BEECOMIST®-APPLIED *BACILLUS SPHAERICUS* FOR THE CONTROL OF RICELAND MOSQUITOES

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ABSTRACT. An aerially applied flowable concentrate (FC) of *Bacillus sphaericus* Neide (isolate 2362) was evaluated against riceland mosquitoes using the Beecomist® spray head. Application of the FC at 0.58 and 1.17 liter/ha in maturing rice fields resulted in a 48 hr mean posttreatment reduction of *Anopheles quadrimaculatus* of 71 and 82%, respectively. A significant reduction in larval populations one week posttreatment was also observed. Treatment rates of 0.29, 0.44, and 0.58 liter/ha in reflooded second crop rice fields resulted in 48 hr posttreatment reduction of *Psorophora columbiae* larvae of 50, 76 and 98% respectively.

INTRODUCTION

The results of several small plot field trials attest to the effectiveness of *Bacillus sphaericus* Neide as a larvicide of vector and nuisance mosquitoes (Mulla et al. 1984a, 1984b; Mulligan et al. 1978, Lacey et al. 1984). It has the added benefits of persisting in nature, recycling under certain conditions, and is safe for nontarget organisms (Mulla et al. 1984b, Davidson et al. 1984). Until recently the lack of sufficient primary powder (spores, cellular inclusions, media residues) and formulated *B. sphaericus* has hampered large scale field testing of this pathogen. Recently, the World Health Organization has distributed several experimental batches of *B. sphaericus* produced by the U.S. Department of Agriculture, Abbott Laboratories and Biochem Products. This has enabled evaluation of *B. sphaericus* against several mosquito species in a variety of habitats.

In order to optimize the effectiveness of this bacterium it will be necessary to adapt both the formulation and the application method to specific situations (Lacey 1984). Flowable concentrate (FC) formulations of *Bacillus thuringiensis* Berliner var. *israelensis* de Barjac (serotype H-14) have provided several application options for the control of black fly and mosquito larvae (Lacey and Undeen 1986). One of the most innovative methods to date is the use of the Beecomist® spray head for the application of undiluted *B. thuringiensis* (H-14) FC to rice fields for the control of *Psorophora columbiae* (Dyar and Knab) and *Anopheles quadrimaculatus* Say at considerably reduced rates from those recommended by the manufacturers (Yates 1984, Sandoski et al. 1985, M. Yates, unpublished data).

The objective of this study was to evaluate an experimental FC formulation of *B. sphaericus* against riceland mosquitoes using the Beecomist® spray head.

METHODS AND MATERIALS

The inoculum used for testing was an experimental flowable concentrate made with 12.3% primary powder of the 2362 isolate (serotype 5a5b) of *B. sphaericus* and formulated in a manner similar to that of the Bactimos® formulation of *B. thuringiensis* (H-14) (D. Ross, personal communication).

Aerial application of the *B. sphaericus* FC was made with a Beecomist model 360 spray head equipped with a 80-100μ perforated stainless steel sleeve. The spray head was mounted on the starboard wing spray boom of either a Piper Pawnee 260 for tests conducted in Stuttgart, AR, or on a Cessna 188 Ag truck for tests conducted in Jennings, LA. The spray system and mounting details were identical to those of Sandoski et al (1985). By varying the amount of CO_2 pressurizing the system, flow rates of 26–52 ml of FC/sec through the spray head were obtained which resulted in treatment rates of 0.29 liter/ha (4 oz/acre) to 0.58 liter/ha (8 oz/acre) when applied in 18.3 m swaths (1 pass/swath) at airspeeds of 161 kph (Piper Pawnee) to 177 kph (Cessna Ag truck). The 1.17 liter/ha rate (16 oz/acre) was obtained by making 2 passes/swath using a flow rate of 48 ml/sec (161 kph). To ensure adequate coverage, applications were started 35 m upwind of each plot. Swath direction was as close to perpendicular to prevailing winds as possible.

For efficacy evaluation against larvae of *An. quadrimaculatus*, the formulation was applied at an altitude of ca. 15 m in maturing (pre-panicle stage) rice fields near Stuttgart, AR between July 8 and 19, 1985. Treatment rates of 0.58 and 1.17 liter/ha were each evaluated in three 0.81 ha (2 acre) plots. The treated plots and nearby control plots were sampled imme-

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diately prior to treatment and again 48 hr and 1 week posttreatment. Sampling consisted of taking 200 dips per plot using a standard 450 ml mosquito dipper along 2 parallel transects running through the plots and 20 m in from the sides of the plots.

