Journal of the American Mosquito Control Association, 11(4):424–427, 1995

FIELD TRIALS WITH METHOPRENE, TEMEPHOS, AND *BACILLUS THURINGIENSIS* SEROVAR *ISRAELENSIS* FOR THE CONTROL OF LARVAL *CULISETA MELANURA*

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ABSTRACT. In 1992, Altosid® (methoprene) pellets, Abate® (temephos) 5 CG, and Bactimos® (*Bacillus thuringiensis* serovar *israelensis*) granules were applied aerially to small field plots within a *Culiseta* breeding swamp. Aspects measured included the deposition and diffusion of larvicides into larval crypts and effects on larval *Culiseta melanura* and nontarget aquatic organisms. Formulations were deposited at approximately 50% of application rates. The presence of methoprene in 8 crypt water samples indicated that this larvicide was able to diffuse into *Cs. melanura* larval crypts. Methoprene was selected for evaluation in a large-scale field trial. In the spring of 1993, methoprene pellets applied to 25% of the study area were an effective larvicide against *Cs. melanura*. The inhibitory effect on pupae exceeded 81% over a 5-wk posttreatment period.

INTRODUCTION

The municipal response to eastern equine encephalitis (EEE) epizootics has traditionally relied on the use of broadscale applications of mosquito adulticides (Grady et al. 1978, Hayes 1981). In central New York, this response has included the application of adulticides to the swamp habitat of *Culiseta melanura* (Coq.) (Morris 1988). Evaluations of spraying efficacy in controlling EEE have produced equivocal results (Grady et al. 1978), and recent data from New York indicate that adulticides were generally ineffective in controlling *Cs. melanura* or EEE virus activity (J. J. Howard, unpublished data).

This study was designed to reinvestigate the feasibility of using larvicides as control strategy for *Cs. melanura* (Hayes 1962). The thick overstory associated with breeding swamps in central New York and the cryptic nature of larval breeding habitat (Woodrow and Howard 1994) precluded the use of liquid larvicide formulations. The goals of the project were to determine 1) if granular and pellet formulations could penetrate to the swamp floor, 2) if active ingredients could diffuse into larval crypts, and 3) the impact of the larvicides on *Cs. melanura* and nontarget organisms.

METHODS AND MATERIALS

Field trials were conducted in the Toad Harbor–Big Bay Swamp complex in the Town of West Monroe, Oswego County, NY. Small-plot field tests were conducted with methoprene, temephos, and *Bacillus thuringiensis* serovar *israelensis* (*B.t.i.*) in 1992. Methoprene was selected for a large-scale field trial in 1993.

1992 methods: Eight 400 × 100-m (4-ha) test plots were designated along accessible edges of the swamp complex in areas with typical *Cs. melanura* breeding habitat. Plots were at least 50 m from each other and established with the long side of the plot parallel with an upland edge. A 400-m longitudinal transect was constructed through the center of each plot 50 m from the upland shore. Five sampling locations were marked at 65-m intervals on the transects. Two treatment plots for each larvicide and 2 control plots were randomly assigned. Altosid® (4% methoprene) pellets and granular formulations of Abate® 5 CG (5% temephos) and Bactimos® (2.5% *B.t.i.*) were applied to treatment plots with a fixed-wing aircraft equipped with a granular spreader at rates of 5, 4, and 10 lb/acre (5.6, 4.5, and 11.2 kg/ha), respectively, on July 1. To facilitate aerial recognition, plot corners were marked with 3 double-fused, white smoke generators (Superior Signal Company, Spotswood, NJ), hooked in series, and suspended from portable 1.5-m platforms. Smoke generators were ignited immediately before application.

Penetration and deposition of larvicides to the swamp floor were determined by collecting pellets or granules in pans located at 5 sites within each treated plot. Temephos and *B.t.i.* were collected in 31.5 × 25.0 × 7.0-cm aluminum pans (Reynolds® Redi-Pan: Reynolds Metals Company, Richmond, VA). Deeper elliptical aluminum pans (Reynolds® Redi-Pan), 44.1 × 33.7 × 7.9 cm, were used to collect the heavier methoprene pellets.

