EFFICACY OF *BACILLUS SPHAERICUS* AND *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* FOR CONTROL OF *CULEX PIPIENS* AND FLOODWATER *AEDES* LARVAE IN IOWA[1]

WILLIAM J. BERRY,[2] MARK G. NOVAK,[2] SAYADETH KHOUNLO,[2,3] WAYNE A. ROWLEY[2] AND GARY L. MELCHIOR[4]

ABSTRACT. Granular and flowable concentrate formulations of *Bacillus thuringiensis* var. *israelensis* provided a 90–100% reduction in *Aedes vexans* and *Culex* spp. larvae in natural larval habitats. A briquet formulation of *B. thuringiensis* was less effective, providing a 12–76% reduction. No residual activity occurred in sites treated with *B. thuringiensis*. Granular formulations of *Bacillus sphaericus* (2.78–8.42 kg/ha) caused a 100% reduction in *Culex pipiens* larvae in natural sites and artificial pools. *Bacillus sphaericus* also controlled (84–98% reduction) a mixed population of *Aedes trivittatus* and *Cx. pipiens* in subplots of a retention pond. In field sites, *B. sphaericus* continued to control *Cx. pipiens* larvae for 96 hr.

INTRODUCTION

Microbial larvicides for mosquito control have been identified and developed in the last 10 years. Since its discovery *Bacillus thuringiensis* var. *israelensis* (serotype H-14) has been tested as a mosquito larvicide in numerous laboratory and field studies against several genera of mosquitoes (Sun et al. 1980, Lacey and Singer 1982, Mulla et al. 1980). A major drawback of *B. thuringiensis* has been a lack of residual activity (Sebastien and Brust 1981, McLaughlin et al. 1982). Recently *Bacillus sphaericus* has been shown to have mosquito larvicidal activity (Lacey and Singer 1982, Mulla et al. 1984). Spores of *B. sphaericus* have been shown to persist for months in larval habitats and cadavers. Larvicidal activity reoccurs if the spores are resuspended (Hertlein et al. 1979, Des Rochers and Garcia 1984). Reportedly, this bacterium is most effective against *Culex* spp. and *Psorophora* spp. with variable activity against *Aedes* spp. *Aedes melanimon* Dyar (Mulla et al. 1986), *Ae. pseudoscutellaris* (Theobald) and *Ae. polynesiensis* (Gardner et al. 1986) and others are susceptible, but *Aedes aegypti* (Linn.) (Lysenko et al. 1985) and *Ae. sierrensis* (Ludlow) (Mulligan et al. 1978) seem unaffected by *B. sphaericus*.

This study was designed to evaluate the efficacy of 9 commercial formulations of *B. thuringiensis* and 2 formulations of *B. sphaericus* as larvicides against common mosquito larvae in central Iowa.

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[2] Department of Entomology, Iowa State University, Ames, IA 50011-3222.

[3] Present Address: 2026 Highwood Drive, Garland, TX 75041.

[4] Abbott Laboratories, 51 Darlyn Drive, Bowling Green, OH 43402.

MATERIALS AND METHODS

Formulations used in this study were provided by Biochem Products, Montchanin, DE 19710; Zoecon, Dallas, TX 75234; and Abbott Laboratories, North Chicago, IL 60064. Products were applied at recommended rates based on potency. Corn-cob granular (2 sizes) and flowable concentrate (FC) formulations were evaluated. Small granules were designated for a 10/14 mesh size and large granules were for a 5/8 mesh size. Additionally, Bactimos® 30-day sustained release briquets were included in the study.

Tests were conducted in: 1) artificial pools, 2) field sites and 3) subplots of a large lagoon. Where practical, granular formulations were applied with a hand-held fertilizer spreader (Ortho Whirlybird®). When amounts to be applied were too small to be manageable, product was mixed with blank granules of the same size to provide a greater volume. Flowable concentrate formulations were mixed with water, to provide greater working volumes, and applied with a hand-held plastic insecticide sprayer (Green Thumb®). Briquets were applied manually.

