Journal of the American Mosquito Control Association, 12(2):220–224, 1996
Copyright © 1996 by the American Mosquito Control Association, Inc.

LABORATORY AND FIELD PLOT BIOASSAY OF *BACILLUS SPHAERICUS* AGAINST ARKANSAS MOSQUITO SPECIES[1]

R. L. GROVES AND M. V. MEISCH

Department of Entomology, University of Arkansas, Fayetteville, AR 72701

ABSTRACT. *Bacillus sphaericus* was evaluated in laboratory bioassays against larvae of 7 Arkansas mosquito species and in rice field plots against a single mosquito species. Laboratory-tested species included *Aedes aegypti, Ae. albopictus, Ae. vexans, Anopheles punctipennis, Culex quinquefasciatus, Cx. restuans,* and *Psorophora columbiae.* Field plot bioassays were evaluated against *Ps. columbiae* only. The laboratory formulation tested demonstrated activity against all 7 species. Mortalities were observed at 24 and 48 h posttreatment. Twenty-four-hour LC_{50} results ranged from 0.84 to 15.04 international toxic units (ITU)/ml for all species and 48-h LC_{50} results ranged from 0.38 to 9.27 ITU/ml. Twenty-four-hour LC_{90} results ranged from 1.90 to 48.21 ITU/ml for all species, whereas 48-h LC_{90} results ranged from 1.30 to 33.83 ITU/ml. Subsequent field plot evaluations yielded control at both dosages (0.45 and 0.9 g/ha) during the initial 48-h exposure period. Mortalities at 48 h posttreatment for both dosages were 87 and 90%, respectively. Posttreatment mortalities never exceeded 16.7% after 24 h and none were significantly different from control mortality ($P \leq 0.05$).

INTRODUCTION

Considerable research has been conducted upon bacterial insecticides as viable biological control agents against the larvae of pestiferous mosquitoes. Numerous strains of *Bacillus thuringiensis* Berliner have been evaluated and subsequently employed as biolarvicides. Additionally, a number of strains of *Bacillus sphaericus* Neide have been identified as entomopathogenic (Singer 1973) and have been evaluated in field and laboratory bioassays (Ramoska et al. 1977; Mulligan et al. 1980; Mulla et al. 1984, 1988; Lacey et al. 1986). These strains have been demonstrated to be pathogenic to medically important genera of mosquitoes including *Aedes, Anopheles,* and *Culex* (Singer 1974).

Differences in mortalities have been observed, however, between field and laboratory bioassays (Mulla 1986, Kellen et al. 1965). Suspended solids in the water column of field plot bioassays may account for some of these differences (Mulligan et al. 1980). Sunlight degradation in shallow suspensions has also been implicated as a factor contributing to these mortality differences (Davidson et al. 1984). Settling of the spore-forming bacteria through the height of the water column may remove the spores from the larval feeding zone (Davidson et al. 1984).

Bacillus sphaericus was evaluated as a potential biolarvicidal control agent in the rice-growing region of eastern Arkansas against 7 mosquito species in laboratory bioassays to establish LC_{50} and LC_{90} estimates. Test organisms for the laboratory bioassays included *Aedes albopictus* (Skuse), *Aedes aegypti* (Linn.), *Aedes vexans* (Meigen), *Anopheles punctipennis* (Say), *Culex quinquefasciatus* Say, *Culex restuans* Theobald, and *Psorophora columbiae* (Dyar and Knab). Once estimates of LC_{50} and LC_{90} values were established in the laboratory for *Ps. columbiae,* field plot bioassays were conducted against this species to evaluate the reliability of these estimates.

MATERIALS AND METHODS

Laboratory bioassay: A stock solution of *B. sphaericus,* (S-2362, Pasteur Institute), at an initial dosage of 1,000 international toxic units (ITU)/ml was prepared by adding 8.33 mg of test preparation (120 ITU/mg) to 1 liter of distilled water. A 10-ml aliquot of this initial dilution was then placed in 1 liter of distilled water. Serial 2× dilutions were subsequently prepared from this preparation by removing a 500-ml aliquot and adding it to 500 ml of distilled water resulting in 6 dilutions (10.0, 5.0, 2.5, 1.25, 0.625, and 0.3125 ITU/ml). Each dilution was replicated 3 times for each of the 7 species tested.

