TESTS OF LARVICIDES FOR CONTROL OF SALT-MARSH SAND FLIES (*CULICOIDES*), 1967

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Salt-marsh sand flies of the genus *Culicoides* comprise one of the more important groups of pests along the coastal areas of Florida. Hydraulic filling or permanent flooding of the salt marshes can reduce the breeding sites by as much as 95 percent (Rogers 1962), but water-edge sites such as dike and ditch banks, natural shores, etc. are not affected by these water management practices. Therefore, tests were conducted to find suitable larvicides for control of sand flies at these water-edge sites and in similar breeding areas. These tests were started at Vero Beach, Florida, during 1963 and were continued at Panama City, Florida, during 1965 through 1967.

Dove *et al.* (1932) reported that creosoted pine sap at high concentration was very toxic to sand fly larvae; whereas, Goulding *et al.* (1953) reported poor results with creosote emulsion but showed that several of the chlorinated hydrocarbon insecticides were effective larvicides for *Culicoides* on the Florida East Coast. As a result of Goulding's work, some of these pesticides, especially dieldrin, were used rather extensively for control of *Culicoides* by some counties in Florida during the 1950's. But Smith *et al.* (1959) found that larvae collected from a marsh in Palm Beach County that had been treated three times with dieldrin, once with malathion, and once with heptachlor, each time at the rate of 1.0 lb. per acre, were more than 100 times as resistant to dieldrin, heptachlor, chlordane, and lindane, and about 10 times as resistant to endrin as those collected from an untreated marsh in St. Lucie County. Evidence of cross-resistance was also demonstrated since the Palm Beach marsh had not been treated with chlordane, lindane or endrin.

In the tests described in this paper, an effort was made to find effective sand fly larvicides which seem less likely to cause resistance problems and also be as safe as possible for fish and wildlife.

METHODS

LABORATORY. Test larvae were collected from field populations for use in screening of experimental larvicides in the laboratory. At Vero Beach there was a mixed population of *Culicoides furens* (Poey) and *C. melleus* (Coquillett), and at Panama City *C. hollensis* (Melander and Bures) and *C. melleus*. Soil samples collected in the field were transported to the laboratory and processed by the method reported by Bidlingmayer (1957). Species were identified by rearing samples of the larvae to the adult stage.

In the laboratory screening tests, stock solutions or emulsions of experimental larvicides were diluted in sea water to obtain the desired concentration of toxicant in parts per million on a wt./wt. basis. Each dosage level was replicated five times, plus checks, with each replication containing 20 third instar larvae. Just prior to treatment the larvae were placed on separate stainless steel, 200-mesh screens and washed into their respective beakers by inverting the screen and pouring 200 milliliters of solution, containing the desired dosage level of toxicant, through the screen into the beaker. The larvae were exposed continuously with mortality recorded at 24, 48, and 72 hour intervals. Larval mortality was determined by touching the head capsule with a stainless steel loop and observing for 30 seconds; if no movement occurred within this time, the larva was considered dead.

FIELD. Small field plots were established on the shores of the Indian River at Vero Beach. The test sites were grouped into two types: those on open shores having heavy wave action were referred to as "rough water" sites; plots in protected areas such as tidal ditches were referred to as "calm water" sites.

Pre-sampling was necessary since breeding along the shoreline was not continuous. Test plots were established only where significant numbers of larvae were found. Each plot contained 1,000 square feet, and extended from the low tide mark to above the high tide mark. No sprayed plot was closer than 300 feet to another, and check plots were more than 500 feet from the nearest treated plot.

A total of 6 test plots were treated in the rough water sites, and 12 in the calm water sites. All treatments were applied at low tide.

Each plot was sampled one day prior to treatment, and at weekly intervals thereafter until the treatment failed to maintain at least 90 percent reduction of larvae. During each period of sampling, four random strip samples of soil 3 inches wide, 8 feet long, and 2 inches deep were collected perpendicular to the edge of the water. The soil was placed in 1-quart oil cans and transported to the laboratory for processing. Each time a treated plot was sampled, the check plot in the respective area was sampled in the same manner.

Emulsifiable concentrates of the toxicants were made by dissolving emulsifying agents in the toxicant. The required formula for each toxicant that produced a reasonably stable emulsion in water ranging in salinity from tap water to ocean water was determined in the laboratory prior to the screening tests. The formulae in parts by volume were as follows:

Coal Tar Creosote	
Coal tar creosote Pl-54	80
Emcol AD5-13	14
Toximul S	6
Panasol AN-5	
Panasol AN-5	90
Triton X171	6
Emcol AD5-13	4
Velsicol AR-60	
Velsicol AR-60	80
Xylene	10
Antarate 9183	10
Coal tar creosote—Panasol AN-5	
Coal tar creosote	45
Panasol AN-5	45
Xylene	5
Emcol AD5-13	5

In each instance, the concentrates were emulsified in the required amount of brackish water from the breeding area and applied at the indicated dosage of toxicant in a gross volume of 220 gallons per acre. All applications were made with a 4-gallon compressed air, hand sprayer equipped with a No. 8002 flat spray nozzle.