Artificial pool studies of *B. sphaericus* efficacy were conducted in 200-liter (1 m diameter) children's wading pools maintained under a wood frame screenhouse with a clear plastic roof. The pools were lined with black plastic, and a 2.5 cm layer of washed coarse sand was spread on the bottom. Pools were filled with 100 liters (15–20 cm depth) of tap water and allowed to settle for at least 12 hr before the addition of larvae. *Culex pipiens* Linn. larvae were collected from a waste retention pond at the swine research unit affiliated with Iowa State University. Larvae (2–4 instar) were counted into groups of 500 and released into the pools. Larvae were allowed to acclimate for 6–12 hr before products were applied. Pretreatment samples (10 dips/pool) were taken by the standard dipper count method. *Bacillus sphaericus* (Strain 2297 Serotype 25) granular formulations were applied at rates of

2.78 and 5.56 kg/ha evenly over the surface of the pool. Each treatment was replicated 2–3 times in each of 3 trials. Posttreatment samples (10 dips/pool) were taken at 24-hr intervals for 72 hr or until 100% mortality occurred. Water temperature in the pools varied from 20 to 40°C, depending on ambient weather conditions. Food was not provided in the pools, though organic material that fell in (grass clippings, insects, etc.) was not removed.

Field trials were conducted in natural mosquito larval habitats, principally ditches and culverts, in or near Ames, IA. Products were randomly assigned to sites and each product was applied to a minimum of 4 different sites. Vectobac G® (200 ITU/mg) was applied at a rate of 5.56 kg/ha and ABG-6197 and ABG-6199 (400 ITU/mg) were applied at 2.78 kg/ha. Formulations were applied at rates of 296 or 593 ml/ha depending on potency. Bactimos briquets were applied at the rate of one briquet per 9.3 sq m. Pretreatment larval counts (10 dips/site) were made at each site before application of the product. Representative samples of larvae from each site were identified to species. Posttreatment counts were taken at 24 hr intervals or until 100% mortality occurred. Plots were examined for reinfestation through 120 hr posttreatment. Larvicidal activity was determined by comparing the number of live larvae in posttreatment samples with the number present in the pretreatment samples. Larvae that were not killed were also identified. Products were evaluated against floodwater *Aedes vexans* (Meigen), and against *Cx. pipiens* and *Cx. restuans* Theobald larvae.

Bacillus sphaericus was also evaluated in field sites. Large and small granular products (ABG-6185A and ABG-6185B) were applied at rates of 2.78, 5.56 and 18.42 kg/ha to sites containing *Culex* larvae. Pre- and posttreatment samples were taken as described.

Subplot trials with *B. sphaericus* were carried out in a 1 ha lagoon with a maximum depth of 0.5 m. The lagoon was primarily fed by overflow water from the Ames Water treatment facility and was richly organic. Subplots were separated from the pond by 1-m diameter chicken wire frames covered with a double layer of heavy black plastic. These were sunk 2–5 cm into the mud on the bottom of the pond. Three groups of 6 subplots were set up. Each was inhabited by natural populations of larvae (*Aedes trivittatus* (Coquillett) and *Cx. pipiens*). Pretreatment larval counts (5 dips/plot) were made. Subplots were randomly assigned to each of 6 treatments with each treatment replicated in each group of subplots. *Bacillus sphaericus* (large and small granules) was applied at 2 rates (2.78 and 5.56 kg/ha) and *B. thuringiensis* (Vectobac-G) was applied at the rate of 5.56 kg/ha. The sixth subplot was an untreated control. Posttreatment counts were taken at 24 hr intervals through 120 hours. Water temperature ranged from 17 to 20°C.

RESULTS

Culex pipiens larvae were controlled by both granular formulations of *B. sphaericus* when applied at rates of 2.78 and 5.56 kg/ha (Table 1) in artificial pools. Mortality (ca. 100%) was observed within 24 hr. Complete reduction of larvae occurred after 24 hr at both rates when the small granular formulation was applied. With few exceptions, 100% mortality was also observed after 24 hr in pools treated with the large granules (ABG-6185A). In every test, 100% mortality occurred within 48 hr. *Culex pipiens* pupae were observed in control pools after 48 hr.