A formulation of *B. sphaericus* with a potency of 120 ITU/mg was produced and supplied by the Novo Nordisk Company, Entotech Inc., Davis, CA. Larval mosquitoes for laboratory bioassays were obtained from naturally occurring populations of *Ae. vexans, An. punctipennis, Cx. quinquefasciatus,* and *Cx. restuans* in the Fayetteville, AR, area and *Ps. columbiae* from the

[1] This study was completed as part of USDA, CSRS Southern Regional Project S-230 on Riceland Mosquitoes and is approved for publication by the Director of the Arkansas Agricultural Experiment Station.

Rice Research and Extension Center (RREC), Stuttgart, AR. *Aedes albopictus* and *Ae. aegypti* were obtained from laboratory colonies at Texas A&M University, College Station, TX. For all laboratory bioassays, 3rd-instar larvae were used.

Twenty larvae were placed in 237-ml wax-impregnated serving cups containing the serial dilutions (approximately 150 ml) for all 3 replications. Cups were maintained at approximately 24°C and exposed to a 12:12 light:dark regime. After a 24-h exposure, larvae were fed a finely ground, 0.5-mg portion of fry meal. Larval mortality was quantified at 24 and 48 h posttreatment for all species.

Mortalities obtained at both 24 and 48 h posttreatment were analyzed by a probit procedure (Proc Probit Log_{10}). Estimates of LC_{50} and LC_{90} were obtained for all mosquito species tested (SAS Institute Inc. 1985).

Field plot bioassay: Experimental rice field plots (9.1 × 9.1 m) were constructed on the RREC. A single liquid formulation of *B. sphaericus* was evaluated for efficacy against 2nd-instar *Ps. columbiae* larvae. Larvae were collected from temporary, rain-filled pools on the RREC. This formulation was initially applied in experimental rice field plots at dosages of 2.0, 1.0, 0.5, 0.25, and 0.125 ITU/plot. After evaluation of mortalities for these unreplicated rates of application, a single formulation was applied at rates of 0.45 kg/ha (0.053 ITU/plot) and 0.9 kg/ha (0.107 ITU/plot). Each dosage, including 3 untreated control plots, was replicated 3 times. Dosages within each treatment were determined using an estimated volume of water in each experimental plot of 270.7 $ft.^3$.

The 2 dosages were applied beginning June 27, 1995, through June 29, 1995, and monitored at 24 and 48 h posttreatment. Each replication of both dosages was applied using a hand-held B&G compressed air sprayer. Sentinel groups of 10 *Ps. columbiae* larvae were placed in floating cages (Sandoski et al. 1986) in all plots. Installation of sentinel larvae took place at the time of initial treatment and also at 24 and 48 h posttreatment.

Mortality within groups of sentinel larvae exposed immediately after treatment was observed at 24 and 48 h (July 27, 1995). Mortality of sentinel larvae was again observed at 24 and 48 h of exposure for sentinels installed in the plots 24 h posttreatment (July 28, 1995). Larval mortality was observed 24 h after exposure for sentinels installed in experimental plots 48 h posttreatment (July 29, 1995). Sentinel larval mortality was not observed 48 h after exposure due to the apparent inactivity of the compound.

Percentage mortality data were subjected to an arcsine, square root transformation and a subsequent analysis of variance (General Linear Models). Mean percent mortalities were corrected by Abbott's formula (Abbott 1925) and mean separation was subsequently conducted using a least squared means (LSmeans) procedure (SAS Institute 1985).

RESULTS AND DISCUSSION

Laboratory bioassay: Results obtained from laboratory bioassays are reported in Table 1. Estimates of LC_{50} values for all species at 24 h posttreatment ranged from 0.84 to 15.04 ITU/ml and at 48 h posttreatment ranged from 0.38 to 9.27 ITU/ml. Estimates of LC_{90} values for all species at 24 h posttreatment ranged from 1.90 to 48.21 ITU/ml and at 48 h posttreatment ranged from 1.30 to 33.83 ITU/ml.

Anopheles punctipennis appeared to be the least susceptible species, with LC_{50} and LC_{90} estimates at 24 h posttreatment of 15.04 and 48.21 ITU/ml, respectively; estimates at 48 h posttreatment were 9.27 and 33.83 ITU/ml, respectively (Table 1). Settling of bacteria through the height of the water column may have removed a large percentage of spores from the larval feeding zone, resulting in the apparent low susceptibility (Davidson et al. 1984).

