EFFICACY AND LONGEVITY OF *BACILLUS SPHAERICUS* 2362 FORMULATIONS FOR CONTROL OF MOSQUITO LARVAE IN DAIRY WASTEWATER LAGOONS[1]

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ABSTRACT. *Bacillus sphaericus* strain 2362 was evaluated for the control of *Culex* larvae in dairy wastewater lagoons. Both initial and long-term efficacy were studied. Two primary powder preparations of ABG-6184 yielded mediocre and short-term control at the rates of 0.25 and 0.5 lb/acre (0.26 and 0.56 kg/ha), while level of control and persistence greatly increased as the dosages were increased to 1.0, 2.0 and 4.0 lb/acre (1.12, 2.24 and 4.48 kg/ha). The 1.0 and 2.0 lb/acre rates yielded almost 100% control for 4 weeks and the 4.0 lb/acre rate yielded control (99%) for 49 days or longer. A flowable concentrate preparation (BSP-2) yielded complete initial and persistent control of larvae for 14-21 days at 2.0, 4.0 and 5.0 lb/acre (2.24, 4.49 and 5.6 kg/ha).

Granular formulations of *B. sphaericus* 2362 (ABG-6185) were also evaluated at 2.5, 5.0, 7.5, 10.0 and 20.0 lb/acre (2.8, 5.6, 8.4, 11.2 and 22.4 kg/ha) of the granules. Some of the formulations were more active than the others, yielding excellent initial and persistent control (80% +) for 14–21 days with one treatment.

INTRODUCTION

For sanitation purposes, dairy herds in California are washed twice daily prior to milking, and milking barns are washed thoroughly after each milking. Wash wastewater which contains high contents of solid and dissolved organic materials is pumped into large ponds or series of ponds depending on the size and cultural practices of each dairy. Wastewater in these ponds is either used for pasture irrigation or allowed to dry through evaporation and percolation. These highly polluted ponds provide ideal larval development habitat for several *Culex* mosquito species (Mulla et al. 1987a, O'Meara and Evans 1983).

The microbial larvicides *Bacillus sphaericus* and *B. thuringiensis* (H-14) have exhibited excellent biological activity against several mosquito species in the laboratory and under field conditions (Davidson et al. 1981, Lacey and Singer 1982; Mulla et al. 1982, 1984, 1986). Efficacy of these materials, however, was found to be highly influenced by quality of water in mosquito development sites. *Bacillus sphaericus* provided a higher level of control in fresh water than in polluted water (Mian and Mulla 1983). In organically enriched ponds, *B. sphaericus* 2362 produced little or no control of larvae at the rate of 0.21 kg/ha, while excellent control was obtained in the unenriched ponds (Mulla et al., 1984). Similar trends were observed with *B. thuringiensis* H-14 against *Culex quinquefasciatus* Say in polluted water in Florida (Rathburn et al. 1984), and in dairy wastewater lagoons in Tulare County, California (Mulla et al. 1982). However, some formulations of *Bacillus sphaericus* 1593 under controlled conditions, yielded excellent extended control of culicine mosquito larvae in containers of fresh and polluted water (Lacey et al. 1984, Silapanuntakul et al. 1983). Control of *Culex* mosquitoes in manure-laden ponds and lagoons is extremely difficult. Present methods of choice consist of applying organophosphate larvicides and/or larvicidal oils on a weekly basis. The cost of equipment, application and surveillance make it economically unfeasible to implement these types of control measures in these habitats. The need for the evaluation of persistent and slow-release formulations is therefore very obvious with a view toward reducing the frequency of treatments. The present studies were implemented to assess the efficacy and longevity of new formulations of *Bacillus sphaericus* 2362 against mosquito larvae in dairy wastewater lagoons, and to determine the optimum rate of application for each formulation.

MATERIALS AND METHODS

Six different formulations of *B. sphaericus* 2362 were utilized in these studies; they were 2 preparations of the primary powder ABG-6184, 4 formulations of corncob granules of various sizes and potencies (designated as ABG-6185, ABG-6185A) and a liquid flowable concentrate formulation BSP-2. The primary powder and granular formulations were provided by Abbott Laboratories (North Chicago, IL) while the liquid BSP-2 was provided by Solvay and Company

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(Brussels, Belgium). The mesh size and potency (spore contents) of each granular formulation are shown in the tables along with plot size treated and rates of application.

