MONOLAYER FILMS AS MOSQUITO CONTROL AGENTS AND THEIR EFFECTS ON NONTARGET ORGANISMS[1]

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ABSTRACT. Arosurf® [polyoxyethylene (2) isostearyl ether (2 mole ethoxylate of isostearyl alcohol)] forms a persistent monolayer film on the surface of water and is believed to alter the surface tension markedly. Film produced by this material is highly detrimental to mosquito pupae and larvae, the former being as much as 60-fold more susceptible than the latter at a LC_{90} dosage level.

Under field conditions, this film-forming substance yielded high level (90%+) control of larvae and pupae of *Culex tarsalis* at the rate of 0.5 to 0.75 gal/acre, with no apparent effect on nontarget organisms such as mayfly naiads, diving beetle adults, ostracods and copepods. Nonanoic and oleic acids, film-forming substances and proven mosquito oviposition repellents, enhanced activity of Arosurf slightly and yielded improved formulation. It is possible that the combination of these two film-forming substances might prolong the efficacy of treatments under field conditions.

INTRODUCTION

The appearance of acquired resistance in larvae of some important mosquitoes to most conventional larvicides stimulated a search for finding alternative methods of mosquito control. The use of monomolecular organic surface films to alter water surface tension and thus to disrupt behavior and normal development of immature mosquitoes, has been investigated in recent years. The role of surface tension of water affecting immature stages of mosquitoes has been studied by several workers. Russell and Rao (1941), Lorenzen and Meinke (1968), Mulla and Chaudhury (1968), Garrett and White (1977), White and Garrett (1977), and White et al. (1978) reported changes in surface tension of water by the addition of surfactants and monomolecular organic surface films which produced substantial mortality in larvae and pupae of mosquitoes, or resulted in the drowning of newly emerging adults on treated water surface. Large numbers of film-forming surface active agents were evaluated as mosquito larvicides (Mulla 1967a, 1967b) and some of these were found to offer potential for practical mosquito control programs.

One of the surface active agents, isostearyl alcohol, containing 2 oxyethylene groups (Arosurf®, ISA-20E), a nonionic surface active agent forming a monomolecular organic surface film on water, was reported to possess good activity against natural populations of *Anopheles quadrimaculatus* Say (White and Garrett 1977),

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[1] These studies were conducted in cooperation of Northwest and Coachella Valley Mosquito Abatement Districts.

Aedes taeniorhynchus Wiedemann, *Culex nigripalpus* Theobald and *Cx. quinquefasciatus* Say in salt marsh and sewage water at rates of 0.3–0.5 ml/m^2 (Levy et al. 1980, 1981a, 1981b). Mortality due to presence of persistent film of this material is attributed to the lowering of surface tension of water to about 29 dynes/cm, causing suffocation by water entering the trachial system (Garrett and White 1977).

The current studies were initiated to determine: 1) activity and longevity of this promising material against *Cx. quinquefasciatus* in the laboratory and against *Cx. tarsalis* Coquillett and *Cx. peus* Speiser in field ponds, and in dairy wash lagoons, and 2) to study its effect on some nontarget organisms present in ponds during the study period. Additionally, we explored the possibility of increasing activity and longevity of the monomolecular organic surface film and improving sprayability by the addition of either oleic or nonanoic acid (also film-forming substances), which in recent studies were found to act as oviposition repellents of *Culex* and *Aedes* mosquitoes (Hwang et al. 1981, Schultz et al. 1982). It was postulated that joint action of both materials might produce better results at lower rates, and prolong effectiveness of a treatment by the elimination of existing larval populations, and at the same time preventing rapid reinfestation due to oviposition repellency of nonanoic and oleic acids.

METHODS AND MATERIALS

LABORATORY EVALUATION. One percent (1%) stock solutions (w/v) were prepared either in acetone or ethyl alcohol (95%), using ISA-20E [polyoxyethylene (2) isostearyl ether] commercially known as Arosurf 66-E2 (provided by Sherex Chemical Co., Dublin, OH), or nonanoic acid, Arosurf with nonanoic acid (1:1). Serial dilutions of each were prepared as needed in the same solvent used in stock solution. Required amount of the diluted solution (0.2–2.0 ml) was added to 10-oz foam bowls No. 102 (Dixie Marathon Products, American Can Company, Greenwich, CT 06830) containing 20 4th-instar larvae or 10 pupae of *Cx. quinquefasciatus* in 200 ml of tap water. Water surface area in the bowls measured 10 × 10 cm and water depth measured 2.7 cm.

