EFFICACY OF *BACILLUS SPHAERICUS* FORMULATIONS AGAINST *PSOROPHORA COLUMBIAE* LARVAE IN SMALL RICE PLOTS[1,2]

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ABSTRACT. *Bacillus sphaericus* formulations were evaluated against *Psorophora columbiae* larvae in small rice plots. All formulations tested provided good control (>84%) for all rates tested at 2 days posttreatment. At 10 days posttreatment, fair control (>70%) was recorded for ABG-6232 (0.184 kg/ha), ABG-6262; 27-242-BA (0.766 and 2.298 liters/ha), and ABG-6262; 29-293-BA (2.298 liters/ha). At 7 days posttreatment mortality was below 66%. Control did not exceed 27% at 15 days posttreatment for any formulation tested. Application rates for all formulations tested had little relationship with observed mortality, and control levels often were greater at the lower dosages tested. *Bacillus sphaericus* provided an effective means for controlling *Ps. columbiae*, but additional research is needed to determine the lower limit of effective treatment dosages and spore persistence in the rice field environment.

INTRODUCTION

Microbial insecticides have become an increasingly popular method for controlling larval mosquito populations because they are often highly effective against the target species, but have few, if any, adverse effects on nontarget macroinvertebrates (Mulla et al. 1984). During the past decade considerable effort has been devoted to the discovery and development of new genetic strains and formulations of microbial agents. Among bacterial agents, varieties of *Bacillus thuringiensis* have been widely tested and employed for larval mosquito control. Although the use and study of *Bacillus sphaericus* has been developing for several years (Chapman 1976), the species has received much less attention than *B. thuringiensis*. Some strains of *B. sphaericus* have demonstrated high levels of insecticidal activity against mosquito larvae, but their effectiveness has varied based on formulations tested, mosquito species, environmental conditions and water quality (Davidson et al. 1984, Des Rochers and Garcia 1984).

The dark rice field mosquito, *Psorophora columbiae* (Dyar and Knab), is considered to be a major pest in the southeastern United States (Steelman et al. 1972). Efficacy of *B. thuringiensis* against *Ps. columbiae* larvae in rice fields has been previously evaluated (Dame et al. 1981, Hembree et al. 1980, Lacey et al. 1986, Meisch et al. 1990, Mulla et al. 1988, Stark and Meisch 1983). Nothing has been reported for *B. sphaericus* in Arkansas ricelands against *Ps. columbiae*. The purpose of this study was to evaluate the effectiveness of various formulations of *B. sphaericus* against *Ps. columbiae* larvae in small rice plots.

MATERIALS AND METHODS

Tests were conducted at the Rice Research and Extension Center, Stuttgart, Arkansas. Plots were constructed to simulate rice field conditions and measured 6 × 6 m between levee centers with 16 m^2 rice growing pans. Commercially available rice varieties and accepted management practices for Arkansas were used throughout the study. Ditch and pan water depths were approximately 23 cm and 10 cm, respectively.

All *B. sphaericus* formulations used in this study were manufactured by Abbott Laboratories (North Chicago, IL). Liquid (ABG-6262®; subformulations 27-242-BA, 29-293-BA, 29-406-CD) and wettable powder (ABG-6232®; 0.184 and 0.367 kg/ha) formulations were mixed with 1 liter of water and applied with a hand-held CO_2 pressurized sprayer. All liquids were tested at 0.766 and 2.298 liters/ha, and subformulation 27-242-BA also was tested at 1.532 liters/ha. The granular formulation, ABG-6185® (0.918, 1.835 and 3.671 kg/ha), was spread evenly by hand across the plot.

Treatment plots were randomized and 3 plots were allocated for each rate of each formulation tested. Three control plots were used for each

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[2] Opinions and assertions contained herein are those of the authors and are not to be regarded as official or as reflecting the views of the Department of the Air Force. Mention of a commercial product does not constitute a recommendation of its use by either the University of Arkansas or the United States Air Force.

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evaluation. Treatments were replicated twice excluding the wettable powder, ABG-6232, ABG-6262; 27-242-BA (1.532 liters/ha) and ABG-6185 (0.918 kg/ha). Second and third instar *Ps. columbiae* were collected from naturally occurring populations near the test site and from nurseries. Floating cages, as described by Sandoski et al. (1986), containing 10 larvae each were placed in rice plots at 1 cage/plot immediately following treatment. Mortality was assessed at 2, 10 and 15 days posttreatment for the first replication and at 2, 7, 10 and 15 days for the second replication.

Percent mortality values were corrected for control mortality by Abbott's formula (Abbott 1925). Data were subjected to ANOVA for testing the hypothesis that mean mortality values among treatments were equal. Mean separations were accomplished using least-square means (LSMEANS) (SAS Institute 1985). For all tests, critical $P = 0.05$.

