EFFICACY OF GRANULAR FORMULATIONS OF *BACILLUS THURINGIENSIS* (H-14) FOR THE CONTROL OF *ANOPHELES* LARVAE IN RICE FIELDS[1]

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ABSTRACT. Three granular formulations of *Bacillus thuringiensis* (H-14) were applied to dense stands of maturing and mature rice for control of *Anopheles crucians* and *An. quadrimaculatus*. Aerial applications of the Vectobac® granule (200 ITU/mg) at 5.6, 11.2 and 22.4 kg/ha to 0.4 ha plots resulted in 92, 94 and 96% reduction 48 hr after application, respectively, in populations predominantly consisting of late instars. The Bactimos® granule (175 ITU/mg) was applied by aircraft to 0.4 ha plots and by a Cyclone® seeder to 100 m² plots at 2.8, 5.6 and 11.2 kg/ha, resulting in 100% reduction after 48 hr in natural populations of predominantly early instar larvae at all 3 rates with each type of application. Older instars confined to sentinel cages responded with 92, 98 and 100% mortality, respectively. Complete mortality was also observed at the same time in natural populations of predominantly young larvae in similar plots treated with Teknar® granules (104 ITU/mg) at 1.7, 3.0 and 7.5 kg/ha. Near complete mortality (98-99%) was also observed in the older larvae used in the sentinel cages.

INTRODUCTION

The efficacy of *Bacillus thuringiensis* (H-14) as a larvicide of mosquitoes is documented for a number of species in a variety of habitats. Experimental ground applications against field populations of *Anopheles* larvae demonstrated a high degree of larvicidal activity of *B. thuringiensis* (H-14) against several species (Dame et al. 1981, McLaughlin et al. 1982, Yu et al. 1982, Stark and Meisch 1983). Anopheline larvae, however, demonstrate lower susceptibility than do culicine larvae to most formulations of this bacterium, principally because of their surface-feeding behavior and the rapid settling of the toxic moiety of *B. thuringiensis* (H-14) (Goldberg and Margalit 1977, de Barjac 1978, de Barjac and Coz 1979, Standaert 1981, Lacey and Singer 1982). In certain habitats, prevailing environmental conditions may further lower larvicidal potential of *B. thuringiensis* (H-14) (Lacey 1985), e.g., a dense canopy of vegetation may adversely affect the efficacy of wettable powders by trapping the applied material.

Formulations that afford greater penetration of dense vegetation and remain in the feeding zone of anopheline larvae may enhance the efficacy of *B. thuringiensis* (H-14). The purpose of this investigation was to evaluate the larvicidal activity of 3 commercially produced granular formulations against *Anopheles crucians* Wiedemann and *Anopheles quadrimaculatus* Say in maturing and mature first crop rice fields.

METHODS AND MATERIALS

Four rice fields in the vicinity of Jennings, Louisiana, were selected for field trials on the basis of the presence of suitable anopheline populations and dense stands of maturing rice. Nine to twelve 0.4 ha (1 acre) plots (30.5 × 132.7 m) were set up and marked with wooden stakes in each field. A control plot was also designated for each field and situated on the other side of a levee and at least 100 m from the nearest treated plot. On the morning of the day treatments were made 25 standard mosquito dipper (350 ml) samples were taken in each plot along both sides of a transect running through the center of the plot starting and ending 10 m inside the plot. Posttreatment samples were taken in an identical manner 48 hr following granule application. The control plots were sampled in a similar manner except that 75 dips/plot were taken for pre- and posttreatment samples. The number of *Anopheles* larvae in each dipper sample was counted in the field and returned to the plots. All instars were counted in pretreatment samples and 1st instars (considered to be unexposed to the treatment) were omitted from posttreatment counts.

In addition to the determination of percent reduction using the pre- and posttreatment dipper samples, 3 screened floating sentinel cages (holding ca. 0.8 liter water; 211 cm² opening) each containing 10 field-collected 3rd or 4th instar *An. crucians*/*An. quadrimaculatus* were placed in the center of each plot prior to treatment. Numbers of living larvae were recorded 48 hr after treatment.

Additionally, a random sample of 100 *Anopheles* larvae from each field was used for

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[1] This paper reflects the results of research only. Mention of a pesticide, commercial or proprietary product in this paper does not constitute a recommendation of this product by the U.S. Department of Agriculture.

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determination of the age composition for the population and 25 older instars from each field were later identified to species in the laboratory. *Culex salinarius* Coquillett, *Culex erraticus* (Dyar and Knab) and *Uranotaenia* sp. Edwards were also taken in the samples but proved to be too heterogeneously distributed to be of value for plot to plot comparison of formulation efficacy.

