FIELD APPLICATION OF A BACTERIAL INSECTICIDE[1]

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ABSTRACT. *Bacillus sphaericus* strains SS11-1, 1404-9 and 1593-4 were applied to field populations of *Culex nigripalpus* larvae. Field populations of *Psorophora columbiae* larvae were treated with strain 1593-4. In the 3 experiments larval populations were reduced by nearly 90% when the bacterial suspensions were applied in 3.1×10^{-4} to 1.9×10^{-3} dilutions of whole preparation. Bite count data suggest a reduction in adult emergence due to the treatments.

INTRODUCTION

The insecticidal ability of *Bacillus sphaericus* strains SS11-1, 1404-9, and 1593-4 against mosquito larvae has previously been demonstrated in bioassays of laboratory-reared and field-collected larvae. Singer (1975) reported that the SS11-1 strain isolated from India was more active against *Culex* species than *Aedes aegypti* and *Anopheles* species. Strains 1593-4 from Indonesia and 1404-9 from the Philippines were shown to be insecticidal against *Culex* species and were more active against *Anopheles* species than was the SS11-1 of the earlier investigation (Singer and Murphy 1976). In a recent study, field-collected larvae of *Psorophora columbiae* and *Culex nigripalpus* were shown to be susceptible to all 3 strains of the *Bacillus* (Ramoska et al. 1977).

Results of the field application of *Bacillus sphaericus* against populations of *Cx. nigripalpus* and *Ps. columbiae* are reported here.

METHODS AND MATERIALS

The *Bacillus sphaericus* strains SS11-1, 1404 and 1593 (Singer 1976) used in this study were prepared at the University of Western Illinois and shipped to Southwest Florida where all tests were conducted. The preparation of *B. sphaericus* contained between 3.3×10^{10} and 4.2×10^{10} viable cells per ml.

The field testing in this investigation was performed on wild populations of *Ps. columbiae* and *Cx. nigripalpus* larvae between the months of May and September, 1976 in Lee County, Florida. Three field tests were performed, the first, experiment 'A' was performed on a roadside ditch which was approximately 100 m long and contained approximately 7.5×10^4 liters of water. Experiment 'B' took place in a flooded field depression .15 m deep. It contained 1.9×10^3 liters of water. The third field test, experiment 'C' was also performed on a field depression. It contained 5.7×10^2 liters of water. Experiment 'A' was performed on naturally occurring field populations of *Cx. nigripalpus* larvae while 'B' and 'C' were performed on wild *Ps. columbiae* larvae. Untreated control sites were chosen for all experiments. Each control site was of the same general size and shape and contained the same mosquito species and ratio of instars as the respective treated site.

Applications of the preparations were made using undiluted bacterial suspensions in a 12-liter hand compression portable sprayer calibrated to deliver 1000 ml per minute at working pressure of 12 to 15

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psi. Treatments were made holding the nozzle tip approximately 1 m above the water surface and uniformly dispersing the preparation over the entire surface area of the water.

The concentration of larvae in treated and control plots was estimated by counting the number of larvae per instar collected in from 20–40 300 ml water samples. These samples were randomly taken from all areas of the plot. In this manner the average number of larvae per unit volume (300 ml) of water was calculated. Post treatment counts were made at 24, 30 and 48 hr after application of the pathogen.

Bite counts were obtained by counting the number of mosquitoes alighting on the exposed arms of a person in a 1 min period. Bite counts were taken after dark.

RESULTS AND DISCUSSION

As indicated by the data in Table 1, the field applications of *B. sphaericus* were quite successful in reducing larval populations in the three tests. It is significant to note that the preparations used in the experiments were titrated for bacterial viability 1 to 2 weeks prior to field testing and may have lost some potency due to storage.

In experiment 'A', larvae per dip counts were reduced from 24.5 to 2.7 in 48 hours. This represents a reduction of 89% in larval population. In the same time frame, the control ditch went from 25.9 larvae per dip to 34.7, an increase of 34% indicating that eggs were hatching in the ditch during the course of the experiment. The instar ratios shown in Table 2 indicates which instars were affected most heavily by the application. While the pre-treatment ratio and post-treatment control ratio of instars remained nearly identical, the ratio of instars between the treated and post-treatment control showed that the number of 2nd and 3rd instars dropped dramatically in the treated ditch, while the number of 4th instar larvae increased by nearly 50%. This increased ratio is the result of the earlier instars' higher susceptibility to the bacteria (Ramoska et al. 1977). The lower susceptibility of 4th instar larvae accounts for a larger 4th instar ratio in the treated larval populations (Table 2). No significant change between the number of 1st instar larvae in the treated and control tests indicated that they were continually hatching during the course of experiment 'A'. Since live post-treatment larvae could not be found in experiments 'B' and 'C', no such instar analysis could be performed on them. The instar ratios of the control areas were taken at a 48-hour interval though, and these demonstrated the synchronous instar progression that is typical of floodwater mosquitoes.