RESULTS

The *B. sphaericus* formulations evaluated in this study provided good control of *Ps. columbiae* larvae (Table 1). No significant differences ($P > 0.05$) were recorded among formulations or application rates at 2 days posttreatment and mortality was >84% in all instances. Some control also was recorded at 10 days posttreatment, but mortality was generally inadequate in comparison to the day 2 reading. Although >90% control was recorded for ABG-6232 and ABG-6262 at 10 days posttreatment, most mortalities were not greater than 70%. In some instances, there were no significant differences in mortalities between 2 and 10 day posttreatment readings for their respective formulations and application rates. There was a marked decrease in observed mortalities at 7 days posttreatment in comparison with either their 2 or 10 day counterparts, but not all of the lower mortality levels were significantly different from their respective higher values. Because of the small sample size used in this study (3 plots/rate/formulation), statistical comparisons may be exaggerated in certain instances and should be cautiously interpreted. Although a maximum of 65% control was recorded for ABG-6262 29-406-CD (0.766 liter/ha) at 7 days, other mortalities were <40%. By 15 days posttreatment, all mortalities were below 28% and, in most cases, were significantly lower than those observed at 10 days. Mean control plot mortality was 13.4% and no control mortality exceeded 20%.

DISCUSSION

All *B. sphaericus* formulations tested clearly provided good control of *Ps. columbiae* larvae 2 days posttreatment for all application rates tested. The high mortality rates observed for the application rates tested suggests that even lower application rates may be effective. Other studies have also reported *Ps. columbiae* larvae to be highly susceptible to *B. sphaericus* (Lacey et al. 1986; Mulla et al. 1985, 1988; Ramoska et al. 1977).

In this study, formulation type and application rate did not play as great a role in larval mortality as did time duration. Mortality was often greater at the lower application rates tested. Although mortality rates at 10 days posttreatment were not often significantly different from those of 2 days posttreatment, the per-

Table 1. Percent mortality of *Psorophora columbiae* larvae after introduction into small rice plots treated with various *Bacillus sphaericus* formulations.[1,2,3]

Formulation	Rate/hectare	Days after treatment			
		2	7	10	15
ABG-6232	0.184 kg	100.0 Aa	—	92.2 Aab	2.6 Ba
	0.367 kg	100.0 Aa	—	62.7 Aab	2.0 Ba
ABG-6185	0.918 kg	100.0 Aa	—	60.8 Aab	0 Bb
	1.835 kg	92.2 Aa	4.5 Cb	53.3 Bab	15.4 Ca
	3.671 kg	96.1 Aa	30.9 BCab	59.3 Bab	6.2 Ca
ABG-6262 (27-242-BA)	0.766 liters	92.3 ABa	21.5 Cab	72.8 Bab	9.5 Ca
	1.532 liters	100.0 Aa	—	68.6 Aab	7.2 Ba
	2.298 liters	84.6 Aa	15.5 Bab	100.0 Aa	22.6 Ba
ABG-6262 (29-293-BA)	0.766 liters	88.4 Aa	3.9 BCb	34.7 Bb	1.8 Ca
	2.298 liters	96.1 Aa	19.9 Bab	76.9 ABab	27.0 Ba
ABG-6262 (29-406-CD)	0.766 liters	96.1 Aa	65.4 Aa	69.2 Aab	1.8 Ba
	2.298 liters	92.3 Aa	40.1 Bab	44.0 ABab	1.8 Ca

[1] Means in the same row followed by the same upper case letter are not significantly different ($P \geq 0.05$) by least-square means.

[2] Means in the same column followed by the same lower case letter are not significantly different ($P \geq 0.05$) by least-square means.

[3] Means corrected for control mortality by Abbott's formula (Abbott 1925).

centages were much broader in range. Control was poor for all formulations and rates at 15 days posttreatment.

Reports of *B. sphaericus* persistence in mosquito habitats are varied, but durations generally are longer than those reported in this study. For example, *B. sphaericus* has been reported to remain persistent in a broad array of aquatic habitats for up to 30 days (Des Rochers and Garcia 1984). Similarly, Mulla et al. (1984) found formulations of *B. sphaericus* provided good control against *Culex tarsalis* Coq. larvae for about 3 weeks.

Because rice fields are shallow, aqueous habitats and often have high levels of suspended solids, *B. sphaericus* activity is probably depleted more rapidly than in aquatic habitats not sharing these characteristics. A high level of suspended solids in the water column also can diminish *B. sphaericus* activity (Mulligan et al. 1980). Also, *Bacillus* spores in shallow, aqueous suspensions (e.g., rice fields) are slowly detoxified and rendered nonviable by sunlight (Davidson 1984). These factors may explain the shorter activity period observed in this study. Furthermore, the lack of long-term residual activity of *B. sphaericus* may be due to settling of the spores, which are denser than water and tend to settle rapidly, removing the spores from the larval feeding zone (Davidson et al. 1984). However, Lacey (1984) and Mulligan et al. (1980) reported that spores of *Bacillus* spp. can retain their insecticidal properties long after settling from the water column. Thus, a single treatment with *B. sphaericus* probably controls only the initial larval mosquito population, and subsequent populations normally are not affected unless the spores are mechanically stirred from the substrate.

The differences in larval mortality at 2, 7 and 10 days posttreatment may be related to the aforementioned settling of *B. sphaericus* spores. Between days 2 and 7 the *B. sphaericus* spores may have settled partially from the water column. Agitation of the substrate in the treatment plots from a heavy rainstorm between days 7 and 10 of the second treatment application may have stirred *Bacillus* spores from the substrate and returned them to the larval feeding zone.

This study indicates that *B. sphaericus* can be an effective control agent of *Ps. columbiae* in rice fields. However, the effects of lower dosages, spore settling, and water quality on the effectiveness of *B. sphaericus* formulations remain to be tested.

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