Arosurf and nonanoic acid were tested separately and in (1:1) combination against 4th-instar larvae and pupae. Each material was tested 2–3 times at 4 different concentrations, utilizing 3 replicates per rate. Along with each test, 3 bowls were treated with each of the solvents used in the stock solution, and 3 bowls were left untreated as check. Treated larvae and the checks were fed brewer's yeast—lab chow mix (1:3) immediately after treatment, and mortality readings were taken daily for the first 5 days, and every 3–4 days thereafter, until all test organisms either died or reached viable adult stage. Dead larvae, pupae and adults were counted and removed and the bowls containing surviving individuals were kept in a controlled temperature room (25.5°C) during the duration of the experiment.

FIELD EVALUATION. Arosurf 66-E2, nonanoic acid, and Arosurf plus nonanoic acid (1:1) were evaluated in experimental ponds at the Aquatic and Vector Control Research Facilities of the University of California at Riverside, and in the Coachella Valley of southern California. These facilities were described by Mulla et al. (1982). In brief, water surface in the ponds at Riverside measures 3.7 × 7.3 m, and is kept free of vegetation. Water loss due to evaporation and percolation is somewhat high (6–8 liters/min), and water pH measures about 8.2. Ponds in the Coachella Valley measure 6 × 6 m, are vegetated, water loss is minimal (1 liter/min), and water pH is about 9.4. At both locations, water depth was maintained constant (30 cm) by the use of float valves.

In one test Arosurf alone was applied at 0.46, 0.69 and 0.93 ml/m^2 (0.5, 0.75 and 1.0 gal/acre) while in another test, Arosurf-nonanoic acid combination (1:1) was applied at total rate of 0.23 and 0.46 ml/m^2 (0.25 and 0.5 gal/acre). Both of these materials were also applied separately at the rate of 0.23 ml/m^2 (0.25 gal/acre).

In order to achieve good coverage, the required amount for each rate was mixed with (95%) ethyl alcohol (100 ml total spray/pond), and applied with a 1-liter all-purpose household sprayer. Aqueous spray was difficult to apply due to the insolubility of Arosurf in water, which tends to form gel, plugging nozzle of the sprayer. In each test, 3 ponds were used per application rate, and 6 ponds were used as check. Three of the check ponds were left untreated, while 100 ml/pond of ethyl alcohol (95%) was applied to the other 3 check ponds. Prior to treatment, and 2, 7, 14 and 21 days after treatment, 5 dips per pond were taken. The 5 dips were combined into one sample, preserved with (95%) ethyl alcohol, and organisms present were counted and identified in the laboratory under a dissecting microscope. Arosurf 66-E2 alone or in (1:1) combination with oleic acid were also tested in dairy wash lagoons at the rates of 0.93, 1.86, 2.79 and 3.72 ml/m^2 (1, 2, 3 and 4 gal/acre). Tests were conducted in the western part of Riverside County in 4 lagoons which are known to harbor heavy populations of *Cx. peus*. These lagoons were vegetated along the perimeter, and contained floating scum and vegetation which moved around the lagoons according to wind direction. Water in these lagoons is used for irrigation; therefore, water depth fluctuated from 1–3 meters. Surface area and location of each of the lagoons utilized in these studies are included in the appropriate table.

The required amount of the material for each test was applied with a 4 liter stainless steel hand sprayer, provided with a straight stream nozzle size 0001 in 2 swaths. The first swath was applied along the sides, while the second swath was directed toward the middle, thus covering the entire surface area. In all field tests percent reduction was calculated by comparing mean number of larvae and pupae in posttreatment counts with those in the pretreatment. Results were statistically analyzed with a Compucorp 145 E computer, and methods of statistical analysis used are included in the tables.

