EVALUATION OF *BACILLUS SPHAERICUS* 2362 AGAINST *CULEX QUINQUEFASCIATUS* IN SEPTIC DITCHES[1]

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ABSTRACT. Four application rates of *Bacillus sphaericus* strain 2362 were tested for efficacy in septic ditches against *Culex quinquefasciatus* 2nd–4th instar larvae. Trials were conducted over a 2-year period. In 1987, all dosages applied to dairy effluent ditches resulted in substantial reductions after 48 h. Differences among dosages did not differ significantly ($P \geq 0.05$). After 5 days, mosquito larval numbers increased with dosage rates of 0.6, 0.9 and 1.2 liters/ha. Residual control was maintained, however, at 2.4 liters/ha for 17 days. In 1988, *B. sphaericus* applied to domestic sewage effluent ditches at rates of 1.2 liters/ha, and 2.4 liters/ha induced >79% suppression for a period of 15 days, and the 1.2 liters/ha rate induced >79% suppression for a period of 20 days. A rate of 0.9 liters/ha provided good to excellent control (>88%) for a period of 10 days. A rate of 0.6 liters/ha produced <50% suppression after 48 h.

INTRODUCTION

Bacillus sphaericus Neide persists in aquatic environments for long periods. Des Rochers and Garcia (1984) demonstrated that *B. sphaericus* 2362 could persist and remain toxic in a variety of water types for up to 30 days, but primary sewer water (80 mg/liter suspended solids) was detrimental to bacterial persistence when compared with secondary sewer water (12 mg/liter suspended solids) and spring water. Similarly, Mian and Mulla (1983) and Mulla et al. (1984) reported that the activity of *B. sphaericus* was adversely affected by high levels of pollution in water. Mulligan et al. (1980) evaluated the effects of microbial activity and suspended solids in raw sewage on *B. sphaericus* 1593-4. They found that raw sewage, particularly the suspended solids fraction, greatly reduced the efficacy of the pathogen after 28 days as compared with dairy effluent and tap water. Moreover, *B. sphaericus* settled to the bottom of street catch basins and did not infect larvae unless resuspended into the feeding zone (Mulligan et al. 1980). Pathogen activity has been shown to be inversely related to the amount of food available to mosquito larvae (Ramoska and Pacey 1979). The extended activity of *B. sphaericus* may be due to recycling of spores in larval cadavers or to persistence of sufficient quantities of toxin in the habitat (Lacey and Undeen 1986).

The objective of this study was to determine the effective dosage level and persistence of *B. sphaericus* flowable concentrate against *Culex quinquefasciatus* Say in septic ditches with a high organic matter level.

[1] This study was accomplished as a cooperative effort among the University of Arkansas Entomology Department, the Rice Research and Extension Center in Arkansas and DUPHAR B. V. Crop Protection Division.

MATERIALS AND METHODS

The *B. sphaericus* formulation BSP-1 (strain 2362)[2] used was a flowable concentrate containing 12.3% fermentation solids and 87.7% inert ingredients. Four application rates of the BSP-1 were evaluated for activity and persistence against *Cx. quinquefasciatus* larvae.

1987 trials: Field trials were conducted in septic ditches in Washington Co., Arkansas, at the University of Arkansas Agricultural Experiment Station in Fayetteville and on a private farm in Farmington, Arkansas, during September and early October 1987. Ditches contained effluent from dairy barns. Treatments were applied with a CO_2 pressurized, hand-held sprayer to 36-m^2 plots and included *B. sphaericus* at 0.6, 0.9, 1.2 and 2.4 liters/ha, a malathion standard (57% EC) at 1.1 liters/ha and an untreated control. All treatments and a control were replicated 4 times.

Mosquito larvae were counted using standard 460-ml dippers. Twenty dips were taken from each plot prior to treatment, at 24 and 48 h post-treatment, and thereafter at 5-day intervals until larval numbers began to increase. Each dip was poured into a 20 × 25 cm enameled pan and the number of each instar and pupae of *Cx. quinquefasciatus* was recorded. A small number of *Anopheles* species were found during sampling but not in sufficient numbers to make a statistical evaluation.

Two open floating cages (Sandoski et al. 1986), each containing ten 2nd–3rd instar larvae obtained from ditches outside of the test area, were placed in each plot following treatment. Larval mortality was determined after 24 and 48 h post-treatment.

[2] The *Bacillus sphaericus* was supplied by DUPHAR B. V. Crop Protection Division, 1381 CP Weesp, Holland.

1988 trials: The persistence of *B. sphaericus* 2362 in domestic septic situations was evaluated at Almyra, Arkansas. Ditches were not paved, and larvicides, other than herbicides or diesel, were not used. Test plots were 6.0 × 1.8 m sections of ditch with an average depth of 0.3 m. Treatments were applied as in 1987. Post-treatment counts were taken 48 h after initial treatment and thereafter at 5-day intervals until larval densities increased to precount levels. Each test and control was replicated 3 times.

A floating cage holding ten 2nd–3rd instar larval *Cx. quinquefasciatus* was placed in each plot immediately following the treatment and at 5-day intervals afterwards. Larval mortality was observed 48 h after cage installation. This procedure was continued until mortality was negligible.

