FIELD TESTS OF DIFLUBENZURON, METHOPRENE, FLIT MLO® AND CHLORPYRIFOS FOR THE CONTROL OF *AEDES TAENIORHYNCHUS* LARVAE IN DIKED DREDGED SPOIL AREAS.[1]

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ABSTRACT. Replicated field tests were conducted with 4 chemicals at various rates of application to control *Aedes taeniorhynchus* larvae in temporary pools of water in depressions within diked dredge spoil disposal areas. Lowest rates of application which were effective for complete control were: diflubenzuron (Dimilin®), 0.01 lb AI/acre (11g/ha.); methoprene (Altosid® SR-10), 0.04 lb AI/acre (45 g/ha); Flit MLO®, 4 gal/acre (15.6 liters/ha). Applications of controlled release pellets of chlorpyrifos (Dursban® 10CR) at the recommended rate (1 oz pellets/850 ft²/1 in. water depth) gave complete control at 2 days posttreatment.

INTRODUCTION

Diked dredged material disposal sites are often breeding habitats for large numbers of *Aedes taeniorhynchus* (Wiedemann) and *Ae. sollicitans* (Walker) mosquitoes (Ezell 1978, Scotton and Axtell 1979). Many disposal sites, necessitated by dredging harbors and waterways, are close to human habitations, and mosquito control is required. Suitable physical (habitat modification) or biological control methods are not available so chemical control (larviciding) of the breeding sites is presently the only practical method. Some mosquito abatement programs treat these dredged spoil disposal sites with Flit MLO®. Recently some areas have been treated with Dursban® 10CR (a controlled release pellet formulation of chlorpyrifos) which has been reported to be effective against a variety of mosquito species in various habitats although data on *Aedes* in dredged spoil areas have not been published (Evans et al. 1975, Keenan 1978, Nelson et al. 1976). The insect growth regulators diflubenzuron (Dimilin®) and methoprene (Altosid®) have shown promise in tests for the control of *Ae. taeniorhynchus* in coastal marsh habitats (Dame et al. 1976; Rathburn and Boike 1975, 1977; Rogers et al. 1976), but tests in diked dredged spoil areas have been limited to a few pilot aerial application trials with Altosid (Ezell 1978). This report presents the results of our replicated field tests of these chemicals for the control of *Ae. taeniorhynchus* in dredged spoil disposal areas in coastal North Carolina.

MATERIALS AND METHODS

The chemicals tested were (1) diflubenzuron (Dimilin®, Thompson-Hayward Chemical Co.), 25% active ingredient (AI) wettable powder formulation. Chemical formula: 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl) urea. (2) methoprene (Altosid® SR-10, Zoecon Corp.), 10% AI liquid microencapsulated formulation. Chemical formula: isopropyl (E,E)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate. (3) Flit MLO®, a refined petroleum oil, Exxon Corp. (4) chlorpyrifos controlled-release formulation (Dursban® 10CR, Dow Chemical Co.), chlorinated polyethylene pellets containing 10.6% active ingredient. Chemical formula: 0,0-diethyl 0-(3, 5, 6-trichloro-2-pyridyl phosphorothioate).

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Tests were conducted by application of the chemicals to the surface of pools of water within diked dredged spoil disposal areas adjacent to the intracoastal waterway in Onslow County, North Carolina in 1975 and 1976. These pools were in depressions resulting from the method of dike construction. The dikes were formed from material removed by a drag-line and as a result, ditches ca. 30 ft wide and 6 ft deep with varying lengths were left inside and parallel to the dikes. When dredged material taken from the channel of the intracoastal waterway was pumped into the diked areas, the ditches filled with fine silt and the subsequent drying and shrinking of this material resulted in depressions of various sizes with many isolated from adjacent depressions. Rain water accumulates in these depressions creating the isolated pools we used. Since these pools are dependent upon rainfall, their size depends upon the amount and sequence of rainfall. During prolonged dry periods, the pools dry completely and the underlying silt becomes deeply cracked. These pools in our test sites often contained large numbers of mosquito larvae all of which during the time of our testing were *Ae. taeniorhynchus*.

