EFFICACY OF SUSTAINED-RELEASE FORMULATIONS OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* AND METHOPRENE FOR CONTROL OF *COQUILLETTIDIA PERTURBANS* IN INDIANA[1]

EDWARD D. WALKER[2]

Immature *Coquillettidia perturbans* (Walker) attach to the roots of emergent vegetation (Smith 1908), making them difficult to sample, although several sampling methods have been applied (Service 1976, Morris et al. 1985, Walker and Crans 1986, Batzer and Sjogren 1986). Consequently, there have been few larvicide evaluations done for this species. Hagmann (1953) found that dieldrin controlled larvae whereas aldrin, BHC and DDT did not. Yap et al. (1968) generated LD_{50} values in the laboratory for temephos, chlorpyrifos, DDT, malathion, and carbaryl; results suggested that *Cq. perturbans* had higher natural resistance to these compounds than did several other mosquito species. Walker et al. (1986) tested flowable *Bacillus thuringiensis* var. *israelensis* (Teknar®), 1% granular temephos (Abate®), and an emulsifiable concentrate of chlorpyrifos (Dursban® 2E) against *Cq. perturbans* in small scale field trials in northern Indiana, and found no reductions in larval numbers for any of the tests. Sjogren et al. (1986) tested temephos on sand and corncob, *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) as wettable powder and flowable concentrate, and methoprene in slow-release briquets, against *Cq. perturbans* in Minnesota. They found that temephos and *B.t.i.* had no consistent larvicidal effects, whereas methoprene reduced adult emergence in comparison with non-treated plots.

Coquillettidia perturbans is an important pest in St. Joseph County, Indiana, and is a suspected vector of eastern equine encephalitis (EEE) virus there (Walker 1984). This species was also suspected as a vector of EEE virus during an outbreak in southwestern Michigan in 1980 (Francy 1982; H. D. Newson, Michigan State University, personal communication). In 1984 the St. Joseph County (Indiana) Mosquito Abatement Program began a project emphasizing surveillance and larvicide evaluations for *Cq. perturbans* (Walker 1984). The goals of this project were to locate larval habitats and experimentally test larvicides, so that *Cq. perturbans* populations could be controlled to prevent EEE virus transmission to horses and humans in the county. The habitats of *Cq. perturbans* are often environmentally sensitive freshwater marshes; thus appropriate larvicides are those which would cause minimal harm to these habitats. This paper reports results of field tests of sustained-release formulations of *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) and methoprene.

Methoprene was tested in June-July 1985. The methoprene (Altosid® SR-10, Zoecon Corporation, Dallas, TX) was formulated into 19 g sinking charcoal briquets with 3.6% AI (by weight), designed to release methoprene at a rate of 0.01 g per day for up to 70 days. Briquets were obtained from Dr. R. D. Sjogren of the Metropolitan Mosquito Control District, St. Paul, Minnesota. Sjogren et al. (1986) had previously shown these briquets to effectively control *Cq. perturbans*.

The study site for the methoprene test was a farm pond in northwest St. Joseph County, Indiana. This site has a large stand of cattails (*Typha* sp.) with an associated population of *Cq. perturbans* larvae (Walker et al. 1986). Maximum depth was ca. 1 m. Twelve non-contiguous 3.08 × 3.08 m plots were measured in the cattail stand, and 6 plots were randomly assigned to either treatment or control groups. Minimum distance between plots was 10 m. A briquet was placed in the center of each treatment plot on June 13. This dosage was slightly higher than the highest dosage tested by Sjogren et al. (1986). No briquets were placed in the control plots. Emergence traps, 1 per plot, were placed over cattail clumps in the plots on June 16. These traps were 0.61 × 0.61 × 0.61 m polyvinylchloride pipe frames over which midge-proof net tents (BioQuip) were stretched. The frames were kept afloat by square Styrofoam bases. Adult *Cq. perturbans* were collected from some traps using a mouth aspirator on June 26 and 27, but mouth aspiration proved much too difficult a method of collection. Instead, a portable 6-volt-battery powered car vacuum (DELUXE model) was modified into an aspirator for use. Collections were made from the emergence traps using this aspirator on July 10, 12, 16 and 19. This period coincided with the peak period of emergence of *Cq. perturbans* in St. Joseph

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[2] Vector Biology Laboratory, Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556. *Present address*: Department of Entomology, Michigan State University, East Lansing, MI 48824.