Application of the granules was made with a Grumman G-164A Ag Cat airplane using a Transland Deep Throat high volume granule spreader in 15.2 m swaths at a height of 4–5 m and an approximate airspeed of 160 km/hr. The delivery rate was calibrated with active granules at 11.2 kg/ha (10 lbs/acre). One pass/swath (each plot was 2 swaths wide) resulted in delivery of 11.2 kg/ha; 2 passes/swath resulted in delivery of 22.4 kg/ha (20 lbs/acre). The 5.6 kg/ha (5 lbs/acre) rate was achieved by mixing inert carrier granules with active granules in a 1:1 ratio and applying the mixture at 11.2 kg/ha.

The 3 granular formulations used for testing were: Teknar® 402 Gr 70 (104 ITU/mg; Zoecon, Inc., lot T315703); Vectobac® (200 ITU/mg; Abbott Laboratories, lot 51004Br); and Bactimos® (175 ITU/mg; Biochem, Inc., lot 614689). The formulations consisted of wettable powders (Vectobac and Bactimos) or spray dried flowable concentrate (Teknar) applied to the surface of various sizes of corn cob grits. Due to the proprietary nature of the 3 commercial products, the identities of the various diluents used in the production of the granules were not provided by the suppliers.

Each application rate was replicated 3 times with each experimental material. The Vectobac and Bactimos granules were aerially applied at 5.6, 11.2, and 22.4 kg/ha. The Teknar granules were applied at 3.0, 7.5, and 14.9 kg/ha. It was originally intended to apply the Teknar formulation at the same rate as the other 2 formulations. However, the exposure to either high humidity and/or the temperature (38°C+) in the airplane's hopper resulted in a calculated reduction of 33% in delivery rate of the 2 highest rates with the active granules. The 1:1 mixture of inert and active granules, however, resulted in about a 34% increase in the lowest delivery rate. The actual delivery rates were determined by weighing the granules remaining in the hopper after applications were made. The originally intended delivery rates of 5.6, 11.2 and 22.4 kg/ha were actually 7.5, 7.5 and 14.9 kg/ha, respectively, for the Teknar formulation. After recalibrating the spreader, a fourth aerial application of a 1:1 mixture of active and inert Teknar granules resulted in a delivery rate of 3.0 kg of active granules/ha.

Further evaluations were conducted with the Teknar and Bactimos formulations at 1.7 and 2.8 kg/ha, respectively, applied manually with a Cyclone-seeder in 100 m^2 plots. The seeder was calibrated to deliver 0.454 kg granules at a uniform rate throughout each plot. Seventeen grams of active Teknar and 28 g of active Bactimos granules were added to each 437 g and 426 g, respectively, of inert material to provide the proper treatment rates.

Three sentinel cages containing ten 3rd or 4th instar *Anopheles* larvae were placed in all the treated 100 m^2 plots. One sentinel cage was placed in each of 3 control plots. As in the 0.4 ha plots, 25 dips were taken per plot. Plots of both sizes were set up in a Latin square when possible, otherwise, a completely randomized block design was used. Field efficacy and laboratory data were analyzed with ANOVA and Duncan's new multiple range test.

The density and flotation properties of the 3 granular formulations were assessed in the laboratory. After the number of granules in three 100 mg samples of each product was counted the theoretical number of granules/m^2 at a treatment rate of 5 kg/ha was calculated. Also, three 100-granule samples of each formulation were gently placed on the surface of well water in separate 1-liter beakers. The number of floating granules was determined at the time of application and again at 24 hr. Identical samples were vigorously agitated after application by stirring for 10 sec with a clean wooden stick and observed for flotation as in the previous test. Qualitative observations were also made on the release of *B. thuringiensis* (H-14)-containing material from the surface of the granules by watching the granules and surface of the water for several minutes after the formulations were added to the beakers.

RESULTS

The results of efficacy testing of the Vectobac granular formulation in 0.4 ha plots are presented in Table 1. Despite the broad range of rates used, no significant differences between rates were found: the 5.6 kg/ha rate appears to be as efficacious as the 22.5 kg/ha rate. The significant decline in numbers of larvae in the control plots may have further obscured any possible differences in reduction due to difference in application rate.

The results of both large and small plot tests for the Bactimos and Teknar granules are presented in Tables 2 and 3, respectively. The highest rates utilized for both formulations are omitted from the tables since 100% mortality in both unconfined and sentinel cage larvae was

Table 1. Evaluation of aerially applied Vectobac granules (10% WP; 200 ITU/mg) against *Anopheles* larvae in mature stands of rice.

