EFFECTIVENESS OF *BACILLUS THURINGIENSIS* SEROTYPE H-14 AGAINST *ANOPHELES CRUCIANS*

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ABSTRACT. *Bacillus thuringiensis* (var. *israelensis*) serotype H-14 was tested for efficacy against *Anopheles crucians* larvae in small ponds at a golf course, a woodland lake and along the edge of a rice field. Two commercial formulations were tested ranging from 0.25 to 6.0 kg/ha (1.5×10^8 to 3.0×10^9 International Toxic Units/ha). The higher rates ($1.8 - 3.0 \times 10^9$ I.T.U./ha) reduced larval counts 80 – 100% in most tests. The lowest rates were always less effective. Variability between test plots was large. Differences in larval reduction between application rates were not statistically significant.

Bacillus thuringieneis (var. *israelensis*) serotype H-14 (*Bti*) (Goldberg and Margalit 1977, deBarjac 1978a) was tested for control of *Anopheles crucians* Wied. larvae. Laboratory assays generally agree that much higher concentrations are needed to kill anophelines (deBarjac 1978b, deBarjac and Coz 1979), than are needed to kill other species. Our tests were conducted at 3 locations: 1) a small clear pond with minimal vegetation, in a golf course, 2) a large woodland lake with very dense mats of surface aquatic plants, and 3) in a rice field in southwest Louisiana. Three formulations from commercial companies were tested. The purpose of these tests was to determine whether *B.t.* H-14 would reduce *An. crucians* larval populations enough to be a potential control agent.

MATERIALS AND METHODS[2]

The *B.t.* H-14 formulations were obtained from Abbott Laboratories[3] and Biochem Products[4]. The Abbott formulation was under the Environmental Protection Agency permit number 27523-EUP-1. The Biochem material was under permit number 43382-EUP-1. Bioassays with *Aedes aegypti* (Linn.) against the International Standard for *B.t.* H-14 (IPS-78), rated the Abbott formulations at 300 Toxic Units/mg (Abbott ABG-6108B), 1800 T.U./mg (Biochem B-666), and 3000 T.U./mg (Biochem 676). The amounts applied varied from 0.23 kg/ha to 6.0 kg/ha (1.5×10^8 to 6.0×10^9 T.U.'s/mg). Conventional usage employs a weight per surface area application rate, although product labels also indicate the potency in T.U./mg as derived from laboratory assays against a standard (IPS-78).

Each test was designed with the plots arranged along the edge of the pond or field. Plots were 10 m long and extended 3 m from the edge. A similar untreated buffer area separated each plot. In addition to *B.t.* H-14 plots an untreated plot and a plot treated with the larvicide Dursban® (chlorpyrifos) at 87 ml/ha (1.2 oz/ac) were included. No chemical plot was used in the rice field because Dursban is not approved for use in rice fields.

Pretreatment larval samples were taken by the standard dipper count method, one per linear meter. The *B.t.* H-14 and Dursban were applied. Posttreatment counts of larvae were made the next day. The sampling was continued every day for 3 days posttreatment. Observations were also made of water temperature and for the presence and relative abundance

[1] Director, Jefferson Davis Parish Mosquito Control District, Jennings, LA.

[2] Mention of a trademark, proprietary product or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

[3] Abbott Laboratories, North Chicago, IL.

[4] Biochem Products, a Division of Salsbury Laboratories, Inc., Monchanin, DE.

of non-target aquatic organisms. Samples were dipped from the buffer areas between plots, and also beyond 3 m from the edge. These samples were intended to determine whether plot sizes were adequate.

The *B.t.* H-14 was premixed as a slurry before adding to the spray tank. The spray system consisted of a 3 gal. (ca. 12 liter) soft-drink syrup canister fitted with a long outlet hose and a spray wand using a flat-spray nozzle. The inlet side of the tank was connected to a CO_2 bottle equipped with a pressure regulator. Highly accurate dispensing of the desired volume per time unit was accomplished.

The test site consisted of 3 distinct environments. The first was a pool at Bayou Oaks Golf Course. It was characterized by open, clear water except for a moderate amount of vegetation about 1 m from the bank. The maximum water depth 3 m out from the bank was 0.5 m. Four treatments were applied from May 6 through June 30, 1980. The second area was a pond ca 1.5–2.0 ha in a pine and mixed hardwood forest. The pond was about half covered with aquatic plants which included a 1–3 m vegetation zone along the pond's edge. The maximum water depth, 3 m from the bank, was approximately 0.67 m. The plant growth was not dense, but provided excellent breeding area for *An. crucians.* Five applications were made from May 16 through July 7, 1980. The third test site was a maturing rice field subjected to a 30 cm flood on July 4. Three treatments were applied from July 16 through July 30, 1980.

RESULTS

BAYOU OAKS. The Bayou Oaks test results are shown in Table 1. The first test on May 6 resulted in 100% mortality of mosquito larvae and all non-target species in the Dursban plot. *Bacillus thuringiensis* serotype H-14 reduced larval counts 63–96%. Application rates did not show great differences in mortality from the dipping count (See Table 1). The water temperature was 28°C. Recovery (new hatch) occurred on subsequent sampling days in the *B.t.* H-14 plots.

