CONTROL OF SALT MARSH *CULICOIDES* AND *TABANUS* LARVAE IN SMALL PLOTS WITH GRANULAR ORGANOPHOSPHORUS PESTICIDES, AND THE DIRECT EFFECT ON OTHER FAUNA [1]

WILLIAM J. WALL, JR. AND VAHE M. MARGANIAN [2]
Bridgewater State College, Bridgewater, Massachusetts

ABSTRACT. Granular formulations of Dursban applied at the rate of 0.2 and 0.05 pound of technical material per acre and Diazinon applied at 0.2 pound per acre to salt marsh mud significantly reduced populations of *Culicoides furens* and *C. hollensis* larvae with low mortality to other organisms. Baytex and Abate applied at 0.2 and 0.3 pound per acre, respectively, resulted in poor control of *Culicoides* larvae. Some dead organisms including *Fundulus* sp., *Apeltes quadracus, Palaemonetes* sp., *Leptochelia* sp. and *Uca* sp., were found in the treated and control areas and in traps placed adjacent to these areas. Gas chromatographic analysis of representative organisms showed the presence of Dursban, Diazinon and Baytex in the tissues of only those exposed to the pesticides employed. Plankton taken from creeks near the treated areas were not noticeably affected by the pesticides.

Granular Dursban, Abate and Diazinon applied at the rate of 0.05, 0.4 and 0.3 pounds per acre, respectively, did not appear to control *Tabanus lineola* and *T. nigrovittatus* larvae breeding in salt marsh sod. Some *Fundulus* confined to screen cages adjacent to the Diazinon treated plots were found dead following treatment while those in the Dursban and Abate treated areas were alive. *Fundulus* confined to potholes in the Dursban plots apparently were unaffected by the pesticides, but those in the Diazinon areas were affected up to the third day following treatment. Gas chromatographic analysis of the dead fish gave positive results. Abate and Dursban apparently did not cause a decrease in the invertebrate fauna of the treated plots, but Diazinon did.

INTRODUCTION. In a previous publication (Wall and Marganian, 1971) we discussed the effect of granular Dursban, Baytex, Abate and Diazinon on *Culicoides melleus* (Coq.) larvae and other organisms breeding in intertidal sand on Cape Cod. This paper presents a continuation of that study to include *Culicoides furens* Poey and *C. hollensis* Melander & Brues breeding in salt marsh mud, and *Tabanus nigrovittatus* Macq. and *T. lineola* Fab. larvae breeding in salt marsh sod.

CULICOIDES FURENS AND *C. HOLLENSIS*

MATERIALS AND METHODS. In this series of experiments, 40 x 10 yard plots and 40 x 15 yard plots were marked out in salt marshes containing drainage ditches or potholes in which the *Culicoides* larvae breed. Each marsh had been sampled prior to selection to locate areas with high larval counts, and over 95 percent of all larvae examined were *C. hollensis*. The mud samples were collected in 9-ounce paper cups which were disposed of after each use to prevent contamination. Larval separation procedure was that described by Wall and Doane (1960). For each test, two plots were treated with a single concentration of a pesticide and a nearby untreated plot was used as a control. All pesticides were applied by hand at low tide, and in some instances, uncoated granules were added to the pesticide-coated granules to provide additional material for better distribution. The Dursban was formulated on Pike's Peak clay granules approximately 24/40 mesh, the Diazinon was formulated on a corn cob base granule approximately 24/40 mesh and the Abate was formulated on sand at 18/40 mesh. The formulation of the Baytex granules was not established. Air, water, sod and mud temperatures of the treated

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[2] Departments of Biology and Chemistry, respectively.

and control plots, and the temperature and salinity readings of the nearest tidal creek or bay were taken at low tide during each sampling.

With the cooperation of personnel from the Massachusetts Division of Marine Fisheries, live animals were placed in screen cages, and the cages were located in drainage ditches or creeks adjacent to the treated areas so that the animals would be exposed to the runoff during the tidal changes following pesticide application. A quantitative survey of the organisms found in the treated and control areas was made before, and at varying intervals following treatment. Plankton tows were also taken in the creeks adjacent to the treated areas during each sampling. Mud, water and organisms taken from the treated and control areas were analyzed by gas chromatographic techniques before, and at varying intervals following treatment. The results of the analysis will be published in the *Pesticides Monitoring Journal*.