All formulations of *B. thuringiensis* were effective (ca. 100% reduction in the number of larvae) in field sites with the exception of Bactimos briquets (1–76% reduction) (Table 2). Sites containing *Culex* larvae were frequently reinfested 48 hr after treatment with *B. thuringiensis*. Newly hatched larvae were responsible for the observed reinfestation.

Table 1. Efficacy of granular formulations of *Bacillus sphaericus* against *Culex pipiens* in artificial pools.

Product[1]	Rate (kg/ha)	Mean no. larvae/dip (±SE)[2] 0 hr	24 hr	48 hr	Mean percent reduction (24 hr)	Corrected % reduction[3]
ABG-6185A	5.56	6.5(±1.9)	0.02(±0.03)	0	97	94
	2.78	6.2(±1.3)	0.03(±0.04)	0	95	90
ABG-6185B	5.56	5.4(±1.1)	0	0	100	100
	2.78	5.6(±0.8)	0	0	100	100
Control	—	7.5(±1.6)	3.8(±0.9)	4.5(±0.8)	51	

[1] ABG-6185A was a large granule formulation and ABG-6185B was a small granule formulation.
[2] Values are means for 6 pools (10 dips/pool) with the exception of the control (3 pools) and ABG-6185A at 0.46 kg/ha (7 pools).
[3] Corrected with Abbott's formula (Abbott 1925).

Bacillus sphaericus provided effective control of *Cx. pipiens* larvae within 24 hr in field sites (Table 3), and residual activity continued through 96 hr, when many of the sites were dry. Reinfestation did not occur in sites treated with either granular formulation of *B. sphaericus* during the 96-hr test period and additional samples taken in the sites that still contained water (120 and 144 hr) indicated that no reinfestation had occurred.

Both formulations of *B. sphaericus* effectively controlled mosquito larvae in subplot trials (Table 4). A mixed population of *Ae. trivittatus* and *Cx. pipiens* larvae was present in the plots. Greater than 90% reduction in the number of larvae (both species) occurred within 48–72 hr.

Table 2. Field trials of *Bacillus thuringiensis* var. *israelensis* in Iowa in 1986.

Formulation	Principal species (No. sites)	Mean no. larvae/dip[1] 0 hr	24 hr	48 hr	Mean % reduction (24 hr)	Corrected % reduction[2]
Bactimos briquet	*Ae. vexans* (4)	30(±12)	23(±12)	13(±2)	24	1
	Cx. pipiens (1)	18.8	11.3	4.5	40	76
ABG-6188	*Ae. vexans* (1)	9.9	0.1	0	99	100
	Cx. pipiens (4)	10(±5)	0.4(±0.3)	0.2(±0.1)	96	96
	Cx. tarsalis (1)	10.7	0.8	3.6	93	93
ABG-6138	*Cx. pipiens* (3)	5.6(±1.9)	1.9(±1.7)	2.3(±1.3)	76	76
	Ae. vexans (1)	15.3	0	0	100	100
ABG-6193	*Ae. vexans* (2)	4.0(±0.2)	0	0	100	100
	Cx. pipiens (1)	18.4	0.1	6.6	99	99
	Cx. restuans (1)	23.2	0.8	0	97	97
Teknar	*Ae. vexans* (3)	6.1(±2.5)	0.1(±0.1)	0	98	97
HPD	*Cx. pipiens* (3)	38(±26)	5(±7)	8(±10)	87	87
ABG-6197	*Cx. pipiens* (3)	7(±3)	1.0(±1)	2(±3)	86	86
ABG-6199	*Ae. vexans* (1)	9.9	0	0	100	100
	Cx. pipiens (3)	7.1(±1.2)	1.1(±0.25)	3(±3)	85	85
Control	*Ae. vexans* (3)	18(±6)	14(±4)	14(±3)	23	—
	Cx. pipiens (4)	14(±8)	16(±17)	21(±11)	0	—

[1] 10 dips/site.
[2] Corrected with Abbott's formula (Abbott 1925).

Table 3. Field trials of *Bacillus sphaericus* against *Culex pipiens* in central Iowa.

