EVALUATION OF METHOPRENE, TEMEPHOS AND *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* AGAINST *COQUILLETTIDIA PERTURBANS* LARVAE IN MINNESOTA

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ABSTRACT. Temephos, *Bacillus thuringiensis* var. *israelensis (B.t.i.)* and methoprene were tested for larval control of *Coquillettidia perturbans*. Neither temephos nor *B.t.i.* treatments at their maximum recommended dosages consistently reduced larval numbers. Larval emergences were reduced 99% in test plots treated with experimental, controlled release methoprene briquets (Altosid SR–10™). Breeding sites of *Cq. perturbans* in a 5,000 km^2 area were treated using methoprene briquets in the 1984 season. Adult populations in a 7,700 km^2 area were 2.5 times higher in untreated areas than treated areas. Methoprene can effectively control larval populations of *Cq. perturbans*.

INTRODUCTION

Coquillettidia perturbans (Walker) females are noted for their aggressive attack on humans (Armstrong 1941). Eastern equine encephalitis virus (Chamberlain 1957; Howitt et al. 1949) and western equine encephalitis virus (Sekla et al. 1980) have been isolated from *Cq. perturbans* females implicating them as a possible vector of these diseases to horses and humans. But efforts to control larvae of this species using larvicides have been ineffective (E. D. Walker, Michigan State University, Department of Entomology, personal communication). *Coquillettidia perturbans* larvae attach to roots of emergent and floating vegetation (Smith 1908), so control agents must reach roots in all zones of the water column to be effective. Dense vegetation and highly organic conditions in larval habitats also reduce effectiveness of control agents. In Minnesota, vegetation is often consolidated into floating mats and larvae are only accessible through openings that occur in such mats (Batzer and Sjogren, 1986). All these factors hinder larval control efforts. Adult control is difficult because emergence takes place continuously over a 2-month period even though emergence is univoltine (Armstrong 1941, Lounibos and Escher 1983).

We evaluated temephos, *Bacillus thuringiensis* Berliner var. *israelensis (B.t.i.)* and methoprene as larvicides for *Cq. perturbans*. These materials were selected because of their low toxicity to nontarget organisms which is an important consideration for treating marsh habitats. We used commercially available formulations of temephos and *B.t.i.*, but methoprene (Altosid SR-10™) was formulated into experimental controlled release briquets. These briquets differed from Altosid briquets that are commercially available in that they were designed to release methoprene over a 70-day period at a rate of 0.01 gm of methoprene per day compared to the 30-day period and 0.014 gm per day release rate of commercial briquets. We had approval from the Environmental Protection Agency (EPA) to use this new formulation provided the application rate did not exceed the label rate for commercial briquets of 1 briquet per 0.283 m^3 which converts to 0.05 gm of methoprene being released per m^3 per day. The highest rate of methoprene release used for our tests was 0.004 gm/m^3/day or 8% of the maximum dosage allowed. Zoecon Industries (Dallas, Texas) has submitted an application for EPA registration of long duration briquets similar to our experimental formulation. The advantage of long duration briquets is that a site need only be treated once to cover the entire emergence period of *Cq. perturbans*.

MATERIALS AND METHODS

All studies were conducted in cattail (*Typha* spp.) marshes in the Minneapolis-St. Paul area of Minnesota. Temephos and *B.t.i.* trials were conducted in 100^2 ml plots. We devised a statistically validated method to measure larval densities of plots by scraping larvae from roots of randomly selected plants (n = 15) using a screen bottomed dipper (Batzer and Sjogren, in press). Pretreatment and 1 week posttreatment densities of treated plots were compared by analysis of variance (ANOVA) and untreated control plots were similarly monitored. Twelve trials were conducted in May 1983 and 3 trials in August 1982 to obtain data on early instars in warmer water.

We applied temephos (Abate™) formulated as 1% sand at 0.224 kg AI per hectare (0.2 lb./acre) with a hand seeder to 6 plots in stands of emergent cattail. The same rate of 1%

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corncob temephos was applied with the seeder to 2 plots of cattail in floating vegetative mats. We applied aqueous Bactimos™ wettable powder *B.t.i.* with a Hudson sprayer at 480 million AA (*Aedes aegypti*) units AI per hectare to 3 emergent plots and 2 floating plots. Aqueous Teknar™ *B.t.i.* concentrate was hand sprayed at 1,130 million AA units AI per hectare over 1 emergent plot and 1 floating plot.

We tested methoprene in paired 900 m^2 plots with similar larval densities in each of 1 emergent cattail marsh and 3 marshes with cattail in floating vegetative mats. Paired plots were located at least 50 m apart to prevent cross contamination. We randomly selected 1 member of each pair for treatment in late May, 1983 with methoprene (Altosid SR-10™) formulated into controlled release briquets. Each briquet weighed 23.3 gm (3.0% AI) and released 0.01 gm of methoprene per day for 70 days. Briquets were placed every 2.16 m in a grid pattern across plots which resulted in a briquet density of 1 briquet/4.65 m^2. In plots with clumps of floating vegetation, briquets were placed in openings closest to the measured coordinates. Water depths in all plots were between 80–100 cm, so our treatments ranged from 4.3–5.4% of the maximum label dosage. We based our dosage on surface area rather than water volume because we anticipated significant fluctuations in the water level over the subsequent 70 days.