The chemicals were applied with a hand-pumped compressed air sprayer with the rate of output calibrated so that we could use time of spraying (with a standard pressure) to regulate the amount of active ingredient applied per pool according to the surface area to give the equivalent of the desired amount per acre. Each isolated pool was a replicate. The treated pools had surface areas of ca. 70-2900 ft^2 with most ca. 300–900 ft^2. The depth of the water was 1–6 in. In 4 tests, diflubenzuron was applied at 0.005, 0.01, 0.02 and 0.04 lb/A (5.6, 11.2, 22.4 and 44.8 g/ha), methoprene at 0.02, 0.027 and 0.04 lb/A (22.4, 30.2 and 44.8 g/ha) and Flit MLO at 1, 2, 4 and 6 gal/A (9.3, 18.7, 37.4, 56.1 liters/ha). In addition, 2 tests were conducted to compare a granular formulation containing 1% diflubenzuron to the wettable powder formulation containing 25% active ingredient when both were applied at the same rates of active ingredient per acre. One test (4 pools) of the controlled-release pellet formulation of chlorpyrifos was conducted and the pellets were distributed by hand.

Numbers of mosquito larvae and pupae were determined immediately before treatment in each pool by obtaining the average from 10 dips taken about equidistant around the pool with a standard 1-pt white enamel dipper. Posttreatment samples were taken in the same manner until cast pupal skins were found in any of the pools which indicated that adult emergence was occurring and hence the completion of that particular brood of mosquitoes. In one test (No. 4), data on 2 successive broods were obtained. The % reduction at various posttreatment intervals was calculated for each pool by using the number of larvae and pupae pretreatment counts and posttreatment counts. The avg % reduction for a treatment was the avg of the % reductions of the replicates (pools) for that treatment.

Concurrently, in the treatments with diflubenzuron and methoprene, at 1-day posttreatment 100–250 (depending upon availability) 4th instar larvae were collected from each treated and untreated (control) pool and held equally divided in water from their source in 4 mesh-topped cups in the laboratory (ca 24.4°C) to determine the presence of adults successfully emerging (i.e. completely separated from the pupal exuviae and able to fly). This was also done at greater posttreatment intervals in test no. 4 and in the comparison of 2 formulations of diflubenzuron. These laboratory-held samples gave the most accurate determination of the effects of the treatments with diflubenzuron and methoprene since those chemicals are insect growth regulators (IGR) and they often have a delayed effect, i.e. the mosquito larvae and pupae are not killed, but adult emergence either does not occur or the emerging adults are deformed. Because of this delayed effect, the field counts are

Table 1. Effects of Flit MLO and the insect growth regulators diflubenzuron and methoprene at various application rates on *Ae. taeniorhynchus* in pools within diked dredged spoil areas.

Chemical & application rate	No. replicates (pools)[a]	Pretreat. avg no. larvae & pupae/dip/replicate	Avg no. larvae & pupae (N)/dip/replicate and avg % reduction at various days postreatment[b]												Avg % adult emergence in lab[c]
			1 day		2 days		3 days		4 days		5 days		6 days		
			N	%	N	%	N	%	N	%	N	%	N	%	
Test no. 1—Treated July 2, 1975, 3rd & 4th instar larvae present.															
diflubenzuron, 0.04 lb/A	6	34.3	4.2	87.0	0										5.3
diflubenzuron, 0.02 lb/A	7	26.5	4.7	64.7	0										15.3
untreated	3	34.8	19.6	34.4	1.6*										97.7
Tested no. 2—Treated July 14, 1975, 3rd & 4th instar larvae present.															
diflubenzuron, 0.01 lb/A	7	51.3	8.0	80.0	17.9	61.3	3.6	88.4	1.0	89.1					0
Flit MLO, 2 gal/A	2	105.6	30.1	70.9	47.0	55.2	288.1	31.2	110.9*	29.3					—
Flit MLO, 1 gal/A	2	18.1	18.9	5.8	67.3	0	31.8	43.6	80.7	0					—
untreated	3	32.7	95.8	0	126.1	22.0	196.9	0	137.4*	0					94.5
Test no. 3—Treated Sept. 10, 1975, 2nd instar larvae present.															
diflubenzuron, 0.005 lb/A	2	158.2	84.2	44.7	107.6	26.3	14.3	87.9	55.3	54.6	57.0	54.3	185.4	0	10.4
methoprene, 0.027 lb/A	3	282.9	472.2	11.6	153.7	28.5	39.9	83.8	552.5	48.5	592.9	8.5	159.0*	42.4	12.8
methoprene, 0.02 lb/A	4	398.2	578.5	14.0	126.7	50.2	45.6	82.9	274.5	52.7	201.4	53.3	60.5*	74.3	31.4
untreated	2	200.2	957.0	0	660.0	16.6	166.6	36.9	1091.0	0	821.2	0	176.7*	18.9	89.4
Test no. 4—Treated Sept. 24, 1975, all larval instars & pupae present.															
diflubenzuron, 0.02 lb/A	2	80.9	116.4	34.5	—	—	3.2	90.6	—	—	0.7	98.5	0	100	0
methoprene, 0.04 lb/A	2	111.8	68.8	20.9	—	—	23.3	61.6	—	—	15.0	75.5	231.3[d]	37.4	0
methoprene, 0.027 lb/A	4	116.5	133.0	26.9	—	—	33.9	60.4	—	—	21.8	78.1	465.3[d]	16.3	0
Flit MLO, 6 gal/A	2	78.6	0	100	—	—	0	100	—	—	0	100	0	100	
Flit MLO, 4 gal/A	2	50.6	1.5	97.6	—	—	3.9	92.3	—	—	1.9	96.1	2.4	96.7	—
untreated	2	176.6	47.9	70.4	—	—	827.1*	0	—	—	763.4	0	362.6*	0	76.0