The site for experiment 'A' lent itself particularly well to bite count analysis, since it was located in an area isolated from other actively breeding sites. The data graphed in Fig. 1 show that after applica-

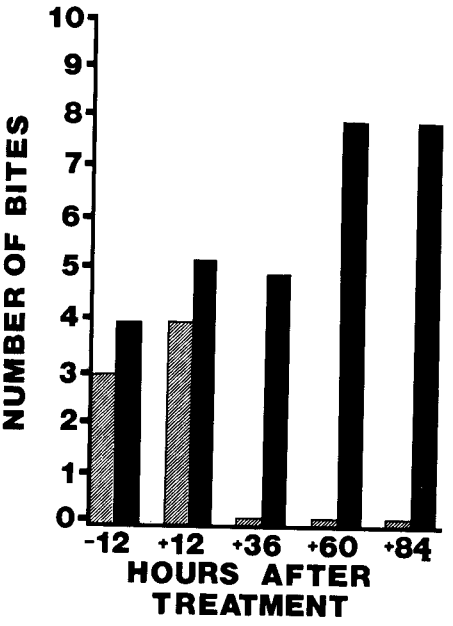

Fig. 1. One-minute bite counts obtained near *B. sphaericus* SSII-1, 1404 and 1593 treated (striped) and control (solid) field *Culex nigripalpus* populations. Each count is the average of 3 trials taken consecutively at one minute intervals.

Table 1. Results of field tests of *B. sphaericus* strains on larvae of *Psorophora columbiae* and *Culex nigripalpus*.

Experiment	Bacterial Preparation	Preparation Viable Cell Count	Dilution of Preparation in Test Site	Average Number of Larvae Per Dip[1]				% Reduction	
				Pre-Treatment		48 hr Post-Treatment			
				Treated $\bar{x}\pm s_{\bar{x}}$[2]	Control $\bar{x}\pm s_{\bar{x}}$	Treated $\bar{x}\pm s_{\bar{x}}$	Control $\bar{x}\pm s_{\bar{x}}$	Treated	Control
A	1593+404 +SSII-1	3.3×10^{10}	3.1×10^{-4}	24.5±10.5	25.9±12.5	2.7±9.2	34.7±17.2	89%	34% increase
B	1593	4.2×10^{10}	1.9×19^{-3}	9.0± 4.5	6.8± 4.0	0.0	8.4± 4.1	100%	24% increase
C	1593	2.9×10^{9}	4.7×10^{-3}	21.7± 5.1	10.4± 4.3	0.0	11.9± 4.2	100%	14% increase

[1] Dip=300 ml H_2O.
[2] $s_{\bar{x}}$=Standard error of the sample mean.

Table 2. Analysis of differences in instar ratio on *Culex nigripalpus* field populations treated with *B. sphaericus* strains 1593, 1404 and SSII-1.

Experiment	Instar	Avg. frequency of occurrence of each instar[1]			Percent of change in frequency of occurrence of each instar		
		24 hr Pre-Treat	48 hr Post-Treat	Control Post-Treat	Pre-Treat Minus Post-Treat	Pre-Treat Minus Post-Treat Control	Pre-Treat Minus Post-Treat Control
A	1	5%	5%	3%	+2%	−2%	0
	2	27%	15%	31%	−12%	+16%	+4%
	3	30%	15%	29%	−15%	+13%	−2%
	4	34%	60%	33%	+26%	−27%	−1%
	P	6%	5%	4%	−1%	−1%	−2%

tion of the bacteria to field populations of *Cx. nigripalpus* larvae, bite count numbers decreased. In the control site, on the other hand, an increased number of bites was recorded over the same time period. Both the 'A' site and its control site were well isolated spatially from other mosquito breeding areas and for that reason the bite count data are accurate indicators of adult emergence from each respective site. The other experiments did not lend themselves to such an assay since their controls were close to the treated ponds.

In experiment 'B' (Table 1) no living larvae were found in the treated pool 48 hr after treatment with strain 1593, while the control plot showed an increase of 24% during the same 48-hour period. Since the larvae treated in this experiment were 3rd and 4th instars (Table 2), it is unlikely that this increase is the result of hatching of larvae as was the case in experiment 'A' but due to evaporation of water in the test and control pools. This loss of water volume resulted in a larger number of larvae per dip in the control pool.

In the final experiment ('C'), no living larvae were found in the treated pond 30 hr after application of *B. sphaericus* strain 1593. The dead larvae found in the treated pool were brought into the lab and bacterial infection was confirmed microscopically. Moreover, experimental infection of healthy larvae in laboratory tests using the dead larvae as inoculum was accomplished within 24 hr.

It should be noted in Table 2 that the standard error figures for Experiment 'A' are approximately twice as high as those for Experiments 'B' and 'C'. This is due principally to the propensity of *Cx. nigripalpus* larvae to aggregate. Although this aggregation occurs in *Ps. columbiae* also, it is not as pronounced. Hence, these larvae are relatively more evenly dispersed than are the *Culex*.

CONCLUSION

B. sphaericus is capable of reducing populations of *Ps. columbiae* and *Cx. nigripalpus* larvae under field conditions. Its speed of activity can prevent mid- to late-instar larvae from pupating and emerging into adults. The two assets of efficacy and rapid action make *B. sphaericus* a promising candidate for biological control of mosquitoes in Southwest Florida.

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