EFFICACY AND FIELD EVALUATION OF *BACILLUS THURINGIENSIS* (H-14) AND *B. SPHAERICUS* AGAINST FLOODWATER MOSQUITOES IN CALIFORNIA[1]

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ABSTRACT. The microbial control agents *Bacillus thuringiensis* (H-14) and *B. sphaericus* were evaluated in laboratory and field against *Psorophora columbiae. Bacillus sphaericus* strain 2362 was also tested in the field against *Aedes melanimon.*

Psorophora columbiae was slightly more susceptible than *Culex quinquefasciatus* to active strains of *B. sphaericus.* The LC_{90} for active strains ranged from 0.013 to 0.069 mg/liter. In field trials, aqueous suspensions of primary powder of *B. sphaericus* 2362 and 1593 yielded 98-99% reduction in larvae at the rates of 0.1 to 0.25 lb/acre of the primary powder. Granular formulations of Bt (H-14) were evaluated against *Ps. columbiae,* yielding 96-99% control of larvae at rates ranging from 1 to 10 lb/acre of the granules, depending on the potency and type of the formulations.

Aedes melanimon was slightly less susceptible than *Ps. columbiae* to *B. sphaericus* 2362. In warmer water a rate of 0.25 lb/acre of the primary powder yielded 88% control, while this same rate in cool weather yielded only 4% reduction. A rate of 0.5 lb/acre of the primary powder was needed to obtain 94% control of larvae in cool weather.

INTRODUCTION

Recently, two microbial larvicides *Bacillus sphaericus* Neide and *B. thuringiensis* Berliner serotype (H-14) have been found to exhibit high level of activity against several species of stagnant and floodwater mosquitoes in the laboratory and under field conditions (Davidson et al. 1981, Lacey and Singer 1982, Mulla et al. 1982b, 1984a, 1984b). Efficacy of these microbial agents, however, was found to be influenced by many factors such as water quality in mosquito breeding sources (Mulla et al. 1982a, 1984a), nutrient availability and ingestion rate of nutrients by mosquito larvae (Ramoska and Pacey 1979), larval density, formulations, potency of formulations (Mulla et al. 1982b), larval instars, temperature (Wraight et al. 1981), and mosquito species (Lacey and Singer 1982, Mian and Mulla 1983, Mulla et al. 1984b).

Several formulations of *Bacillus thuringiensis* H-14 were reported to be highly active against several species of stagnant and floodwater mosquito larvae in irrigated pastures, rice fields, and experimental outdoor ponds. These species include *Aedes nigromaculis* (Ludlow), *Psorophora columbiae* (Dyar and Knab), *Anopheles quadrimaculatus* Say, *Culex tarsalis* Coquillett and *Cx. peus* Speiser (McLaughlin and Billodeaux 1983, Mulla et al. 1982b, Stark and Meisch 1983). *Bacillis sphaericus* 1593 also yielded satisfactory results against *Cx. tarsalis,* but failed to produce adequate control in a mixed larval population of *Ae. nigromaculis* and *Ae. melanimon* in irrigated pastures (Mulligan et al. 1978). This batch of 1593 was produced in a pilot plant long before use and may have lost some activity.

In order to improve activity and longevity of these biocontrol agents under various field conditions, Davidson (1984) and Lacey (1984) suggested further research on finding, testing, and developing new strains and formulations to prolong their shelflife, and improve their stability and residual activity in the field.

Recently, we studied the efficacy of several new strains and formulations of *B. sphaericus* and *B. thuringiensis* (H-14), against stagnant water mosquitoes in laboratory and in outdoor ponds at the Aquatic and Vector Control Research Facility, University of California, Riverside, and in the Coachella Valley of southern California. Some of these newly developed strains and formulations produced excellent results against *Cx. tarsalis* and *Cx. peus* with no apparent effects on the nontarget biota (Mulla and Darwazeh 1984, 1985; Mulla et al. 1984a, 1984b). The present studies evaluated these highly active strains and formulations against the floodwater mosquitoes *Ae. melanimon* Dyar and *Ps. columbiae* under field conditions in various parts of southern California. Both these flood-water mosquitoes constitute important pest species in many agricultural and rural communities in California.