One-liter water samples for residue analyses were collected from the methoprene, temephos, and control plots with a modified chemical

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transfer pump (Woodrow and Howard 1994). Twenty samples, 5 from each treatment and control plot, were collected according to the following schedule. Open-water samples were collected from the temephos and control plots at 4 and 24 h posttreatment and from the methoprene and control plots at 24 and 120 h posttreatment. Larval crypt water samples were collected from the temephos plots at 120 h and from the methoprene and control plots at 120 and 264 h posttreatment. Water samples were collected in 1-liter, wide-mouth, brown glass bottles. Chemical residues were fixed in the water samples by pouring off 100 ml of the sample and replacing it with 80 ml of methylene chloride. The bottles were vigorously shaken for 1 min and refrigerated on wet ice until analyzed at the New York State Department of Environmental Conservation (NYSDEC) Hale Creek Laboratory. Samples were analyzed for temephos on a Tracor 222 gas chromatograph with a flame photometric detector in the phosphorus mode and for methoprene on a Waters 600E liquid chromatograph equipped with a Waters 990 photodiode array detector. Detection limits were 0.2 μg/liter for temephos and 10 μg/liter for methoprene.

Larval density in breeding crypts was monitored by following procedures used in the development of the modified chemical transfer pump. Five 1-liter pump samples were obtained weekly from randomly selected sites within each plot for 4 wk prior to and 5 wk after the application of larvicides on July 1.

Field studies on nontarget animals were conducted in the methoprene, temephos, and control plots by personnel from the NYSDEC, Field Toxicant Research Unit[2] and will not be further discussed in this report.

1993 methods: On May 7, methoprene (Altosid) pellets were applied at the rate of 5 lb/acre (5.6 kg/ha) to 260 ha (1 sq. mi.) of Toad Harbor Swamp. Posttreatment efficacy data on adult emergence inhibition were collected from May 10 through July 1. Cicero Swamp, Town of Cicero, Onondaga County, was the nontreatment area. Collection of adult emergence inhibition efficacy data followed the protocol suggested by Zoecon Corporation[3]. *Culiseta melanura* pupae were collected along accessible edges in the treatment and control areas with the modified chemical transfer pump. Contents of individual breeding crypts were pumped through 30-mesh sieves. The sieve contents were resuspended in plastic wash tubs containing clear swamp water. Pupae were hand-pipetted from the tubs and transferred to 120-ml glass vials fitted with plastic snap caps. Vials were labeled by collection site and date and returned to the encephalitis field station, Town of West Monroe, for observation. At the field station, up to 10 pupae/collection site were placed in 150 ml of distilled water in 250-ml glass beakers labeled by site and date. Beakers were covered with nylon netting held in place with a rubber band to prevent escape of emerging adults. Food for adults was a 10% sugar solution soaked into a cotton ball placed on top of the netting. A lid from a 1-pint ice cream carton was placed on the nylon netting to prevent the cotton ball from drying. Beakers were checked daily and status of pupae or adults recorded until all pupae had died or emerged. The inhibitory effect of methoprene was determined by Abbott's formula (1925):

$$\% \text{ inhibition} = X - Y/X \cdot 100$$

where X = % living in the control site and Y = % living in the treated site.

Analysis of variance (ANOVA) and Student's *t*-test were conducted with version 6.08 of the Statistical Analysis System (SAS Institute 1989) at Syracuse University.

RESULTS AND DISCUSSION

1992: Deposition rates and theoretical concentrations of agents in 4 in. (10.2 cm) of water based on the expected and observed depositions are presented in Table 1. Observed deposition rates for Altosid, Abate, and Bactimos were 40, 55, and 69% of expected values, respectively. All larvicides reached the ground at rates with theoretical concentrations within the toxicity range for mosquito larvae.

