FIELD TEST OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* AGAINST *PSOROPHORA COLUMBIAE* LARVAE IN SMALL RICE PLOTS[1]

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ABSTRACT. A field trial for the control of *Psorophora columbiae* with *Bacillus thuringiensis* var. *israelensis* was conducted in small rice plots. Forty-eight hour mortality increased from 61.5% at a dose of .44 parts per million (ppm) to 100% at a dose of 4.4 ppm when 2nd stage larvae were placed in the plots immediately after treatment. When 3rd and 4th stage larvae were placed in the plots 48 hr after treatment, 72 hr mortality varied from 26.3% at a dose of .44 ppm to 75% at a dose of 8.0 ppm.

INTRODUCTION

A new bacterial mosquito pathogen was recently isolated in Israel (Goldberg and Margalit 1977) and was designated *Bacillus thuringiensis* var. *israelensis*, Serotype 14 of *B. thuringiensis* by H. de Barjac (1978a). Laboratory bioassays with the new agent have demonstrated encouraging biological control potential against several mosquito species (Goldberg and Margalit 1977, de Barjac 1978 a,b,d, de Barjac and Coz 1979), and studies of its pathology and mode of action have been initiated (de Barjac 1978c).

With the objective of evaluating efficacy of *B. thuringiensis* var. *israelensis* against *Psorophora columbiae* larvae under field conditions, a field trial was conducted in small plots planted in rice at the University of Arkansas Rice Branch Experiment Station.

METHODS AND MATERIALS

Nine test plots and 3 control plots (Fig. 1) were used. Each plot was 550 cm square at the water line. Emergent rice about 25 cm tall was growing in a 366 cm square in the center of the plots. Water in the 92 cm wide open area between the rice and the dike averaged 23 cm deep. Depth of water in the rice planted area was about 10 cm. Volume of water in each plot was 5220 liters. Three-fourths of this volume was in the open area between the rice and the dike. The water temperature at midmorning was 24°C, and the pH was 6.8. The water was quite turbid but a quantitative measurement of turbidity was not made.

Unseasonally cool, dry weather prevailed during the test. Prevailing winds were from the northeast rather than from the southwest as was usual for that time of year. Time from hatch to first pupation of the *Ps. columbiae* used in the test was 6 days.

The *B. thuringiensis* var. *israelensis* material tested was a wettable powder formulation, Lot No. 6406–125, an experimental preparation provided by Dr. Terry Couch of Abbott Laboratories. The applications were quantitated as parts per million (ppm) (weight/volume). Two plots were treated at 0.44 ppm; 3 plots were treated at 0.88 ppm; 3 plots were treated at 4.4 ppm; and 1 plot was treated at 8.0 ppm. The material to be applied to each plot was weighed on an electronic bal-

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Figure 1. Small rice plots used for field test of *Bacillus thuringiensis* var. *israelensis* against *Psorophora columbiae*.

ance. Sixteen hours before application each aliquot was thoroughly mixed with one liter of well water in a blender for 1 min. The mixture was held in plastic bottles at 4°C overnight. The following morning the bottles were examined carefully for settling of the material. Only in the container with the highest dose was there faint visual evidence of settling. All containers were vigorously shaken by hand for 30 seconds before the material for each plot was further diluted to 4 liters.

The material was applied with a new stainless steel, 3 gal capacity compression sprayer equipped with a pressure gauge and an adjustable jet. The sprayer was pumped to a pressure of 30 psi, and the time required to spray out one liter was determined. Approximately the first liter of each treatment was applied to the rice-covered area of the plot by timing the application, and the remaining 3 liters were applied to the open area between the rice and the dike. Thus, the amount of material applied to each area of the plot was proportional to the volume of water in that area. The material was applied as a coarse spray to avoid wind drift. The nozzle of the sprayer was held below the top of the rice. The 100 mesh strainer in the hand-piece of the sprayer was removed to avoid clogging. About 12 min were required for actual treatment of each plot.

