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Experimental formulations of *Bacillus sphaericus* for the control of anopheline and culicine larvae

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SUMMARY

Four experimental formulations of *Bacillus sphaericus* Neide (2362 isolate) were evaluated for larvicidal activity against culicine and anopheline larvae in several natural and artificial habitats. A granular formulation (5% primary powder) was tested against natural populations of mosquitoes in two simulated habitats in Florida and in maturing and reflooded rice fields in Louisiana. Larvae of *Culex quinquefasciatus* Say were reduced by 97 and 99% after application of the granules at the rate of 10 kg/ha to polluted tanks and 2.5 kg/ha to sod-lined potholes, respectively. Anopheline and *Psorophora columbiae* (Dyar and Knab) larvae were reduced by 68 and 92–100%, respectively, after application of 5 kg granules/ha to rice fields. A flowable concentrate (12.8% primary powder) applied to unpolluted and organically enriched habitats in Florida at 0.25 kg/ha reduced populations of *Culex* spp. by 93–100% and 99%, respectively. Sustained-release briquets (5% primary powder) applied at the rate of one half briquet/1.8 m^2 sod-lined potholes reduced larval populations of *Cx. quinquefasciatus* by 88–95% for up to 2 weeks in open sunlight. Sustained-release pellets (30% primary powder) applied to small woodland pools in Memphis, TN at the rate of four pellets/pool virtually eliminated larval populations of *Cx. restuans* Theobald for over 8 days. Variable persistence of larvicidal activity was noted for the other treatments depending on the formulation, target species and habitat.

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INTRODUCTION

Certain isolates of *Bacillus sphaericus* Neide, most notably those in the 5a5b serotype, are highly efficacious as larvicides against several species of mosquitoes [3,18]. Perusal of the literature reveals a variety of parameters that influence efficacy and degree of residual larvicidal activity, persistence of spores and toxins, and recycling of *B. sphaericus*.

Initial larvicidal activity of an efficacious isolate of *B. sphaericus* may be influenced by treatment rate, target species susceptibility, feeding strategy, density and age of larvae, temperature, water quality and other environmental factors as well as formulation and efficiency of application [6,10,12–14,17]. Even when high initial reduction of larvae is attained, a target population may rebound to pretreatment density due to settling of larvicidal entities from the feeding zone of subsequent cohorts [4,11,12,14] or inactivation of toxins and spores by solar radiation [14]. Residual activity may be due to persistence of accessible toxin in the habitat and/or due to recycling of the bacterium in larval cadavers with subsequent amplification of spores and toxins. In either case, accessibility of toxin to the target mosquito larvae is one of the major determinants for practical and especially residual control. Accessibility can be greatly enhanced and prolonged through appropriate formulation.

Despite the larvicidal activity of *B. sphaericus* there has been limited interest in its commercial development. As a consequence, there is a paucity of commercially produced formulations of this microbial control agent. Until recently most field trials have been conducted with unformulated whole cultures, primary powders and one wettable powder [6]. The objective of this study was to produce and/or evaluate granular, briquet, and flowable concentrate (FC) formulations of *B. sphaericus* against anopheline and culicine mosquitoes under natural and simulated natural conditions.

MATERIALS AND METHODS

The *B. sphaericus* (isolate 2362) primary powder used in our studies for the formulation of granules, pellets and briquets was grown in a 250 liter fermentor on B-16 medium* at 30°C, 300 rpm agitation, and aerated at 0.666 vvm. Fermentation proceeded until sporulation was complete (average age = 53.5 h). The fermentation beer was then centrifuged through a Sharples continuous flow centrifuge, Model AS-16, at a feed rate of approximately 6 l/min. In the preparation of the slurry for spray drying, 10%, by weight, of lactose was added along with 1%, by weight, of Petro® Morwet EFW, a wetting agent. The slurry was brought to a total of 20% solids for spray drying. Spray drying was done in a Bowen Laboratory Spray-Aire® drier with a direct fired gas heater. Atomization was performed by a centrifugal atomizer using a CSE wheel. Spray drying was conducted with an inlet temperature of 150°C and an outlet temperature of 75–80°C, at a feed rate of 125 ml/min. A total of 6.55 kg of primary powder was produced in this manner. A mixture of primary powder from six fermentation runs was bioassayed in the laboratory (27°C) against 48 h second instar *Culex quinquefasciatus* Say in the manner described by Lacey [6]. Comparative bioassays were also performed with lyophilized primary powder of the International Standard, RB 80 (isolate 1593; [1]), in order to assign a toxicity rating to our primary powder.