RESULTS AND DISCUSSION

Laboratory evaluation. The monomolecular organic surface film formed by Arosurf exhibited a high level of activity against 4th-instar larvae and pupae of *Cx. quinquefasciatus* in the laboratory (Table 1). Pupae had greater susceptibility (60-fold) to this material than the 4th-instar larvae at the LC$_{90}$ level. The LC$_{90}$ against pupae was in the range of 0.0008 ul/cm^2 (0.4 mg/liter), compared to 0.05 μl/cm^2 (25 mg/liter) against the larvae. Nonanoic acid alone did not show any noticeable activity against both pupae and larvae at the rate of 0.08 μl/cm^2 (40 mg/liter). The combination of Arosurf and nonanoic acid did not show any synergistic activity against either larvae or pupae, and Arosurf alone, was 2- and 12-fold more effective against larvae and pupae respectively than the two materials combined (Table 1). However, combination of the 2 materials displayed an increase in the activity of Arosurf under field conditions (Tables 2 and 3). From these laboratory studies it is apparent that Arosurf has lower activity against *Cx. quinquefasciatus* as compared with *An. quadrimaculatus* (White and Garrett 1977). Our findings are more or less in agreement with those of Levy et al. (1980, 1981a, 1981b) in evaluating this material against *Aedes* and *Culex* species. It appears that *Anopheles* larvae are highly susceptible to the films of isostearyl alcohol with 2 mole ethoxylates.

Table 2. Efficacy of monomolecular organic surface film (Arosurf 66-E2) on *Culex tarsalis* in experimental ponds.

Rate		Larvae/dip	Mean (%) reduction posttreatment (days)[c]		
gal/acre	ml/m^2	Pretreatment	2	7	14[d]
Test A—University of California, Riverside, October 1981[a]					
0.50	0.46	34 a	86 b	96 b	91 a
0.75	0.69	46 a	84 b	94 b	72 a
1.00	0.93	40 a	95 b	98 b	87 a
Check	—	25 a	0 a	6 a	3 b
Test B—Coachella Valley, October 1981[b]					
0.50	0.46	16 a	80 b	80 b	61 b
0.75	0.69	19 a	94 b	99 b	93 c
1.00	0.93	12 a	90 b	98 b	93 c
Check	0	9 a	0 a	0 a	0 a

[a] Water temperature range 13–23°C, mean minimum 13.8°, mean maximum 22.7°C.

[b] Water temperature range 13–25.5°C, mean minimum 15.3°, mean maximum 23.8°C.

[c] Mean (%) mortality followed by the same letter(s) in a column are not significantly different from one another, using Duncan's multiple range test ($P = 0.05$). Statistical analysis for each experiment was conducted separately.

[d] Reduction based on 2 replicates on day 14 of test A. All others based on 3 replicates.

Mortality in 4th-instar larvae treated with Arosurf and the combination of Arosurf and nonanoic acid at the rates of 2 and 4 μl/cm^2 (20 and 40 mg/liter) began to occur 2–4 days after

Table 1. Activity of Arosurf 66-E2 and nonanoic acid against 4th-instar larvae and pupae of *Culex quinquefasciatus* in the laboratory.

Formulation	LC$_{50}$ and LC$_{90}$[a] (ul/cm^2)	LC$_{50}$ and LC$_{90}$[a] (mg/l)	Correlation coefficient	Slope
4th-instar larvae				
Nonanoic acid	>0.080– —	>40.0– —	—	—
Arosurf®	0.02–0.05	10.8–24.6	0.95	3.58
Arosurf-nonanoic (1:1)	0.04–0.11	18.1–52.7	0.87	2.76
Pupae				
Nonanoic acid	>0.08– —	>40.0– —	—	—
Arosurf	0.0004–0.0008	0.20–0.40	0.93	4.35
Arosurf-nonanoic (1:1)	0.003–0.012	1.52–6.00	0.85	2.14

[a] Values were obtained through log probit regression analysis by using Compucorp 145 E computer.

Table 3. Joint action of Arosurf 66-E2 and nonanoic acid against *Culex tarsalis* in experimental ponds. University of California at Riverside, November 1981.[a]

Rate (gal/acre)		Larvae/dip	Mean (%) reduction post-treatment (days)[b]	
Arosurf	Nonanoic	Pretreatment	2	7
0.125	0.125	29	69 a	50 a
0.25	0.25	38	94 a	79 a
0.25	0.00	10	70 a	62 a
0.00	0.25	41	26 b	18 b
Check	(ethyl alcohol)	13	31 b	0 b
Check	—	19	9 b	33 b

[a] Water temperature range 13–23°C, mean minimum 13.8°, mean maximum 22.7°C.

