CONTROL OF *CULICOIDES MELLEUS* (COQ.) (DIPTERA: CERATOPOGONIDAE) WITH GRANULAR ORGANOPHOSPHORUS PESTICIDES, AND THE DIRECT EFFECT ON OTHER FAUNA [1]

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Much information is available on the direct and indirect effects of organochlorine pesticides on intertidal biota, including bloodsucking dipterous larvae, but few data are available on the direct and indirect effects of organophosphorus pesticides on other marine organisms. Ludwig *et al.* (1968) reported that no deleterious effects on associated fauna were produced by 0.025 pound active Dursban per acre when applied as a spray to a salt marsh for control of *Aedes sollicitans* larvae.

This paper describes preliminary studies on Cape Cod. These were an attempt to identify one or more organophosphorus pesticides formulated on granules, capable of controlling bloodsucking Diptera breeding in the intertidal areas, while providing a minimum of ecological disturbance. *Culicoides melleus* larvae were selected as the target species since they are numerous, easy to sample, and are associated with a number of other intertidal organisms.

PRELIMINARY FIELD TESTS

MATERIALS AND METHODS. The preliminary field tests were conducted during June and July of 1967 in small intertidal sand plots located on sandy beaches having high and relatively uniform *C. melleus* larval populations. The width of each plot extended from low tide level to about high tide level and uniform plots 25 yards long and 8 yards wide were employed. The pesticides were distributed by hand at about low tide, and, at very low concentrations, inert granules were mixed with the treated granules to insure a more even distribution of the materials. Sand samples were collected in glass jars holding approximately 7 fluid ounces, and the samples were collected shortly before treatment and at varying intervals thereafter. The method of larval separation and counting was that employed by Jamnback and Wall (1958). A general survey of the intertidal biota was conducted before and after treatment to determine the lethal effect of each pesticide on these organisms. An intertidal control area close to the treated areas was also included in our studies.

RESULTS AND DISCUSSION. The results of our initial tests are presented in Table 1. Dursban (O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate) and Diazinon (O,O-diethyl O-[2-isopropyl-4-methyl-6-pyrimidinyl] phosphorothioate) provided excellent control of the larvae, while Abate (O,O,O′,O′-tetramethyl-O,O′-thiodi-p-phenylene phosphorothioate) formulated on sand, and Baytex (O,O,-dimethyl O-[4-(methylthio)-m-tolyl] phosphorodiothioate) were also quite effective. Malathion (di-ethyl mercaptosuccinate, S-ester with O,O-dimethyl phosphorodithioate) produced some larval reduction, but the Bay 77488 [3] (O,O-Diäthylthiophosphoryl-a-oximinophenyl-essigsäurenitril) and Bay 78182 [3] (O,O-Diäthylthiophosphoryl-a-oximino-o-chlorophenyl-essigsäurenitril) were quite ineffective, and the two tests using Abate formulated with celatom produced poor and inconsistent results. A general

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survey of the fauna in the treated areas showed that a number of Collembola were killed by the Baytex, Dursban and both concentrations of the Diazinon, and a number of small fish known as "silversides" (*Menidia*) were killed by the Diazinon applied at 0.2 pound per acre.

Because the Abate formulated with celatom gave poor results and the Abate formulated with sand gave good results, we conducted another series of tests using three concentrations of each formulation to compare their effectiveness. Each formulation was tested at 0.2, 0.3, and 0.4 pound of technical pesticide per acre, and samples were collected 1, 2, 3 and 9 days following

TABLE 1.—Results of field tests using granular organophosphorus pesticides to control *Culicoides melleus* larvae in 0.04 acre intertidal sand plots.

