EFFICACY OF *BACILLUS SPHAERICUS* 2362 FORMULATIONS AGAINST FLOODWATER MOSQUITOES[1]

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ABSTRACT. Four new formulations of *Bacillus sphaericus* 2362 yielded excellent control of floodwater mosquitoes *Psorophora columbiae* and *Aedes nigromaculis* in irrigated fields in Kings and Riverside counties of California. A primary powder formulation (ABG-6184) was the most active, producing excellent control of *Ps. columbiae* and *Ae. nigromaculis* at the rates of 0.05 to 0.5 lb/acre (0.055–0.56 kg/ha). A liquid formulation (BSP-2) was slightly less active, but was effective against the same species in the range of 1.0–1.5 lb/acre (1.12–1.68 kg/ha). Activity of the two corn cob granular formulations was largely dependent on potency (spores/gram). The high spore count granules (1.5×10^9 spores/gram) yielded 91 and 98% reduction of *Ae. nigromaculis* at the rates of 2.5 and 5.0 lb/acre (2.8 and 5.6 kg/ha), respectively. Against the same population, the lower spore count formulation (7.6×10^8 spores/gram) produced complete control at the rate of 10 lb/acre (11.2 kg/ha), but poor results were obtained at the rate of 5 lb/acre (5.6 kg/ha).

INTRODUCTION

Several strains of *Bacillus sphaericus* were reported to exhibit high activity against several mosquito species in the laboratory and under field conditions (Lacey and Singer 1982, Mulla et al. 1984b). A powder preparation of strain 2362, however, was the most active preparation tested, yielding excellent control of stagnant and floodwater mosquitoes such as *Culex tarsalis* Coquillett, *Cx. peus* Speiser, *Psorophora columbiae* (Dyar and Knab), *Aedes nigromaculis* Ludlow, and *Ae. melanimon* Dyar (Mulla et al. 1984a, 1985).

Efficacy of these microbial larvicides was reported to be highly dependent on the availability of toxin in the larval feeding zone (Aly 1983) as well as on the feeding behavior of the target mosquito species (Ramoska and Hopkins 1981). In order to enhance the activity of *B. sphaericus* 2362 against a wide spectrum of mosquito species, these factors were taken into consideration in the preparation of several new formulations. The following studies were initiated to evaluate these new formulations in irrigated fields and to determine their optimum rates of application against *Ae. nigromaculis* and *Ps. columbiae*.

MATERIALS AND METHODS

Formulations of *B. sphaericus* 2362 evaluated included ABG-6184 primary powder (1.5×10^{11} spores/gram), BSP-2 liquid (2.0×10^7 spores/gram) and two fine mesh (1 mm) corn cob granules, ABG-6185 (7.6×10^8 spores/gram) and ABG-6185 (1.5×10^9 spores/gram).

These new formulations were evaluated against *Ae. nigromaculis* and *Ps. columbiae* in irrigated fields in Kings and Riverside counties in California. Tests in Kings County were conducted against *Ae. nigromaculis* in alfalfa fields and irrigated pastures. Test sites were within 15–30 km radius from the city of Hanford, California. In Riverside County, tests were conducted against *Ps. columbiae* in irrigated pasture in Blythe, California, and in O'Rourke date garden in the Coachella Valley of southern California.

Plot size in each test varied according to the size of the mosquito breeding area, and ranged in size from 1/16 to 1/2 acre (253 to 2023 m^2). Two plots were used per application rate, and along with each test, two plots were left untreated as checks. Plot size, water temperature and mosquito species prevailing during each test are shown in the tables.

The required amount for each rate of application of the primary powder (ABG-6184), and the liquid formulation (BSP-2) were suspended in tap water, and applied at the rate of 8 gals of the aqueous suspension per acre (66.5 liter/ha). The aqueous suspension was applied with a 4 liter stainless steel pressurized hand sprayer equipped with 004 size fan jet nozzle. The required amount of the corn cob granules (2.5 and 5.0 lb/acre) were mixed with blank granules of similar size and texture, and applied at the rate of 8 lb of the mix per acre (8.96 kg/ha). The 10 lb/acre rate was sufficient in quantity to apply without mixing with blank granules. The granules were applied with a PCB model B (US Borax Co., Los Angeles, California).

To assess the effectiveness of the new formulations against mosquito larval populations, 20 dips per plot were taken prior to treatment

[1] These studies were conducted in cooperation with Coachella Valley Mosquito Abatement District, Thermal, California, and Kings County Mosquito Abatement District, Hanford, California. Studies supported by funds from state of California, Special Fund for Mosquito Research.

and 24 and 48 hr after treatment. Percent reduction calculation was based on number of larvae in posttreatment counts vs. pretreatment.

RESULTS AND DISCUSSION

Psorophora columbiae larvae were highly susceptible to the microbial larvicide *B. sphaericus* 2362. The primary powder (ABG-6184) produced complete control of larvae at all rates applied (0.1–0.5 lb/acre) in date garden plots (0.112–0.560 kg/ha), within 24 hr after treatment (Table 1). The minimum rate yielding satisfactory control was not determined, but we speculate to be in the range of 0.025–0.05 lb/acre (28–55 g/ha). The liquid formulation BSP-2, which contains lower spore count per gram than the primary powder, was 5–10 fold less active, but yielded complete control of *Ps. columbiae* larvae in Palo Verde Valley at the rate of 0.5–1.0 lb/acre (0.56–1.12 kg/ha). In another test in the Coachella Valley, 89% and complete control was obtained at the rates of 0.5, 1.0 and 2.0 lb/acre (0.56, 1.12 and 2.24 kg/ha) (Table 1). These findings indicate both formulations, ABG-6184 primary powder and BSP-2 liquid, could be used effectively against *Ps. columbiae* at the rates of 0.05–0.1 and 0.5–1.0 lb/acre (55–112 kg/ha and 0.56–1.12 kg/ha), respectively.