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[1] These studies were supported by UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases, and State of California Special Fund for Mosquito Research.

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METHODS AND MATERIALS

Prior to field evaluation, five strains and preparations of *B. sphaericus* which displayed good biological activity against *Culex* and *Anopheles* larvae in the laboratory (Mulla et al. 1984b) were evaluated in the laboratory against field collected 4th instar *Ps. columbiae*. These were: 2362 (IF–117), 2362 (IF–97), 1593 (IF–94), 2013–4, and 2013–6. Along with these strains and preparations, the international standard *B. sphaericus* strain 1593 (RB80) provided by H. deBarjac, Institut Pasteur, Paris, France, was also tested and used as a standard.

Methods utilized in the evaluation of these materials in the laboratory are described elsewhere (Mulla et al. 1982a). In brief, 1% stock suspension of each preparation (w/v) was prepared in tap water, and serial dilutions in tap water were prepared as needed. The required amounts of the proper strength dilutions were added to 4 oz disposable ice cream cups (Sweetheart Cup Division, Baltimore, MD), containing 20 4th-instar larvae of *Ps. columbiae* in 100 ml of tap water. Each material was tested at 4–5 concentrations on 2–3 different occasions, utilizing 3 replicates per concentration. Along with each test, 3 cups were left untreated as checks. All test organisms were kept at a temperature of 27°C. After 48 hr of exposure, mortality readings were taken. Values obtained were subjected to log probit regression analysis to obtain the LC_{90} estimates (mg/liter) and 95% confidence limits.

Procedures utilized in field studies were as follows:

PALO VERDE VALLEY. Studies were conducted in irrigated pastures at the Ox Bow Ranch, located 5 miles south of the town of Palo Verde in Imperial County, California. At time of treatment, larval population consisted of 3rd and 4th instars. Water level (pH 7.9) in test plots was either above or at vegetation growth level, and ranged in depth from 8 to 14 in. (20–36 cm). Bermuda grass in the plots was dense and ranged in length from 6 to 8 in. (15–20 cm).

Materials tested included 2 powder preparations of *B. sphaericus* (1593–IF 94 and 2362–IF 117) and several corn cob granules of *B. thuringiensis* H–14. The granular formulations were: ABG–6143 (100 IU/mg), ABG–6138 (200 IU/mg), ABG–6141 (300 IU/mg), Sandoz 402 GR70 (260 IU/mg), and (5%) Bactimos (175 IU/mg). In addition, an aqueous suspension (ABG–6145 containing 600 IU/mg) also was included in these studies.

The corn cob granules were evaluated either in 0.25 or 0.5 acre plots of irrigated pastures, depending on amount of material available, utilizing 2 replicates per application rate. Each rate was applied in a separate irrigation check, and in each test, 2 plots in the same field were left untreated as checks. The granules were applied with a PCB Model B spreader (US Borax, Los Angeles, CA).

The aqueous suspension of *B. thuringiensis* H–14 (ABG–6145), and the 2 powder preparations of *B. sphaericus* (1593–IF 94 and 2362–IF 117) were tested in ⅛ acre irrigated pasture plots, utilizing 2 replicates per rate in the same irrigation check. Two plots in the same field were left untreated as checks. The required amount of material for each rate was mixed in 4 liters of tap water and applied with a 4 liter stainless steel hand sprayer, equipped with Spraying Systems 004 fan jet nozzle.

OWENS VALLEY. Studies were conducted in Mark John's pastures on Reynolds Ave., west of highway 395 in Big Pine, and in Jack Tatum's pastures on highway 6, south of Dixon Lane in Bishop, California.

Second, 3rd, or 4th instars were present at time of treatment. Vegetation was extremely dense, and consisted of a mixture of several grasses and legumes such as Bermuda grass, water grass, alfalfa and clover. Some sagebrush and other perennial weeds were also present, but had only spotty distribution. Water level (pH 6.5) was above vegetation growth in test plots and ranged in depth from 10 to 14 in. (25–35 cm).