[b] Mean (%) mortality followed by the same letter(s) in a column not significantly different from one another, using Duncan's multiple range test ($P = 0.05$).

treatment, prior to or upon pupation. Dead pupae from larvae treated with Arosurf were observed to be deformed and albino (Fig. 1), and emerging adults from surviving pupae mostly drowned on the water surface. Treated pupae were affected 2 days after treatment, and mortality occurred in the pupal stage or they died as partly eclosed adults. Adults from treated pupae were observed to be partially emerged out of the pupal skin and dying on water surface (Fig. 2). From these findings, Arosurf, due to its effects on water surface tension (Garrett and White 1977), appears to interfere with the normal development of larvae, pupae, and emergence of adult mosquitoes. It shows higher activity against pupae than larvae, as 90% mortality occurred in pupae at the rate of 0.0008 μl/cm^2 (0.4 mg/liter), while 0.05 μl/cm^2 (24.6 mg/liter) was required to achieve the same results in the larval stage. The combination of Arosurf and nonanoic acid in laboratory tests caused 90% mortality in the larval stage at the high concentration of 0.11 μl/cm^2 (52.7 mg/liter), while only 0.012 μl/cm^2 (6 mg/liter) was required to obtain similar results against pupae.

FIELD EVALUATION. Arosurf 66-E2 produced excellent control of *Cx. tarsalis* larvae in experimental ponds at the Aquatic and Vector Control Research Facilities at the University of California, Riverside, and the Coachella Valley

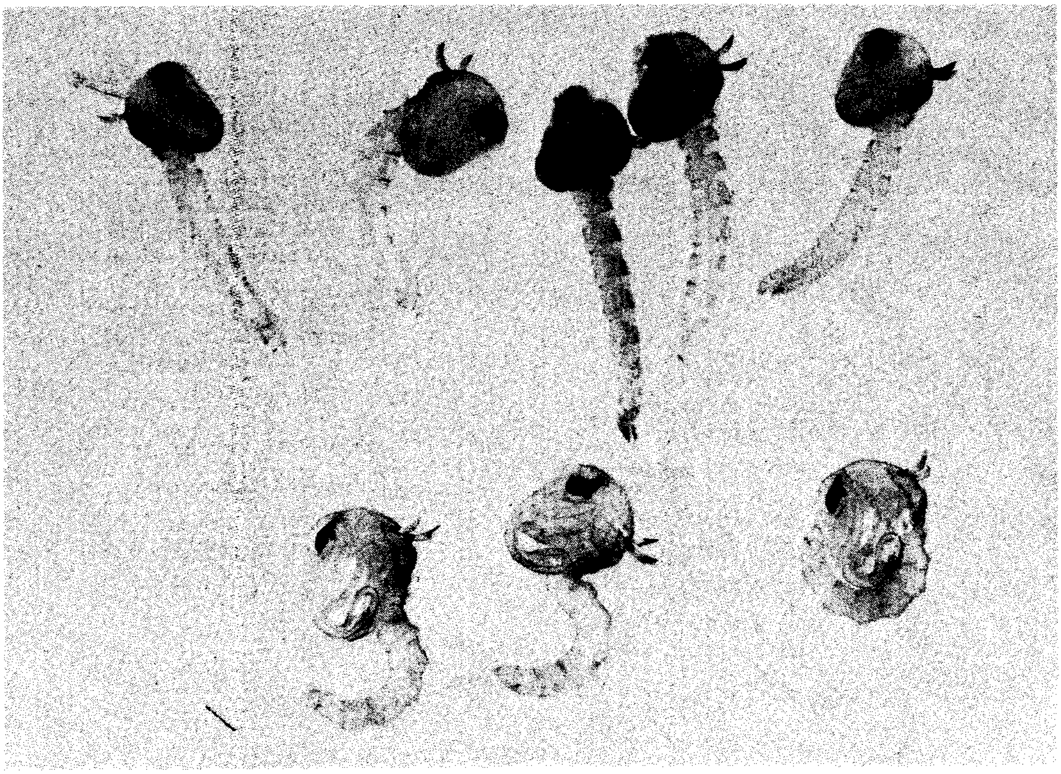

Fig. 1. Morphogenetic aberrations in pupae of *Culex quinquefasciatus* treated in the larval stage with Arosurf 66-E2, forming monomolecular film on water surface. Top row treated, bottom row untreated normal pupae.