Rate (kg/ha)	Mean number larvae/dip ± S.E.[a] Pretreatment	48 hr posttreatment	Mean % reduction Dip determined	Sentinel cages[b]
5.6	1.49 ± 0.14	0.12 ± 0.05	92 a	93 a
11.2	2.07 ± 0.46	0.12 ± 0.06	94 a	89 a
22.5	2.23 ± 0.25	0.09 ± 0.06	96 a	96 a
Control	2.11 ± 0.67	1.32 ± 0.01	37 b	37 b

[a] *Anopheles crucians* (76%) and *An. quadrimaculatus* (24%) [16% 2nd instars; 84% 3rd and 4th instars; age and species composition determined from 100 larvae taken at random in pretreatment samples]; water temperature 27–34°C; water depth 15–30 cm; dense mature rice; 18–20 July 1983.

[b] 3rd-4th instar *Anopheles* spp., means followed by the same letter are not significantly different at the 0.05 level of probability.

Table 2. Evaluation of Bactimos granules (5% WP; 175 ITU/mg) against *Anopheles* larvae in maturing stands of rice.

Rate	Mean number larvae/dip ± S.E. Pretreatment	48 hr posttreatment	Mean % reduction Dip determined	Sentinel cages[a]
		Hand-applied (8–10 Aug 1983)[b]		
2.8	2.28 ± 0.30	0	100 a	92 a
Control	2.0 ± 0.69	1.0 ± 0.04	50 b	43 c
		Aerially applied (26–28 July 1983)[c]		
5.6	1.15 ± 0.15	0	100 a	98 ab
11.2	1.49 ± 0.31	0	100 a	100 b
Control	1.73 ± 0.38	1.72 ± 0.24	1 c	38 c

[a] See Footnote b, Table 1.

[b] *Anopheles crucians* and *An. quadrimaculatus* (88% 1st and 2nd instars, 12% 3rd and 4th instars; age and species composition determined from 100 larvae taken at random in pretreatment samples); water temperature 27–34°C; water depth 5–15 cm; dense sub-heading rice.

[c] *Anopheles crucians* (75%) and *An. quadrimaculatus* (25%) [88% 1st and 2nd instars, 12% 3rd and 4th instars]; water temperature 27–34°C; water depth 0–15 cm, dense young rice (0.5m).

Table 3. Evaluation of Teknar granules (104 ITU/mg [=260 AA units/mg]) against *Anopheles* larvae in maturing stands of rice.

Rate	Mean number larvae/dip ± S.E. Pretreatment	48 hr posttreatment	Mean % reduction Dip determined	Sentinel cages[a]
		Hand-applied (8–10 Aug 1983)[b]		
1.7	2.52 ± 0.42	0	100 a	99 a
Control	2.0 ± 0.69	1.0 ± 0.56	50 b	43 b
		Aerially applied (26–28 July 1983)[c]		
3.0	1.55 ± 0.25	0	100 a	—
Control	1.73 ± 0.38	1.72 ± 0.24	1 c	38 b
		Aerially applied (21–23 July 1983)[d]		
7.5	1.99 ± 0.23	0	100 a	98 a
Control	1.61 ± 0.39	1.25 ± 0.23	22 d	10 c

[a] See Footnote b, Table 1.

[b] *Anopheles crucians* and *An. quadrimaculatus* (88% 1st and 2nd instars, 12% 3rd and 4th instars; water temperature 27–34°C; water depth 0–15 cm; dense young rice (0.5 m).

[c] *Anopheles crucians* (75%) and *An. quadrimaculatus* (25%) [88% 1st and 2nd instars, 12% 3rd and 4th instars; age and species composition determined from 100 larvae taken at random in pretreatment samples]; water temperature 27–34°C; water depth 0–15 cm; dense young rice (0.5 m).

[d] *Anopheles crucians* (73%) and *An. quadrimaculatus* (27%) [88% 1st and 2nd instars, 12% 3rd and 4th instars]; water temperature 27–34°C; water depth 15–30 cm, dense mature rice.

produced with both the highest and the next to the highest rates. No significant difference between rates was observed for the 2 granular formulations using dipper samples. The lowest rate for the Bactimos granule, however, was significantly less effective against older instars in the sentinel cages than the highest rate (Table 2). High control mortality in the sentinel cages and high reduction in the control plots was common, which made comparisons between formulations tenuous at best. The most reliable and tenable evaluations of penetration and insecticidal activity of the Bactimos and Teknar granules were on those dates when there was a negligible reduction of the natural population in control plots (26–28 July) or when there was low sentinel cage mortality in the control plots (21–23 July). Excellent control was obtained with both formulations at comparable rates.