Water samples were taken from both trials subsequent to treatments. Samples were analyzed at the University of Arkansas Soil Testing Laboratory for PH, turbidity, dissolved oxygen, suspended solids and nitrogen.

Percentage data from the trials were corrected for control mortality by Abbott's formula (Abbott 1925), arc sin transformed and subjected to an ANOVA. Mean separation was accomplished by LSMEANS (SAS Institute 1985).

RESULTS

1987 trials: The highest reductions in larval counts were observed at 48 h (Table 1). Larval reduction was >80% for all rates and 93.8% for the 2.4 liters/ha rate. Larval populations began to increase at 5 days for all rates. However, some residual control was apparent at 5 days in the 0.6 and 2.4 liters/ha treatments. The 2.4 liters/ha treatment at the Farmington site produced residual suppression over a period of 17 days. Larval density in controls increased except at the 10-day observation in which a reduction of 17% was recorded for 5 and 10 days, respectively.

Sentinel observations at a rate of 0.9 liters/ha were significantly ($P < 0.05$) lower than observed in other *B. sphaericus* treatments after 48 h (Table 2). Control mortality remained 3% or less for all sentinel installations.

1988 trials: All rates produced excellent control at 48 h post-treatment (Table 1). The 2.4- and 1.2-liter/ha rates produced significantly greater mortality than the lower rates at 5 days post-treatment. The 2.4- and 1.2-liter/ha rates exhibited little decrease in suppression at 10 days post-treatment with 94.3% and 96.6% recorded, respectively. A resurgence in mortality was observed with the 0.9-liter/ha rate in which

Table 1. Percentage reduction in dip counts of *Culex quinquefasciatus* larvae from septic ditches treated with *Bacillus sphaericus* strain 2363 in Washington Co., Arkansas, 1987 and Almyra, Arkansas, 1988.[1]

Rate (liters/ha)	Mean no. larvae/dip (0 h)	% reduction — Sampling period post-treatment: 48 h	5 days	10 days	15 days	20 days
Wash. Co. 1987[2]						
0.6	82.2	87.7[3,4] Aa	47.5 Aa	—	—	—
0.9	87.2	82.2 Aa	19.9 Ba	—	—	—
1.2	25.2	88.9 Aa	32.2 Ba	—	—	—
2.4	57.6	93.8 Aa	52.4 Aa	—	—	—
Standard 1.1	29.1	96.7 Aa	31.3 Ba	—	—	—
Almyra 1988[5]						
0.6	13.0	92.2 Aa	35.2 Bb	3.5 Cb	—	—
0.9	24.2	99.0 Aa	65.5 Bb	88.5 ABa	—	—
1.2	28.9	99.8 Aa	99.2 ABa	96.6 ABa	91.4 ABa	79.1 Ba
2.4	42.7	99.9 Aa	99.6 Aa	94.3 ABa	79.3 BCa	64.5 Ca

[1] Data from each year was analyzed separately.

[2] Larval density in controls increased except at the 5-day observation in which a reduction of 17% was recorded.

[3] Mean percent reduction in the same row followed by the same upper case letter are not significantly different ($P \geq 0.05$) by LSMEANS.

[4] Mean percent reduction in the same column followed by the same lower case letter are not significantly different ($P \geq 0.05$) by LSMEANS.

[5] Larval density in controls increased with the exception of the 0.9 liters/ha treatment in which reductions of 48.1% and 28.4% were recorded for 5 and 10 days, respectively.

Table 2. Percent reduction of *Culex quinquefasciatus* in sentinel cages placed in septic ditches treated with *Bacillus sphaericus* in Washington Co., Arkansas, 1987 and Almyra, Arkansas, 1988.[1]

Treatment rate (liters/ha)	Installation period post-treatment[2]				
	0 h	5 days	10 days	15 days	20 days
Wash. Co. 1987[3]					
0.6	78.6[4, 5] Aab	—	—	—	—
0.9	69.1 Ab	—	—	—	—
1.2	92.0 Aab	—	—	—	—
2.4	93.8 Aab	—	—	—	—
Standard 1.1	97.7 Aa	—	—	—	—
Almyra 1988[6]					
0.6	94.4 Aa	0.0 Bc	1.9 Bb	—	—
0.9	100.0 Aa	11.1 Bbc	2.8 Bb	—	—
1.2	97.9 Aa	36.5 Bb	33.3 Bab	25.0 Ba	3.5 Ba
2.4	73.4 ABa	96.5 Aa	52.2 Ba	23.0 Ba	20.8 Ba

[1] Data from each year was analyzed separately.
[2] Larvae were counted 48 h after installation.
[3] Control mortality remained 3% or less for all installations.
[4] Mean percent reduction in the same row followed by the same upper case letter are not significantly different ($P \geq 0.05$) by LSMEANS.
[5] Mean percent reduction in the same column followed by the same lower case letter are not significantly different ($P \geq 0.05$) by LSMEANS.
[6] Control mortality remained 10% or less for all installations except for the 0.6 liters/ha treatment control at 5 days (15%).

a level of 88.5% was recorded. Suppression was minimal for the 0.6-liter/ha plots. Plots treated with the 1.2-liter/ha rate exhibited higher mortality than those treated with 2.4-liter/ha at 15 and 20 days post-treatment, but the differences were not significant ($P > 0.05$). The 1.2-liter/ha rate maintained excellent suppression through 15 days with mortality declining to 79.1% at 20 days post-treatment. Larval densities in control ditches increased during tests except for the 0.9-liter/ha control ditch in which 48.1% and 28.4% reductions were recorded for the 5 and 10 day counts, respectively.