Culex restuans and *Cx. quinquefasciatus* were the most susceptible species evaluated (Table 1). Estimates of LC_{50} and LC_{90} values for *Cx. restuans* at 24 h posttreatment were 0.84 and 1.90 ITU/ml, respectively; estimates of LC_{50} and LC_{90} values at 48 h posttreatment were 0.38 and 1.30 ITU/ml, respectively. Estimates of LC_{50} and LC_{90} values for *Cx. quinquefasciatus* at 24 h posttreatment were 0.96 and 4.53 ITU/ml, respectively; estimates of LC_{50} and LC_{90} values at 48 h posttreatment were 0.58 and 5.53 ITU/ml, respectively.

Psorophora columbiae was slightly less susceptible than both *Culex* species tested (Table 1). Estimates of LC_{50} and LC_{90} values at 24 h posttreatment were 2.32 and 15.80 ITU/ml, respectively; estimates of LC_{50} and LC_{90} values at 48 h posttreatment were 0.50 and 4.23 ITU/ml, respectively. Singer and Murphy (1976) demonstrated the tendency of *Culex* species to produce high lethal responses to low dosages of a variety of strains of *B. sphaericus*. Also, *Ps. columbiae* larval susceptibility to strains of *B. sphaericus* is dependent upon the larval age assayed (Ramoska et al. 1977). Mortality differences between 2nd- and 3rd-instar larvae in the assay by Ramoska et al. (1977) were 76.6 and 25.0%, respectively, a mortality difference of 51.6%. The 3rd-instar larvae used in our laboratory assay may have been significantly less susceptible to

Table 1. Toxicity of *Bacillus sphaericus* to 7 mosquito species evaluated in the laboratory. One hundred eighty larvae were used for each time interval.

Species	Time (h) posttreatment	LC_{50} (95% CL) (ITU/liter)	LC_{90} (95% CL) (ITU/liter)	Slope ± SE
Aedes aegypti	24	7.06 (4.79–9.26)	12.79 (9.67–26.92)	4.9 ± 1.03
	48	4.72 (4.00–5.40)	9.38 (8.05–11.60)	4.3 ± 0.54
Aedes albopictus	24	4.08 (3.35–5.07)	10.52 (7.90–16.43)	4.0 ± 0.54
	48	2.36 (1.99–2.81)	4.91 (3.94–6.87)	3.1 ± 0.42
Aedes vexans	24	4.40 (3.73–5.24)	8.66 (6.96–12.25)	4.4 ± 0.64
	48	0.39 (0.18–0.60)	3.32 (2.13–7.39)	1.4 ± 0.26
Anopheles punctipennis	24	15.04 (12.54–19.81)	48.21 (31.79–110.93)	2.5 ± 0.45
	48	9.27 (7.63–11.24)	33.83 (23.85–64.03)	2.3 ± 0.37
Culex quinquefasciatus	24	0.96 (0.74–1.33)	4.53 (2.81–10.07)	1.9 ± 0.27
	48	0.58 (0.40–0.84)	5.53 (2.86–19.64)	1.1 ± 0.46
Culex restuans	24	0.84 (0.71–1.04)	1.90 (1.41–3.34)	3.6 ± 0.62
	48	0.38 (0.30–0.47)	1.30 (0.95–2.19)	2.4 ± 0.35
Psorophora columbiae	24	2.32 (1.71–3.26)	15.80 (9.16–39.97)	1.5 ± 0.23
	48	0.50 (0.26–0.74)	4.23 (2.70–9.13)	1.4 ± 0.24

the bacilli than younger instars would have been had they been assayed.

All 3 *Aedes* species tested against *B. sphaericus* demonstrated moderate susceptibility, with *Ae. aegypti* being the least susceptible. The estimates of LC_{50} and LC_{90} values at 24 h posttreatment were 7.06 and 12.79 ITU/ml, respectively; whereas estimates of LC_{50} and LC_{90} values at 48 h posttreatment were 4.72 and 9.38 ITU/ml, respectively. These results parallel those of Singer (1974), whose laboratory-reared *Ae. aegypti* and *Ae. albopictus* showed relatively low susceptibility to many strains of *B. sphaericus* when compared to members of the *Culex* and *Psorophora* genera. Berry et al. (1993) identified patterns of larvicidal activity against different mosquito species from differing strains of *B. sphaericus*. Specifically, a binary toxin (41.9 kDa) produced by different strains of *B. sphaericus* showed differential activity towards *Cx. quinquefasciatus* and *Ae. aegypti*, with the latter being less susceptible to the binary toxin. Thanabalu et al. (1993) also investigated the role of the binary toxin against larvae and cells in culture in which they showed a marked difference between the susceptibility of *Cx. quinquefasciatus* and *Ae. aegypti*. Davidson (1988) suggested that the specificity of the toxin for glycoprotein receptors in the midgut of larval mosquitoes is responsible for the differences in susceptibility among species.