Temephos was detected in open water samples but not in crypt samples. The mean concentrations of temephos in open water samples (n = 10) were 13 μg/liter (range, 0–78 μg/liter) and 0.8 μg/liter (range, 0–4 μg/liter) for samples collected at 4 and 24 h posttreatment, respectively. Methoprene was not detected at measurable levels in open water samples but was detected at measurable levels in 2 crypt water samples. One sample collected at 120 h posttreatment had a concentration of 20.9 μg/liter, and the other collected at 264 h posttreatment had a concentration of 233.0 μg/liter. Additionally, trace amounts of methoprene were detected in 6 crypt and 3 open water samples collected at 24 (n = 6), 120 (n = 1), and 240 h (n = 2) posttreatment. The limit of detection for methoprene

[2] Simonin, H. A., E. A. Paul, R. E. Foley, J. Symula, T. Martin and S. Jackling. 1992. The effects of several mosquito larvicides on non-target organisms. Abstract, 13th annual meeting, Society Environmental Toxicology and Chemistry, Cincinnati, OH.

[3] Zoecon Corporation. Fact sheet: protocol for evaluation of Altosid® pellets.

Table 1. Expected and observed deposition rates of aerially applied methoprene (Altosid) pellets and granular temephos (Abate 5-CG) and *Bacillus thuringiensis israelensis* (*B.t.i.*) (Bactimos) to Toad Harbor Swamp on July 1, 1992.

	Methoprene		Temephos		*B.t.i.*	
	Exp.	Obs.[1]	Exp.	Obs.[1]	Exp.	Obs.[1]
Application rate (lb/acre)	5.0	2.0	4.0	2.2	10.0	6.9
Application rate (kg/acre)	2.3	0.9	1.8	1.0	4.5	3.1
Active ingredient (g/acre)	90.9	36.7	95.0	50.8	7.95×10^{8}[2]	5.5×10^{8}[2]
Theoretical concentration 4 in. (10.2 cm) of water (μg/liter/day)	7.38[3]	2.92[3]	231.0[4]	123.8[4]	1,936.0[2,4]	1,354.0[2,4]

[1] Mean of 10 samples.
[2] *Aedes aegypti* international toxic units/acre or liter.
[3] Assuming a constant 30-day release.
[4] Assuming agent completely dissolved.

(10 μg/liter) exceeded both the theoretical concentration (2.92 μg/liter) and the minimum toxicity for mosquito larvae—as low as 0.04 μg/liter for *Aedes aegypti* (Linn.) (Schaefer and Wilder 1973). It is possible that methoprene was present at effective concentrations in the 9 samples with trace amounts.

The detection of methoprene in larval crypts is attributed to the formulation tested. The methoprene was a pellet with a 30-day residual, whereas temephos was a granule with a 24–48-h residual. The sustained release capability of methoprene allowed sufficient time for it to diffuse into larval crypts. Once within the dark larval crypts, methoprene, which photo-degrades (Quistad et al. 1974), would remain active longer than temephos, which degrades by hydrolysis and photolysis (Tinsley 1979).

Table 2. Pooled (n = 10) weekly means [$\log_{10}(n + 1)$] of *Culiseta melanura* larvae collected from the control, *Bacillus thuringiensis israelensis* (*B.t.i.*), and temephos plots June 11–August 7, 1992.

Dates	Week[1]	Control	*B.t.i.*	Temephos
Pretreatment				
June 11–12	1	0.18	—	—
June 15–19	2	0.07	0.57	0.28
June 22–26	3	0.42	0.16	0.39
June 29–31	4	0.09	0.21	0.33
Posttreatment				
July 6–10	5	0.26	0.14	0.25
July 13–17	6	0.45	0.46	0.25
July 20–24	7	0.60	0.16	0.19
July 27–31	8	0.39	0.16	0.29
August 3–7	9	0.32	0.46	0.17

[1] No significant differences within sites for any weekly mean (ANOVA, Duncan grouping, P = 0.05).