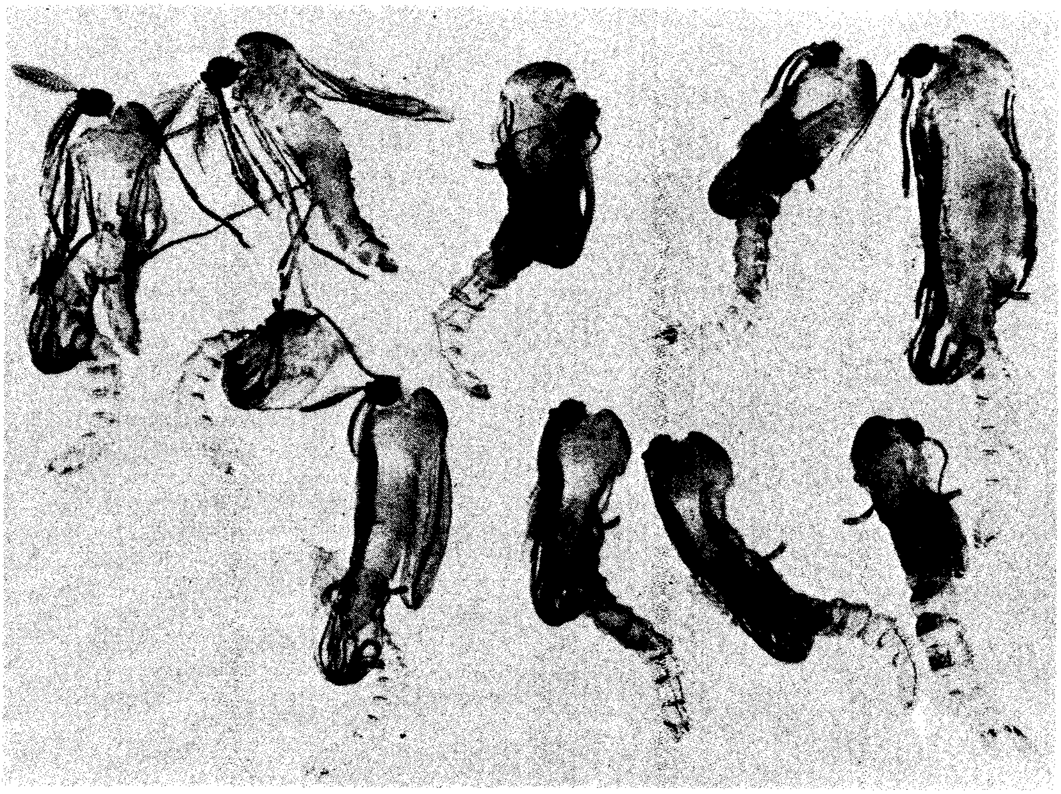

Fig. 2. Incomplete eclosion and drowning of adult mosquitoes *(Culex quinquefasciatus)* emerging from pupae treated with Arosurf 66-E2, a film-forming substance.

of southern California (Table 2). At the rates of 0.46, 0.69 and 0.93 ml/m^2 (0.5, 0.75 and 1.0 gal/acre), essentially similar results were obtained at both locations 2, 7, and 14 days after treatment. Mosquito larval populations began to recover slightly 2 wk after treatment, but still excellent control prevailed for more than 2 wk at the two higher rates of 0.69 and 0.93 ml/m^2. Reduction in the larval population at both locations began 2 days post-treatment, and reached a peak 1 wk after treatment. In the ponds, at Riverside, 96, 94, and 98% control was obtained at 0.46, 0.69 and 0.93 ml/m^2, while the same rates produced 80, 99, and 98% control in the Coachella Valley, 7 days after treatment (Table 2), high level of control prevailing for 2 wk at the two higher rates in the Coachella Valley ponds. These results are generally in agreement with those of Levy et al. (1981a) against *Ae. taeniorhynchus,* although control of larvae in their studies was poor in some of the tests.

Arosurf and nonanoic acid combined (1:1) produced 94% control at the combined rate of 0.5 gal/acre 2 days after treatment, which is higher than that obtained with Arosurf alone at one-half the combined rate. At the rate of 0.25 gal/acre, nonanoic acid caused only 26% reduction, while the same rate of Arosurf produced 70% (Table 3). Nonanoic acid appears to enhance the activity of Arosurf when applied in combination (1:1) under field conditions at the rate of 0.5 gal/acre, which consist of 0.25 gal/acre of each material. At this low rate, no satisfactory control was possible when either material was applied alone.

At all rates applied (0.5, 0.75 and 1.0 gal/acre), Arosurf 66-E2 showed no drastic adverse effects on the nontarget organisms, and their numbers remained rather stable in the ponds during these studies. Nontarget biota assessed include mayfly naiads *Callibaetis pacificus* (Seeman), beetle adults *Berosus metalliceps* Sharp (Dytiscidae) and copepods (Table 4). Dead adult chironomid midges and mosquitoes (drowned) were observed in large numbers on water surface in the treated ponds. The adult mosquitoes and midges either drowned upon emergence or during oviposition and visitation. Additional studies are warranted to determine the effect of Arosurf on larvae and emergence of adult chironomid midges.