Tests were conducted at Midhill, Lakerkirk and Kasbergen dairies in Riverside and San Bernardino counties, California. At Lakerkirk, according to our instructions, available wastewater was pumped daily into the test plots. Since we had no control over water flow at Kasbergen and Midhill dairies, 7 and 3 holding ponds, respectively, were used in rotation for various periods. Water temperature during these studies (July–August, 1987) was in the range of 23°C minimum to 33°C maximum.

The primary powder ABG-6184 was evaluated at rates of 0.25, 0.5, 1.0, 2.0 and 4.0 lb/acre (0.28, 0.56, 1.12, 2.24 and 4.48 kg/ha). The liquid formulation BSP-2 was applied at the rates of 0.5, 1.25, 2, 4 and 5 lbs/acre (5.6, 1.4, 2.24, 4.48 and 5.6 kg/ha) of the formulation. The required amount for each rate of the primary powder and the liquid formulations was mixed with tap water, and applied at the rate of 8 gals (30 liters) of the aqueous suspensions/acre. The material was applied with a 1 gal (4 liters) pressurized hand sprayer, equipped with a 0006 Teejet nozzle. Rates of application for the corn cob granules were based on contents of spore count/gram of each formulation. Two formulations of the corn cob granules (ABG-6185, 1–2 mm and ABG-6185 A, 3 mm) were broadcasted by hand, while the third formulation (fine mesh granules less than 1 mm in size, ABG-6185 20/40 mesh), was applied with a knapsack type, gasoline powered, granular blower (Model MD 150X, Maruyama Mfg. Co. Inc., Tokyo, Japan).

During each test, one pond was used per application rate, and an adjacent pond was left untreated as check. With the limited number of ponds available for testing, replication of treatments was not feasible. Mosquito larval population at all locations consisted mostly of *Culex peus* Speiser. *Culex quinquefasciatus* were also present, but in small numbers (5–10%).

To assess initial efficacy and longevity of each formulation against larvae, 20 dips (5/each side) were taken per pond prior to treatment, and 3 and 7 days after treatment and every week thereafter, until the larval population recovered to the pretreatment level. The percent reduction was based and calculated on the number of 3rd and 4th-instar larvae in posttreatment counts vs. pretreatment in the treated ponds.

RESULTS AND DISCUSSION

Bacillus sphaericus 2362 primary powder (ABG-6184) yielded mediocre control of larvae at the rate of 0.25 lb/acre (0.28 kg/ha) against *Cx. peus* larvae in dairy wastewater lagoons (Table 1). At the next higher rate of 0.5 lb/acre (0.56 kg/ha), good initial control was obtained 3 days after treatment but the population began to recover 7 and 14 days after treatment. However, at the rates of 1 and 2 lbs/acre (1.12 and 2.4 kg/ha), excellent control was obtained for more than 28 days. At the highest rate of 4 lb/acre (4.48 kg/ha), 99% reduction continued for 49 days.

The liquid formulation BSP-2 produced mediocre control at the lowest rate of 0.5 lb/acre (0.56 kg/ha), however, good initial control was obtained at the higher rates of 1.25 and 2.0 lb/acre (1.4 and 2.24 kg/ha), but effectiveness declined 14 days after treatment (Table 2). At the higher rates of 2.5, 4 and 5 lbs/acre (2.8, 4.48 and 5.6 kg/ha), excellent control of larvae was obtained for 21 days or longer.

According to the data presented here, an increase in application rates of formulations was directly correlated with an increase in the initial activity and longevity of control by *B. sphaericus* 2362 in polluted water. For optimum results, the

Table 1. Efficacy of two batches of *Bacillus sphaericus* 2362 primary powder (ABG-6184) against *Cx. peus* larvae in dairy wastewater lagoons.