The second test on June 17 took place during much warmer weather. Water temperature was 34°C the day of the test. This temperature remained constant for the entire series. Dursban again killed all organisms. The *B.t.* H-14 failed to produce high mortality in any plots on June 18. The plot receiving the lowest rate 0.5

Table 1. Effect of *Bacillus thuringiensis* H-14 formulation ABG 6108B (Abbott Laboratories) and of Dursban® on *Anopheles crucians* larvae applied to a golf course water hazard pond, Bayou Oaks Country Club, Sulphur, LA. Data are larvae per 10 dips pretreatment/1 day posttreatment and the percent reduction or (increase) adjusted for untreated plot counts. Duncan's multiple range test scores are shown by letter a and b in the totals column.

	Dates of sampling									
	5/6/80		6/17/80		6/24/80		6/30/80		Totals	
Rate kg/ha	Larvae	%	Larvae	%	Larvae	%	Larvae	%	Larvae	%
Dursban 87 ml/ha	27/1	97	38/0	100	24/0	100	10/0	100	99/1	99 a
B.t. H-14 (ABG 6108B)										
6.0	—		—		43/8	72	19/1	95	62/9	83 ab
4.0	—		—		52/22	37	49/26	48	101/48	44 ab
3.0	24/6	77	22/14	(71)	—		—		46/20	28 b
2.0	12/5	62	58/10	54	26/11	37	24/5	80	120/31	65 ab
1.0	23/1	96	22/7	14	36/16	34	31/10	69	112/34	59 ab
0.5	22/1	96	1/18	(470)	—		—		23/19	(37)
Untreated	20/22		43/16		43/29		20/41		146/108	

kg/ha yielded only 1 larva in the dipper samples on the day of the treatment preventing evaluation of that rate. Newly hatched larvae were the only ones found in all *B.t.* H-14 plots for the 3 days following application.

Application rates were doubled for the last 2 applications on June 24 and 30. Larval counts increased the 3 days following the treatment because of newly hatched larvae. Very few 3rd or 4th instars were present up to 3 days after treatment, although they were present in moderate numbers before treatment. The analysis of treatments by Duncan's multiple range test showed Dursban different from 3.0 kg/ha *B.t.* H-14, but no other separations were possible due to a high standard deviation of 29.5.

WOODLAND POND. The results are presented in Table 2. The first 2 applications of *B.t.* H-14 reduced larval counts 57–92%. Water temperature were ca. 30–31°C. Dursban was not applied in the second test (June 19) because few larvae (3 per dip) were present and we wished to determine how long the residual effect from the June 16 application would persist. Significant survival of newly hatched larvae occurred in the Dursban plot the 4th day after application. This concurs with the observations at Bayou Oaks. The last 3 applications of *B.t.* H-14 were at the doubled rate. *Bacillus thuringiensis* serotype H-14 was as effective at 6.0 kg/ha as was Dursban one day after treatment. Analysis by Duncan's multiple range test show Dursban and the rates of *B.t.* H-14 from 3.0 to 6.0 kg/ha have the same effect (see Table 2). A definite decrease in effectiveness occurred with only 0.5 kg/ha.

RICE FIELD TESTS. This environment differed from the 2 ponds. The edge of the rice field had rice stems emerging from the water, broad-leafed weeds either emerging from the surface or fallen off the plant and lying on the surface, various amounts of organic debris from the wooded edge of the field, and a brownish to reddish algal growth in some of the plots. Another difference was that 3 formulations of *B.t.* H-14 were tested.

Table 3 presents the results. The highest rate of Abbott material (6.0 kg/ha) gave the most consistent and greatest kill. Certainly if chemicals were not desirable, as in the case of the rice field test, *B.t.* H-14 would be quite effective and acceptable. Statistical analysis of the tests did not show significant differences between formulations or rates because of large

Table 2. Effect of *Bacillus thuringiensis* H-14 formulation ABG 6108B (Abbott Laboratories) and of Dursban® on *Anopheles crucians* larvae in a woodland pool, Sulphur, LA. Data are larvae per ten dips pretreatment/2 day posttreatment and percent reduction of (increase) adjusted for untreated plot counts. Results of Duncan's multiple range test are shown by letters after the totals. Totals with the same letter are not different at P = .05.

	Dates of sampling											
	6/16/80		6/19/80		6/24/80		6/30/80		7/7/80		Totals	
Rate kg/ha	Larvae	%	Larvae	%	Larvae	%	Larvae	%	Larvae	%	Larvae	%
Dursban 87 ml/ha	24/0	100	—		29/0	100	46/0	100	42/0	100	141/0	100 a
B.t. H-14												
6.0	—		—		31/0	100	27/0	100	35/0	100	93/0	100 a
4.0	—		—		32/1	95	41/3	93	47/3	96	120/6	96 ab
3.0	25/6	81	33/2	92	—		—		—		58/8	87 a bc
2.0	24/6	74	20/3	79	20/0	100	33/8	77	28/10	79	128/29	77 cd
1.0	24/8	74	28/3	85	28/3	83	18/3	84	24/4	90	122/21	83 bc
0.5	25/11	66	16/5	57	—		—		—		41/16	64 d
Untreated	80/103		44/32		65/41		35/37		25/42		249/255	

Table 3. Effects of 3 formulations of *Bacillus thuringiensis* H-14 on *Anopheles crucians* larvae. Data are larvae per 10 dips pretreatment/1 day posttreatment and percent reduction or (increase) adjusted for the untreated plot counts. Data are totals of results from 3 replicate plots on each treatment day (6 untreated plots).