Studies on efficacy of Arosurf in dairy la-

Table 4. Effect of monomolecular organic surface film Arosurf 66-E2 on nontarget organisms in experimental ponds. (Coachella Valley, October 1981)[a]

Pre- and Posttreatment (days)	Mean no. of nontarget organisms/ 5 dips at indicated rates (gal/acre)			Untreated (check)
	0.5	0.75	1.0	
	Mayfly naiads			
Pretreatment	5	3	2	3
2	11	13	12	3
7	7	19	7	5
14	18	30	9	15
21	21	61	20	25
	Diving beetle adults			
Pretreatment	5	2	3	7
2	10	8	12	11
7	3	3	3	4
14	15	5	7	5
21	6	4	2	4
	Ostracods			
Pretreatment	10	18	7	36
2	34	66	8	50
7	58	37	43	55
14	102	104	143	45
21	200	234	512	64

[a] Water temperature as in Table 2. Copepods were not effected at all rates applied.

goons was carried out in several ponds. At the rate of 1 gal/acre (8.96 kg/ha), this material produced excellent control of *Cx. peus* larvae in Hettinga and DeGraff dairy wash lagoons (Table 5). Hettinga lagoon was smaller in size (0.25 acre), and water was pumped and used for irrigation more frequently. Satisfactory control was achieved for a period of 2 wk only with Arosurf alone. However, in the larger lagoon (DeGraff), which measured 2 acres, and water level remained relatively constant during the experiment, mosquito larvae were eliminated for 21 days, and began to appear 28 days after treatment. The combination of Arosurf 66-E2 and oleic acid (1:1) also yielded good results in Hettinga dairy lagoon at the combined rate of 1 gal of the mixture/acre. The combination showed an increase in the activity and longevity of the materials under septic conditions, which could be attributed to the oviposition repellency of oleic acid. To substantiate these findings, additional tests were conducted with another lot of Arosurf 66-E2, but these tests failed to produce satisfactory results when applied at 2, and 4 gal of the mixture/acre (Table 5).

As shown in Table 5, the material used in the first 3 tests was obtained directly from the manufacturer (Sherex Chem. Co.) and was stored under room temperature (22±1°C) in the laboratory at the University of California, Riverside, remained stable and uniform in color at all times. The new shipment which was obtained from Northwest Mosquito Abatement District, Riverside, and used in the last 5 tests, was stored in a chemical shed, where temperature was in excess of 38°C during the summer, was found to be separated into two layers, and turbid in color. The material was mixed thoroughly prior to testing, but, even then failed to produce satisfactory results, similar to those obtained with the stable material in the first 3 tests. There are a number of uncontrollable factors in the breeding sources which can lead to variation in the level of control obtained. Inactivation of the material does not seem to be the sole factor for variation in the level of efficacy.

From the foregoing field studies, it is clear that monomolecular surface films produced by

Table 5. Joint action of the monomolecular film (Arosurf 66-E2), and oleic acid against *Culex peus* in dairy wash lagoons. (Riverside County, 1982).

Rate (gal/acre)		Location	Pretreatment larvae/dip	Percent reduction after treatment (days)				
Arosurf	Oleic			3	7	14	21	28
		Shipment[a] to University of California, Riverside						
1.0	0	Hettinga	10	97	95	78	0	0
1.0	0	DeGraff	18	96	100	100	100	80
0.5	0.5	Hettinga	15	93	89	93	99	93
		Shipment[b] to Northwest Mosquito Abatement District						
2.0	0	Vandemaer	143	88	57	0	—	—
3.0	0	Vandemaer	61	84	70	0	—	—
1.0	1.0	Echevaria	73	85	78	40	0	—
4.0	0	Vandemaer	24	4	0	—	—	—
2.0	2.0	Echevaria	44	0	0	—	—	—

[a] Material (lot 255K) stored at 22°C.

[b] Material (lot 255K) stored at 38°C.

Arosurf 66-E2 alone or in combination with nonanoic or oleic acid can result in excellent control of mosquito larvae and pupae in fresh and septic open water as well as in water with slight vegetation. Addition of acids to Arosurf not only enhances its activity and longevity, but the combination is also desirable because it yields a good formulation that can be easily applied with standard available equipment. Arosurf alone when diluted with water becomes jellified and clogs spray equipment, making it extremely difficult to make operational treatments in a short period of time.

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