County (Walker and Craig 1986), although emergence began before this time and continued after it. Adults were placed into cardboard cups, killed by freezing, and sorted.

The *B.t.i.* test was conducted during October–November, 1985, in a cattail marsh in Granger, northeast St. Joseph County. The formulation used was a floating, sustained-release, Bactimos briquet (marketed by Biochem Products, Montchanin, DE) containing 10% AI by dry weight. The briquets release *B.t.i.* for 30 days and the active ingredient gradually sinks. The recommended rate of application is 1 briquet/100 ft^2 regardless of depth, but higher rates (up to 4 briquets/100 ft^2) are recommended in highly organic situations.

Thirty 3.08 × 3.08 m non-contiguous plots were measured in the site for the *B.t.i.* test. These plots traversed the deepest sections of the site (up to 1 m). The plots were 3 m apart. Ten plots were randomly chosen as control plots, 10 plots were randomly chosen to receive 1 briquet (the labeled dosage), and 10 plots were randomly chosen to receive 2 briquets. The briquets were anchored into the treated plots with flagged metal stakes on October 2–3. Water temperature then was 21.1°C as measured with a soil temperature probe (depth, 0.6 m) and a Yellow Springs Instrument battery-operated temperature gauge.

Larval sampling for the *B.t.i.* test was conducted on November 6, 7, and 8. By this time the briquets had disintegrated. Larvae were sampled from the base of each of 10 cattail stems within each plot with a modified, hand-operated bilge pump (Walker and Crans 1986). The effluent from pump samples from each plot was put into a 16 liter plastic container for laboratory sorting. Samples were poured in small amounts through sieves of 5 × 5 mm, 2.5 × 2.5 mm, and 1 × 1 mm mesh. Larvae trapped on the sieves were collected with a pipette and counted. The 3 treatments were compared using a 1-way analysis of variance (ANOVA).

In the methoprene test, 105 *Cq. perturbans* (15 males, 90 females) were collected from the control plots, and 19 (3 males, 16 females) were collected from the treated plots (Table 1). This represents an 82% difference in emergence between treated and control plots. Earlier treatment in the methoprene experiment may have prevented even the small emergence that did occur into the traps over treated plots. There was a significant difference in total *Cq. perturbans* emergence between treated and control plots (Mann-Whitney *U*-test, $U = 30.5$, $P < 0.05$).

In the *B.t.i.* test, there were a median of 12 larvae in control plots (range, 0–90), 13.5 larvae in plots treated with 1 briquet (range, 0–67), and 9.5 larvae in plots treated with 2 briquets (range, 0–177). All larvae collected were 3rd or 4th instars. There was considerable variation in numbers of larvae among plots. ANOVA on $\log_{10}(X + 1)$-transformed data showed no significant difference in numbers of larvae among the 3 treatments ($F = 0.84$, $P > 0.05$). The results of the *B.t.i.* experiment showed that sustained-release *B.t.i.* was not efficacious as a larvicide for *Cq. perturbans* even though the larvae were presumably exposed for up to 30+ days. Temperatures during the experiment were not low (average high for October 17.4°C, average low for October 7.7°C; National Weather Service, Michiana Regional Airport, South Bend, IN), so low temperature was not a factor affecting the outcome of the experiment. Gut analysis of larvae collected at the beginning and middle of the experiment showed that they were feeding on particulate material and algae. Possibly some aspect of the feeding ecology of *Cq. perturbans* larvae, in relation to the formation of the *B.t.i.* used in the experiment, prevented effective action of the *B.t.i.*.

The 2 experiments reported here are not strictly comparable because in one the number of emerged adults was used whereas in the other numbers of larvae were determined. However, the results of the methoprene experiment contrast with the *B.t.i.* test, since in sustained-release formulation methoprene effectively prevented emergence of *Cq. perturbans*, whereas in sustained-release formulation *B.t.i.* did not effectively kill larvae.

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Table 1. Total *Coquillettidia perturbans* collected from 6 methoprene treated and 6 non-treated 3.08 × 3.08 m plots at Hurwich farm pond, St. Joseph County, Indiana, July 1984.

	Treated plots			Control plots		
	Male	Female	Total	Male	Female	Total
Median	0.5	2.5	3	2	14	16
Range	0–1	0–6	0–7	0–6	0–36	0–42
Total	3	16	19	15	90	105

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