* Pupated and adults emerged.

[a] Each replicate was an isolated pool within the diked dredged spoil area. Pools varied from 100–2900 ft^2 and 1–6 in deep.

[b] Avg no. larvae and pupae per dip based on 10 dips per replicate. Avg % reduction for a treatment was the avg of the % reductions of the replicates for that treatment and consequently cannot be calculated directly from the values given in this table.

[c] Avg adult emergence from 100 to 250 4th instar larvae collected 1 day posttreatment from each replicate and held in the laboratory equally divided in 4 cups of water from the replicate.

[d] Nearly all 3rd & 4th instar larvae which did not pupate and produce adults as determined by counts on the 9th and 12th days posttreatment.

less accurate than the laboratory data. Nevertheless, some mortality did occur in the field. This was especially true at higher rates of application and was more evident in treatments with diflubenzuron than with methoprene. Diflubenzuron interferes with chitin synthesis during the molting process and mortality can occur in any larval instar and in the pupal stage whereas methoprene is a juvenile hormone mimic and primarily the 4th instar larvae are most susceptible to it with the mortality being expressed by the failure of adult emergence. The field counts underestimated the degree of control, but did indicate whether or not there was some reduction in mosquito abundance and whether or not adult emergence occurred (as evident by presence of pupal cast skins) in the treated areas when it occurred in the untreated areas.

RESULTS AND DISCUSSION

The results of the 4 tests comparing diflubenzuron, methoprene and Flit MLO are summarized in Table 1. In the initial test with diflubenzuron (test No. 1), 3rd and 4th instar larvae were present at the time of treatment, (July 2, 1975) and the depressions had been flooded for 5 days (a 3 in. rainfall on June 27–28). At 1 day posttreatment large numbers of dead larvae and deformed dead pupae were observed in the treated sites where avg reduction was 87% at the 0.04 lb/A rate (range 76–97% for 6 replicates) and 64% at 0.02 lb/A (range 0-100% for 7 replicates). From larvae retrieved at 1 day posttreatment and held in the laboratory, adult emergence was 5% for the 0.04 lb/A rate and 15% for the 0.02 lb/A rate. By the 2nd day posttreatment adult emergence occurred in the untreated pools while complete control (death of all mosquito larvae and pupae) resulted from the diflubenzuron treatments at both 0.02 and 0.04 lb/A rates.

The pools in test no. 1 were mostly dried by the time a 4.6 in. rainfall on July 11–12, 1975, reflooded the depressions. In test no. 2 (treated July 14) mostly 3rd and 4th instar larvae were present at the time of treatment with diflubenzuron and Flit MLO. Diflubenzuron at 0.01 lb/A gave a high level of control. The field counts showed an avg reduction of 80% at 1 day posttreatment with a range of 0.97% reduction for the 7 replicates. There was no adult emergence from larvae retrieved at 1 day posttreatment and held in the laboratory. By the 4th day posttreatment there were very few live larvae or pupae found in the diflubenzuron treatments while in the untreated pools they were abundant and adult emergence was occurring. There was some reduction in mosquitoes in the Flit MLO treatments at the higher rate (2 gal/A), but not at the lower rate (1 gal/A). The Flit MLO treatments were inadequate, however, for pupation and emergence of adults occurred on the 4th day posttreatment at the same time as in the untreated pools.