Formulation[1]	Rate (kg/ha) (No. trials)	Mean no. larvae/dip[2] 0 hr	24 hr	48 hr	Mean % reduction (24 hr)
ABG-6185A	2.78(4)	20(±10)	0.3(±0.6)	0	99
	5.56(4)	24(±7.4)	0.4(±0.5)	0	98
	8.42(3)	10(±3.1)	0.2(±0.2)	0	98
ABG-6185B	2.78(1)	8.4	0.5	0	94
	5.56(4)	21(±9.9)	0	0	100
Control	—(4)	14(±8.6)	16(±16.5)	21(±11.0)	0

[1] ABG-6185A was a large granule formulation and ABG-6185B was a small granule formulation.
[2] 10 dips/site.

Table 4. Evaluation of *Bacillus sphaericus* and *Bti* (Vectobac G) against *Culex pipiens* and *Aedes trivittatus* in subplot trials.

Formulation	Rate (kg/ha)	No. of larvae/dip[1] 0 hr	24 hr	48 hr	72 hr	% reduction 24 hr	48 hr	72 hr
ABG-6185A	2.78	51	25	9	4	50	81	92
	5.56	74	18	6	12	76	93	83
ABG-6185B	2.78	42	32	10	1	24	77	98
	5.56	60	13	7	5	79	89	92
Vectobac G	5.56	97	31	44	15	69	55	84
Control	—	83	41	74	59	51	11	29

[1] Larval counts are means of 3 plots, 5 dips/plot.

There was not a concomitant reduction in the number of larvae in untreated control plots. Plots treated with Vectobac-G were reinfested after 48 hr. Early instar *Cx. pipiens* larvae also appeared in plots treated with B. sphaericus after 48 hr, but it was not determined if these larvae died in later instars.

DISCUSSION

Bacillus sphaericus provided extremely effective control of *Cx. pipiens* larvae in all situations. This microbial larvicide seems to have at least some residual activity, particularly in highly organic sites with large numbers of larvae (Hertlein et al. 1979, Des Rochers and Garcia 1984). In artificial pools, a lack of food may have enhanced *B. sphaericus* activity (Ramoska and Pacey 1979) leading to 100% control in 24 hr at low application rates. Nevertheless, 100% control also occurred in field sites within 24 hr of treatment. *Bacillus sphaericus* is effective against a number of genera of mosquitoes, including *Culex* and *Psorophora* (Mulla et al. 1984, 1985; Lacey and Singer 1982), but some species of *Aedes* larvae are not susceptible to *B. sphaericus* (Lysenko et al. 1985, Mulligan et al. 1978). An 80 to 90% reduction in the number larvae occurred in the subplot tests. The larval population in these plots consisted of 60% *Cx. pipiens* and 40% *Ae. trivittatus.* Though remaining larvae in the plots (after treatment) were *Ae. trivittatus*, up to 90% reduction occurred in some plots indicating that this species is somewhat susceptible to *B. sphaericus*. It would be valuable to test *B. sphaericus* against other floodwater species such as *Ae. vexans.*

All formulations of *B. thuringiensis* were effective at controlling mosquito larvae in central Iowa, with the exception of Bactimos® briquets. No attempt was made to anchor the floating briquets, and they were noted to drift to one end of the site, depending on wind direction. Few or no larvae were detected in the vicinity of briquets, but many were often detected in other locations within the site. Incomplete coverage caused by drifting briquets is the probable explanation for this failure. Briquets may have been more effective in containers or catch basins where movement would be restricted. Both floodwater (principally *Ae. vexans*) and stagnant water (principally *Cx. pipiens*) mosquito larvae were controlled by *B. thuringiensis*. Similar results have been observed by numerous other authors. Rapid (24–48 hr) reinfestation of stagnant water sites by *Cx. pipiens* is a significant drawback of *B. thuringiensis* in community mosquito control programs. Sites must be treated frequently throughout the mosquito season until long-lasting formulations of *B. thuringiensis* are developed (McLaughlin et al. 1982, Sebastien and Brust 1981). This already important agent could become invaluable by the development of such a formulation.

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