The granular formulation was produced by Mount Pulaski Products (Mount Pulaski, IL) under the supervision of D. Ross using primary powder of *B. sphaericus,* 12/14 U. S. mesh corn cob grit, and the identical binder and process that are used for commercial production of the Bactimos® granular formulation of *B. thuringiensis* Berliner var. *israelensis* de Barjac (serotype H-14). The resultant formulation was 5% primary powder by weight. The briquets were produced by Summit Manufacturing (Baltimore, MD) using primary powder of *B. sphaericus* and the same process and diluents used for making Bactimos *B. thuringiensis* (H-14) briquets. Each donut-shaped briquet weighed ca. 14.26 ± 0.08 (S.E.) g and measured 5.5 cm in diameter, including a 1.0 cm center hole, and contained 5% primary powder by weight. A flowable concentrate (FC) of *B. sphaericus* was produced by Solvay and Cie, S. A. (Brussels, Belgium) using primary powder (2362; 12.8% by weight) and the same adjuvants and diluents used to produce the Bactimos *B. thuringiensis* (H-14) FC. Bioassays of the

* Fermentor medium B-16: Nutrisoy® (30.0 g/l), yeast extract (2.0 g/l), $CaCO_3$ (2.0 g/l), HySoy® (15.0 g/l), corn steep (10.0 ml/l), $MgSO_4 \cdot 7H_2O$ (0.3 g/l), $MnSO_4 \cdot H_2O$ (0.02 g/l), $ZnSO_4 \cdot 7H_2O$ (0.02 g/l), $FeSO_4 \cdot 7H_2O$ (0.02 g/l).

FC were conducted against *Cx. quinquefasciatus* as described above to determine toxicity rating. Due to the proprietary nature of the ingredients and processes used to produce the Bactimos formulations, they cannot be disclosed in this paper.

The sustained-release pellets (1.25 cm diameter, 0.7 cm high, 0.66 ± 0.006 g/pellet total weight) were made with primary powder, sifted powdered sugar as a wetting and releasing agent and polypropylene powder (Accurel® powder, 70% void, Armak Co., McCook, IL) as a flotation agent in a 1:1:1.3 ratio, respectively, using the same methods as described by Lacey et al. [11]. Each pellet contained 0.2 g of primary powder. Control pellets were made in an identical manner except with autoclaved primary powder.

The 1.8 m^2 sod-lined potholes described by Focks and Bailey [5], located at the USDA Insects Affecting Man and Animals Research Lab (IAMARL), were used for exposing natural populations of *Cx. quinquefasciatus* to primary powder and the granular, briquet and FC formulations. Samples were taken before application of the formulations, at 48–72 h posttreatment and at varying intervals afterwards for up to 3 weeks. Samples consisted of ten dips taken with a standard mosquito dipper (250 ml), eight in the corners and two on the sides of each pothole. Concomitant comparison of the granules with primary powder was made using the spray-dried 2362 primary powder applied at a rate of 0.25 kg/ha with a CO_2 pressurized sprayer equipped with a flat fan nozzle. The granules were applied at 2.5 and 5.0 kg/ha by evenly spreading the required amount of granules over the surface of each pothole. Each treatment and control were replicated three or four times. Replicates were begun on separate dates within a 3 week period.

