EVALUATION OF GRANULAR *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* (SEROTYPE H-14) FORMULATIONS AGAINST MOSQUITO LARVAE IN CENTRAL IOWA[1]

J. L. CLARKE III[2] AND W. A. ROWLEY[2]

ABSTRACT. Three granular formulations of *Bacillus thuringiensis* var. *israelensis* were assessed in the summer of 1983. Evaluations included artificial pool studies, field trials and a prehatch/preflood trial in artificial pools. All products provided good control, especially of the floodwater species *Aedes vexans* and *Ae. trivittatus*. Residual activity was lacking in all products. Reinfestation was common in *Culex* breeding areas within 72 hr posttreatment. Prehatch treatments provided good control when flooded 1 hr after application; poor control occurred when pools were flooded 144 hr (6 days) after application.

INTRODUCTION

Bacillus thuringiensis variety *israelensis (B.t.i.)* was isolated from a mosquito larval habitat in Israel (Goldberg and Margalit 1977) and was designated *Bacillus thuringiensis* var. *israelensis* (serotype H-14) by de Barjac (1978). Since the discovery of *B.t.i.*, numerous laboratory and field studies have demonstrated its effectiveness against a number of species in at least 7 genera, including mosquito species reportedly resistant to conventional insecticides (Sun et al. 1980). *Anopheles* is the only genus in which the efficacy of *B.t.i.* is in question, and this seems to be a function of the surface feeding habits of *Anopheles* larvae (Goldberg and Margalit 1977, Mulla et al. 1980).

The *B.t.i.* formulations used since 1977 have been either wettable powders or flowable concentrates. Satisfactory granular formulations of *B.t.i.* were not available until 1983. The objective of this study was to evaluate 3 recently developed granular formulations of *B.t.i.* against mosquito larvae in central Iowa. To accomplish this, efficacy was assessed in artificial pools against *Aedes trivittatus* (Coquillett) and in a variety of natural mosquito habitats. The prehatch treatment potential of these formulations also was examined in artificial pools.

MATERIALS AND METHODS

FORMULATIONS. Formulations of *B.t.i.* tested in this study were produced by Biochem Products, Montchanin, DE 19710; Sandoz, Inc., Wasco, CA 93280; and Abbott Laboratories, North Chicago, IL 60064. The Biochem products used were Bactimos granules, a corncob granular formulation containing 5% (by weight) Bactimos 50% wettable powder, and Bactimos-S, Bactimos granules coated with carbon as a sun block. Both formulations had an estimated potency of 175 *Aedes aegypti* International Toxic Units (AA ITU) per milligram. Bactimos-S was tested as a prehatch-applied insecticide. Teknar-70 (Sandoz) was a granular formulation consisting of a corncob core, coated with wax, then coated with *B.t.i.* It had an estimated potency of 104 AA ITU/mg.[3] Abbott Laboratories formulation (ABG-6138) also was a corncob granular that had an estimated potency of 200 AA ITU/mg. Teknar (Sandoz), a water-dispersible concentrate (600 AA ITU/mg), was used in field trials to compare efficacy and residual activity with those of the 3 granular formulations. A 5% Abate (temephos) clay granule was used in artificial pool and prehatch studies as a standard, and blank corncob granules were used in check plots.

ARTIFICIAL POOL STUDY. Tests were conducted in 209-liter children's wading pools. A 1.5 to 2.0 cm thick layer of washed coarse sand was spread on the bottom of each pool. Approximately 100 liters of tap water were added and allowed to settle for several hours before addition of larvae. *Aedes trivittatus* eggs were obtained from field-collected females. Eggs were hatched, and early 2nd instar *Ae. trivittatus* (375/pool) were released into each pool and allowed to acclimate for 12 hr before treatment. Two pools were used per formulation. Pretreatment larval samples were taken by the standard dipper count method, 5 dips/pool. The standard dip method was used rather than caged larvae because it is possible that caged larvae may be prevented from ingesting as many spores as free-roaming larvae (Dame et al. 1981, Stark and Meisch 1983). Each formulation was applied evenly over the water surface

[1] Journal Paper No. J-11402 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA 50011, Project 2277.

[2] Department of Entomology, Iowa State University, Ames, IA 50011.

[3] Teknar granular has a stated label potency of 260 AA units. Teknar water dispersible concentrate has a stated label potency of 1500 AA units, which is equivalent to 600 AA ITU/mg. With a conversion factor of 0.4 (600/1500), the Teknar granules would have a potency of 104 AA ITU/mg.

of each pool at 2.78 and 5.56 kg/ha. Posttreatment counts (5 dips/pool) were taken at 9 hr and 24 hr. Water temperature in the pools varied from 22 to 35°C.