Only *B. sphaericus* 2362 (IF–117) was evaluated against *Ae. melanimon* in irrigated pastures. Most pastures in the area are considered wild range pastures with no irrigation borders, but the entire field is irrigated by means of flood irrigation. Therefore, excess water accumulates in the low spots providing ideal breeding sites for *Ae. melanimon*. Test plots (1/16 acre each) were marked with flags, and each low spot was treated with one rate, and an adjacent low spot in the same field was used as check. In each test, 2 plots were used per rate, and 2 plots were left untreated as checks.

ASSESSMENT TECHNIQUES. To assess the effect of the microbial larvicides on the larval population, 10 dips per plot, in all tests, were taken prior to treatment and 24 and 48 hr after treatment. Due to the substantial increase in the number of larvae in the untreated plots (checks) in the posttreatment counts, the formula (Mulla et al. 1971) was utilized in the calculation of percent reduction:

$(\%R) = 100 - \left(\frac{C1}{T1} \times \frac{T2}{C2} \right) 100$, where C1 = mean number of larvae in check pretreatment, T1 = mean number of larvae in treated pretreatment, T2 = mean number of larvae in treated posttreatment, and C2 = mean number of larvae in check posttreatment. The increase in the larval population counts in the checks 24 and 48 hr after treatment is attributed mainly to water loss due to evaporation and percolation, which forced the larval population to concentrate in large numbers in small puddles of water. At time of treatment, the larval population was scattered evenly over the entire plots. In all tests, water temperature was monitored with a mini-maximum recording thermometer during the duration of each test.

RESULTS AND DISCUSSION

In the laboratory, *B. sphaericus* strain 2362 (IF-117) was the most active one tested, causing 90% mortality in 4th-instar larvae of *Ps. columbiae* at a concentration of 0.013 mg/liter (Table 1). The standard international preparation 1593 (RB80), and 2362 (IF-97) were equal in activity, but were slightly less active than 2362 (IF-117), producing 90% mortality at 0.021 and 0.02 mg/liter respectively. Strains 1593 (IF-94) and 2013-6 were about 5 times less active than 2362 (IF-117), with an LC_{90} of 0.065 and 0.069 mg/liter. Strain 2013-4 was the least active, with an LC_{90} of 0.126 mg/liter (Table 1). *Psorophora columbiae* larvae appear to be 2-3 fold more susceptible to both preparations of 2362 and the international standard 1593 (RB80) than *Cx. quinquefasciatus* larvae. Strains 2013-4 and 2013-6 were less active against *Ps. columbiae* than *Cx. quinquefasciatus*, while 1593 (IF-94) showed similar activity against the two species (Mulla et al. 1984b).

The three Abbott corn cob granules of *B. thuringiensis* (H-14) were similar in size (0.15-0.5 cm), and were highly active against *Ps. columbiae* field populations. However, effective application rates varied according to the potency of each formulation (IU/mg). ABG-6143 (100 IU/mg) yielded 98% control at the rate of 10 lb/acre of granules, while the more potent formulation ABG-6138 (200 IU/mg) produced similar results at half this rate (Table 2). ABG-6141 (300 IU/mg) produced 96% control at 5 lb/acre, while 76% control was obtained at the low rate of 2.5 lb/acre. The aqueous suspension ABG-6145 (600 IU/mg) also displayed excellent activity against *Ps. columbiae* larvae, causing 87, 95 and 98% control at the rates of 0.25, 0.50 and 1.0 lb/acre respectively (Table 2).

The granular formulations provided by Abbott showed similar efficacy against larvae of *Ps. columbiae* as they did against *Cx. tarsalis* and *Cx. peus* larvae in experimental ponds. The aqueous suspension, however, was more effective against *Culex* species (10 fold), yielding excellent reduction in *Culex* population at the low rate of 0.05 lb/acre (Mulla and Darwazeh 1985) as compared to a rate of 0.5 lb/acre against *Ps. columbiae* found here.

