CONTROL OF *CULEX* SPP. MOSQUITOES IN SEWAGE TREATMENT SYSTEMS OF SOUTHWESTERN FLORIDA WITH MONOMOLECULAR ORGANIC SURFACE FILMS

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ABSTRACT. The efficacy of 2 types of monomolecular organic surface films were evaluated at varying dosages against natural populations of *Culex nigripalpus* Theobald and *Cx. quinquefasciatus* Say in settling, polishing, and evapo-percolation ponds of an industrial sewage treatment plant. Results of spray application at a dosage as low as 0.33 ml/m^2 water surface (0.35 gal/acre) indicted that one of the films was significantly more effective than the other formulations in controlling larvae and pupae of the *Culex* spp.

Monomolecular organic surface films can modify the physical properties of water surfaces in ways which interfere with the normal activities and development of mosquito larvae, pupae, and emerging adults. These films can significantly reduce the surface tension of a mosquito habitat and subsequently kill larvae and pupae by inhibiting proper orientation at the air-water interface and/or by increasing the wetting of tracheal structures. Monomolecular films are biodegradable and have been shown to have no adverse affects on mammals (Reynolds, personal communication) and several species of vertebrate and invertebrate aquatic organisms (White and Garrett 1977). Therefore, these materials are expected not to insult the environment or pose a health hazard to man.

Although this approach to mosquito control has been shown to have practical application, significant data have not been generated to indicate the field efficacy of monomolecular surface films against a wide variety of mosquito species and stages of development. Garrett and White (1977) developed criteria for selection of film-forming organic chemicals with optimum properties for practical field effectiveness. In both laboratory and field studies several film-forming materials provided essentially 100% control of 4th instar larvae of *Anopheles quadrimaculatus* Say at a surface concentration of 0.04 ml/m^2 (White and Garrett 1977). At the same surface concentration, none of the films was effective in controlling larvae of *Aedes taeniorhynchus* Wiedemann. However, 3 of the surface films caused 100% cumulative mortalities to pupae and emerging adults of this species (Garrett 1976). White et al. (1978) reported 90% and greater control of 4th instar larvae of *An. quadrimaculatus* in laboratory studies using additional surface films selected on the basis of the criteria established by Garrett and White (1977).

The control of mosquitoes in polluted water environments is a major concern to mosquito control districts of southwestern Florida. Sewage settling, aeration, and decomposition ponds have been constructed as an alternative to sewers for many schools, shopping centers, trailer parks, and industrial complexes. These ponds contain sewage effluent in various stages of processing and subsequently provide the rich nutrient base for breeding of extremely large population of *Culex quinquefasciatus* Say and *Cx. nigripalpus* Theobald throughout the year. These species are a serious nuisance and are also potential vectors of St. Louis encephalitis in southwestern Florida. Based on the aforementioned studies a program was developed to evaluate surface-film control of *Culex* mosquitoes in sewage treatment habitats of Lee County, Florida.

METHODS AND MATERIALS

Isostearyl alcohol containing 2 oxyethylene groups (ISA-2OE) and 2

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sorbitan monooleate-base formulations were investigated as mosquito-controlling, film forming agents in an active sewage treatment plant servicing a pharmaceutical manufacturing complex. Sorbitan monooleate (SMO) was formulated with either 2-ethyl butanol (2EB) or 2-propanol (2P) (isopropyl alcohol) at the rate of 75% SMO and 25% of a particular solvent (SMO 75/2EB and SMO 75/2P). These materials are liquids, less dense than water and spread spontaneously and rapidly on a water surface lowering the surface tension to 29 dynes/cm or lower.

These nonionic surface-active films spread into uniform, nearly monomolecular layers over the water, and thus can not be seen because they are too thin to absorb light or cause iridescence due to reflective interference. However, it was necessary to determine the presence of a film and insure that it existed at a sufficiently high film pressure (surface tension reduction) to be physically effective against larvae, pupae, and emerging adults. To accomplish this, a refined grade of oleyl alcohol (9-octadecen-1-ol, cis isomer) was used as a spreading oil to indicate the completeness of coverage by the mosquito control film.

In all tests, a few drops of the indicator (oleyl alcohol) were applied with a pipette around the perimeter of the pond 24 and/or 48 hr post-treatment to indicate control-film depletion. The dosages of ISA-2OE and SMO formulations utilized in the tests were expected to persist on the surface as a "lens" of excess material and release film to replace that degraded by natural processes, thereby re-establishing film pressure and uniform coverage.