After a period without substantial rain, the pools dried until a 2.2 in. rainfall on Sept. 8–9, 1975 caused a flooding of the deeper depressions. In test no. 3 (treated Sept. 10) mostly 2nd instar larvae were present at the time of treatment with diflubenzuron and methoprene. Diflubenzuron at the low rate of 0.005 lb/A gave a fair level of observable control in the field and there was only 10% emergence of adults from larvae retrieved at 1 day posttreatment and held in the laboratory. Adult emergence did not occur in the diflubenzuron treated pools at the time (7 days posttreatment) it was occurring in the untreated pools and in the pools treated with methoprene. The effects of methoprene were not, of course, as obvious in the field. However, some reduction in the number of larvae and pupae was evident although adult emergence occurred at 7 days posttreatment at the same time as it occurred in the untreated pools. Adult emergence in the laboratory from larvae retrieved at 1 day posttreatment was 12% and 31% for the 2 rates of methoprene while emergence from the controls was 89%. Also, during test no. 3 a rain the 2nd day posttreatment very likely flushed additional larvae into the pools and initiated additional egg hatching and a new brood of larvae as well as diluting the treatments. The increase in the avg no. of larvae and pupae on the 4th day posttreatment reflects this situation. It should be noted that methoprene is most effective when applied against 4th instar larvae and our treatments were made when mostly 2nd instar larvae were present which undoubtedly reduced the effectiveness of methoprene. Diflubenzuron, however, is effective against all instars. Under these field conditions the degree of mosquito control by the rates of application of diflubenzuron and methoprene which we used was not satisfactory.

Test no. 4 was conducted following a 3.5 in. rainfall on Sept. 22–23, 1975, which reflooded all of the depressions. At the time of treatment (Sept. 24) all larval instars were present in the pools. Flit MLO at 4 and 6 gal/A gave essentially 100% control at 1 day posttreatment and for the duration of the test (until adult emergence occurred on the 7th day posttreatment in the untreated pools). Diflubenzuron gave 34% observable control in the field at 1 day posttreatment and ultimately gave 100% control for the duration of the test, i.e. no adult emergence occurred. Larvae retrieved from the diflubenzuron treatments at 1 and 3 days posttreatment and held in the laboratory produced no adults. The methoprene treatments at 0.04 and 0.02 lb/A also gave 100% control during the duration of this test, i.e. the larvae observed in the field on the 7th day posttreatment did not pupate and produce adults as determined by field counts on the 9th and 12th days posttreatment. Larvae retrieved from the methoprene treatments at 1 day posttreatment and held in the laboratory produced no adults. In addition, (Table 2), larvae were retrieved from the methoprene treatments at 3, 5, 7 and 9 days posttreatment and the adult emergence was low (0.2–17.1%) indicating the effectiveness of these treatments during the duration of this test.

Table 2. Duration of effectiveness of methoprene against *Ae. taeniorhynchus* in test no. 4 (see table 1) as determined by adult emergence from 4th instar larvae and pupae in the laboratory in water from the test sites.

Rate of application lb AI/acre	Avg. % adult emergence at days posttreatment			
	3	5	7	9
0.04	4.4	1.6	17.1	0.8
0.027	7.0	13.6	10.6	0.2
Untreated	93.4	89.9	96.0	84.0

In test no. 4 and the previous tests with diflubenzuron and methoprene, observations were made in the field on organisms other than mosquitoes in the pools. Although an occasional dead water-boatman and water beetle were found, large numbers were alive in the pools. Live fiddler crabs (*Uca* sp) and minnows were observed in the treated pools and no evidence of toxic effects of the chemicals on these non-target organisms was observed. These compounds, however, will kill certain non-target organisms under specified conditions and applications rates (Breaud et al. 1977, Julin and Sanders 1978).

Diflubenzuron formulated as a wettable powder (25% AI) and granular (1% AI) when applied at the same rates of active ingredient per acre (0.025 and 0.04 lb/A) both gave effective mosquito control (Table 3). Applications were made when 3rd and 4th instar larvae were present and adult emergence occurred in the untreated pools at 3 days posttreatment in test no. 1 and at 4 days posttreatment in test no. 2. Concurrently, live larvae and pupae were nearly completely absent in the pools treated with diflubenzuron. Larvae were retrieved from the treated pools and held in the laboratory for adult emergence whenever sufficient numbers of larvae or pupae could be found in the

Table 3. Comparison of effectiveness of wettable powder (25% act. ingred.) and granular (1% act. ingred.) formulations of diflubenzuron at 2 rates for the control of *Ae. taeniorhynchus* in pools within diked dredged spoil areas.