Pesticide	Dosage (technical lbs./acre	No. Days after Treatment	Average Number of Larvae		Percent* Reduction
			Treated Plot	Check Plot	
Dursban	0.2	(before tr.)	41.0 (4)	12.0 (4)**	–
1%		1	1.0 (4)	21.0 (4)	98.6
		2	0.0 (3)	22.0 (4)	100.0
		6	0.0 (4)	31.0 (4)	100.0
Diazinon	0.3	(before tr.)	50.0 (3)	50.0 (3)	–
1%		2	0.0 (3)	50.0 (3)	100.0
		3	0.0 (3)	50.0 (3)	100.0
Diazinon	0.2	(before tr.)	18.0 (5)	12.0 (4)	–
1%		1	1.0 (4)	21.0 (4)	96.8
		2	0.0 (2)	22.0 (4)	100.0
		6	3.0 (4)	31.0 (4)	93.5
Abate	0.2	(before tr.)	42.0 (3)	31.0 (4)	–
1% (sand)		1	50.0 (4)	45.0 (2)	21.8
		2	4.0 (4)	25.0 (4)	88.2
		5	9.0 (4)	50.0 (4)	86.7
Baytex	0.2	(before tr.)	22.0 (4)	31.0 (4)	–
5%		1	3.0 (4)	41.0 (4)	89.7
		2	3.0 (4)	31.0 (4)	86.4
		8	5.0 (4)	50.0 (4)	85.9
Malathion	0.3	(before tr.)	46.0 (3)	50.0 (3)	–
5%		2	25.0 (3)	50.0 (3)	45.7
		3	22.0 (3)	50.0 (3)	52.2
Bay 77488	0.2	(before tr.)	50.0 (4)	50.0 (4)	–
1%		1	50.0 (4)	49.0 (4)	0.0
		2	45.0 (4)	50.0 (4)	10.0
		6	50.0 (4)	50.0 (4)	0.0
Bay 78182	0.2	(before tr.)	46.0 (4)	50.0 (4)	–
1%		1	42.0 (4)	49.0 (4)	6.9
		2	49.0 (4)	50.0 (4)	0.0
		6	50.0 (4)	50.0 (4)	0.0
Abate	0.2	(before tr.)	42.0 (4)	31.0 (4)	–
1% (celatom)		1	44.0 (4)	41.0 (4)	20.9
		2	50.0 (4)	31.0 (4)	0.0
		3	50.0 (2)	50.0 (4)	26.1
Abate	0.2	(before tr.)	13.0 (6)	13.0 (4)	–
1% (celatom)		1	14.0 (4)	28.0 (4)	50.0
		2	10.0 (4)	17.0 (4)	41.2
		7	16.0 (4)	9.0 (4)	0.0

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

treatment. Over the 9-day period, the celatom formulations gave an average control of 14, 39, and 33 percent respectively, and the sand formulations gave an average control of 45, 90, and 100 percent respectively, for the concentrations tested.

The results clearly showed that the quicker release sand formulation of Abate provided greater control of *C. melleus* larvae than the slower release celatom formulation. These results also indicated that concentrations of at least 0.3 pound of technical Abate per acre were necessary to effectively reduce the numbers of *Culicoides* larvae in the sand. Other than the *Culicoides* larvae, the smaller fauna in the Abate treated areas did not appear to be adversely affected by this pesticide.

SMALL PLOT TESTS

MATERIALS AND METHODS. In our next series of experiments, Dursban, Baytex, Abate and Diazinon were employed as the test materials. The length of each of the treated plots was increased to 75 yards, thereby creating an area of 0.12 acre. Two treated plots and a nearby untreated control area were utilized to test each pesticide. Sand samples, a plankton tow, water, sand, and air temperatures, and salinity readings were taken during low tide at each test site, before and at varying intervals following treatment. The sand samples were taken to a depth of approximately one inch, washed as previously described, and the numbers and kinds of organisms were separated, counted, and recorded. The sand samples were collected in paper cups holding 9 fluid ounces, and the cups were used only once to prevent contamination. With the cooperation of personnel from the Massachusetts Division of Marine Fisheries, screen cages containing living organisms netted in the area were placed in the bay below tide level and directly in front of the treated and control areas. All areas were treated by hand at approximately low tide, and these experiments were conducted over a period of two summers.

Organisms and sand from the treated and control plots, and organisms taken from screen cages, were analyzed before and following treatment by means of a gas chromatograph to determine the presence or absence of pesticide residues. No reliable technique for analysis of Abate was available for our F & M 400 Series Biomedical Gas Chromatograph when our experiments were started. However, in anticipation of the development of an analysis technique prior to the termination of the project, we continued to test Abate during the first two summers. The procedure employed and the results obtained from the gas chromatograph analysis will be published in another paper.

RESULTS AND DISCUSSION. The results of the tests employing the four pesticides are shown in Table 2, and the average temperature and salinity readings taken during these tests are given in Table 3.

Excellent larval control was obtained using 0.2 pound of technical Dursban per acre, but numerous small, dead *Fundulus* spp. (killifish) and a few small dead, unidentified crabs were found in the treated plots the day following treatment. *Fundulus* spp. and *Palaemonetes* sp. (prawns) placed in the cages in front of the treated and check areas, however, appeared to be unaffected by the pesticide up to 4 days following treatment when they were released. Except for collembolans, none of the other small organisms normally found in intertidal sand such as ciliates, turbellarians, nematodes, oligochaetes, polychaetes, copepods, isopods, amphipods, etc., appeared to be affected by the pesticide. A few dead fiddler crabs (*Uca* sp.) were found on the sand at low tide, and it was noted that the majority of the fiddler holes were located in the beach sand above the treated areas.