The corn cob granules Sandoz 402 (GR-70), and Bactimos (5%) were fine in texture (14/20 and 12/14 mesh), and exhibited excellent activity against *Ps. columbiae.* At the rates of 5 and 10 lb/acre, Sandoz 402 (GR-70) yielded 99% and complete control of larvae in open water, while 97% and complete control was obtained in heavy vegetation (Table 3). At the rates of 5 and 8 lb/acre, Bactimos (5%) granules caused 97 and 92% reduction in heavy vegetation, while the low rate (5 lb/acre) eliminated the larval population completely in open water. The high rate of 8 lb/acre caused 97% control in open water, where larval density in test plots was extremely heavy (Table 3). It seems that both these granular formulations yielded extremely good control of *Ps. columbiae* larvae in both open and vegetated breeding sources at the same rates of application. These as well as Abbott granular formulations of *B. thuringiensis* (H-14) were effective in the range of 5-10 lb/acre against larvae of this mosquito.

In other studies (Mulla and Darwazeh 1985), Sandoz 402 GR-70 was reported to be highly active against larvae of *Cx. tarsalis* and *Cx. peus* at the low rate of 1.0 lb/acre. This Sandoz formulation appears to be more effective (5 fold) against larvae of *Culex* species than larvae of the floodwater mosquito *Ps. columbiae.*

Bacillus sphaericus 2362 (IF-117) primary powder produced better results against *Ps. columbiae* larvae than *B. sphaericus* 1593 (IF-94).

Table 1. Susceptibility of field collected 4th-instar larvae of *Psorophora columbiae* to various strains and preparations of *B. sphaericus* in the laboratory.

Preparation	Preparation method	mg/liter LC_{90}	(95%) CL Lower	(95%) CL Upper
2362 (IF-117)	Spray dried	0.013	0.007	0.02
2362 (IF-97)	Acetone precipitation	0.020	0.008	0.03
1593 (RB80)	Lyophilized	0.021	0.015	0.03
1593 (IF-94)	Acetone precipitation	0.065	0.045	0.08
2013-6	Lyophilized	0.069	0.035	0.10
2013-4	Lyophilized	0.126	0.087	0.16

At the rates of 0.05, 0.1 and 0.25 lb/acre, strain 2362 (IF–117) produced 66, 98 and 99% reduction respectively, while 1593 (IF–94) caused 84 and 99% control at 0.25 and 0.5 lb/acre (Table 4). Strain 2362 was reported earlier to be more effective against larvae of *Culex* spp. than strain 1593. At the rate of 0.05 lb/acre, complete control of *Cx. tarsalis* and *Cx. peus* was obtained with 2362, while 0.1 lb/acre was required to obtain similar results with 1593 (Mulla

Table 2. Evaluation of various formulations of *B. thuringiensis* H-14 against *Psorophora columbiae* larvae in irrigated pastures, Palo Verde Valley, California.[a]

Materials and formulations	Rate lb/acre	Rate kg/ha	Mean no. larvae/10 dips Pretreatment	Mean no. larvae/10 dips 24 hr posttreatment	% reduction
			Aug. 13, 1983 (26–40°C)		
ABG-6143	5.0	5.6	330	250	64
Corn cob gr.	10.0	11.2	430	20	98
(100 IU/mg)					
Check	—	—	320	670	—
			July 20, 1983 (27–38°C)		
ABG-6138	5.0	5.6	120	7	97
Corn cob gr.	10.0	11.2	147	4	99
(200 IU/mg)					
Check	—	—	47	87	—
			Aug. 13, 1983 (26–40°C)		
ABG-6141	2.5	2.8	280	140	76
Corn cob gr.	5.0	5.6	510	40	96
(300 IU/mg)					
Check	—	—	320	670	—
			July 20, 1983 (27–38°C)		
ABG-6145	0.25	0.28	64	15	87
Aqueous susp.	0.50	0.56	62	6	95
(600 IU/mg)	1.00	1.12	110	4	98
Check	—	—	47	87	—

[a] Water temperature, minimum and maximum are given for each test.