Evaluations of the granules were also made against *Cx. quinquefasciatus* in settling tanks (2 × 0.7 × 0.8 m) near the IAMARL. The tanks are normally used to catch soil and organic matter washed from plant samples. Consequently they are extremely enriched and support large numbers of *Cx. quinquefasciatus* (≥300 larvae/dip). Three tanks were used for treatments and one was kept as a control (a.m. temp. 22°C). The granules were applied by hand at 10 kg/ha. Pretreatment and 72 h posttreatment larval samples were taken as in the pothole studies. During the course of the exposure period the tanks were not in operation but after 72 h their use was required, precluding assessment of residual activity. Comparative tests were made using aqueous suspensions of the primary powder in the same tanks. Three rates, 0.1, 0.25 and 0.5 kg/ha were applied to the three treated tanks on three separate occasions over a 3 week period (mean temp., 9–10 a.m., 27°C). Assignment of treatment rates and controls was rotated such that tanks were not used twice for the same treatment or control during the test period. Complete recovery of larval populations 1 week after each replicate test was evident. Pre- and 72 h posttreatment larval samples were taken as in the pothole tests.

The granules were also evaluated against natural populations of larval *Anopheles quadrimaculatus* Say and *An. crucians* Weidemann in maturing first-crop rice fields near Jennings, LA. Two rates, 2.5 and 5.0 kg/ha, were each applied to three 1 ha plots in the manner described by Lacey and Inman [9] using a Grumman Ag Cat airplane equipped with a Transland spreader. Larval samples (50 dips/treated plot and 100 dips/control plot) were taken as in the study of Lacey and Inman [9] immediately prior to treatment, 48 h after treatment and again after 1 week in order to assess any possible residual activity. Control plots were placed in each of the treated fields at a sufficient distance from the treated plots to prevent contamination.

Tests were also conducted with granules applied with a hand-powered Cyclone® seeder at 1.0, 2.5 and 5.0 kg granules/ha against *Psorophora columbiae* (Dyar and Knab) in 400 m^2 plots in reflooded second crop rice fields in an identical manner to that described by Lacey and Inman [9]. Three replicate plots were used for each treatment rate and control. Larval samples were taken immediately before and 24 h after treatment as described above. In addition to dipper samples, three floating sentinel cages, each containing 10 field-collected late second and third instar larvae, were placed in each plot to aid in the efficacy assessment of the granules.

The FC was evaluated in several habitats at 0.25

kg/ha using the CO_2 pressurized sprayer described above to apply an aqueous suspension of the formulation. The required amount of FC was suspended in 250 ml of water and applied evenly to the surface of each treated plot. In the pothole habitat (IAMARL) each treatment and control was replicated three times. Tests of the FC were also conducted in plastic wading pools against natural populations of *Cx. quinquefasciatus* larvae. Eight pools (1.5 m in diameter, 1.8 m^2, 17–26 cm deep depending on rainfall) were placed in a cypress dome in a rural setting near Jacksonville, FL. The pools were shaded for most of the day. The bottom of each was covered with soil and leaf mold (ca. 8 cm) and flooded with well water to within 7–10 cm from the rim of the pool. After 1 week floating debris was removed from the pools and additional water was added. Pretreatment larval samples were taken the following day as in the potholes (10 dips/pool). Four pools were treated and the remaining four were kept as untreated controls. Sampling continued for 3 weeks until control populations declined considerably.

The FC was also evaluated in small plots in full sun in an extremely turbid and organically enriched spray field inhabited by *Cx. nigripalpus* Theobald. The spray field was used for the oxidization of waste water from an orange juice processing plant near Fort Pierce, FL. Enclosures (1 m^2; three enclosures for each treatment and controls; water in enclosure 60 cm deep) were made using pieces of thick plastic sheeting (1.2 m high; 4 m long) attached to four PVC pipes (7.5 cm diameter; 1.5 m high) with heavy-duty staples. The pipes were then driven into the soft substratum of the lagoon until they were firmly anchored in place and the sheeting was also driven several centimeters below the surface of the substratum. In this manner larvae as well as *B. sphaericus* were restricted from entering or leaving the enclosures. Pretreatment samples were taken as in the potholes. Posttreatment samples were taken at 48 h and at weekly intervals for 2 weeks.