Immediately following treatment of each plot, containers for holding mosquito larvae were attached to stakes in the plot. The containers were sections of white polyvinyl chloride irrigation pipe 30 cm long by 10 cm diameter. A nylon screen of about 35 mesh was glued to the bottom. Just above the bottom on opposite sides of the containers were three 1 cm diameter screened holes to facilitate flow of water through the container. Four containers were placed in each test plot. One was placed in the center of the rice and 3 were placed in the open area between the rice and the dike but at the edge of the rice. One of the latter group was at the northeast corner of the rice, and the remaining 2 were one-third of the way around the rice on the south and west sides, respectively. Four containers for larvae were used in each plot to test the possibility that position, either in the rice or in relation to the prevailing wind, would influence results. One larval container was placed at the edge of the rice in each of the 3 control plots.

Larvae for the test were acquired by damming a dry roadside ditch and flooding it with water. This produced a generation of larvae fairly homogeneous in age and size. Ten second stage *Ps. columbiae* larvae approximately 36 hr old were placed in each cup immediately after treatment for Test I. Cheese cloth covers were placed over the cups. Mortality counts were made after 6, 24 and 48 hrs. After 48 hrs, the first group of larvae was removed, and Test II was initiated by replacing them with late 3rd and early 4th stage larvae from the same hatch as used for Test I. Mortality counts were made on Test II larvae at 24, 48 and 72 hrs. During the 2nd test, some pupation occurred.

RESULTS

Of the 3 groups of 10 larvae used as controls in each of the 2 tests (total of 60 larvae), 5 larvae (8.3 percent) died. The percentages of mortality given below for the treated plots are uncorrected for control mortality.

In Test I (Table I), 48-hr mortality increased from 61.5% at a dose of 0.44 ppm to 100% at a dose of 4.4 ppm, when 2nd stage larvae were placed in the plots immediately after treatment. Ninety-five% mortality was achieved at 0.88 ppm. In Test II (Table I), 72 hr mortality varied from 26.3% at a dose of 0.44 ppm to 75% at a dose of 8.0 ppm. Mortality increased with dose and duration of exposure in both tests. There was considerable variability in mortality among the plots treated with the same dose. This cannot be explained.

Mortality occurring during Test II, during which older larvae were used, was markedly less than in Test I. Bioassay studies still underway in our laboratory indicate that older larvae are less susceptible to the agent (unpublished data). Although residual activity of the agent for several days was detected in tests conducted against *Culex quinquefasciatus* in

Table 1. Percentage mortality in *Psorophoro columbiae* treated with *Bacillus thuringiensis* var. *israelensis*. (40 larvae per plot).

Dose	0.44 ppm		0.88 ppm			4.4 ppm			8.0 ppm
Plot	1	2	1	2	3	1	2	3	1
Test 1 (Second Stage Larvae)									
Mortality									
6 hr	2.5	20.0	17.5	45.0	67.5	100	100	100	100
24 hr	27.5	76.5	85.0	87.5	100	100	100	100	100
48 hr	42.5	80.0	85.0	100	100	100	100	100	100
Summary	61.5		95.0			100			100
Test II (Third and Fourth Stage Larvae)									
24 hr	0	20.0	5.0	22.5	7.5	35.0	20.0	0	45.0
48 hr	2.5	40.0	35.0	45.0	32.5	57.5	50.0	5.0	60.0
72 hr	5.0	47.5	35.0	52.5	40.0	67.5	57.0	12.5	75.0
Summary	26.3		42.5			45.8			75.0

Panama (personal communication, Lieutenant Colonel Ray Parsons and Dr. James Nelson), the factors of increased age of test larvae and reduced activity of the agent should account for the reduced mortality in Test II.

During the experiment, a total of 180 larvae were treated in cups at each of the 4 locations within the plots. Mortality among all larvae treated at each location was: center cups = 66.1%; northeast corner cups = 66.1%; west side cups = 63.9%; south side cups = 70.5%. Mean mortality in all cups at all positions was 66.7%. There was a slight suggestion that greater mortality occurred at the down wind position (south side). Larvae positioned within the rice did not appear to have less mortality than larvae in more exposed positions.

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