Five settling, polishing and/or evapo-percolation ponds (Fig. 1) of an industrial sewage treatment plant containing sewage and industrial effluent and high populations of all immature stages of *Cx. nigripalpus* and lesser populations of *Cx. quinquefasciatus* were treated with several

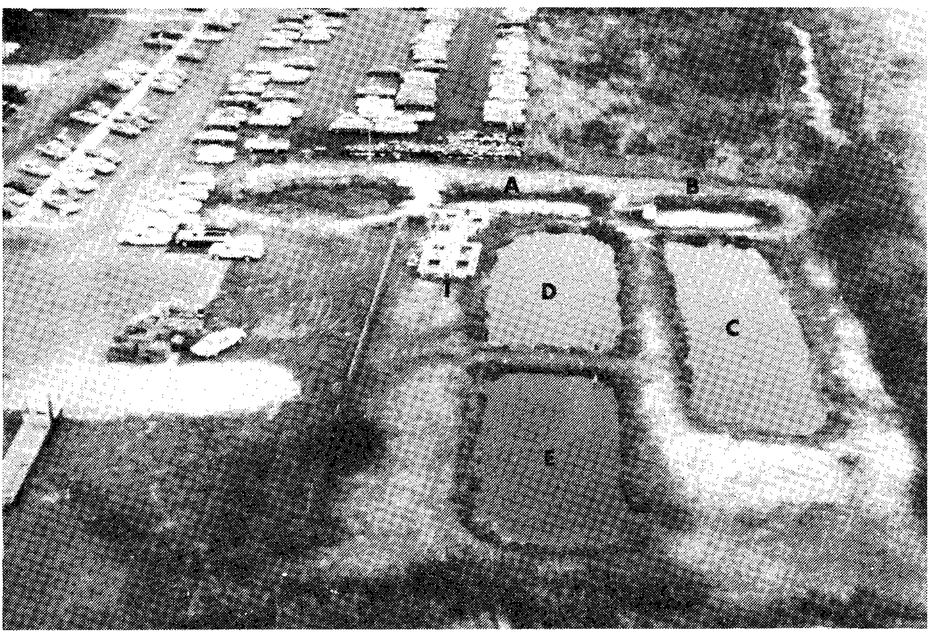

Fig. 1. Industrial sewage treatment system used to evaluate the mosquito control effectiveness of monomolecular surface films (1 = main pumping station; A and B = sewage settling ponds; C = polishing pond; D and E = evapo-percolation ponds).

concentrations of the monomolecular surface films. The ponds were designated A (ca. 160 m^2 water surface) , B (ca. 150 m^2), C (ca. 540 m^2), and D and E (ca. 404 m^2). The amount of organic scums and floating debris, surface water agitation and level of water fluctuated daily in each pond depending on the pumping and draining sequences, wind velocity and rainfall. Settling ponds A and B always contained a significantly greater concentration of floating mats of organic debris and scum as well as surface agitation due to pumping than was observed in polishing pond C and evapo-percolation ponds D and E.

Sixteen test series were conducted at the sewage treatment plant using ISA-2OE and two SMO formulations. Each material was dispensed around the vegetative perimeter of a pond with a hand-activated pump sprayer (651 ml capacity) adjusted to deliver a pin-stream spray pattern. The number of ponds sprayed in a test depended on the concentration of immature mosquitoes present at the time of sampling.

Larvae and pupae were sampled from the grassy perimeter of each test pond ca. 1 hr before treatment by taking 6–11 dips with a pint dipper. For example, extrapolations from the number of larvae and pupae collected per dip in several tests from the grassy 0.61 m area bordering pond C indicated that an average of 4,575,145 (2,544,093–8,443,372) immatures were present before treatment. Similar mosquito populations were also present in ponds D and E. For the most part, larvae and pupae were highly concentrated between mats of floating scum and debris throughout ponds A and B. Larval and pupal populations of 2,631,472 were estimated to be present in pond A before treatment in 1 test. Similar numbers were observed in settling pond B.

Percentage reduction of larvae according to instar and pupae was determined 24 and/or 48 hr post-treatment in tests 1–6 and was used as the criterion to evaluate the effectiveness of the monomolecular films. In tests 7–16 the evaluation was based on percent reduction in total mosquito biomass (i.e. the number of grams (± 0.05) of total larvae (1st-4th instar) and pupae sampled in 6–16 dips at 24 and/or 48 hr post-treatment due to the extremely high concentrations of immatures present in all the sewage ponds at the time of spraying. In tests 7–16, larvae and pupae were usually pooled, washed and cleaned of debris, blotted with absorbent paper towels to remove excess water and allowed to air-dry at 22–24 C (ambient) in wax-coated containers for ca. 24 hr before being weighed.