The *B. sphaericus* briquets were compared with Bactimos *B. thuringiensis* (H-14) briquets against *Cx. quinquefasciatus* in the sod-lined potholes at the IAMARL at the rate of 1/2 briquet/pothole (ca. 7.13 g of briquet/pothole) in the manner described for evaluation of the granules and FC. Sampling continued for 3 weeks at which time a severe reduction in control populations masked any possible effect of the treatments.

Woodland pools near Memphis, TN (1.5–3.1 m diameter) were utilized for evaluation of the slow-release pellets against natural populations of mostly late instar *Cx. restuans* Theobald. Pretreatment samples consisted of six standard dipper samples from each pool. Four pellets were placed into each of four pools and six pools were left as controls. The water temperature at the time of treatment was 18.6°C. Due to the slow initial release of *B. sphaericus* from the pellets (Lacey, unpublished data), posttreatment samples were not taken until 4 days after treatment. Samples were taken again 8 days after treatment, but the pools had dried before a 2 week posttreatment sample could be taken.

The data from the laboratory bioassays of RB-80, the FC and the 2362 primary powder were subjected to probit analysis. The percentage reduction of larvae in treated and control field plots was calculated using pre- and posttreatment larval numbers. The effect of reduction of larval numbers in control plots was accounted for using Abbott's formula prior to statistical analysis. Analysis of variance and Duncan's New Multiple Range test were performed on all field trial data after correcting for control mortality and arcsine transformations of percentages.

RESULTS

The LC_{50} and LC_{95} for the 2362 primary powder and for the RB-80 International Standard against *Cx. quinquefasciatus* were 0.0068 and 0.021 mg/l and 0.0049 and 0.016 mg/l, respectively. The LC_{50} and LC_{95} for the FC were 0.0272 and 0.092 mg/l, respectively. Bourgouin et al. [1] assigned an arbitrary toxicity rating of 1000 toxin units/mg to RB-80 when tested against *Cx. pipiens pipiens*. Multiplying this factor by the LC_{50} value for RB-80 divided by the LC_{50} value for the 2362 powder re-

Table 1

Comparative efficacy of *B. sphaericus* granules (5% primary powder) and primary powder against late instar *Cx. quinquefasciatus* larvae in sod-lined potholes[a]

Treatment	% Reduction ± S.E. (time after treatment)[b]			
	72 h	1 week	1.5 week	2 week
Granules				
2.5 kg/ha	99.5 ± 5.0 (a)	96.0 ± 4.0 (a)	78.5 ± 13.5 (a)	50.0 ± 6.0 (a)
5.0 kg/ha	100 (a)	94.3 ± 5.2 (a)	62.7 ± 21.5 (a)	63.0 ± 12.0 (a)
Primary powder				
0.25 kg/ha	100 (a)	91.0 ± 2.6 (a)	63.0 ± 12.0 (a)	42.5 ± 19.5 (a)
Control	9.0 ± 3.0 (b)	13.0 ± 3.0 (b)	24.0 ± 24.0 (a)	35.0 ± 14.0 (a)

[a] Mean pretreatment larval density: 31/dip (27°C, 24 April–18 May 1984).
[b] Means in the same column followed by the same letter are not significantly different at the 0.05 level.

sults in a relative toxicity rating of 721 toxin units/mg for the primary powder. Repeating this calculation with the LC_{50} of the FC yields a toxicity rating of 180 units/mg of FC.

The results of comparative trials between the *B. sphaericus* granule at 2.5 and 5.0 kg/ha and primary powder at 0.25 kg/ha in the pothole habitat are presented in Table 1. Similar results were obtained with both treatments (0.25 kg primary powder/ha is equivalent to 5 kg granules/ha). High levels of control for all treatments were apparent for up to 1 week. Effective abatement of larvae in the treated plots was still observed 1.5 weeks posttreatment but a significant decline in control populations did not permit further assessment of residual activity beyond this point.

Larval reduction in the organically enriched tanks was 97% 72 h after treatment at a rate of 10 kg granules/ha (control reduction was 6%). A 99% reduction of larvae was observed in tests run with aqueous suspensions of 2362 primary powder at 0.5 kg/ha (equivalent to 10 kg granules/ha) in the same tanks. Reductions of 95% and 96% were obtained using the 0.1 and 0.25 kg/ha rates, respectively, of the primary powder. Increases in larval populations in the control tanks were observed during the testing period.

