PRELIMINARY OBSERVATIONS ON THE EFFECT OF ALCOHOL AND OIL FILMS ON THE OVIPOSITION OF *CULEX PIPIENS QUINQUEFASCIATUS* (SAY) [1]

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While testing various candidate compounds as attractants for *Culex pipiens quinquefasciatus* (Say), Fay and Perry noted that increasing numbers of adults were found dead in hemoglobin solutions that were progressively aged at room temperature in the laboratory prior to testing. Fresh solutions of hemoglobin did not exhibit this phenomenon, and it was thought that bacterial action on the components of the solutions may have altered either the surface tension or the wetting properties. The tests described in this paper were initiated to determine the effect of various surface active agents in preventing oviposition by *C. p. quinquefasciatus* through trapping of adults at the time they contact the water.

MATERIALS AND METHODS. The relative effectiveness of various surface active agents was determined by adding the test formulation to water, either as a fast-breaking emulsion or a surface film. Known dosages were applied to 1 liter of distilled water having an 8-inch diameter surface. Tests were made on both fresh preparations and preparations held for 1 week at 80° F. and 60 percent relative humidity to check limitations on residual action. Acetone-killed male and female adult mosquitoes in approximately equal numbers were dropped in groups of 100 on the test preparations. The species used were *C. p. quinquefasciatus, Aedes aegypti, Anopheles albimanus,* and *A. quadrimaculatus.* Since orienting tests showed that a portion of the surface film is removed with each mosquito that becomes submerged, evaluations were based on the number of freshly killed mosquitoes that submerged completely in 5 seconds.

Several alcohols, i.e., 1-Hexanol, 1-Octanol, 1-Decanol, and 1-Dodecanol, were tested as fast-breaking emulsions by adding 1 ml. of the test alcohol to a liter

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of water containing 1 ml. of a 1 percent aqueous solution of sodium lauryl sulfate, and then agitating. The spreading action of the sodium lauryl sulfate held a film of alcohol on the water in a matter of a few minutes.

Candidate oils, WSX 6979, 6980, 6981, 6983, 6984, 7031, 7034, and WSX 7035,[2] were tried with various surfactants as spreaders. Atlox 1087[3] with a hydrophile-lipophile balance of 9.2 was selected as a standard.

Following the initial screening tests, a given formulation was further evaluated over a period of several days using reared specimens of *C. p. quinquefasciatus*. An 18-hour test period from mid-afternoon until the following morning was used from Monday through Friday of weeks 2 and 3 of the adult life of the specimens. Four colonies of adults, each containing 600 males and 600 females, were used in each experiment. Four black plastic pint cups, each containing 100 ml. of distilled water with a surface area of 0.034 sq. ft., were used in each test colony. The water surface was treated in two of the cups. One cup was placed in each corner of the cage with the two treated containers located diagonally across from one another. The positions of the treated and control cups were rotated daily to eliminate any bias due to position. For each test in each colony, the combined counts of egg rafts and dead males and females in the treated containers and in the check containers were tabulated. Two criteria of evaluations in each overnight colony test were used: (1) the percent of the total egg rafts that were deposited on the treated cups, and (2) the percent of the dead adults found in the treated cups only, as opposed to the total dead adults found in all the cups.

RESULTS. The alcohols applied alone to water failed to spread and did not wet the mosquitoes. Sodium lauryl sulfate, when applied alone at 0.01 percent, was likewise ineffective. A combination of both components—0.1 to 0.001 percent alcohol with 0.001 percent sodium lauryl sulfate—is necessary to produce effective results (Table 1). Tests showing complete exclusion of egg rafts at 0.1 percent alcohol were further tested at a dilution of 0.01 percent, and in the case of 1-Dodecanol at 0.001 percent concentration. Above 1-Dodecanol the alcohols are solid at room temperature and below 1-Hexanol the alcohols are too soluble in water to form effective films.

TABLE 1.—Effect of alcohols on deposition of egg rafts and mortality of *C. quinquefasciatus* adults. Values (averages of four replications) represent percentages of totals in all containers.

Alcohol[1]		Percent alcohol concentration		
		0.1	0.01	0.001
1-Hexanol	Rafts	11	..	..
	Males	2	..	..
	Females	12	..	..
1-Octanol	Rafts	0	7	..
	Males	32	11	..
	Females	39	8	..
1-Decanol	Rafts	0	10	..
	Males	83	94	..
	Females	80	88	..
1-Dodecanol	Rafts	0	0	25
	Males	98	76	23
	Females	100	72	13

[1] All alcohol concentrations contained 0.001 percent sodium lauryl sulfate.

On the basis of the numbers of egg rafts laid in both the alcohol-treated containers and the plain distilled water containers, as well as the number of dead adults found in the containers, the colony tests (Table 1) show that only the heavier alcohols are effective at reduced concentrations. In these colony tests, failure was indicated by the presence of egg rafts in the alcohol-treated containers.

The results using films of long-chain alcohols, especially 1-Dodecanol, appear promising. 1-Dodecanol, used at a con-

[2] Furnished through the courtesy of Esso Research and Engineering Company, Baytown, Texas.

[3] Use of trade names is for identification purposes only and does not constitute endorsement by the Public Health Service or U. S. Department of Health, Education, and Welfare.

centration of 0.01 percent with 0.001 percent sodium lauryl sulfate, was effective for at least 5 days and other spreaders may increase its efficiency even further.