Methoprene controls larvae by preventing adult emergence, so we used emergence cages to monitor effects of the material. Ten pyramid shaped cages of wood and nylon mesh screen which sampled a 1 m^2 surface area were randomly placed in each plot. We placed cages for sampling emergent vegetation over plants and cages for sampling floating vegetative mats over openings in the mats. Some coordinates in floating mats were too treacherous to approach, so the closest feasible locations were then selected.

We removed and counted emerged *Cq. perturbans* adults from ages weekly from June 1 through August 17, although collections were conducted twice weekly during peak emergence. Seasonal totals in paired treatment and control plots were compared using ANOVA.

We then determined optimal dosage rates of methoprene briquet treatments in 1 emergent and 1 floating cattail marsh. We selected eight 100 m^2 plots of similar larval densities in each marsh, 2 plots were randomly selected as controls and each of the remaining 6 randomly assigned a rate of 1 briquet per 3.3, 4.9, 7.5, 11.5, 16.6, or 25.1 m^2 (note: 1 briquet/3.3 m^2 is the highest dosage). Five emergence cages were randomly placed in each plot as previously described. Emerged *Cq. perturbans* were collected from cages weekly from May 15 through September 1. Emergence levels of the plots were compared using an ANOVA followed by Duncan's (1955) multiple range test.

The Metropolitan Mosquito Control District (MMCD) supported a prototype *Cq. perturbans* larval control program in the Minneapolis/St. Paul metropolitan area in 1984. We treated 224 breeding sites (330 ha) identified within a 5000 km^2 area with methoprene briquets between March 15 and June 10. Sites that were difficult or too deep to walk through were treated in March while still frozen over using briquets designed to release 0.01 gm of methoprene per day for 150 days. We used briquets designed to release for 100 days for treatments in May and 70 day briquets for subsequent treatments. Emergent vegetation was treated at a rate of 1 briquet/13.5 m^2 and floating vegetation at a rate of 1 briquet/10.0 m^2. These rates would not exceed label rates for commercial briquets even if the water in a site virtually dried up.

We measured the success of treatments on the ice in 2 plots where the pretreatment emergence levels in 1983 were known and the 1983 larval densities were similar. Emergence for 1983 and 1984 were monitored using emergence cages and levels compared using ANOVA. An indicator of the success of the prototype control program was MMCD monitoring of adult mosquito populations from early May through September, 1984 in a 7700 km^2 area in the Minneapolis/St. Paul vicinity which included the treated area. Mosquito landing counts were collected twice weekly between 800 and 1000 hr by 55 field workers of the MMCD at 220 locations spaced every 6.4 km across the area. Each designated location was a spot in a wooded harborage area and each collector collected all mosquitoes attracted to them over a 2 min period with a battery operated vacuum. Seasonal numbers of *Cq. perturbans* females collected at locations in controlled and uncontrolled areas were compared using Wilcoxon's rank sum test.

RESULTS AND DISCUSSION

Temephos formulated on sand was used in emergent cattail plots to penetrate through debris to roots on the bottom. The floating corncob formulation was used in floating cattail to slow descent of the temephos and allow it to disperse laterally under cattail mats where larvae were concentrated. A significant level of larval control was achieved in only 2 of 8 temephos treatments, and 25–35% of larvae in the two plots still survived (Table 1). One of 7

Table 1. Results of temephos (0.224 kg AI/hectare applications in test plots.

Plot type	Formulation	Mean no. larvae/sample ±SD (n = 15) Pre-treatment	Post-treatment
emergent	1% sand	11.1 ± 6.1	14.3 ± 9.3
emergent	1% sand	21.4 ± 16.8	14.5 ± 9.3
emergent	1% sand	7.4 ± 7.4	5.6 ± 4.2
emergent	1% sand	14.5 ± 9.3**	5.3 ± 5.0**
emergent	1% sand	5.7 ± 4.2**	1.4 ± 1.6**
emergent*	1% sand	18.9 ± 14.3	22.4 ± 15.2
floating	1% corncob	10.5 ± 5.1	10.2 ± 6.9
floating	1% corncob	7.6 ± 4.5	7.8 ± 8.3

* This plot was treated in late August whereas others were treated in May.

** Posttreatment densities are significantly lower than pretreatment levels (F-test, $P \leq .05$).

plots treated with *B.t.i.* resulted in a significant reduction of larvae (Table 2). Temephos and *B.t.i.* were considered unsatisfactory for operational *Cq. perturbans* control.

Seasonal emergences in 4 plots treated with methoprene briquets were 1% of that in paired untreated plots (Table 3). Excellent control occurred over the entire emergence period from early June through mid-August.

In comparing different dosage rates of methoprene, we found a significant increase in emergence when dosage was lower than 1 briquet/11.5 m^2 in plots with floating vegetation (Table 4). All treatments in the emergent test marsh were equally effective (Table 4).