The results of tests conducted in rice fields against the anopheline species are presented in Table 2. The granules enabled good penetration of the dense vegetative canopy in the rice fields which may have impeded other formulations. The control achieved using the 5.0 kg/ha rate was considered operationally inadequate. The larvicidal activity

Table 2

Field efficacy of aerially applied *B. sphaericus* granules against larvae of *An. quadrimaculatus* (55%) and *An. crucians* (45%) in maturing rice fields near Jennings, LA[a]

Rate (kg/ha)	% Reduction ± S.E.	
	48 h posttreatment	1 week posttreatment
2.5[b]	17.3 ± 11.1 (a)	47.7 ± 21.5 (a)
5.0[c]	67.6 ± 13.7 (b)	53.4 ± 27.8 (a)
Control	0	0

[a] Means in the same column followed by the same letter are not significantly different at the 0.05 level. Mean pretreatment larval density: 1.6 larvae/dip.
[b] July, 1984 (24–32°C); dense maturing rice; age composition before treatment: 51% 1st instar, 23% 2nd instar, 14% 3rd instar, and 12% 4th.
[c] Sparse to dense rice, some portions of field recently inundated. At the 48 h posttreatment count the water level dropped considerably necessitating some sampling from ruts running through the field. Age composition before treatment: 44% 1st instar, 30% 2nd instar, 20% 3rd instar, and 6% 4th instar.

Table 3

Efficacy of a granular formulation of *B. sphaericus* (5% primary powder) for the control of *Ps. columbiae* larvae in (reflooded) second-crop rice fields

Rate (kg/ha)	% Reduction ± S.E.[a]	
	dip determined	sentinel cages
1.0[b]	61.4 ± 10.2 (a)	87.5 ± 11.4 (a)
2.5[c]	89.0 ± 3.0 (b)	88.9 ± 10.5 (a)
5.0[d]	91.5 ± 0.9 (b)	100.0 (a)
Control	9.3 ± 9.3 (c)	14.4 ± 5.9 (b)

[a] Means in the same column followed by the same letter are not significantly different at the 0.05 level. Mean pretreatment larval density: 3.2 larvae/dip, August 1984, Jennings, LA.
[b] 33.3°C; 61% 2nd instars, 39% 3rd instars.
[c] 28.9°C; 100% late 2nd instars.
[d] 30.6°C; 44% 2nd instars, 56% 3rd instars.

observed for the same formulation applied to second-crop rice fields against *Ps. columbiae* was considerably higher (Table 3). Due to the small size of the plots it was suspected that some invasion by untreated larvae may have occurred. However, the dosage-correlated reduction of larvae and general agreement between sentinel cage- and dipper-derived data indicate that peripheral invasion of the sampling transect was not excessive. In most cases the water-soaked chaff remaining from the first rice crop held larvae close to the surface and restricted movement.

The results of field trials with the FC formulation are presented in Table 4. Although initial effective larval abatement was observed at all three test sites, significantly longer residual activity was only noted in the shaded Cypress dome habitat (Jacksonville). In each habitat sampling was suspended after either control populations and/or re-

Table 4

Activity of the Biochem Products' flowable concentrate (12.8% primary powder) of *B. sphaericus* (2362) applied at the rate of 0.25 kg/ha against *Culex* spp. under various field conditions in Florida

Location/treatment	% Reduction ± S.E. (time after treatment)			
Fort Pierce[b]	48 h	6 days		
FC	99.7 ± 0.2	69.8 ± 26.1		
Control	0[a]	44.3 ± 13.1[e]		
Gainesville[c]	48 h	4 days	1 week	
FC	87.0 ± 12.7	93.2 ± 6.8	0[a,e]	
Control	48.4 ± 10.4[e]	0[a]	0[a]	
Jacksonville[d]	72 h	1 week	10 days	2 weeks
FC	100	100	97.8 ± 2.2	66.7 ± 33.3
Control	0[a]	0[a]	0[a]	29.3 ± 32.9[e]