Field plot bioassay: Initial, nonreplicated

Table 2. Percent mortality of *Psorophora columbiae* exposed to 2 concentrations of *Bacillus sphaericus* after 24 and 48 h of exposure at the Rice Research and Extension Center, Stuttgart, AR.

Treatment (g/ha)	Length of exposure[1] (h)				
	June 27		June 28		June 29[2]
	24	48	24	48	24
0.45	23.3aB	87.0aA	3.3aA	3.3aB	0.0aA
0.9	60.0bA	90.0aA	3.3aA	16.7aA	0.0aA
Control	0.0cC	0.0bB	0.0aA	6.7aB	3.3aA

[1] Means reported from retransformed data analyzed by general linear models (GLM). Means not followed by the same lowercase letter within columns are significantly different (α = 0.05), by least squared difference. Means not followed by the same uppercase letter within rows are significantly different (α = 0.05), by least squared difference. Means have been corrected for control mortality according to Abbott (1925).

[2] The 48-h exposure test was not conducted on June 29, 1995.

field plot bioassays of 2.0, 1.0, 0.5, 0.25, and 0.125 ITU/plot resulted in mortalities of 100, 100, 100, 100, and 90% at 24 h posttreatment, respectively, against 2nd-instar *Ps. columbiae*. These field plot dosages and subsequent mortalities did not parallel the laboratory bioassay of the same species, in which the reported estimate of the LC_{50} value was 2.32 ITU/ml. As previously discussed, larval age may have a significant effect on mortality.

Replicated treatment dosages of 0.053 ITU/plot (0.45 g/ha) and 0.107 ITU/plot (0.9 g/ha) yielded significant control of *Ps. columbiae* larvae at 24 and 48 h posttreatment (Table 2). Larvae exposed immediately after treatment with *B. sphaericus* yielded mortalities of 23.3 and 60.0% at 24 h for dosages of 0.053 ITU/plot and 0.107 ITU/plot, respectively (exposure date = June 27, 1995). The same larvae observed at 48 h posttreatment resulted in cumulative mortalities of 87.0 and 90.0% at dosages of 0.053 ITU/plot and 0.107 ITU/plot, respectively.

Sentinel larvae installed in the same experimental plots 24 h posttreatment yielded mortalities of 3.3 and 3.3% for both 0.053 ITU/plot and 0.107 ITU/plot, respectively, when exposed for 24 h on June 28, 1995. After 48-h exposure, cumulative mortalities for these sentinels were 3.3 and 16.7% for 0.053 ITU/plot and 0.107 ITU/plot, respectively.

Sentinel larvae installed in the experimental plots 48 h posttreatment yielded mortalities of 0.0 and 0.0% for both 0.053 ITU/plot and 0.107 ITU/plot, respectively, when exposed for 24 h on June 29, 1995. Mortality readings for these same sentinels exposed 48 h after installation were not recorded. These field plot experiments demonstrated that the maximum effect of *B. sphaericus* on *Ps. columbiae* larvae occurs within 24 h posttreatment preceding additional cumulative mortality. Those sentinel installations past 24 h posttreatment yielded no greater than 16.7% mortality at any exposure time.

The dosage levels applied and replicated in this study were chosen to target a mid-lethal dosage range (LC_{30}–LC_{70}). Higher dosages have been demonstrated to evoke a rapid or immediate lethal response due only to bacilli toxin (Ramoska et al. 1977). Larvae exposed to mid- or sublethal dosages will acquire bacilli that are able to multiply in the larval gut, resulting in increased mortality of a population (Davidson et al. 1975).

These experiments demonstrate the potential of *B. sphaericus* as an effective larvicidal control agent for the rice-growing regions of southeast Arkansas. The lowest concentration required to evoke a 90% mortality against *Ps. columbiae* is approximately 0.1 ITU/ml. The rapid response obtained at these minimal dosages further enhances the effectiveness of *B. sphaericus* as a suitable biocontrol agent.