Formulation	Rate of application lb AI/acre	No. replicates (pools)	Pretreat. Avg no. larvae & pupae/dip/replicate	Avg no larvae & pupae (N)/dip/replicate, avg % reduction (%) and avg % adult emergence (E) in laboratory at various days posttreatment.[a]								
				1 day			2 days			3 days		
				N	%	E	N	%	E	N	%	E
Test no 1—Treated June 1, 1976, 3rd & 4th instar larvae present.												
wettable powder	0.04	3	28.3	19.7	30.6	0	2.0	89.2	—	0	100	0[b]
	0.025	3	21.7	8.0	59.0	0	0.4	97.7	—	1.2	94.6	—
granular	0.04	3	15.0	10.0	24.3	0	0.2	98.1	—	0	100	—
	0.025	3	25.0	26.7	10.0	0	23.0	16.0	—	0.3	98.8	0
untreated		2	24.5	15.8	40.5	93.0	19.2	31.1	—	15.3*	31.7	96.0
Test no 2—Treated June 24, 1976, 3rd & 4th instar larvae present.												
wettable powder	0.04	3	84.7	1.4	98.5	0	0	100	10.0	0.6	99.3	92.7
granular	0.04	3	123.0	1.4	95.0	0	0.1	99.9	0	0.1	99.9	95.6
untreated		3	63.9	122.2	0	77.3	100.2	0	89.8	40.5*	47.7	95.8

* Pupated and adults emerged on 4th & 5th days posttreatment.

[a] Avg no. larvae and pupae per dip based on 10 dips per replicate. Avg % reduction for a treatment was the avg of the % reductions of the replicates for that treatment and consequently cannot be calculated directly from the values given in this table. Avg % adult emergence was based on 150 4th instar larvae collected if present 1–3 days posttreatment from each replicate and hold in the laboratory equally divided in 10 cups in water from the replicate.

[b] No larvae or pupae were found in the dip counts. However, with extensive searching 150 larvae were obtained from 1 of the replicates and held in the laboratory with no adult emergence resulting.

field. In test no. 1, no adult emergence occurred in the laboratory from larvae collected at 1 and 3 days posttreatment. In test no. 2, 0%, 10.9–13.8% and 92.7–95.6% emergence occurred from larvae collected at 1, 2 and 3 days posttreatment, respectively. Very few live larvae or pupae were left in the pools at 2 and 3 days posttreatment. This loss of effectiveness of the diflubenzuron treatments at 3 days posttreatment in the 2nd test was very likely due to dilution by rain (0.6 in) occurring on the night of the 2nd day posttreatment.

A test (treated Oct. 11, 1976) with controlled-release polyethylene pellets of chlorpyrifos (Dursban 10CR) included 4 pools (400–840 ft^2) varying from 1 to 4 in. deep with mostly 2nd instar larvae present at the time of treatment at the recommended rate of 1 oz. pellets per 850 ft^2 per 1 in. water depth. In all replicates there was complete (100%) control at 2 days posttreatment (Table 4). The onset of cold weather and lack of further mosquito production prevented obtaining data on the subsequent effectiveness of this controlled-release formulation. Mosquito breeding was observed in the treated areas the next summer, however, suggesting that the pellets had lost their effectiveness by then.

Table 4. Efficacy of controlled-release chlorpyrifos pellets (Durshan® 10CR) at recommended rate (1 oz. pellets/ 850 ft^2/1 in. water depth) for control of *Ae. taeniorhynchus* in pools within diked dredged spoil areas.

Replicate (pool)	Avg no. larvae/dip[a] Pretreat	2 da. Posttreat
1	42.4	0
2	15.3	0
3	9.3	0
4	2.7	0
Untreated	10.1	3.9

[a] Based on 10 dips per replicate.

SUMMARY AND CONCLUSIONS

Ae. taeniorhynchus larvae in pools in diked dredged spoil areas were controlled readily by diflubenzuron at an application rate of 0.01 lb/A or higher. Wettable powder and granular formulations of diflubenzuron were equally effective. Methoprene at 0.04 lb/A gave complete mosquito control; at 0.027 lb/A control was complete in one test and inadequate in another. Flit MLO gave excellent control at 4 gal/A and higher and little or no control at 1 and 2 gal/A. Treatments with controlled-release chlorpyrifos pellets (Dursban® 10CR) gave 100% control in 2 days. Data were not obtained on the longevity of effectiveness of these pellets.

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