[a] Increase over pretreatment larval density.
[b] *Cx. nigripalpus*, mean pretreatment density: 32 larvae/dip; age structure: 4.7% 1st instar; 9.0% 2nd instar; 29.8% 3rd instar; 49.3% 4th instar; 10.2% pupae; water temp. 26°C, 3 July 1985. Organically enriched highly turbid (32 NTU) wastewater in full sunlight.
[c] *Cx. quinquefasciatus*, mean pretreatment density: 22 larvae/dip; mostly late instars; water temp. 27–31°C, 21 May 1985. Sod-lined potholes in full sunlight.
[d] *Cx. quinquefasciatus*, mean pretreatment density: 2.6 larvae/dip; age structure: 26.9% 1st instar; 23.1% 2nd instar; 13.8% 3rd instar; 41.4% 4th instar; 16 August 1985, 25.5°C; partially to fully shaded wading pools with natural substrate.
[e] Significantly different from other means in the same row.

Table 5

Efficacy of briquet formulations of *B. sphaericus* and *B. thuringiensis* (H-14) against late instars of *Cx. quinquefasciatus* in sod-lined potholes[a]

Treatment	% Reduction ± S.E. (days posttreatment)[b]			
	3	7	10	14
B. sphaericus	88.0 ± 4.3 (a)	78.9 ± 11.0 (a)	93.1 ± 3.8 (a)	94.5 ± 2.8 (a)
B. thuringiensis	25.8 ± 23.1 (b)	86.6 ± 4.5 (a)	94.9 ± 1.8 (a)	82.6 ± 7.6 (a)
Control	0 (c)	0 (b)	0.9 ± 0.9 (b)	15.0 ± 8.0 (b)

[a] Mean pretreatment larval density: 41 larvae/dip (26°C at treatment time, September 1984).

[b] Means in the same column followed by the same letter are not significantly different from one another at the 0.05 level.

sidual larvicidal activity of the FC declined dramatically.

The briquets of *B. thuringiensis* (H-14) and *B. sphaericus* and the floating pellets all provided significant residual larval control. The results of the pothole tests of the briquet are presented in Table 5. The *B. sphaericus*-based briquets initially provided significantly better control than the *B. thuringiensis* (H-14) briquets, but the activities of the two were comparable for the remainder of the test. Effective larval control was obtained until termination of the test 3 weeks posttreatment. Further sampling was discontinued due to a sharp decline in the control populations.

Larval populations of *Cx. restuans* in the Woodland pools near Memphis were reduced by 87–99% (mean reduction 91.6 ± 2.7% S.E.) 4 days after application of pellets. Larvae were almost completely eliminated 8 days posttreatment (94.7–100% reduction; mean 98.7 ± 1.3% S.E.). A net gain in larval numbers was observed in the control pools during both sampling periods. It was not possible to determine any additional residual activity due to the drying of the pools before the 2 week posttreatment sample could be taken.

DISCUSSION

The microbial control of mosquitoes breeding under vegetative cover or in deep water habitats may be enhanced by formulating *B. sphaericus* to penetrate the cover and remain within the feeding zone of target mosquito larvae. The granules evaluated in this study provided both penetration and a limited amount of flotation in non-polluted habitats. Although there was no apparent enhancement of residual activity in the granular formulation when used in open clear water habitats, neither was there a decrease in efficacy due to formulation as evidenced by the comparable activity of granules and an equivalent amount of primary powder in the pothole studies.

The lack of effective control of the anopheline larvae is probably due to the decreased susceptibility of these species to *B. sphaericus* [8,10] and not to the granular formulation. The high degree of susceptibility of *Ps. columbiae* to formulated *B. sphaericus* in the rice field habitat is comparable to that reported for unformulated primary powders evaluated under laboratory [10] and small plot conditions [13]. Similar susceptibility of *Ps. columbiae* was also reported by Lacey et al. [8] after Beecomist®-application of *B. sphaericus* FC to second-crop rice fields.

The depth, turbidity, and decreased surface tension of the water, and rapid settling of the granules in the soil washing tanks, ostensibly influenced the observed decrease in efficacy of the granules compared with that of the primary powder in the same system. It appears that in this setting the granular formulation only serves to augment rapid settling.