RESULTS AND DISCUSSION. The results obtained using Baytex, Dursban, Abate and Diazinon are given in Table 1. The average soil, water and air temperatures all ranged from 72° to 81° F and salinity readings from 19 to 26 percent during the field tests. Baytex applied at the rate of 0.2 pound of technical material per acre appeared to control the *Culicoides* larvae in one plot, but produced erratic results in the other. This may have been due to difficulties encountered in treating this type of environment uniformly with the small amount of 5.0 percent granules that were used to cover each plot. Other causes

TABLE 1.—Results of tests using organophosphorus pesticides to control *Culicoides furens* and *C. hollensis* larvae in 0.08 and 0.12 acre salt marsh plots. Two replicates averaged for each pesticide.

Pesticide	Dosage (technical) lbs./acre	No. days after treatment	Average number of larvae		Percent * reduction
			Treated plot	Check plot	
Baytex 5%	0.2	(before tr.)	45.0 (12)	40.0 (3)**	....
		1	7.0 (12)	22.0 (3)	72.0
		2	12.5 (12)	11.0 (3)	0.0
		6	8.0 (12)	9.0 (3)	20.7
Dursban 1%	0.2	(before tr.)	48.0 (12)	21.0 (3)	....
		1	17.0 (11)	22.0 (3)	66.2
		2	10.0 (12)	19.0 (3)	77.0
		8	9.0 (12)	24.0 (3)	83.6
		18	11.0 (12)	24.0 (3)	80.0
Dursban 1%	0.05	(before tr.)	48.0 (12)	27.0 (4)	....
		1	25.0 (12)	29.0 (4)	51.6
		2	13.0 (12)	4.0 (4)	0.0
		3	14.0 (12)	34.0 (4)	76.8
		9	5.0 (12)	20.0 (4)	86.0
		16	5.0 (12)	10.0 (4)	71.9
Abate 1%	0.3	(before tr.)	28.0 (12)	37.0 (4)	....
		1	14.0 (12)	15.0 (4)	0.0
		2	27.0 (12)	41.0 (4)	12.9
		5	22.0 (12)	46.0 (4)	36.8
		7	22.0 (12)	47.0 (4)	38.2
Diazinon 2%	0.2	(before tr.)	20.0 (15)	30.0 (6)	....
		1	3.0 (12)	23.0 (5)	80.4
		2	4.0 (12)	23.0 (4)	73.9
		4	4.0 (12)	27.0 (4)	77.8
		7	4.0 (12)	50.0 (4)	88.0
		14	12.0 (12)	26.0 (4)	30.6

* Adjusted using Abbott's formula.

** Number of samples given in parentheses.

may have been the overhanging banks of old ditches which prevented the pesticide from reaching the mud, or the presence of small pools of water in the ditches which prevented the pesticide granules from penetrating the mud.

On the day following treatment, numerous dead fiddler crabs (*Uca* sp.) were found in one plot treated with Baytex and some reduction of *Leptochelia* spp. (tanaidacean) and amphipods was also noted. Several *Palaemonetes* sp. (prawn) and *Uca pugnax* confined in screen cages in drainage ditches adjacent to the treated area were not noticeably affected by the pesticide up to 2 days following treatment. However, one of three *Fundulus heteroclitus* (killifish) confined in a screen cage was found dead the second day following treatment. Gas chromatographic analysis of the dead fiddler crabs and *Fundulus* revealed the presence of Baytex in the tissues.

Due to the number of fiddler crabs killed, and to the erratic results obtained from these plots and from the intertidal sand plots previously treated with this same pesticide, it was decided not to test the Baytex further.

Dursban applied at the rate of 0.2 pound of technical material per acre resulted in reduced numbers of *Culicoides* up to 18 days following treatment. *Modiolus demissus* (ribbed mussel), *Palaemonetes* sp., *Uca pugnax,* and *Fundulus* spp. confined in screen cages in a creek immediately adjacent to the treated areas, were not noticeably affected by the drainage of the pesticide at the low tide following treatment. These organisms were examined the first and second days following treatment and released. A slight decline in the numbers of *Leptochelia* spp. indicated that these forms might have been affected by the pesticide in one plot.

Dursban applied at a concentration of 0.05 pound of technical material per acre also considerably reduced the numbers of *Culicoides* larvae up to 16 days following treatment. It was our opinion that the percent reduction figure of zero obtained on the second day following treatment was due to poor sampling in the check area. This sampling procedure which we have employed over a period of many years has shown that the larvae are not necessarily distributed uniformly throughout the mud of a drainage ditch. Hence, it is occasionally possible to obtain several samples containing few or no larvae. In the case in question, a more representative group of samples was taken on the following day.