Table 2. Results of *Bacillus thuringiensis* var. *israelensis* treatments in test plots.

Plot type	Formulation*	Mean no. larvae/sample ±SD (n = 15) Pre-treatment	Post-treatment
emergent	Bactimos	9.7 ± 9.5	6.9 ± 6.8
emergent	Bactimos	12.9 ± 11.1	7.9 ± 5.5
emergent	Teknar	14.3 ± 9.3	11.5 ± 8.8
floating	Teknar	10.2 ± 6.9**	4.7 ± 4.1**
floating	Bactimos	8.9 ± 5.8	8.6 ± 5.5
floating	Bactimos***	19.7 ± 14.9	18.3 ± 15.2
emergent	Bactimos***	14.0 ± 8.9	19.1 ± 11.3

* Aqueous Bactimos wettable powder dosage was 0.48 billion AA units AI/hectare. Aqueous Teknar concentrate dosage was 1.13 billion AA units AI/hectare.

** Post- and pre-treatment densities differ significantly (F-test, $P \leq 0.05$).

*** These plots were treated in late August whereas the others were treated in May.

Table 3. Comparison between paired 900 m^2 plots with one randomly treated with methoprene briquets.

Plot type	Mean no. of adults collected/cage ±SD (n = 10)* Treated	Control
emergent	2.0 ± 4.0	123.3 ± 112.4
floating	3.5 ± 4.9	363.2 ± 314.9
floating	12.6 ± 15.7	1492 . ± 1406.4
floating	8.2 ± 7.8	664.7 ± 335.0

* All paired means are significantly different (F-test, $P \leq 0.01$).

The irregular spacings of openings in floating vegetative mats prevent even spacing of briquets, so slightly higher dosages may be required in these sites compared to emergent vegetation. One cage in a plot treated at 1 briquet/3.3 m^2 resulted in 106 adults. This indicates that in rare cases inadequate control may occur even at high dosages, which is not unexpected because of the complexity of larval habitats.

Our efforts to correlate spacing of briquets in the above plots to methoprene levels in water were unsuccessful. Water samples analyzed for methoprene by liquid-liquid extraction followed by gas chromatographic analysis of flame ionization (Schaefer and Dupras 1973) yielded similar readings in all treated or untreated plots and did not match the observed bioassay. Isolation of methoprene from marsh waters is difficult because numerous terpenes similar to methoprene are commonly

Table 4. *Coquillettidia perturbans* emergences in a series of 100 m^2 plots with increasing methoprene briquet densities in two cattail marshes.

Dosage*	Mean no. mosquitoes/cage ±SD (n = 5)** Emergent marsh plots	Floating marsh plots
control	29.8 ± 30.8a	227.8 ± 185.5c
control	36.6 ± 38.0a	181.4 ± 157.7c
1/25.1	3.2 ± 5.0b	77.4 ± 91.2d
1/16.6	0.6 ± 0.9b	65.2 ± 56.0cd
1/11.5	0.4 ± 0.9b	3.8 ± 2.5e
1/ 7.5	0.6 ± 0.9b	8.6 ± 14.4e
1/ 4.9	0.6 ± 0.9b	8.4 ± 9.1e
1/ 3.3	1.8 ± 2.7b	23.4 ± 46.2e

* Dosage is expressed as 1 briquet/m^2 and listed from lowest to highest.

** Means of $\log 10(x+1)$ transformed data followed by the same letter are not significantly different at the $P \leq 0.05$ level (Duncan's (1955) multiple-range test).

present in organic waters (Zoecon Industries, personal communication). Our failure to isolate methoprene requires us to express dosage in the unorthodox scale of one briquet per unit area. Theoretical levels can be extrapolated from the 0.01 gm/day release rate (1 briquet/10 m^3 yields 1.0 ppb), but rapid breakdown of methoprene under natural conditions (Schaefer and Dupras 1973) complicates projections.

The treatments used in the prototype treatment program of 1 briquet/10 m^2 in floating vegetation and 1 briquet/13.5 m^2 in emergent vegetation should have been effective from the findings in Table 4. Population monitoring by the MMCD resulted in a seasonal total of 28.9 *Cq. perturbans* females per location (SD = 52.3, n = 137) in untreated areas which was significantly higher than the 11.4 females per location (SD = 11.0, n = 82) in treated areas (Wilcoxon rank sum test, $p \leq 0.05$). This single season's data show adult populations were 2.5 times higher in untreated areas than treated areas, but significantly more monitoring must be done to accurately assess the effectiveness of regional control. Treatments done on ice showed significant reductions from 123 adult per cage (SD = 110) to 0 adults in one site and from 1492 adults per cage (SD = 1406) to 3.8 adults per cage (SD = 3.3) in the other (F test, $p \leq 0.01$). Treatments on the ice can be an effective way to control sites not practical to treat by walking through them.

The studies completed indicate that methoprene briquets can be a valuable tool to significantly reduce *Cq. perturbans* populations. Our findings indicate that the label rates of currently available briquets should be effective. If the EPA registration application for long duration briquets similar to our experimental briquets is approved, then a single treatment per year will yield season long control for a typical emergence pattern.

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