Table 3. Evaluation of various formulations of *B. thuringiensis* H-14 against *Psorophora columbiae* larvae in irrigated pastures, Palo Verde Valley, California (August 1984).[a]

Materials and formulations	Rate lb/acre	Rate kg/ha	Mean no. larvae/10 dips Pretreatment	Mean no. larvae/10 dips 24 hr posttreatment	% reduction
			Open water		
Sandoz-402	5.0	5.6	113	2	99
GR-70	10.0	11.2	160	0	100
(260 IU/mg)					
Bactimos (5%)[b]	5.0	5.6	97	0	100
Corn cob gr.	8.0	8.9	500	29	97
(175 IU/mg)					
Check	—	—	96	192	—
			Vegetated water		
Sandoz-402	5.0	5.6	130	10	97
GR-70	10.0	11.2	252	0	100
(260) IU/mg)					
Bactimos (5%)[b]	5.0	5.6	131	10	97
Corn cob gr.	8.0	8.9	90	16	92
(175 IU/mg)					
Check	—	—	63	139	—

[a] Water temp. ranged 24–29°C.

[b] Formulation prepared from Bactimos WP (3500 IU/mg, Bio-Chem Products).

Table 4. Evaluation of *B. sphaericus* as primary powder against *Psorophora columbiae* larvae in irrigated pastures, Palo Verde Valley, California.

Pathogen	Rate lb/acre	Rate kg/ha	Mean no. of larvae/100 dips and (%) reduction: Pretreatment	48 hr posttreatment	(%R)
			Test A, July 16, 1983 (water temp. 27–41°C)		
2362	0.25	0.28	505	2	99
(IF-117)	0.50	0.56	473	1	99
1593	0.25	0.28	740	117	84
(IF-94)	0.50	0.56	710	2	99
Check	—	—	865	1450	—
			Test B, Aug. 21, 1983 (water temp. 24–29°C)		
2362	0.10	0.11	480	4	99
(IF-117)	0.25	0.28	460	2	99
Check	—	—	160	580	—
			Test C, Aug. 25, 1984 (water temp. 29–34°C)		
2362	0.05	0.06	440	232	66
(IF-117)	0.10	0.11	377	13	98
Check	—	—	303	473	—

et al. 1984b). *B. sphaericus* 2362 appears to be equally effective against the *Culex* spp. and *Ps. columbiae*. In general, satisfactory control of these species could be obtained at the low rate of 0.1 lb/acre of the primary powder of this bacterial agent.

Larvae of the pasture mosquito *Aedes melanimon* were more tolerant to *B. sphaericus* 2362 (IF-117) than *Ps. columbiae* in irrigated pastures. At the rate of 0.1 lb/acre (the effective rate for the control of *Ps. columbiae*), only 90% control of 2nd and 3rd instars, and 80% of 3rd and 4th instars was obtained when field water temperature was in the range of 9°–22°C (Table 5). At the same water temperature, 88% reduction was possible at the rate of 0.25 lb/acre. The same rate, at a lower water temperature (6°–22°C), produced only 4 and 31% control, while the high rate of 0.5 lb/acre, caused only 94% control in 3rd and 4th instar larvae (Table 5).

These findings indicate that the activity of *B. sphaericus* 2362 against *Ae. melanimon* larvae was lower, probably due to cold temperature during the test period. Water pH in the Owens Valley was in the range of 6.5 compared to 7.9 in the Palo Verde Valley of southern California. Additional studies, however, are needed during the summer months (June–August 1985) to fully assess the efficacy of this microbial control agent against *Ae. melanimon*.

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Table 5. Evaluation of *B. sphaericus* 2362 (IF-117) against *Aedes melanimon* larvae in irrigated pastures, Owens Valley, California.

Rate lb/acre	Rate kg/ha	Larval instar	Mean no. of larvae/100 dips and (%) reduction: Pretreatment	48 hr posttreatment	(%R)
		Test A, Sept. 22, 1984 (water temp. 9–22°C)			
0.10	0.11	2–3	83	28	90
		3–4	77	52	80
0.25	0.28	3–4	495	203	88
Check	—	3–4	81	280	—
		Test B, Sept. 25, 1984 (water temp. 7–22°C)			
0.25	0.28	3–4	255	290	31
Check	—	3–4	142	235	—
		Test C, Sept. 26, 1984 (water temp. 6–22°C)			
0.25	0.28	3–4	130	550	4
0.50	0.56	3–4	98	25	94
Check	—	3–4	48	213	—

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