FIELD TRIALS. Sixteen tests were conducted throughout the summer of 1983. Application rates for the granular formulations were 5.56 and 11.12 kg/ha and 0.7 and 1.4 liters/ha for the water-dispersible concentrate. The quantity of material applied was determined by the surface area of the water at each site. All granular material was weighed on an analytical balance and applied with an Ortho Whirlbird Spreader (Ortho Chemical Company). The water-dispersible concentrate was mixed with enough water to cover the area and was applied with a Hudson Climax Sprayer (Model No. 3256654).

Ten random samples were taken before treatment, and three of these were returned to the laboratory for species determination. Posttreatment counts were taken at 24, 48, 72 and 168 hr (7 days). Control was measured by comparing differences in the number of larvae remaining at the various sampling intervals.

PREHATCH STUDY. Tests were conducted in 209-liter children's wading pools described earlier. Formulations were applied to the sand bottom of the pool before flooding to simulate a prehatch treatment. All materials were applied at 5.56 kg/ha. One set of pools (2 pools/formulation and 2 control pools) was flooded 1 hr posttreatment. Immediately after flooding, 400 early 2nd instar *Ae. trivittatus* were released in the pools. One hour after release, samples were taken (5 dips/pool). These were considered pretreatment (premortality counts). In previous experiments, no mortality was observed after 1 hr exposure (Clarke and Rowley, unpublished data). Posttreatment counts (5 dips/pool) were taken 24 hr postflooding. The second set of pools remained unflooded for 144 hr (6 days) with full exposure to local climatic conditions (average daily temperature ranged from 14 to 27°C; 0.15 and 0.05 cm of rain were recorded on day 2 and day 6, respectively).

RESULTS

ARTIFICIAL POOL STUDY. *Aedes trivittatus* was controlled by all 3 granular formulations at both 2.78 and 5.56 kg/ha (Table 1). High mortality was observed as early as 9 hr posttreatment. One hundred percent mortality occurred in all pools treated at 5.56 kg/ha within 24 hr. All pools treated with *B.t.i.* at the 2.78 kg/ha level showed more than 93% mortality at 24 hr. An analysis of variance (ANOVA) showed no significant differences between formulations at 24 hr posttreatment, but there was a significant difference ($P = 0.05$) between rates within formulations. There was no mortality in control pools. Ramoska and Pacey (1979) found that increased amounts of food available to mosquito larvae reduced mortality in larvae exposed to *Bacillus sphaericus*. Food availability was minimal in the pool study, which may help to explain the near 100% mortality at the 2.78 kg/ha application rate.

FIELD TRIALS. Control of *Aedes* and *Culex* spp. occurred within 24 hr at all sites treated with *B.t.i.* granules (Table 2). Nevertheless, *Culex* reinfestation occurred within 72 hr, although granules were still visible in the treated areas. *Aedes* reinfestation was minimal except when significant rainfall occurred 24 hr posttreatment. In these situations, newly hatched *Aedes* larvae were not killed.

Field trials with the *B.t.i.* water-dispersible concentrate were similar to those for the 3 granular products tested (Table 2). *Ae. trivittatus* and *Ae. vexans* (Meigen) were highly susceptible to this product. Existing *Culex* populations also

Table 1. Evaluation of selected granular formulations of *Bacillus thuringiensis* var. *israelensis* and Abate (Temephos) against *Aedes trivittatus* in artificial pools.

Rate (kg/ha)	Formulation	Larvae/dip[a]			% reduction	
		0 hr[b]	9 hr[c]	24 hr[d]	9 hr	24 hr
2.78	Abate	2.9	0.1	0.0	96.5	100.0
5.56	Abate	4.3	0.2	0.0	95.3	100.0
2.78	Teknar-70	5.0	2.0	0.2	60.0	96.0
5.56	Teknar-70	4.6	0.3	0.0	93.5	100.0
2.78	Abbott-6138	2.9	1.3	0.2	55.2	93.1
5.56	Abbott-6138	5.0	0.3	0.0	94.0	100.0
2.78	Bactimos	5.6	0.3	0.2	94.6	96.4
5.56	Bactimos	4.7	0.4	0.0	91.5	100.0
Control	Blank cob	4.6	7.8	5.0	0.0	0.0

[a] Mean of 2 pools, 5 dips/pool.
[b] 95% CI = ±1.2.
[c] 95% CI = ±0.82.
[d] 95% CI = ±0.19.

Table 2. Evaluation of selected formulations of *Bacillus thuringiensis* var. *israelensis* against mosquito larvae in the field.