In several tests using a weight reduction criterion larvae and pupae were separated with a series of sieves, grouped by instar and pupae, weighed, and related to total reduction in mosquito biomass. Data were evaluated (analyses of variance) according to pond, film type and dosage (ml film/m^2 of water surface). Tests were conducted at wind velocities ranging from 3.2–48.3 kmph and in water having temperatures ranging from 16–30 C.

RESULTS AND DISCUSSION

Data indicating the effect of varying dosages of SMO 75/2EB and ISA-2OE on larvae and pupae of *Cx. nigripalpus* and *Cx. quinquefasciatus* in sewage polishing and settling ponds are presented in Table 1. Initial tests were mainly performed in polishing pond C because of consistently high mosquito populations. Spray application in pond C at surface dosages of 0.55 and 0.71 ml SMO 75/2EB per m^2 resulted in 84.8 and 96.9% control of all larvae and pupae within 24 hr respectively. However, when the dosage of SMO 75/2EB was reduced to 0.44 ml/m^2 little or no control was obtained. In these tests SMO 75/2EB was detected in certain areas of pond C at high film pressure at 24 hr post-treatment at surface dosages of 0.55 and 0.71 ml/m^2 but was not detected at the lowest dosage. This suggested a relationship between dosage and film

. Percent reduction of *Culex* spp. larvae and pupae 24 and 48 hours after spray application of SMO-75/2EB and ISA-2OE in sewage settling and polishing ponds.

Pre-treatment mosquito samples: No. larvae by instar and pupae (P); Total (T)						Post-treatment 24 hr: No. larvae by instar and pupae						24 hr: % reduction of larvae by instar and pupae						48 hr: No. larvae by instar and pupae						48 hr: % reduction of larvae by instar and pupae					
1	*2*	*3*	*4*	*P*	*T*	*1*	*2*	*3*	*4*	*P*	*T*	*1*	*2*	*3*	*4*	*P*	*T*	*1*	*2*	*3*	*4*	*P*	*T*	*1*	*2*	*3*	*4*	*P*	*T*
14	382	1484	347	108	2335	6	28	8	31	0	73	57.1	92.7	99.5	91.1	100	96.9	—	—	—	—	—	—	—	—	—	—	—	—
269	1147	362	2547	3305	7630	27	279	183	280	394	1163	90.0	75.7	49.4	89.0	88.1	84.8	—	—	—	—	—	—	—	—	—	—	—	—
144	748	1338	2559	410	5199	123	1421	1438	2056	506	5544	14.6	0*	0*	19.7	0*	0*	—	—	—	—	—	—	—	—	—	—	—	—
54	694	841	408	302	2299	4	20	35	52	18	129	92.6	97.1	95.8	87.3	94.0	94.4	12	5	0	4	2	23	77.8	99.3	100	99.0	99.3	99.0
45	2412	1313	743	62	4575	76	57	183	138	0	454	0*	97.6	86.1	81.4	100	90.1	36	65	9	20	2	132	20.0	97.3	99.3	97.3	96.8	97.1
47	564	363	135	691	1800	0	132	89	267	82	570	100	76.6	75.5	0*	88.1	68.3	10	55	65	260	55	445	78.7	90.2	82.1	0*	92.0	75.3

ond designation.
/2EB (Test 1S–3 respectively).
. (Tests 4–6 respectively).
over pre-treatment sample.

persistence as well as the significance of prolonged surface tension reduction to larval and pupal mortality. Fourth instar larvae of *Cx. quinquefasciatus* appeared moribund ca. 24 hr post-exposure to 0.5 ml/m² of SMO 75/2EB in bioassays conducted in 400 ml beakers (Levy et al. unpublished). However, these larvae revived and pupated when transferred to clean water. The laboratory observations, and mortality and film persistence data from the field trials, indicated that at the lowest dosage (0.44 ml/m²) the natural rate of biodegradation of this material was too rapid to produce larval and pupal mortality.

Tests in pond C with ISA-2OE (Table 1) at 0.44 ml/m² resulted in 90.1–94.4% mortality of larvae and pupae at 24 hr post-treatment and 97.1–99.0% mortality of immatures at 48 hr. In general, preliminary results in pond C with ISA-2OE indicated that this monomolecular surface film would persist in the habitat for 48 hr at high film pressure and subsequently control larval and pupal populations of *Cx. nigripalpus* and *Cx. quinquefasciatus*.

