used to record the activity and numbers of the various predators; three traps were placed in each of Pools 1 and 2, and six in Pool 3. The traps were set in the pool in one of three positions: on the bottom (B), at the top, just below the surface (T), and in shallow water at the margin (M). All were examined and reset twice daily, 1 hour after sunrise and 1 hour before sunset, from April 13 to June 18 in the smaller pools, and until June 26 in Pool 3, and catches preserved in 80 percent alcohol. Water temperatures were taken with Taylor maximum-minimum thermometers at the margin and water depths checked at each visit to the trap site. Trap collections were also obtained from a permanent pond near Belleville from March 31 to August 25.

RESULTS AND DISCUSSION. The traps