Similar small-plot tests and several large-scale larvicide tests were conducted at Panama City during 1965–1967. For the large-scale tests, sprays were applied by power sprayer mounted in a boat. Areas treated at Panama City were shores of bays and lagoons, canal banks, and one small salt marsh. Sites for the small-plot tests at Panama City were classified as "clean" shores and "weedy" shores, the latter being overgrown by black rush, *Juncus roemerianus* Scheele. All test plots at Panama City were in areas of relatively calm water.

RESULTS

LABORATORY TESTS. Twenty-three experimental compounds were tested at dosage levels ranging from 1 to 60 parts per million (wt./wt.) concentration. The results (Table 1) showed only three compounds toxic enough at the lowest dosage levels to produce 90 percent or higher larval mortality after 24 hours of continuous exposure. These were coal tar creosote and two aromatic solvents, Panasol AN-5 and Velsicol AR-60. However, pyrethrins (Pyrenone) also gave 100% kill at 72 hours both at 5 and 10 p.p.m.

FIELD TESTS. Panasol AN-5, Velsicol AR-60, coal tar creosote, and a 1/1 combination of Panasol and coal tar creosote were further tested in small field plots at Vero Beach. The results of these tests are shown in Table 2. Coal tar creosote was effective for a longer period than the other materials. In general, treatments also were effective for a longer period in areas of calm water than on open shores that were washed by waves. Velsicol AR-60 was the least effective of the materials tested. It should also be noted that larval populations were much higher in the "calm water" areas as shown by the data for the untreated plots.

TABLE 1.—Results of larvicide screening tests of various compounds against third instar sand fly larvae (*Culicoides*).

Toxicant	P.p.m. (wt./wt.)	Percent mortality at indicated hours of exposure		
		24	48	72
Coal Tar Creosote	10	94	100	100
Panasol AN-5	10	89	89	94
	30	100	100	100
Velsicol AR-60	10	71	..	78
	30	100	100	100
Pyrenone 10–1	5	48	..	100
	10	56	..	100
U. C. 10854	5	50	..	92
	10	86	..	98
Bayer 28589	5	38	..	46
	10	84	..	85
Velsicol AR-55	30	60	70	95
PSE-112 (Std. Oil)	60	53	74	100
Sevin	10	9	..	65
Vapam	10	0	40	63
U. C. 20047	10	2	..	53
Bayer 39007	20	15	24	27
Sun Aromatic Oil S	60	26	35	65
Nicotine Sulfate	60	0	..	63
Diesel Oil Em.	30	7	..	20
D-D	10	0	0	8
TFNP (Nitrophenol) 30% Sol.	10	2	2	4
Sun Aromatic Oil #1756	60	10	18	20
Xylene Em.	30	4	4	4
Ortho Diquat	10	0	0	0
Ortho Paraquat	10	0	0	0
Benzene Em.	60	0	0	0

TABLE 2.—Results of larvicide tests against sand flies (*Culicoides*) in small plots at Vero Beach, Florida, 1964.[1]

Treatment	Percent Reduction at Indicated Number of Weeks [2]					
	1	2	3	4	5	6
Coal Tar Creosote Em.						
Calm Water	99	99	99	99	99	96[3]
Rough Water	99	99	97	99	8	..
Panasol AN-5 Em.						
Calm Water	97	..	99	94	89	58
Rough Water	93	..	69	..	..	..
Coal Tar Creosote Em. Panasol AN-5 Em. (50–50)						
Calm Water	96	97	84	+29	..	..
Rough Water	99	95	88	32	..	..
Velsicol AR-60 Em.						
Calm Water	90	75	73	69	..	..
Checks						
Calm Water	+12	+51	+58	+88	+130	+95
Rough Water	+5	+10	6	53	44	25

[1] All treatments 4 gallons active ingredient per acre.
[2] Corrected to check mortalities by Abbott's Formula.
[3] Percent reduction remained above 87 percent through 11 weeks.

In a large scale test conducted in the spring of 1965 at Panama City, the treatment failed in less than 2 weeks. In this test, coal tar creosote was applied at a rate of 8 gallons per acre in a total volume of 220 gallons per acre. Since this was the first test against sand flies in this area it was suspected that a problem of species susceptibility might be indicated. Sand fly larvae were obtained from the Vero Beach area and were compared in the laboratory with larvae collected at Panama City. The tests showed no significant difference in kills of the two samples when exposed to various concentrations of coal tar creosote emulsion.

Small plot tests were then conducted at Panama City to determine effective dosage in that area. Results of these tests are shown in Table 3. These data indicate that both dosage and volume might have contributed to the poor showing of creosote in the initial large scale test. The period of effective kill was nearly doubled by increasing the dosage of coal tar creosote emulsion from 8 to 12 gallons per acre in the clean shore plots, and more than doubled in the weedy plots. Although the increased dosage of creosote prolonged the period of effectiveness in both areas, volume also appeared to be an important factor. In these tests, as well as in all previous field tests, the dilute sprays were applied at a gross volume of 220 gallons per acre. This volume appeared to be inadequate for creosote in the weedy plots since the number of weeks of effective control was approximately half that obtained in the clean shore plots.