Weekly larval density data were transformed [$\log_{10}(n + 1)$]. The analyses of transformed data indicated no significant differences (t-test, P = 0.05) between replicates within treatments except for the 2 temephos plots before July 1. Therefore, the data were pooled by treatment. There was no significant impact of *B.t.i.* or temephos on larval density during any of the weeks (Table 2, ANOVA, Duncan grouping, P = 0.05). The inhibition effect of methoprene on emerging adults could not be determined as no pupae were collected in the weekly pump samples.

The detection of methoprene in larval crypts at levels toxic to mosquito larvae was considered the most significant factor for its selection for a large-scale field trial. Theoretically, a spring application of methoprene targeting overwintering *Cs. melanura* larvae could have a season-long impact on the population of this species. Additionally, there were no nontarget mortality or other effects observed with methoprene (Simonin et al. 1992[2]). Temephos was not found in crypts and did not cause target mortality but was observed to cause mortality to some nontarget invertebrates (Simonin et al. 1992[2]). There was no target mortality with *B.t.i.* and it is unlikely that it would diffuse into crypts before degradation.

1993: Data from the large-scale trial are expressed as weekly (5-day) intervals from the treatment date. Week 1 posttreatment was May 8–12, and week 8 posttreatment was June 28–July 2.

Adult emergence inhibition: From weeks 1 through 5 posttreatment, 254 pupae were collected from the treatment (n = 93) and control (n = 161) areas (Table 3). The inhibitory effect of methoprene was 81.8%. A majority (97%, n = 247) were collected within the first 3 wk posttreatment. The highest mortality from the treatment area was recorded during week 2 posttreat-

Table 3. Emergence inhibition of pupae collected from methoprene treated and non-treated control areas May 8–June 9, 1993.

Posttreatment		Total no. pupae collected	Dead			Emerged live adults[1]
Dates	Week		Pupae	Adults[1]	% dead	
Treatment area (Toad Harbor Swamp)						
May 8–14	1	58	41	8	84.5	9
May 13–21	2	13	13	0	100.0	0
May 22–28	3	19	17	1	94.7	1
May 31–June 4	4	0	—	—	—	—
June 7	5	3	1	0	33.3	2
Total		93	72	9	87.1[2]	12
Control area (Cicero Swamp)						
May 8–14	1	63	6	4	15.9	53
May 15–21	2	37	9	5	37.8	23
May 22–28	3	57	18	3	36.8	36
May 31–June 4	4	0	—	—	—	—
June 9	5	4	2	0	50.0	2
Total		161	35	12	29.2[2]	114

[1] All adults identified as *Culiseta melanura.*
[2] Inhibitory effect of methoprene = 81.8% (Abbott's formula).

ment with 100% mortality occurring in all 13 pupae collected. In the treatment area, mortality averaged 93.1% for the first 3 wk posttreatment. In the control area, mortality averaged 30.1% for this period. The inhibitory effect of methoprene for the first 3 wk posttreatment was 62.9%. The smaller number of pupae collected from the treatment area during posttreatment weeks 2 and 3 may be an indirect measure of the methoprene efficacy as affected pupae would die in the treatment area and could not be collected. We concluded that the application of methoprene in the form of Altosid pellets applied to 25% of an established *Culiseta* breeding swamp was an effective larvicide against *Cs. melanura.*

ACKNOWLEDGMENTS

This research was supported by grant number CO 10447 from the New York State Department of Health and was submitted by R. J. Woodrow in partial fulfillment of the requirements for the M.S. degree from the State University of New York, College of Environmental Science and Forestry, Syracuse, NY 13210. We thank R. A. Wink, J. Oliver, and personnel from the NYSDEC, Rome Field Toxicant Research Unit, for field and laboratory assistance.

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