Table 1. Evaluation of *Bacillus sphaericus* 2362 formulations against *Psorophora columbiae* in irrigated fields.

Formulations	Rate lb/acre	Larvae/dip Pretreatment	(%) reduction (hr) 24	48
	Test A			
ABG-6184	0.10	8	100	100
Primary powder	0.25	7	99	100
	0.50	7	100	100
Check	—	12	0	0
	Test B			
BSP-2	0.5	58	93	100
Liquid	1.0	13	92	100
Check	—	7	0	0
	Test C			
BSP-2	0.5	10	76	89
Liquid	1.0	10	91	100
	2.0	5	94	100
Check	—	4	0	0

Test A: Plot size 1/10 acre (404 m^2) in date garden, Coachella Valley, California. Water temperature range 21–30°C.

Test B: Plot size 1/16 acres (253 m^2) in tailwater ditch, Palo Verde Valley, California. Water temperature range 22–33°C.

Test C: Same as Test A.

The pasture mosquito *Ae. nigromaculis* was less susceptible to *B. sphaericus* 2362 than *Ps. columbiae*. The ABG-6184 primary powder produced poor results against *Ae. nigromaculis* at the rates of 0.1 and 0.25 lb/acre (0.112 and 0.228 kg/ha), but complete control was obtained at the high rate of 0.5 lb/acre (0.56 kg/ha), which is more than 5-fold the effective rate against *Ps. columbiae* (Table 2). A similar trend was observed in earlier studies against *Ae. melanimon* in Owens Valley of the eastern Sierras of California (Mulla et al. 1985, 1986). However, lack of control in Owens Valley could be attributed to low water temperature, which was in the range of 6–26°C. High temperature prevailed during the day, but dropped drastically after sunset. Additional studies, therefore, are needed to determine the effect of cool temperature on the efficacy of *B. sphaericus* against mosquito larvae under natural field conditions. Temperature was reported to be a determining factor on the activity of Bt (H-14) and *B. sphaericus* 1593 against *Ae. stimulans* (Walker) larvae in the laboratory (Wraight et al. 1981).

The liquid formulation BSP-2 produced 93% control of *Ae. nigromaculis* at the rate of 1.0 lb/acre (1.12 kg/ha), while poor results were obtained at 0.5 lb/acre (0.56 kg/ha) (see Table 2).

Activity of the two corn cob granules against *Ae. nigromaculis* was largely dependent on the potency of each formulation. Formulation which contained 7.6×10^8 spores/gram produced complete control at the rate of 10 lb/acre (11.2 kg/ha). The high spore count granules (1.5×10^9

Table 2. Evaluation of *Bacillus sphaericus* 2362 formulations against *Aedes nigromaculis* in irrigated fields in Kings County, California.[a]

Formulations	Rate lb/acre	Larvae/dip Pretreatment	(%) reduction (hr) 24	48
	Test A			
ABG-6184	0.10	8	0	0
Primary Powder	0.25	6	50	83
	0.50	5	92	100
Check	—	12	0	0
	Test B			
BSP-2	0.5	5	20	40
Liquid	1.0	3	90	93
Check	—	3	0	0

Test A: Conducted in 1/8 acre (506 m^2) plots in alfalfa field tailwater. Water temperature range 21–31°C.

Test B: Low rate was conducted in 1/8 acre (506 m^2) plots, and the high rate was conducted in 1/16 acre (253 m^2) plots in irrigated pasture tailwater. Water temperature range 21–31°C.

Table 3. Evaluation of corn cob granules of *Bacillus sphaericus* 2362 against *Aedes nigromaculis* larvae in irrigated pastures.[a]

Formulations spores/gram	Rate lb/acre	Larvae/dip Pretreatment	(%) reduction (hr) 24	48
ABG-6185	5.0	35	66	66
7.6×10^8	10.0	7	86	100
ABG-6185	2.5	34	88	91
1.5×10^9	5.0	41	93	98
Check	—	28	0	0

[a] Test conducted in ½ acre plots (2023 m^2) in Kings County, California. Water temperature, mean minimum 20°C, mean maximum 32°C.

spores/gram) produced 91 and 98% reduction in the population at the rates of 2.5 and 5.0 lb/acre (2.8 and 5.6 kg/ha), respectively (Table 3).

From the above studies, it is clear that the primary powder (ABG-6184) was the most active formulation tested, and could be used effectively at the rates of 0.1 and 0.5 lb/acre (0.112 and 0.56 kg/ha) against *Ps. columbiae* and *Ae. nigromaculis*, respectively. The liquid formulation BSP-2 was less active, but could be utilized for the control of floodwater mosquitoes *Ps. columbiae* and *Ae. nigromaculis* at the rate of 1.0–2.0 lb/acre (1.12–2.24 kg/ha).

Both corn cob granules tested penetrated vegetative growth into the mosquito breeding areas, and were easy to apply with adequate coverage. The more potent formulation (1.5×10^9 spores/gram) however, appears to be more suitable for mosquito control operation due to its effectiveness at the low rates of 2.5 and 5.0 lb/acre (2.8 and 5.6 kg/ha). The less potent formulation (7.6×10^8 spores/gram) was only effective at the high rate of 10 lb/acre (11.2 kg/ha). Granular formulations of *B. sphaericus* as tested here are quite promising for the control of floodwater mosquitoes.

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