In the screening tests using oils alone, only WSX 6980 and 6981 spread over the surface of the water, while WSX 6979 and 6981 were the only oils to wet the mosquitoes without the aid of a surfactant. Thus the addition of Atlox 1087 had a twofold purpose: to spread the oil over the surface of the water and to facilitate the wetting of the mosquito. WSX 7031 and 7035 were not tested extensively since they failed to wet the mosquitoes at most of the concentrations used and had no residual effectiveness. Results of adding Atlox 1087 to the other oils (Table 2) show that the greatest

TABLE 2.—Numbers of freshly-killed adult mosquitoes submerged in 5 seconds by fresh preparations (0.35 sq. ft.) of oils and Atlox 1087 or by 7-day-old films (in parentheses).

Oil Gal./A.	% Concentration of Atlox 1087			
	0.5	1.0	2.0	5.0
WSX 6979	2500	1500	1900	2500
5	(0)	(0)	(200)	(0)
10	2500	2000	3300	4000
	(0)	(0)	(1600)	(650)
WSX 6980	700	1250	1300	2200
5	(250)	(0)	(750)	(2200)
10	1400	1700	2500	4000
	(0)	(0)	(3000)	(2000)
WSX 6981	3500	5000	6500	4000
5	(100)	(4200)	(3000)	(2000)
10	4500	4000	5000	4000
	(5000)	(4600)	(2500)	(3700)
WSX 6983	1200	2500	3600	1200
5	(0)	(0)	(0)	(1500)
WSX 6984	1600	3000	2500	3500
5	(900)	(900)	(700)	(1550)
10	2900	3000	4000	5700
	(1100)	(2000)	(1200)	(5100)
WSX 7034	900	2100	3800	3250
5	(300)	(1100)	(500)	(2500)
10	6000	4000	2900	3500
	(2700)	(3250)	(2000)	(5500)

number of mosquitoes sank in WSX 6981, 6984, and 7034, all of which had good residual effectiveness at 1 week after application.

In colony tests (Table 3) with the more promising formulations, WSX 6981 at 5 gal./A. successfully prevented egg laying in the treated containers for 3 days and trapped a total of 245 males and 132 females. These adults represented 8 percent of all mosquitoes in the four test colonies. The addition of 2 or 5 percent Atlox 1087 to WSX 6981 did not improve its effectiveness. WSX 6984 applied at 5 gal./A. with various combinations of Atlox 1087 was also effective for 3 days. WSX 7034 applied as above was effective for only 1 day.

It was subsequently found that when dead mosquitoes were removed from the containers, a portion of the oil film came away with each one, which resulted in a depletion of the oil. For example, WSX 7034 applied at 20 gal./A. with 1 percent Atlox 1087 lasted for 2 days when the dead mosquitoes were removed from the containers, whereas the same film lasted for 10 days when the mosquitoes were left in the cups.

Promising results were obtained when a combination of equal parts of WSX 6981 and 6984 was used at 10 gal./A. with 5 percent Atlox 1087. This oil film was effective for 7 days even though the dead mosquitoes were removed daily. It killed 672 males and 278 females, or 20 percent of the adults in the four test colonies.

In these colony tests, the numbers of dead females in the treated containers were closely comparable to the numbers of egg rafts laid in the distilled water checks. This observation is interpreted to mean that the oil films were not exerting any repellent effect on ovipositing females.

These preliminary results show that application of certain oil films on water surfaces in the laboratory does not repel adult mosquitoes, but prevents oviposition by trapping the females. Males are also taken when they alight on the treated surfaces, presumably to obtain water. The proportion of a total population which is affected on any given day is under study.

In these laboratory experiments the oils

TABLE 3.—Daily percent of egg rafts and of dead adults found in treated containers as opposed to those in all containers.

Oil Formulation (5 gal./acre)		Age of film (days)					
		1	2	3	4	5–7	8
WSX 6981	Rafts	0	0	0	10	..	..
	Males	66	97	77	93	..	..
	Females	73	100	71	100	..	.
WSX 6981	Rafts	0	0	0	3	..	..
2% Atlox 1087	Males	98	100	94	97	..	..
	Females	98	98	76	59	..	..
WSX 6981	Rafts	0	1	0	18	..	..
5% Atlox 1087	Males	99	99	100	97	..	..
	Females	92	92	77	35	..	..
WSX 6984	Rafts	0	0	0	2	..	..
5% Atlox 1087	Males	99	100	100	100	..	..
	Females	100	97	100	100	..	..
WSX 7034	Rafts	0	5	56	..	..	..
5% Atlox 1087	Males	100	94	50	..	..	..
	Females	96	97	56	..	..	..
WSX 6981:6984 (1:1)	Rafts	0	0	0	0	..	..
5% Atlox 1087	Males	99	98	97	95	..	..
	Females	100	100	92	100	..	..
WSX 6981:6984* (1:1)	Rafts	0	0	0	0	0	1
5% Atlox 1087	Males	98	96	98	98	94	92
	Females	99	95	94	96	83	75

* Applied at 10 gal./A.

were used primarily to interrupt the life cycle of *C. p. quinquefasciatus* at oviposition. They proved successful in interrupting egg laying and killed the gravid female, preventing her from laying any more egg rafts. These particular oils have the additional effect of killing any larvae or pupae already present in the water. If an egg raft has already been deposited when the oil is applied it will not sink and the eggs will hatch; however, once the larvae have emerged they will die. Thus, with a single application, all stages of the life cycle are affected. If these oils prove to have sufficient longevity in field trials, they may prove useful in filariasis control by reducing *Culex* populations in indigenous areas to such a level that effective transmission of the microfilariae might be interrupted.

These studies are being continued through limited field evaluations in water containing various amounts of pollution.

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