The density (no./100 mg), theoretical distribution (no./m^2), and flotation properties of the granules are presented in Table 4. The percentage that floated initially after aerial application was probably higher than that observed in the laboratory after vigorous stirring. Under laboratory conditions the release rates of the Vectobac and Teknar formulations were similar in that large amounts of material were shed from the granules immediately after becoming wet. The Vectobac material was released as relatively fine particles, over 50% of which sank to the bottom of the beaker. The Teknar active material exfoliated from the surface of the granule carrier and sank, for the most part as relatively large particles. After being liberated, some of the finer particles of Teknar were supported by the surface tension and held between aggregates of the floating carrier granules. Despite the reduced flotation of the Bactimos granule some of the wettable powder portion and what appeared to be a lightweight oil were released onto the surface of the water as the granules penetrated the surface tension. A distinct veneer of oil and powder was observed on the surface of the water immediately following introduction of the Bactimos granules in the laboratory tests and remained in evidence for over 2 days on the surface of the unagitated water.

DISCUSSION

The interpretation of the data from our field evaluations of the 3 formulations should be tempered with the accuracy of the dipper sampling method. Although the dipper method is reliable in determining gross changes in larval numbers, its sensitivity for accurately determining *Anopheles* populations is fairly low (Service 1976). When comparing the efficacy of the formulations other factors should also be considered, most notably larval age.

The high degree of control provided by the Teknar and Bactimos formulations compared to the slightly lower effect with the Vectobac formulation was undoubtedly influenced by the age structure of the target larval populations. Several investigations (reviewed by Lacey 1985) on the effect of larval age on larvicidal activity of *B. thuringiensis* (H-14) consistently demonstrate a negative correlation between age and susceptibility. Additionally, the greater number of granules per unit area provided by the Teknar and Bactimos formulations enabled more contact between mosquito larvae and *B. thuringiensis* (H-14) than was possible with the larger, heavier Vectobac granules. The sustained flotation of the Teknar granule and wettable powder from the Bactimos granule also provided maximum contact of the toxic moiety with the surface feeding *Anopheles* spp. Drift may be a problem with the lighter Teknar granule under windy conditions. No such problem was noted under the conditions of our field trials. Improvement of the 3 formulations might be possible by slowing the release rates of Teknar and Bactimos and decreasing the size of the Vectobac carrier granules.

Botanical granules characteristically only permit surface adhesion of particulate active ingredients and consequently, due to settling of both the granules and active moiety, offer limited residual capacity. In addition to the benefits normally associated with botanical carriers (i.e., penetration, flotation, decreased drift during application), a matrix granule, similar in

Table 4. Density and flotation properties of 3 granular formulations of *Bacillus thuringiensis* (H-14).

Formulation	Mean no. granules ± S.E.[a]		Mean % floating ± S.E. (no agitation)		Mean % floating ± S.E. (agitated)	
	per 100 mg	per m^2[b]	initially	after 24 hr	initially	after 24 hr
Vectobac	4 ± 0.25 a	20 ± 1.25 a	75 ± 2.89 a	5 ± 1.86 a	71 ± 2.00 a	4 ± 1.33 a
Bactimos	41 ± 1.10 b	205 ± 5.40 b	59 ± 2.33 b	30 ± 2.33 b	24 ± 2.19 b	4 ± 1.33 a
Teknar	153 ± 7.24 c	765 ± 36.20 c	99.7 ± 0.33 c	98.7 ± 0.33 c	63 ± 3.79 a	62 ± 4.16 b

[a] Means in the same column followed by the same letter are not significantly different at the 0.05 level.
[b] Theoretical no./m^2 at a rate of 5 kg/ha.

structure and composition to the sustained release pellets formulated by Lacey et al. (1984), might provide residual larvicidal activity in permanent and semipermanent breeding sites.

Earlier work on aerially applied suspensions of *B. thuringiensis* (H-14) wettable powders to open water in recently flooded 2nd crop rice fields demonstrated good control of *Psorophora columbiae* Dyar and Knab (McLaughlin and Billodeaux 1983). Fairly high rates of application (1.1 kg/ha) of an unspecified wettable powder in California rice fields (height and density of rice unspecified) provided good but temporary suppression of *Culex tarsalis* Coquillett (Stewart et al. 1983). Considering the lower susceptibility of anopheline mosquitoes, the dense, tall canopy of rice and the relatively small amount of *B. thuringiensis* (H-14) on the granules (5-17% of the weight of the formulation), the level of control attained in our study was remarkably high. Additional research on the duration of control with granular formulations of *B. thuringiensis* (H-14) is warranted.

Although economic considerations may limit the use of *B. thuringiensis* (H-14) for the control of *Anopheles* larvae in rice fields in the southeastern U.S., efficacious formulations such as those presented here may find use in the suppression of malaria vectors where there are high levels of resistance to conventional residual insecticides.

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