B.t. H-14 formulations	Dates of sampling						Totals	
	7/16/80		7/22/80		7/30/80			
	Larvae	%	Larvae	%	Larvae	%	Larvae	%
Abbott 6108B								
300 T.U./mg								
6.0 kg/ha	70/8	91	86/2	98	50/1	98	206/11	98
3.0 kg/ha	43/6	89	47/10	61	50/1	98	140/25	79
1.5 kg/ha	71/11	88	86/6	93	49/8	86	206/25	90
Biochem-Bactimos 666								
1800 T.U./mg								
1.0 kg/ha	106/11	92	—		—		106/11	92
0.5 kg/ha	72/21	77	—		—		72/21	77
0.25 kg/ha	108/29	79	—		—		108/29	79
Biochem-Bactimos 676								
3000 T.U./mg								
1.0 kg/ha	—		64/20	68	80/6	94	144/26	84
0.67 kg/ha	—		93/36	61	88/8	92	181/44	79
0.33 kg/ha	—		86/89	(5)	80/17	82	166/106	44
Untreated	133/171	(29)	116/132	(15)	108/126	(17)	357/429	(20)

amounts of within-plot variability. The most consistent data (lowest coefficient of variation between replications) was provided by the ABG-6180B formulation at 1.8×10^9 T.U./ha. Biochem 666 was used only once and could not be judged. The Biochem 676 material was not consistent between the 2 replicates. An important factor for control is that the rate used be consistently effective.

CONCLUSIONS

Ninety percent or more reduction of sampled *An. crucians* can occur by proper application of *B.t.* H-14. The total T.U./ha are important. The optimum rate will probably be in the range of 2.0×10^9 T.U./ha. Actual kg/ha will have to be determined from the potency rating of the formulation to be used. Concentration of active ingredient (potency) is not the only important factor, however. The length of time the material remains in the larval feeding zone and the ingestion rate by larvae are 2 major factors. Proper formulation to increase feeding rates and to prolong availability in the feeding zone could increase efficacy more than could great increases in the amount of active ingredient. The Bayou Oaks test site had the least vegetation and the least control by *B.t.* H-14 in our tests. Surface material such as emergent vegetation or plant debris, forms a water meniscus. This probably retains the *B.t.* H-14 crystals longer than if *B.t.* H-14 were applied to open water. Moreover, anopheline larvae tend to feed in these covered areas. Therefore, vegetated or debris covered areas are more likely to allow successful use of *B.t.* H-14 formulations for anopheline control.

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COLONIZATION OF THAILAND STRAINS OF *ANOPHELES NIVIPES* AND *ANOPHELES PHILIPPINENSIS*

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ABSTRACT. Two Thailand strains of *Anopheles nivipes* and one of *An. philippinensis* have been colonized by the artificial mating technique. The essential methods and colonization techniques are described. *Anopheles philippinensis* was more easily colonized than *An. nivipes*. Differences were detected in the laboratory feeding propensity and ovipositional behavior of these species. Other small biological differences also were noted.

INTRODUCTION

For years the name *Anopheles philippinensis* Ludlow has been used for a common mosquito with a wide distribution in the Orient. Recently, Knight and Stone (1977) listed this distribution as the Philippines, Borneo, Java, Sumatra, Malaya, French Indochina, Thailand, Burma, India, Andaman Islands, China and Hainan Island. However, Reid (1967) elevated the name *An. nivipes* (Theobald), previously considered a synonym of *An. philippinensis*, to species status based on specimens from Malaya, Burma and the extreme southern peninsular region of Thailand. Separation of these 2 species was based on slightly overlapping morphological characters in several life stages (Reid 1967, 1968). Reid suggested the need for progeny studies to confirm the species status of these 2 taxa. Since Reid's studies, there has been no further elucidation of the status or distribution of *An. nivipes*.

Anopheles philippinensis has been incriminated as a vector of human malaria parasites in India (Covell 1944), and recently (Harinasuta et al. 1976), has been considered a suspected vector in Thailand. The role of *An. nivipes* in malaria transmission has not been examined. Furthermore, the presence of *An. nivipes* in Thailand has not been recognized by the Thailand Malaria Division (unpublished reports), even though Reid (1967) discussed Thai specimens.

In late 1978, studies were initiated by the Department of Medical Entomology, Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, to clarify (1) the specific status of *An. philippinensis* and *An. nivipes* by cross-mating and cytogenetic studies; (2) the distributions and bionomics of these 2 species in Thailand; and (3) the susceptibility of the 2 species to malaria parasites.

To date, preliminary cross-mating and cytogenetic studies (unpublished data) at our laboratory have firmly established

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