Rate (kg/ha)	Site	Species composition	Larvae/dip[a] 0 hr	24 hr	48 hr	72 hr	168 hr	Percent reduction 0–24/24
ABG-6138								
5.56	ISC B5	*Culex* spp.	10.6	0.2	0.7	19.6	15.1	98.1
5.56	Bourns E	*Aedes vexans*	19.2	0.0	0.0	dry	57.5	100.0
11.12	Airport	*Aedes vexans*	19.9	0.0	2.4	0.7	2.8	95.4
11.12	ISC B5	*Culex* spp.	5.5	0.0	0.2	0.2	0.6	100.0
Bactimos								
5.56	Hickory	*Aedes vexans*	85.4	0.1	0.0	0.3	0.0	99.9
5.56	Bourns M	*Aedes vexans*	21.2	0.1	0.0	dry	90.5	99.9
11.12	Time	*Aedes trivittatus*	3.0	0.0	1.8	1.5	2.6	100.0
11.12	Bourns	*Culex* spp.	8.0	0.0	0.0	2.0	2.4	100.0
Teknar-70								
5.56	Bourns W	*Culex* spp.	13.4	0.2	20.1	24.8	7.0	99.9
5.56	Hickory	*Aedes vexans*	42.7	0.0	0.0	dry	dry	100.0
11.12	Haps	*Aedes vexans*	15.9	0.0	0.0	0.0	dry	100.0
Teknar[b]								
0.5	13th	*Aedes trivittatus*	2.8	0.0	0.1	0.0	0.0	100.0
1.0	ISC C5	*Culex* spp.	6.5	0.0	0.0	0.2	0.1	100.0
1.0	Bourns W	*Culex* spp.	19.7	0.7	6.1	64.9	53.5	96.4
1.0	Emma	*Aedes vexans*	24.0	0.0	0.0	0.2	3.9	100.0

[a] Mean of 10 dips/site.
[b] Water-dispersible concentrate, rate in liters/ha.

were controlled, but *Culex* reinfestation occurred within 72 hr.

PREHATCH TRIAL. Good control occurred when pools were flooded 1 hr after application (Table 3). However, less control (32–76%) was achieved when pools were flooded 144 hr (6 days) after granular application. No mortality was observed in pools treated with Bactimos-S, the material treated with carbon as a sunblock. The 5% Abate granular product gave 100% control at both 1 and 144 hr.

DISCUSSION

The advantages of a granular larvicide include ease of handling, ease of dispersal, reduced drift and better canopy penetration. Furthermore, granules offer the potential of slow release (residual activity) which, in the case of *B.t.i.* granules, would prolong the presence of toxin in the larval feeding zone. Commercially available *B.t.i.* products (i.e., wettable powders and flowable concentrates) lose efficacy in 24–48 hr as a result of settling out or inactivation of spores and crystals by ultraviolet light or biodegradation (Dame et al. 1981, Lacey and Singer 1982, Ignoffo et al. 1981). The need for a *B.t.i.* formulation that prolongs availability of the product in the larval habitats has been expressed by a number of investigators (McLaughlin et al. 1982, Sebastian and Brust 1981, Dickeson 1983). Sebastian and Brust (1981) believed that development of formulations that extended residual activity could

Table 3. Evaluation of selected granular formulations of *Bacillus thuringiensis* var. *israelensis* and Abate (Temephos) applied as prehatch treatment against *Aedes trivittatus* in artificial pools.

Rate (kg/ha)	Formulation	1 hr[a] 0 hr[c]	24 hr[c]	% reduction	144 hr[b] 0 hr[d]	24 hr[d]	% reduction
5.56	Abate	6.5[e]	0.0	100.0	1.5	0.0	100.0
5.56	Teknar-70	5.0	0.0	100.0	2.5	0.6	76.0
5.56	Abbott 6138	7.6	0.0	100.0	1.9	1.3	31.6
5.56	Bactimos	6.9	0.5	92.8	2.0	0.9	55.0
5.56	Bactimos-S	7.7	0.0	100.0	2.4	2.4	0.0
Control	Blank cob	5.1	6.1	0.0	2.4	2.9	0.0

[a] Applied to sand bottom 1 hr before flooding.
[b] Applied to sand bottom 144 hr before flooding.
[c] 95% CI = ±4.47, ±0.12 for 0 hr and 24 hr, respectively.
[d] 95% CI = ±0.83, ±2.63 for 0 hr and 24 hr, respectively.
[e] Mean of 2 pools; 5 dips/pool.

make *B.t.i.* an important agent in the management of mosquitoes. Residual activity is essential if *B.t.i.* is to compete with commonly used chemical insecticides. Granular formulations can be used in areas where a prehatch/preflood product is desired. This is often the case in mosquito abatement districts containing large amounts of periodically inundated flood plains or commercial rice fields where intermittent flooding is common.