Seven dead fiddler crabs were found in one 0.05 pound-per-acre treated plot on the day following treatment, but many live, active fiddler crabs were noted in both plots on the same day. *Uca* sp., *Modiolus demissus,* and *Nassarius obsoletus* (mud snail) placed in screen cages in the ditches were alive and active up to two days following treatment, when they were released. Twelve *Fundulus* spp. placed in two screen cages at the outlets to drainage ditches in the treated area were found dead on the day following treatment and gas chromatographic analysis revealed the presence of Dursban in their tissues. No other organisms found in any of the areas treated with both concentrations of Dursban appeared to be reduced in number or visibly affected by this pesticide.

As shown in Table 1, Abate granules applied at the rate of 0.3 pound of technical material per acre provided poor control of the larvae. A few dead fiddler crabs were found in the treated area, but none of the other organisms noted appeared to be affected by the pesticide. Several *Fundulus* spp. were confined in three screen cages in ditches draining the treated areas. On the day following treatment, all the *Fundulus* spp. in two traps were dead, while those in a third were alive and active, and remained alive for 7 days following treatment when they were released. Since we were unable to develop a gas chromatographic technique for Abate residue analysis, we could not determine the cause of the fish mortality. No mortality appeared to have occurred among the other organisms observed in

the treated areas, including *Nassarius obsoletus, Modiolus demissus* and *Littorina littorea* (periwinkle).

Diazinon applied at the rate of 0.2 pound of technical material per acre greatly reduced populations of *Culicoides* larvae up to 7 days following treatment. Numerous small larvae were found in increasing numbers on the 14th, 32nd and 45th days following treatment, and since these tests were conducted early in July when numerous adults are emerging and reproducing, this rapid repopulation was anticipated.

Prior to the treatment with Diazinon, 20 *Fundulus* spp., 20 *Apeltes quadracus* (four-spine stickleback) and 10 *Palaemonetes* sp. were placed in traps at the mouths of ditches draining the treated areas into a tidal creek, and only 4, 0, and 1, respectively, were alive the day following treatment. Five *Carcinus maenas* (green crab) confined in similar cages in comparable locations apparently were not affected by the pesticide drainage. However, several confined *Fundulus* spp. and *Palaemonetes* sp. placed in the creek adjacent to areas not treated and not subject to the run-off were also found dead on the day following treatment. Representative dead specimens were analyzed and a small amount of Diazinon residue was found only in the tissues of those confined in cages adjacent to the treated areas. The cause of death of the organisms not exposed to pesticide run-off could not be established. Some slight reduction in the numbers of ciliates, nematodes and enchytraeids also occurred in the test plots. No sudden plankton population reductions correlated with the four pesticides distributed were noted in any of the samples taken from the tidal creeks during the experimental period.

TABANUS LINEOLA AND *T. NIGROVITTATUS*

MATERIALS AND METHODS. The initial tests were conducted in small salt-marsh plots where the *Spartina alterniflora* was of medium height. The marshes selected had been sampled during previous years and a fairly large number of *Tabanus* larvae were found in this type of habitat. Over 95 percent of the larvae counted were *T. nigrovittatus*. Very few *Chrysops* larvae (often associated with *Tabanus* larvae) were found in the previous sod samples, hence the tests were conducted to determine the effect of the pesticides on *Tabanus* larvae only.

The plots, 40 yards by 30 yards, were marked with stakes and located with a drainage ditch on two sides and a creek on the third side, in an attempt to keep potential larval migration from one plot to another to a minimum. The pesticides were distributed by hand at low tide, and at low concentrations, inert granules were mixed with the treated granules to provide additional material for better distribution.

Square yard samples as described by Wall and Jamnback (1957) were used to estimate *Tabanus* larval population in the treated and control areas. Berlese funnel samples as described by these same authors were also taken from the treated and control areas, but our sod samples were cut in a circle, having a diameter of approximately 12 inches, rather than cut as a 12-inch square.

Fundulus spp. were confined in screen cages in drainage ditches immediately adjacent to the treated areas, and a complete quantitative survey of the organisms found in the treated and control areas was made before, and at varying intervals after treatment. Soil temperature readings were taken whenever the area was sampled. Sod, water and organisms taken from the treated and untreated areas were analyzed for the presence of Dursban and Diazinon by gas chromatographic techniques before and at varying intervals following treatment, and the results will be published in the *Pesticides Monitoring Journal*. These tests were conducted over a period of two summers.

RESULTS AND DISCUSSION. The results of the field tests using granular Dursban,

Abate and Diazinon are given in Table 2. The average sod temperature ranged from 74° F to 79° F and the average air temperature from 77° F to 81° F during the test periods.