An additional test was performed with ISA-2OE in sewage settling pond A at a surface dosage of 0.56 ml/m² (Table 1). In this test, 68.3 and 75.3% mortality of all larvae and pupae resulted 24 and 48 hr post-treatment. Data indicated that total percent reduction of immatures in pond A was ca. 25% less than was observed in pond C. This significant reduction in mortality was attributed to the pocketing of larvae and pupae in the dense mats of floating debris and scum and in densely vegetative areas projecting from the perimeter where the mosquito control film could not penetrate. These extreme surface-disrupting factors were not observed in pond C. Nevertheless, ISA-2OE was detected at 48 hr post-treatment and caused substantial mortality of *Culex* larvae and pupae in an abnormally severe film-stressing environment. ISA-2OE was not detected in pond C or pond A 72 hr after application.

In all tests (Table 1) conducted at surface dosages of 0.55 and 0.71 ml SMO 75/2EB per m² and 0.44 ml ISA-20E per m² high concentrations of moribund and dead pupae were recovered 2–4 hr post-application. However, only a few dead larvae (mainly 4th instar) were observed during this period. High concentrations of dead larvae of all instars and pupae were found in pond C 48 hr post-treatment. In addition, numerous partially and fully emerged adults were observed floating dead on the surface 24 and 48 hr after spray application. Similar findings were also recorded during the test in pond A.

In general, these findings agreed with data from bioassays with ISA-2OE and SMO 75/2EB at 0.5 ml/m² against pupae and 4th instar larvae of *Cx. nigripalpus* and *Cx. quinquefasciatus*; i.e., pupae died at a significantly faster rate than was observed for larvae (Levy, et al. unpublished). In addition, the surface orientation and hatching sequence of egg rafts of *Cx. quinquefasciatus* were not adversely affected by a 24 hr exposure to ISA-2OE or SMO 75/2EB at a surface dosage of 0.5 ml/m² (Levy et al. unpublished). When compared to controls, no significant difference in the number of 1st instar larvae recovered per egg raft was noted. Therefore, film-induced effects on egg rafts of *Cx. quinquefasciatus* exposed to monomolecular films in beakers was not evident. In addition, the numerous *Culex* eggs observed before and after application of film in some tests probably matured and hatched to 1st instar larvae at various intervals throughout an experiment. Consequently, the continuous hatching of eggs could account for the erratic percent control of 1st instar larvae that was observed in several tests (Table 1). However, pond tests did indicate that 1st instar larvae were highly susceptible to the films. For the most part, data from field trials (Table 1) indicated that pupae appeared to be more sensitive to the films than larvae at 24 hr post-treatment; however, this was not as evident when results were evaluated at 48 hr post-treatment.

Also intar susceptibility to the films at 24 and 48 hr post-treatment followed no consistent trend (Table 1).

Additional tests (Table 2) with the monomolecular films were conducted in pond C and pond A as well as settling pond B and evapo-percolation ponds D and E. These evaluations (Table 2) were based on the percent reduction in total mosquito biomass since pre-treatment population estimates of the immatures of *Cx. nigripalpus* and *Cx. quinquefasciatus* were extremely high, and subsequent post-treatment reduction in mosquito biomass could be easily observed. For example, the number of larvae and pupae sampled before treatment in several tests in pond C indicated that 15,391 g (8,963–20,464 g) of total mosquito tissue were present in 0.61 m grassy band around the perimeter.

Spray application of ISA-2OE in pond A at 0.56 ml/m^2, in pond C at 0.44 and 0.33 ml/m^2, and in ponds D and E at 0.44 ml/m^2 resulted in 89.6–98.2% and 96.5–>99.6% mortality of all *Culex* spp. larvae and pupae at 24 and 48 hr post-treatment, respectively (Table 2). At the lowest dosage evaluated for ISA-2OE (0.33 ml/m^2) (Tables 2 and 3), high mortality and film persistence was observed at 24 and 48 hr post-treatment. Furthermore, no significant difference in the susceptibility of immature stages to 0.33 and 0.44 ml/m^2 ISA-2OE was indicated (Table 3). Field observations concerning the post-treatment sensitivity of larvae and pupae were consistent with observations in the initial tests. It should be noted that a significantly higher percent mortality was achieved in the second test in pond A (Table 2) when compared to data from the initial test (Table 1). The increased mortality was attributed to a more careful application of the film.