Because of the large number of dead *Fundulus* spp. found in the treated areas, a second test was conducted in another locality using 0.1 pound of technical Dursban per acre. Again excellent control of the *Culicoides* larvae occurred, but numerous dead fiddler crabs were found in the treated areas the first and second days fol-

TABLE 2.—Results of field tests using granular organophosphorus pesticides to control *Culicoides melleus* larvae in 0.12 acre intertidal sand 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	
Dursban 1%	0.2	(before tr.)	47.0 (16)	34.0 (12)**	–
		1	0.0 (16)	31.0 (12)	100.0
		2	0.0 (16)	25.0 (8)	100.0
		6	3.0 (16)	25.0 (8)	91.3
		13	1.0 (16)	31.0 (8)	100.0
Dursban 1%	0.1	(before tr.)	23.0 (16)	39.0 (8)	–
		1	1.0 (12)	19.0 (8)	91.1
		2	0.0 (12)	32.0 (7)	100.0
		4	0.0 (12)	20.0 (8)	100.0
		12	0.0 (12)	14.0 (10)	100.0
Dursban 1%	0.05	(before tr.)	26.0 (12)	31.0 (8)	–
		1	13.0 (12)	23.0 (8)	32.6
		2	4.0 (12)	27.0 (8)	82.3
		3	4.0 (12)	41.0 (8)	88.3
		7	2.0 (12)	37.0 (8)	93.5
Baytex 5%	0.2	(before tr.)	31.0 (16)	27.0 (8)	–
		1	27.0 (16)	37.0 (8)	36.5
		2	25.0 (16)	17.0 (8)	00.0
		7	22.0 (16)	8.0 (8)	00.0
Abate 1%	0.4	(before tr.)	41.0 (16)	50.0 (8)	–
		1	4.0 (16)	47.0 (8)	89.6
		2	1.0 (12)	50.0 (8)	97.6
		3	1.0 (12)	50.0 (8)	97.6
		7	0.0 (12)	50.0 (8)	100.0
Diazinon 2%	0.2	(before tr.)	18.0 (24)	44.0 (12)	–
		1	7.0 (13)	41.0 (6)	58.3
		2	3.0 (12)	50.0 (6)	85.4
		3	6.0 (12)	50.0 (6)	71.2
		7	4.0 (12)	50.0 (6)	80.5

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

lowing treatment. *Pseudopeluronectes americanus* (winter flounder), *Fundulus majalis, Palaemonetes pugio, Nassarius obsoletus* (mud snail), and *Mya arenaria* (soft-shelled clam) confined in screen cages were not noticeably affected by the pesticide up to 4 days following treatment when they were removed. Other than Collembola and possibly naidid worms, no apparent reduction of the typical intertidal sand organisms appeared to have occurred.

A third test using Dursban applied at the rate of 0.05 pound per acre was undertaken at another locality. Good control of the *Culicoides* larvae was obtained, and no mortality of *Uca* spp. or *Fundulus* spp. occurred in or near the treated areas. A number of *Palaemonetes* sp., *Syngnathus fuscus* (northern pipefish), *Pseudopleuronectes americanus, Uca* spp., *Mugil* sp. (mullet), and *Fundulus majalis* confined in screen cages remained alive and active up to 7 days following treatment when they were removed. When the area was examined on the 36th day after treatment, many, very small *C. melleus* larvae were found, indicating that repopulation had taken place sometime during the previous few weeks.

The Baytex granules applied at the rate of 0.2 pound of technical material per acre gave poor and inconsistent results. This may have been due to the use of 5.0 percent Baytex granules rather than a 1.0 or

TABLE 3.—Average temperature and salinity readings taken at low tide during small plot field tests to control *Culicoides melleus* larvae.

Pesticide	Dosage (technical) lbs./acre	Sand Temp. °F.	Water Temp. °F.	Air Temp. °F.	Salinity °/oo
Dursban 1%	0.2	80	78	76	17
Dursban 1%	0.1	84	77	79	18
Dursban 1%	0.05	79	78	78	30
Baytex 5%	0.2	77	79	79	32
Abate (sand) 1%	0.4	77	78	77	18
Diazinon 2%	0.2	76	71	71	24

2.0 percent formulation which would have been easier to distribute in a uniform manner. No mortality occurred among *Nassarius obsoletus, Mya arenaria, Limulus polyphemus* (horseshoe crab), *Fundulus* spp., *Sphaeroides maculatus* (northern puffer), *Syngnathus fuscus,* and *Brevoortia tyrannus* (Atlantic menhaden), placed in screen cages near the treated areas and released 2 days following treatment. No reduction of the general intertidal sand fauna was recorded from the samples examined.