Although the results of our square-yard tests using Dursban were erratic and difficult to interpret, the data show that applications of this material resulted in poor control of the *Tabanus* larvae. In one plot, a reduction of 61 percent appeared to have occurred by the tenth day following treatment, but this figure was basically due to the large number of larvae taken in the check area on that date. Three square-yard plots were sampled in the treated areas a year after treatment and an average of 28 larvae were found in each square yard.

Fundulus spp. confined in screen cages in the drainage ditches of one area were alive and active up to 9 days following treatment when they were released. Several other *Fundulus* noted in a pothole in one treated area were alive and active up to 4 days following treatment, when the evening high tide washed them out. In the other plot, five *Fundulus* spp. were confined in screen cages, and all but one were found dead on the second day following treatment. Gas chromatographic analysis showed the presence of Dursban in the tissues. Five other *Fundulus* spp. confined to a pothole in the treated area apparently were not affected by the pesticide and escaped during the high tide on the third day following treatment. None of the other organisms in the treated area appeared to be directly affected by the pesticide.

The data obtained by both square-yard and Berlese-funnel sampling showed that Abate applied at the rate of 0.4 pound technical material per acre caused little or no reduction of the *Tabanus* larvae in both plots up to 15 days following treatment. The average reduction in the treated plots, as shown by the Berlese-funnel samples, was approximately 3.0 percent. One set of square-yard samples taken in these treated areas the following summer gave high larval counts (22 per sq. yd.), supporting our conclusion that the Abate treatment did not control the larvae.

TABLE 2.—Numbers of *Tabanus* larvae collected by the square yard sampling method from 0.25 acre salt marsh plots treated with organophosphorus pesticides. Two replicates averaged for each pesticide.

Pesticide	Dosage (technical) lbs./acre	No. days after treatment	Average number of larvae		Percent * reduction
			Treated plot	Check plot	
Dursban 1%	0.05	(before tr.)	17.0 (12)	11.0 (6)**	....
		1	17.0 (12)	12.0 (6)	8.1
		2	20.0 (12)	11.0 (3)	0.0
		3	17.0 (6)	15.0 (3)	26.7
		7	20.0 (6)	6.0 (3)	0.0
		10	24.0 (9)	27.0 (3)	42.5
Abate 1%	0.4	(before tr.)	11.0 (18)	6.0 (3)	....
		1	10.0 (6)	9.0 (6)	39.4
		2	16.0 (12)	7.0 (6)	0.0
		4	11.0 (6)	8.0 (3)	25.2
		7	10.0 (6)	4.0 (3)	0.0
		15	10.0 (6)	5.0 (3)	0.0
Diazinon 2%	0.3	(before tr.)	12.0 (15)	7.0 (6)	....
		1	4.0 (12)	6.0 (6)	61.2
		2	4.0 (12)	8.0 (6)	70.8
		4	5.0 (12)	7.0 (6)	58.3
		7	6.0 (12)	7.0 (6)	50.0

* Adjusted using Abbott's formula.
** Number of samples given in parentheses.

Fundulus spp. confined in traps adjacent to one treated area were unaffected by the pesticide up to 5 days following treatment when they were released. No noticeable reduction of other organisms in the treated areas was recorded, but there did appear to be a considerable increase in the number of pseudococcids, *Rhizoecus maritimus* found on the roots of *Spartina alterniflora*. This same phenomenon was noted by Jamnback and Wall (1959) in some Dieldrin-treated plots on Long Island.

The results of our tests with granular Diazinon were somewhat difficult to interpret since the square-yard and Berlese-funnel samples appeared to give conflicting results. In the square-yard samples, up to 7 days following treatment, there was an average reduction in *Tabanus* larvae of approximately 61 percent in the treated plots, while the sod samples showed approximately a 13 percent reduction in the number of larvae during the same period of time.

Ten *Fundulus heteroclitus* confined in wire cages and placed in drainage ditches immediately adjacent to the treated areas and in the control area apparently were unaffected by the Diazinon up to 7 days following treatment. However, 15 *Fundulus* spp. placed in each of two potholes in the treated areas before treatment were dead the day following treatment. Thirty live fish were then introduced into each of the treated area potholes; these also were found dead the day following introduction, or the second day following treatment. Fifteen new fish were then added to each treated area pothole and these were found dead when examined on the fourth day following treatment. The fish originally placed in the control area pothole were still alive, active and apparently unaffected at this time. *Fundulus* spp. again introduced into the treated area potholes on the fourth day following treatment were alive and active on the seventh day following treatment. Analysis of all dead fish revealed the presence of Diazinon in the tissues. Some reduction of Collembola, Dolichopididae, Amphipoda, Pseudococcidae, Stratiomyiidae, Dasyhelea and Chloropidae appeared to have taken place during the 7-day sampling period.

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