SMO 75/2EB (Table 2) was also evaluated on the basis of biomass reduction at dosages of 0.56, 0.59, and 0.44 ml/m^2 in the sewage settling, polishing, and evapo-percolation ponds. In addition, 2 field tests were conducted with SMO 75/2P (Table 2) since laboratory tests indicated that this formulation was significantly more effective against larvae and pupae of *Cx. quinquefasciatus* (Levy, et al. unpublished). Test results with 0.56 and 0.59 ml SMO 75/2EB per m^2 in ponds A and B (Table 2), respectively, were erratic, producing mortality at 24 hr post-treatment ranging from 7.8–86.8%. However, 85.9 and 96.0% mortality at 48 hr post-treatment was achieved in ponds A and B, respectively, in another test. This fluctuation was attributed to the physical nature of these test ponds, i.e. the thick surface scum and debris, and periodic pumping and draining, as well as to the pocketing of larvae and pupae in the densely grassed and scummed areas that were not sprayed. However, SMO 75/2EB was detected in these tests in some areas of ponds A and B 24 and/or 48 hr post-treatment. These findings agreed with film persistence data obtained in the original tests at the same dosages.

Two evaluations in pond C with 0.44 ml SMO 75/2P (Table 2) per m^2 resulted in 35.2 and 56.3% reduction of all larvae and pupae 24 hr post-treatment. Data indicated that a 35–56% increase in mortality of all immatures was achieved with this formulation when compared to the initial tests with SMO 75/2EB at 0.44 ml/m^2 in pond C (Tables 1 and 2). Mortality was significantly enhanced when SMO was formulated with 2-propanol instead of 2-ethyl butanol in bioassays (Levy, et al. unpublished) and could indicate a solvent-related synergism with this compound. However, SMO 75/2P was not detected in pond C 24 hr post-treatment. This rapid degradation was also observed to occur with SMO 75/2EB and was assumed to be a major factor contributing to the low percent mortality when compared to data from tests with ISA-2OE at the same dosage.

ISA-2OE and SMO materials were not usually detected via the oleyl alcohol indicator on all sides of a sewage pond 24 and/or 48 hr post-treatment even though film was initially sprayed around the entire perimeter. Failure to detect material was attributed to wind velocity and direc-

Table 2. Percent reduction of *Culex* spp. larvae and pupae 24 and 48 hours after spray application of monomolecular surface films in sewage settling, polishing and evapo-percolation ponds.

				Post-treatment			
				24 hr		48 hr	
Sewage pond	Film type	Film dosage (ml/m^2)	Pre-treatment Total mosquito biomass (g)	Total mosquito biomass (g)	% reduction	Total mosquito biomass (g)	% reduction
A	SMO–75/2EB	0.56	3.8	0.5	86.8	—	—
A	ISA–20E	0.56	31.5	3.1	90.2	1.1	96.5
A	SMO–75/2EB	0.56	7.8	—	—	1.1	85.9
B	SMO–75/2EB	0.59	37.4	—	—	1.5	96.0
A	SMO–75/2EB	0.56	6.4	5.9	7.8	—	—
B	SMO–75/2EB	0.59	7.6	6.1	19.7	—	—
C	SMO–75/2P	0.44	43.8	28.4	35.2	—	—
C	SMO–75/2P	0.44	28.4	12.4	56.3	—	—
D	ISA–20E	0.44	31.6	3.3	89.6	—	—
C	ISA–20E	0.44	27.7	0.5	98.2	<0.1	>99.6
D	ISA–20E	0.44	22.6	1.7	92.5	<0.1	>99.6
E	ISA–20E	0.44	24.8	2.6	89.5	<0.1	>99.6
C	ISA–20E	0.44	18.9	1.8	90.5	0.4	97.9
C	ISA–20E	0.33	22.0	—	—	0.7	96.8

Table 3. Percent reduction of *Culex* spp. larvae and pupae after spray application of ISA-2OE in sewage polishing pond.