A very small percentage of the granules remained floating shortly after treatment of the tanks compared with granules in the pothole tests reported in Table 1 and the laboratory studies conducted on Bactimos granules by Lacey and Inman [9] in distilled water. In less polluted situations the granules release significant quantities of inoculum onto the surface tension of the water even when they sink [9].

Settling of the toxic moieties of *B. sphaericus* and the failure of subsequent generations of larvae to come into contact with them interfere with possible residual activity in many habitats [4,11,12,14]. Settling may be accelerated or accessibility of settled spores reduced in polluted or turbid habitats. The influence of organic enrichment on the reduction of residual larvicidal activity of *B. sphaericus* has been demonstrated under both laboratory and field conditions [12,14,16].

The prolonged larvicidal activity of the FC formulation in the sparsely populated Cypress dome pools was probably a function of persistence and accessibility of the applied toxin rather than recycling of *B. sphaericus* and amplification of toxin. Davidson et al. [4] indicated that a minimum larval density may be necessary to enable larva to larva recycling of the bacterium. The low larval density (pretreatment, 2.6 larvae/dip) and apparent lack of continuous colonization at this site probably would not have supported sustained recycling. On the other hand, the clear, shallow water and shaded habitat probably enhanced the accessibility and persistence of toxin.

Liquid FC formulations of *B. thuringiensis* (H-14) have offered the best options for control of mosquitoes in a variety of habitats [7]. Beecomist®-applied *B. thuringiensis* (H-14) FC provided effective control of *An. quadrimaculatus* in rice fields at rates considerably lower than those required using other application methods [15]. Although Beecomist-applied *B. sphaericus* FC did not provide adequate control of *An. quadrimaculatus* in rice fields, it provided effective control of the more susceptible *Ps. columbiae* that was equal to or better than that reported for *B. thuringiensis* (H-14) [8].

A relatively large amount of *B. sphaericus*/unit area is required for effective larval control using the briquets and pellets in small pools. This is partially balanced by the increased residual control and ease of treatment. In a previous study, *B. sphaericus* formulated as sustained-release pellets enabled prolonged control (≥ 8 weeks) of container-breeding *Cx. quinquefasciatus* larvae [11]. Maximum mortality is usually observed 48 h after exposing larvae to the *B. sphaericus* spores when an excessive concentration is not utilized. The onset of mortality, however, may be delayed in older larvae, at low temperatures, or when very low concentrations of inoculum are present. The use of sustained-release pellets in a control program may be at an initial disadvantage due to a delay in release of sufficient quantities of the larvicidal moieties for immediate kill of older instars. Modification of the pellets that will enable an initial rapid release of *B. sphaericus* followed by a sustained release of toxin that is sufficient for the control of recently eclosed larvae should overcome some of the current disadvantages of the pellets. The activity of the prototype sustained-release formulations evaluated in this study indicate that extended control is possible using a matrix-type formulation. Matrix granules may provide sustained control in a number of habitats, enable penetration of dense cover, allow more thorough coverage than the pellets and permit use of conventional application equipment.

Further improvements in the formulation of *B. sphaericus* are warranted before it will be competitive with chemical larvicides in terms of ease of application, cost, efficacy and host range. Increase in the percentage of toxin in *B. sphaericus* formulations through discovery of strains with even greater toxin-producing potential, genetic engineering of existing strains, improved fermentation and more efficient extraction and refinement of toxin will increase formulation options. Use of purified toxin could enable formulation of *B. sphaericus* toxin as an emulsifiable concentrate with even greater application potential than the FC. Micro-lipid-droplet encapsulation of purified toxin of *B. thuringiensis* (H-14) has already been reported by Cheung and Hammock [2] with subsequent improvement in activity against anopheline larvae. Additional re-

search into the use of feeding attractants in formulations, improvement of sustained-release matrices, and use of protectants from UV light and other toxin-denaturing factors is warranted. By tailoring a variety of formulations to the habitats and behavior of the target mosquito, additional improvements in efficacy can be expected.

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