Rate lb/acre	Spores/gram	Plot size acre	Plot size location	Mean no. larvae/dip pretreatment	(%) Reduction after treatment (days)[a] 3	7	14	21	28
0.25	1.4×10^{11}	0.16	Lakerkirk[b]	27	70	22	—	—	—
0.50	1.4×10^{11}	1.25	Midhill[c]	7	94	76	63	0	—
1.00	7.6×10^{10}	0.30	Kasbergen[c]	19	100	62	93	100	100*
2.00	7.6×10^{10}	0.30	Kasbergen	7	100	88	96	100	100*
4.00	7.6×10^{10}	0.10	Lakerkirk	133	99	100	100	100	99**

[a] No reduction in check plots was observed during these tests, therefore larval densities of check plots omitted.

[b] Water pumped into the pond daily.

[c] Water allowed to dry after pond was filled.

* Drying

** 99% control was obtained for 49 days.

Table 2. Efficacy of *Bacillus sphaericus* 2362 flowable concentrate (BSP-2) against *Cx. peus* larvae in dairy wastewater lagoons.[a]

Rate lb/acre	Plot size acre	Plot size location	Mean no. larvae/dip pretreatment	(%) Reduction after treatment (days)[b] 3	7	14	21	28
0.5	0.16	Lakerkirk	21	74	44	—	—	—
1.25	0.16	Lakerkirk	9	99	89	49	—	—
2.0	0.25	Kasbergen	8	100	88	50	0	—
2.5	0.16	Lakerkirk	24	99	99	99*	—	—
4.0	0.30	Kasbergen	34	99	98	98	100	67
5.0	0.16	Lakerkirk	14	100	100	100	100*	—

[a] Formulation contains 2.0×10^7 spores/gram.
[b] No reduction in check plots was observed during these tests, therefore check data omitted.
* Drying.

primary powder ABG-6184 and the liquid BSP-2 need to be used at the rates of 1.0 to 2.0 and 2.5 to 4.0 lb/acre (1.12–2.24 and 2.8–4.48 kg/ha), respectively, for long-lasting control of *Cx. peus* and *Cx. quinquefasciatus* larvae in dairy wastewater lagoons. At these rates, larval control could be achieved for 3 to 4 weeks or longer, depending on the dairy water management practices. These rates are approximately 5 to 10 fold the effective rates against *Cx. tarsalis* Coquillett and *Cx. peus* larvae in clear and less polluted water habitats (Mulla et al. 1987b).

During the course of these studies, oviposition was observed to occur in the newly flooded ponds, and continued for as long as the water was added daily to these ponds. Egg rafts were found in large numbers in vegetation along the edges of the ponds. However, when water was shifted to a new pond, oviposition ceased in the old ponds and began to occur in the new ponds. Under these conditions, reduction in larval population was initially caused by *B. sphaericus*, but absence of larvae in the ponds subjected to drying could be attributed to either lack of oviposition or due to the efficacy of the treatments or both. In dairies, where water was pumped daily into the ponds, extended control was due to *B. sphaericus* persistence in the treated wastewater lagoons. These ponds contained many egg rafts but no larvae were noted. In lagoons where water was turned off, the absence of larvae could be either due to lack of oviposition or due to the activity of *B. sphaericus*.

Efficacy of the corn cob granules was highly influenced by mesh size and number of spores/gram of each formulation. Granules with a low spore count (1.9×10^9 spores/gram) produced lower reduction when used at the rate of 10 lb/acre (11.2 kg/ha), while 87% reduction was obtained at the high rate of 20 lb/acre (22.4 kg/ha) (Table 3). The more potent formulations, with various mesh sizes, but containing equal number of spores/gram (5.0×10^9), produced variable results. ABG-6185 (1–2 mm) yielded 81% reduction at the rate of 5 lb/acre (5.6 kg/ha) after 3 days, while excellent control (98–99%) was obtained for 14 days at the rate of 10 lbs/acre (11.2 kg/ha). The larger size granules (ABG-6185A 3 mm) yielded poor control at the low rate of 2.5 lbs/acre (2.8 kg/ha), however, excellent control was obtained for 14 and 21 days at the rates of 5 and 7.5 lbs/acre (5.6 and 8.4 kg/ha), respectively. The fine mesh granules ABG-6185 (1 mm or 20/40 mesh) caused excellent reduction in the larval population for 14 days at the rate of 10 lbs/acre (11.2 kg/ha) (Table 3).