Film dosage (ml/m^2)	Pre-treatment mosquito samples: Biomass (g) of larvae according to instar and pupae (P); Total (T)					Post-treatment 24 hr: Biomass (g) of larvae according to instar and pupae (P); Total (T)					24 hr: % reduction					48 hr: Biomass (g) of larvae according to instar and pupae (P); Total (T)					48 hr: % reduction				
	1	*2–3*	*4*	*P*	*T*	*1*	*2–3*	*4*	*P*	*T*	*1*	*2–3*	*4*	*P*	*T*	*1*	*2–3*	*4*	*P*	*T*	*1*	*2–3*	*4*	*P*	*T*
0.44	—	—	3.5	15.4	18.9	—	—	0.4	1.4	1.8	—	—	88.6	90.9	90.5	—	—	0.1	0.25	0.35	—	—	97.1	98.4	98.1
0.33	—	1.3	19.1	1.6	22.0	—	—	—	—	—	—	—	—	—	—	—	<0.1	0.4	0.2	<0.7	—	>92.3	97.9	87.5	>96.8

tion. Periodic observations indicated that wind velocity as low as 3.2 kmph would push a film over the water surface to the downwind side or corner of a pond within minutes after treatment, thereby establishing areas of highly compressed film and areas of essentially no film. This was even more pronounced in heavy winds. However, frequent shifts in the direction and speed of the wind were noted to occur within a 24 hr period, therefore redistributing the film uniformly over the water surface or compressing the film in another area of a pond. This frequent redistribution of the films around a pond was presumed to reduce the surface tension in areas where most mosquito larvae and pupae were concentrated and subsequently produce high mortality 24 and 48 hr post-treatment even in winds gusting up to 48.3 kmph.

Shortly after spray application, high concentrations of larvae and pupae were usually observed to be physically translocated with the film. Mosquito larvae and pupae trapped in the area of highly compressed film were pushed together in extremely high concentrations. The low surface tension in this area as well as additional stress from population pressure were assumed to have contributed to the high mortality of pupae and larvae 2 and 24 hr post-treatment, respectively.

Also, ca. 99% mortality of mosquitoes was achieved in one test after application of ISA-2OE at a surface dosage 0.44 ml/m^2 in the rain. This observation along with data from tests conducted at temperature from 16–30 C and in winds fluctuating from 3.2–48.2 kmph suggested that these environmental factors did not appreciably affect the larval and pupal mortality induced by ISA-2OE in sewage pond habitats. Furthermore, the data indicated that with careful spray application ISA-2OE can be used to control large mosquito populations in densely grassed areas of a sewage pond as well as in pond areas of heavy organic surface scum and debris. The fluctuating water levels and periodic surface agitation from the pumping of sewage and industrial effluent into some ponds did not appear to inhibit the action of ISA-2OE on larvae and pupae. However, these factors may have accounted for the rapid degradation of SMO and subsequently low mortality of mosquitoes that were observed to occur within 24 hr after treatment at the lowest dosage tested (0.44 ml/m^2).

Data from tests comparing the mosquito control effectiveness of ISA-2OE, SMO 75/2EB, and SMO 75/2P in sewage settling, polishing and evapo-percolation ponds of an industrial sewage treatment system indicated that ISA-2OE was more effective than the two SMO compounds, particularly at the lowest dosages evaluated.

Adverse effects on invertebrate and vertebrate non-target aquatic organisms exposed to ISA-2OE were not observed. Mammalian studies (Reynolds, personal communication) with ISA-2OE have indicated that this material is non-irritating to the eyes and skin and that the toxicity is extremely low (acute oral LD_{50} = 20,000 mg/kg). Furthermore, film-induced mortality of immature mosquitoes is thought to be produced by physical factors, i.e., habitat surface tension reduction with subsequent wetting of tracheal structures and anoxia, and therefore resistance to ISA-2OE is not expected to develop. These factors as well as the ease of application, susceptibility of larvae and pupae of *Cx. nigripalpus* and *Cx. quinquefasciatus* at a surface dosage as low as 0.33 ml/m^2 (0.35 gal/acre) make this monomolecular surface film an excellent candidate for practical mosquito control in sewage treatment habitats of southwestern Florida.

Additional field tests (Levy et al. unpublished) to determine the efficacy of ISA-2OE against natural populations of larvae and pupae of *Ae. taeniorhynchus, Ae. infirmatus* Dyar and Knab, *An. quadrimaculatus, An. crucians* Wiedemann, *Cx. nigripalpus, Cx. quinquefasciatus, Psorophora columbiae* Dyar and Knab, *Ps. ciliata* (Fabricius), *Uranotaenia lowii* Theobald, and *Ur. sapphirina* (Osten Sacken) at surface dosages of 0.20–0.45 ml/m^2 have resulted in 90–100% control of larvae and pupae 24–72 hr post-application. Preliminary results indicate that ISA-2OE may be useful in controlling several species of mosquitoes in their natural habitats at application rates which are significantly lower than currently used for petroleum-based larviciding oils.

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