Increased dosage also appeared to extend the period of effective control by Panasol AN-5 in the clean shore plots in these tests but not in the weedy areas. These results indicated that volume (coverage) as well as dosage might have been inadequate in these small plot tests.

As a result of these tests, two large-scale tests were made at Panama City during 1967, using 12 gallons per acre of creosote and 20 gallons of Panasol. These treatments were applied in a total volume of 400 gallons per acre. Approximately 3 acres of shore were sprayed in one test and 5 acres in the other. Results are shown in Table 4. The data show that coal tar creosote at approximately half the dosage was more effective than Pan-

TABLE 3.—Results of larvicide tests against sand flies (*Culicoides*) in small plots at Panama City, Florida, 1966.

Treatment	Percent Reduction at Indicated Number of Weeks [1]					
	1	2	3	4	5	6
Coal Tar Creosote Em.						
8 gals./acre						
Clean Shore	97	98	98	77	4	..
Weedy Shore	98	56	+57	..	..	..
12 gals./acre						
Clean Shore	99	99	97	91	92	+23
Weedy Shore	99	94	92	65	..	..
Panasol AN-5 Em.						
8 gals./acre						
Clean Shore	98	97	98	+152	..	..
Weedy Shore	91	55	+65	..	..	..
12 gals./acre						
Clean Shore	99	99	98	91	78	+135
Weedy Shore	98	77	85	65	..	..
Checks						
Clean Shore	+59	+100	+189	+222	+307	+338
Weedy Shore	+39	+3	+119	+161	..	..

[1] Corrected to check mortalities by Abbott's Formula.

asol in both test areas. It is not fully understood why creosote remained effective for 10 weeks in Test Area 1 and only 5 weeks in Test Area 2. However, differences in habitat conditions were noted between the two areas. Test Area 2, the smaller of the two areas, contained more shore covered with vegetation, which might have prevented good coverage of the soil. An increase in numbers of larvae was first noted in the samples collected from the black rush areas. The samples collected from the clean shore areas at that time revealed no increase. In the area treated with Panasol, samples showed that larvae increased in number simultaneously in both the weedy and clean shores.

No special studies were conducted to determine the effects of these larvicides

TABLE 4.—Results of large-scale larvicide tests against sand flies (*Culicoides*) at Panama City, Florida, 1967.

Treatment	Percent Reduction at Indicated Number of Weeks [1]								
	1	2	3	4	5	6	8	10	12
Coal Tar Creosote Em.									
(12 gals./acre)									
Test Area 1	95	99	99	99	97	96	94	91	30
Checks	41	36	11	18	24	20	+9	5	+44
Test Area 2	99	98	96	93	91	89	76	..	..
Checks	+66	+550	+567	+510	+418	+212	+105	..	..
Panasol AN-5 Em.									
(20 gals./acre)									
Test Area 1	96	92	99	85	89	57	..	..	..
Checks	13	38	+58	2	+5	3	..	..	..
Test Area 2	98	97	95	92	82	45	..	..	..
Checks	+66	+550	+567	+510	+418	+212	..	..	..

[1] Corrected to check mortalities by Abbott's Formula.

on fish and wildlife. However, careful observations were made in the large-scale treatments and as far as visual observations could determine, there were no adverse effects along the open shores of the canals and bayous.

In several instances, minnows were killed where these larvicides were applied to small landlocked, shallow pools, and to narrow, shallow waters of hand-dug tidal ditches. In this situation, it is very difficult to apply larvicides without treating some of the water in which the minnows are trapped.

DISCUSSION. Results of these tests show that emulsions of coal tar creosote and Panasol AN-5 are effective for the control of *Culicoides* sand fly larvae, provided they are used in adequate dosage and with good coverage. Results also indicate differences in dosage and volume required in different types of habitats, which suggests preliminary trials in a particular area to determine the required operation for control in that area.

These larvicides are indicated only for shoreline or ditchbank treatment, because the large volume of spray required per acre is not considered a practical operation for treatment of large salt marshes and similar areas.

Due to the limited knowledge about the ecology of sand flies, no routine time interval for larval treatments can be stated at this time. However, based on the seasonal incidence of sand flies on the southeast coast of Florida, as reported by Shields and Hull (1943), it would appear that applications should be made in March and November just prior to the peaks of emergence of sand fly adults. In West Florida, based on two years of light trap collections (unpublished data), adult flies start to increase in number in February, reach a peak in April, and fall off sharply in May and June. Therefore, larviciding in this area should be most effective when done in February and April.

When used as indicated, these larvicides should present no significant threat to fish and wildlife, and, hopefully, will not create serious problems of resistance in the sand fly population.

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