Abate granules applied at the rate of 0.4 pound of technical material per acre provided excellent larval control up to 7 days following treatment, but numerous dead fiddler crabs were found in one treated area on the first and second days following treatment. The replicate area contained very few fiddler crabs and very few fiddler crab holes. Several *Nassarius obsoletus, Uca pugilator,* and *Fundulus* spp, confined in cages adjacent to the treated areas were not visibly affected by the pesticide up to 7 days following treatment when they were released. Some of the *Nassarius* noted in the treated areas appeared to have slower reactions than those examined in the untreated area. The treated areas appeared quite normal on the sixth day following treatment with numerous active fiddlers present, and many new fiddler holes were noted in the test plot containing the larger number of crabs.

Diazinon applied at the rate of 0.2 pound per acre reduced the numbers of *C. melleus* larvae for approximately 2 weeks at which time numerous, very small larvae were found in the treated and untreated plots. Since these tests were conducted during the early part of July when adult emergence and egg laying appeared to be at their peak, this rapid reinfestation did not appear unusual. Although the samples from the second treated plot gave higher counts than the first, most of the larvae from the second plot were taken from one small area, which apparently was not well covered by the pesticide. It was concluded, therefore, that effective control was obtained by the Diazinon, and that repopulation occurred rather rapidly because of the small area treated, the limited residual action of this pesticide, and the flushing action of the tides.

Some reduction of enchytraid and naidid worms, collembolans, and dolichopodid larvae occurred in the Diazinon treated areas. Three small *Fundulus* spp. from a total of 15 were found dead in the screen traps the day following treatment, while *Palaemonetes* spp., *Uca* spp., *Carcinus maenas* (green crab), and *Pagurus longi-*

carpus (hermit crab), were not affected up to 7 days following treatment when they were released.

Plankton tows made immediately adjacent to all treated areas were very difficult to assess since the number and species of organisms varied from day to day in the samples taken from a single locality. Therefore, as our experiments progressed, we continued to take plankton tows, and considered them relatively unaffected by the pesticide if they contained a good number and variety of organisms. No sudden planktonic population reductions correlated with pesticide distributions were noted in any of the samples taken during the entire experimental period. None of the pesticides employed in our tests caused any apparent toxicity to *Modiolus demissus* (ribbed mussel), *Mya arenaria* and *Mercenaria mercenaria* (quahog), found in the treated plots.

SUMMARY. The effect of granular formulations of organophosphorus pesticides on *Culicoides melleus* larvae and other intertidal sand organisms was studied over a period of 2 summers. Preliminary experiments conducted in 0.4 acre intertidal sand plots indicated that Baytex, Dursban, Abate (formulated on sand) and Diazinon applied at low concentrations effectively killed *C. melleus* larvae without appreciably reducing most other intertidal fauna in or adjacent to the treated areas. Numerous collembolans were killed by the Baytex, Dursban and Diazinon, and numerous silversides were killed by the higher concentration of Diazinon. Malathion was somewhat effective as a larvicide, but Bay 77488 and Bay 78182 were ineffective at the concentrations used.

Dursban applied to 0.12 acre intertidal sand plots at the rate of 0.2 pound technical material per acre provided good *C. melleus* larval control but killed numerous *Fundulus* sp., collembolans, and a few *Uca* sp. Applications of 0.1 pound per acre provided excellent *Culicoides* control, but killed numerous *Uca* sp., collembolans and possibly naidid worms. Applied at the rate of 0.05 pound per acre, Dursban effectively reduced the *Culicoides* larvae and was not toxic to fiddler crabs or other organisms in or adjacent to the treated plots. Organisms confined in screen cages placed beyond low tide level were not visibly affected by any of the Dursban applications.

Baytex applied at 0.2 pound per acre was quite ineffective, and caused no apparent mortality to confined organisms or to small intertidal organisms. Abate applied at 0.4 pound per acre provided excellent larval control, but killed numerous fiddler crabs, and Diazinon applied at 0.2 pound per acre appeared to be an effective larvicide, but killed some of the small sand organisms including enchytraids, naidids, collembolans and dolichopodid larvae. Repopulation of the Diazinon plots by *C. melleus* larvae occurred during the second week following treatment. A few confined *Fundulus* spp. may have been killed by the Diazinon, but most of the confined organisms were not affected by the Abate or Diazinon applications. None of the pesticides employed in our tests appeared to directly affect bivalves or plankton in or adjacent to the treated areas.

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