ACKNOWLEDGMENTS

We thank both the Director, John Robinson, and the field crew of the Rice Research and Extension Center, Stuttgart, AR, for their assistance and cooperation in the successful completion of this study. We also thank J. K. Olson, Texas A&M University, College Station, TX, for supplying eggs of *Ae. aegypti* and *Ae. albopictus* used in these tests.

REFERENCES CITED

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Berry, C., J. Hindley, A. F. Ehrhardt, T. Grounds, I. de Souza and E. W. Davidson. 1993. Genetic determinants of host ranges of *Bacillus sphaericus* mosquito larvicidal toxins. J. Bacteriol. 2:510–518.

Davidson, E. W. 1988. *In:* R. M. Faust and J. Margalit (eds.). Proceedings of the workshop on, "Bacterial control of agricultural and medically important insects. A global perspective." Isr. J. Entomol. 23:9–15.

Davidson, E. W., S. Singer and J. D. Briggs. 1975. Pathogenesis of *Bacillus sphaericus* strain SSII-1 infections in *Culex pipiens quinquefasciatus* (=*Cx. pipiens fatigans*) larvae. J. Invertebr. Pathol. 25:179–184.

Davidson, E. W., M. Urbina, J. Payne, M. S. Mulla, H. Darwazeh, H. T. Dulmage and J. A. Correa. 1984. Fate of *Bacillus sphaericus* 1593 and 2362 spores used as larvicides in the aquatic environment. Appl. Environ. Microbiol. 47:125–129.

Kellen, W. R., T. B. Clark, J. E. Lindegren, B. C. Ho, M. H. Rogoff and S. Singer. 1965. *Bacillus sphaericus* Neide as a pathogen of mosquitoes. J. Invertebr. Pathol. 7:442–448.

Lacey, L. A., C. M. Heitzman, M. Meisch and J. Billodeaux. 1986. Beecomist®-applied *Bacillus sphaericus* for the control of riceland mosquitoes. J. Am. Mosq. Control Assoc. 2:548–551.

Mulla, M. S. 1986. Efficacy of the microbial agent *Bacillus sphaericus* Neide against mosquitoes (Diptera: Culicidae) in southern California. Bull. Soc. Vector Ecol. 11:247–254.

Mulla, M. S., H. A. Darwazeh and N. S. Tietze. 1988. Efficacy of *Bacillus sphaericus* 2362 formulations against floodwater mosquitoes. J. Am. Mosq. Control Assoc. 4:172–174.

Mulla, M. S., H. A. Darwazeh, E. W. Davidson, H. T. Dulmage and S. Singer. 1984. Larvicidal activity and field efficacy of *Bacillus sphaericus* strains against mosquito larvae and their safety to nontarget organisms. Mosq. News 44:336–342.

Mulligan, F. S., III, C. H. Schaefer and W. H. Wilder. 1980. Efficacy and persistence of *Bacillus sphaericus* and *B. thuringiensis* H-14 against mosquitoes

under laboratory and field conditions. J. Econ. Entomol. 73:684–688.

Ramoska, W. A., S. Singer and R. Levy. 1977. Bioassay of three strains of *Bacillus sphaericus* on field-collected mosquito larvae. J. Invertebr. Pathol. 30: 151–154.

Sandoski, C. S., W. C. Yearian and M. V. Meisch. 1986. Swath width determination for Beecomist® applied *Bacillus thuringiensis* (H-14) against *Anopheles quadrimaculatus* larvae in rice fields. J. Am. Mosq. Control Assoc. 2:461–468.

SAS Institute, Inc. 1985. SAS user's guide: statistics. SAS Institute, Cary, NC.

Singer, S. 1973. Insecticidal activity of recent bacterial isolates and their toxins against larvae. Nature (Lond.) 224:110–111.

Singer, S. 1974. Entomogenous bacilli against mosquito larvae. Dev. Ind. Microbiol. 15:187–194.

Singer, S. and L. J. Murphy. 1976. New insecticidal strains of *Bacillus sphaericus* useful against *Anopheles albuminus* larvae. *In*: Proceedings, Annual Meeting of the American Society for Microbiology, Atlantic City.

Thanabalu, T., C. Berry and J. Hindley. 1993. Cytotoxicity and ADP-ribosylating activity of the mosquitocidal toxin from *Bacillus sphaericus* SSII-1: possible roles of the 27- and 70-kilodalton peptides. J. Bacteriol. 8:2314–2320.