The fine mesh formulations, as reported earlier (Mulla et al. 1988), also produced excellent control of *Aedes nigromaculis* (Ludlow) in irrigated pasture. Based on the data presented here, it can be concluded that the large size corn cob granules ABG-6185A (3 mm), containing 5.0×10^9 spores/gram, was the most active and most suitable for usage against *Cx. peus* and *Cx. quinquefasciatus* larvae in dairy wastewater lagoons.

In a concomitant study (Matanmi et al. 1989), laboratory bioassays of surface water obtained 1 day after treatment from ponds treated with the primary powder ABG-6184 at the rates of 1 and 2 lbs/acre (1.12 and 2.24 kg/ha), showed high toxicity against 4th-instar larvae of *Cx. quinquefasciatus*. However, no biological activity was detected in treated surface water samples obtained 3, 7, 14, 21 and 28 days after treatment. Water samples obtained from the bottom, however, exhibited a high level of activity for 14 days, but activity at both rates began to decline 21 days after treatment. The persistence of the flowable concentrate formulation (BSP-2) was different than that of the primary powder preparation (ABG-6184). In the surface water, good activity was noted up to 3 days posttreatment but little or no activity was detected at the rates of 4 and 5 lb/acre (4.48 and 5.6 kg/ha) 7 days after treatment. This is in contrast to ABG-6184, where no activity was detected in surface water, 3 days posttreatment. Similarly, in the bottom water of BSP-2 treatment, high level of activity was detected up to 3 days posttreatment

Table 3. Efficacy of *Bacillus sphaericus* corncob granules against *Culex peus* larvae in dairy wastewater lagoons[a].

Rate lb/acre	Plot size acre	Mean no. larvae/dip pretreatment	% reduction after treatment (days) 3	7	14	21
		ABG-6185 (1-2 mm mesh) 1.9 × 10^9 spores/gram[b]				
10	0.16	10	0	—	—	—
20	0.16	10	87	48	—	—
		ABG-6185 (1-2 mm mesh) 5.0 × 10^9 spores/gram[b]				
5	0.16	15	81	8	—	—
10	0.16	47	99	98	99	61
10	1.30	54	99	82	90	drying
		ABG-6185A (3 mm) 5.0 × 10^9 spores/gram[b]				
2.5	1.00	11	67	6	—	—
5.0	1.25	20	96	95	88	49
7.5	1.00	11	92	89	drying	
7.5	0.16	16	80	85	91	88
		ABG-6185 (<1 mm) 5.0 × 10^9 spores/gram[c]				
10	1.30	55	99	83	90	drying

[a] No reduction in check plots were observed. Tests in small plots (0.16 acre) were conducted at Lakerkirk, while tests in larger plots (1.0–1.3 acre) were conducted at Midhill dairy.
[b] Broadcasted by hand.
[c] Applied with a knapsack type power granular applicator.

only. This, again, is in contrast to ABG-6184, where persistent activity in bottom water was detected for 14 days posttreatment. This data supports the report of Davidson et al. (1984) that the powder preparation sinks to the bottom more rapidly.

In a companion study where microbiological assessments were carried out (Matanmi et al. 1989), spores from both formulations persisted in bottom water and soil samples for 28 days, and provided mosquito control in the field for 28 days or longer. In laboratory bioassays using field water, insufficient numbers of spores were found in surface water (sampled 7 days posttreatment) causing little or no mortality in the laboratory reared 4th-instar larvae exposed to the field water for 48 hr. The bottom water in ponds treated with WP contained sufficient spores to cause high mortality in lab bioassay for up to 14 days. Although lab bioassays of surface and bottom water indicated presence of sublethal concentrations of the toxins, in actual field situations control of wild populations persisted for 28 days or longer. This discrepancy between field and laboratory findings is probably due to the continuous exposure of younger instars, which are more susceptible to *B. sphaericus*, to sublethal concentrations for longer periods.

From our studies, it is clear that *B. sphaericus* 2362 holds great promise for the control of *Culex* mosquitoes in polluted waters. However, for good initial and persistent control, the rate of application has to be increased by 5–10 fold over those needed in clear-water situations. This increase in application rates is feasible and justified in view of the long-lasting control obtained with one treatment, saving on the cost of inspection and repeat treatments with less persistent agents providing control for only 3 to 7 days.

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