Due to slightly greater winds (calm to 8 km/hr) in Jennings, LA, applications were made at an altitude of 8–10 m to minimize drift. Treatment rates of 0.29, 0.44 and 0.58 liter/ha were evaluated in recently reflooded second crop rice fields against *Ps. columbiae* larvae. Each rate was applied to two 0.81–1.0 ha plots. Sampling of treated and nearby control plots was identical to the Stuttgart tests in terms of time of pretreatment sampling, number of dips per plot, and relative location of transects. Posttreatment samples, however, were taken 24 and 48 hr after application of *B. sphaericus* due to the advanced age (over 50% 3rd and 4th instars) of the larvae.

The data were corrected for control mortality and analyzed using the SAS general linear models procedure and probit analysis for calculation of lethal field dosages (LFD_{50}–LFD_{90}).

RESULTS

Reduction of *An. quadrimaculatus* larval populations in mature rice fields 48 hr and 1 week after Beecomist application of *B. sphaericus* FC is presented in Table 1. Although a significant reduction in larval numbers was observed in the treated plots, no significant difference in the treatment rates was observed. Continued reduction in larval populations was observed in the treated plots one week after treatment. The level of continued reduction, however, was not positively correlated with treatment rate and the degree of reduction was highly variable from plot to plot (5.7–90.3%).

Data on the reduction of *Ps. columbiae* larvae in reflooded rice fields after aerial application of *B. sphaericus* FC with the Beecomist spray head are presented in Table 2. A positive correlation between treatment rate and degree of control was observed ($R^2=0.96$). Surviving larvae observed 24 hr after treatment appeared sluggish. Additional mortality 48 hr posttreatment was apparent, but due to the breeding habits of *Ps. columbiae* it was not possible to observe additional residual activity. The calculated LFD_{50} and LFD_{90} were 0.30 liter/ha and 0.52 liter/ha, respectively.

Several surviving larvae at the highest treatment rate were sampled adjacent to the leeward side of an overgrown rice field levee that traversed one of the plots. Although the lower treatment altitude provided adequate coverage, the combination of the overgrown levee, mild breeze and perhaps aircraft elevation resulted in a narrow treatment shadow in this particular situation.

DISCUSSION

Comparison of the efficacy of Beecomist-applied *B. thuringiensis* (H-14) FC (Yates 1984, Sandoski et al. 1985) and that of *B. sphaericus* FC in this study clearly indicates the superiority of *B. thuringiensis* (H-14) as a microbial control agent of *Anopheles* larvae. Earlier bioassays that were conducted in the Gainesville laboratory with both bacteria demonstrated a broader host range and greater activity of *B. thuringiensis* (H-14) against *An. quadrimaculatus* (Lacey and Singer 1982). Difference in innate susceptibility levels of *Anopheles* larvae to the two bacteria, rather than differences in application methods or formulation, is apparently responsible for the observed decrease in efficacy. Use of dye cards in rice fields treated with Beecomist-applied *B. thuringiensis* (H-14) FC indicate excellent penetration of even dense foliage (Yates 1984, Sandoski et al. 1985). The fine mist of FC provided by this method of

Table 1. Reduction of *Anopheles quadrimaculatus* larvae in maturing rice fields after Beecomist® application of an undiluted flowable concentrate of *Bacillus sphaericus* (Biochem FC, July 9–24, 1985, Stuttgart, Arkansas).[a]

Rate (liter/ha)	Mean % posttreatment reduction + S.E.[b c] 48 hr	1 week
0.58	70.8a±4.3 (25.6)	62.6a±16.3 (11.2+)
1.17	81.8a±3.4 (31.2+)	39.5a±17.3 (13.0+)

[a] Average pretreatment age composition (all treated plots): 56.9% first instar; 31.8% second instar; 9.2% third instar; 2.0% fourth instar.

[b] Reduction in control plots in parentheses ("+" indicates % increase). Means in the same column followed by the same letter are not significantly different at the 0.05 level.

[c] Wind 0–3.2 kph; air temperature 25°C; water temperature 26°C at treatment time; average maximum water temp. 36°C.

Table 2. Reduction of *Psorophora columbiae* larvae in recently flooded rice fields after Beecomist® application of an undiluted flowable concentrate of *Bacillus sphaericus* (Biochem FC, August 24–29, 1985, Jennings, Louisiana).[a b]

	Mean % posttreatment reduction + S.E.[c d]	
Rate (liter/ha)	24 hr	48 hr
0.29	55.5a±0.7 (12.3)	49.7a±0.3 (18.5+)
0.44	61.1a±2.8 (12.3)	76.1ab±3.5 (18.5+)
0.58	87.1b±2.3 (100+)	97.8b±0.6 (14.0)

[a] Age composition for 0.29 and 0.44 liter/ha plots: 2.2% first instar; 29.7% second instar; 16.2% third instar; 51.7% early fourth instar.