Our experience with granular formulations of *B.t.i.* indicated good control of Iowa mosquitoes, in particular the floodwater species, *Ae. vexans* and *Ae. trivittatus*. Reinfestation was common within 72 hr in *Culex* breeding areas.

The granular formulations did not provide better residual activity than the water-dispersible concentrate. Prehatch treatment studies demonstrated that good control can be achieved only if the sites are flooded shortly after application (i.e., 1 hr). Application 6 days before flooding provided inadequate control. The lack of residual control may be a result of inactivation of *B.t.i.* spores and crystals by ultraviolet light and/or cooler water temperatures that may have reduced feeding rates. The possible inactivation of the *B.t.i.* granules by sunlight (particularly in reference to prehatch treatments) could possibly be alleviated by incorporation of a sun block when formulating the product. However, our initial trials using carbon as a sunblock were not encouraging. Morris (1983) found that the addition of ultraviolet absorbers prolonged the insecticidal activity of *Bacillus thuringiensis* var. *kurstaki (B.t.k.)* when applied to coniferous trees. The result was greater effectiveness against spruce budworms as compared with *B.t.k.* without protectants.

CONCLUSIONS

The granular formulations used in this study are excellent products in terms of ease of handling, ease of dispersal, and penetration of dense canopies. The products tested provide good control of existing mosquito larvae, particularly floodwater *Aedes*. Extending the residual activity of these products would make them more acceptable as a standard insecticide for mosquito control.

References Cited

Dame, D. A., K. E. Savage, M. V. Meisch and S. L. Oldacre. 1981. Assessment of industrial formulations of *Bacillus thuringiensis* var. *israelensis*. Mosq. News 41:540–546.

de Barjac, H. 1978. Une nouvelle variete de *Bacillus thuringiensis* tres toxique pour les moustiques, *B. thuringiensis* var. *israelensis* serotype 14. C. R. Acad. Sci. Paris, Ser. D., 286:797–800.

Dickeson, R. L. 1983. Studies of *Bacillus thuringiensis* subsp. *israelensis* against *Aedes vexans* and *Culex pipiens pipiens*. Unpublished M.S. thesis. Iowa State University Library, Ames. 67 pp.

Goldberg, L. J. and J. Margalit. 1977. A bacterial spore demonstrating rapid larvicidal activity against *Anopheles sergentii, Uranotaenia unguiculata, Culex univittatus, Aedes aegytpi*, and *Culex pipiens*. Mosq. News 37:355–358.

Ignoffo, C. M., C. Garcia, M. J. Kroha, T. Fukuda and T. L. Couch. 1981. Laboratory tests to evaluate the potential efficacy of *Bacillus thuringiensis* var. *israelensis* for use against mosquitoes. Mosq. News 41:85–93.

Lacey, L. A. and S. Singer. 1982. Larvicidal activity of new isolates of *Bacillus sphaericus* and *Bacillus thuringiensis* (H-14) against anopheline and culicine mosquitoes. Mosq. News 42:537–543.

McLaughlin, R. E., T. Fukuda, O. R. Willis and J. Billodeaux. 1982. Effectiveness of *Bacillus thuringiensis* serotype H-14 against *Anopheles crucians*. Mosq. News 42:370–374.

Morris, O. N. 1983. Protection of *Bacillus thuringiensis* from inactivation by sunlight. Can. Entomol. 115:1215–1227.

Mulla, M. S., B. A. Federici and H. A. Darwazeh. 1980. Effectiveness of the bacterial pathogen *Bacillus thuringiensis* serotype H-14 against mosquito larvae. Proc. Calif. Mosq. Vector Control Assoc. 48:25–27.

Ramoska, W. A. and C. Pacey. 1979. Food availability and period of exposure as factors of *Bacillus sphaericus* efficacy on mosquito larvae. J. Econ. Entomol. 72:523–525.

Sebastien, R. J. and R. A. Brust. 1981. An evaluation of two formulations of *Bacillus thuringiensis* var. *israelensis* for larval mosquito control in sod-lined simulated pools. Mosq. News 41:508–511.

Stark, P. M. and M. V. Meisch. 1983. Efficacy of *Bacillus thuringiensis* serotype H-14 against *Psorophora columbiae* and *Anopheles quadrimaculatus* in Arkansas ricelands. Mosq. News 43:59–62.

Sun, C., G. P. Georghiou and K. Weiss. 1980. Toxicity of *Bacillus thuringiensis* var. *israelensis* to mosquito larvae variously resistant to conventional insecticides. Mosq. News 40:614–618.