High sentinel mortality was recorded in plots treated with 0.6-, 0.9- and 1.2-liter/ha rates of *B. sphaericus* at 0 h (Table 2). The 2.4-liter/ha rate displayed lower mortality than other rates at 0 h but mortality increased to 96% in the 5-day cage installation reading, significantly greater than that obtained with other rates. Mortality declined sharply to 36.5% in the 1.2-liter/ha installations. Mortality recorded in plots treated with the 0.6- and 0.9-liter/ha rates was negligible at 5 days and for all subsequent sentinel installations. Sentinel mortality in the 2.4- and 1.2-liter/ha treated plots declined at 10 day post-treatment installations to 52.2% and 33.3%, respectively, and continued to decline in succeeding installations. Control mortality remained 10% or less for all installations except for the 0.6-liter/ha treatment control at 5 days.

Differences existed in suspended solids concentrations at the two locations. Plots containing 0.9, 1.2 and 2.4-liters/ha *Bacillus sphaericus* at the Washington Co. site had concentrations of 140, 150 and 245 ppm suspended solids, respectively. Plots at the Almyra site containing 0.9, 1.2 and 2.4 liters/ha *B. sphaericus* had lower concentrations of suspended solids with 105, 15 and 45 ppm, respectively.

DISCUSSION

1987 trials: A reduction in larval densities was observed for all treatment rates. This reduction was especially pronounced 48 h post-treatment. Larval cadavers were evident at all rates 48 h post-treatment. Larval suppression was not observed at 5 days except for 2.4 liters/ha at the Farmington site where larvae populations remained very low, >90% suppression, for 17 days post-treatment. Several reasons for extended suppression at this site are possible. There was little or no water movement, whereas there was slight water movement through the ditch at other sites. Moreover, the Farmington ditch was deeper (ca. 3 m as compared with ca. 0.3 m), stagnant and not flushed by rains. This ditch also was well shaded whereas the other localities were exposed to substantial sunlight. Possibly reduced sunlight and water current had some effect on activity of microbial agent. Mulligan et al. (1980) demonstrated that sunlight reduced *B. sphaericus* activity. Also, 1st instar larval counts were high at this location for most samples taken, but there were few 2nd, 3rd and 4th instar larvae counted after initial treatment with *B. sphaericus*. Egg rafts were prevalent at both sites, and so the potential for large population increases in treated plots appeared to be present.

1988 trials: A rapid reduction in larvae number was observed at all rates 48 h post-treatment. The 2.4-liter/ha rate demonstrated excellent suppression for 10 days and good suppression at 15 days. Percent reduction at 15 and 20 days was significantly lower ($P<0.05$) at the 2.4-liter/ha rate as compared with the 1.2-liter/ha rate. The lower level of control in the 2.4-liter/ha rate plots could be due to the treatment ditch having higher concentrations of suspended solids than the ditch used for the 1.2-liter/ha rate. The 1.2-liter/ha rate displayed excellent suppression for 15 days and good suppression for 20 days. No substantial residual control was evident in dipper counts at 5 days either for the 0.6- or 0.9-liter/ha rates, although the 0.9-liter/ha rate had excellent control at 10 days.

In sentinel cages, adequate control could not be maintained for more than 48 h except at the 2.4-liter/ha rate. No application rate of *B. sphaericus* tested provided acceptable control after 5 days. Inadequate control could be a result of *B. sphaericus* spores settling below the sentinel container portals or from screens on the sides of the containers becoming clogged with the solids prevalent in the ditches. Mulla et al. (1984) found that on ponds treated with a cream preparation of *B. sphaericus*, spores in the larval feeding zone dropped below 100/ml after the second day. The dipper method of evaluation appeared to be superior to sentinel cages although dipping was much more time consuming.

Bacillus sphaericus provided effective extended control against *Cx. quinquefasciatus* at 1.2-liter/ha or greater rates. Water quality test results were comparable for the 2 trials except for the suspended solids portion, which was higher in dairy effluent ditches. This may have contributed to differences in suppression persistence recorded between the 2 trials and between the high *B. sphaericus* rates in 1988. Mulligan et al. (1980) concluded that the activity of *B. sphaericus* was lowered by the suspended solids portion of the sewage.

Bacillus sphaericus appears to be an excellent alternative to chemical larvicides for control of *Cx. quinquefasciatus*. It provides extended suppression without repeated treatments required at recommended rates for most chemical insecticides. This should provide substantial savings in labor and material costs.

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