[b] Age composition for 0.58 liter/ha plots: 50% late second instar, 50% early third instar.

[c] Control data in parentheses ("+" indicates % increase). Means in the same column followed by the same letter are not significantly different at the 0.05 level.

[d] Temperature: air, 22.8–24.5°C at treatment; water 25.5–30°C. Wind at treatment: 0–3.2 kph for 0.58 l/ha rate; 3.2–8.1 kph for 0.29 and 0.44 liter/ha plots.

Relative humidity: 90–95%.

application apparently enables good coverage of the feeding zone of *An. quadrimaculatus*.

The increased efficacy of Beecomist-applied *B. sphaericus* FC against *Ps. columbiae* over that observed for *An. quadrimaculatus* parallels results obtained in laboratory assays using another isolate of the same serotype of *B. sphaericus* (Lacey and Singer 1982). Our field data were especially encouraging considering the advanced age (52% fourth instars) of the larval populations in plots where the two lower rates were evaluated.

Recently derived laboratory data indicate continued larval death after the initial wave of mortality in exposed larval populations. A 48 hr exposure to *B. sphaericus* (0.005 ppm) that initially resulted in 60% mortality in second instars of *Culex quinquefasciatus* Say yielded cumulative total mortality of 90% when the population was observed through to successful emergence (Lacey et al., unpublished data). Considering the rigors of natural habitats, continued mortality in natural populations of mosquito larvae exposed to *B. sphaericus* may play a considerable role in the true efficacy of this microbial control agent.

Psorophora columbiae is a significant pest of man and cattle and a potential vector of disease in the Southeast United States, especially in rice growing regions (Steelman et al. 1972, Olson and Grimes 1974). Control strategies currently rely primarily on application of conventional chemical adulticides for the suppression of this pest species. Further improvement in *B. sphaericus* formulations, especially with respect to concentration of toxic moiety will permit application of even lower rates of material making treatment of large areas more feasible. Commercial production of an affordable formulation of *B. sphaericus* will provide abatement districts and other public agencies with a viable alternative to chemical insecticides for the control of *Ps. columbiae*.

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References Cited

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.

Lacey, L. A. 1984. Production and formulation of *Bacillus sphaericus*. Mosq. News 44:153–159.

Lacey, L. A. and S. Singer. 1982. Larvicidal activity of new isolates of *Bacillus sphaericus* and *Bacillus thuringiensis* (H-14) against anopheline and culicine mosquitoes. Mosq. News 42:537–543.

Lacey, L. A. and A. H. Undeen. 1986. Microbial control of black flies and mosquitoes. Annu. Rev. Entomol. 31:265–296.

Lacey, L. A., M. J. Urbina and C. M. Heitzman. 1984. Sustained release formulations of *Bacillus sphaericus* and *Bacillus thuringiensis* (H-14) for control of container breeding *Culex quinquefasciatus*. Mosq. News 44:26–32.

Mulla, M. S., H. A. Darwazeh, E. W. Davidson and H. T. Dulmage. 1984a. Efficacy and persistence of the microbial agent *Bacillus sphaericus* for the control of mosquito larvae in organically enriched habitats. Mosq. News 44:166–173.

Mulla, M. S., H. A. Darwazeh, E. W. Davidson, H. T. Dulmage and S. Singer. 1984b. 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 T. Miura. 1978. Laboratory and field evaluation of *Bacillus sphaericus* as a mosquito control agent. J. Econ. Entomol. 71:774–777.

Olson, J. K. and J. E. Grimes. 1974. Mosquito activity in Texas during the 1971 outbreak of Venezuelan equine encephalitis (VEE), II. Virus incidence in mosquito samples from the south Texas plains. Mosq. News 34:178–182.

Sandoski, C. A., M. M. Yates, J. K. Olson and M. V. Meisch. 1985. Evaluation of Beecomist®-applied *Bacillus thuringiensis* (H-14) against *Anopheles quadrimaculatus* larvae in rice fields. J. Am. Mosq. Control Assoc. 1:316–319.

Steelman, C. D., T. W. White and P. E. Schilling. 1972. Effects of mosquitoes on the average daily gain of feedlot steers in southern Louisiana. J. Econ. Entomol. 65:462–466.

Yates, M. M. 1984. A portable system for aerial applications of very low volumes of technical grade concentrates of *Bacillus thuringiensis* var. *israelensis*. Mosq. News 44:583–587.