Journal of the American Mosquito Control Association, 20(4):429–433, 2004

EFFICACY STUDIES OF VECTOBAC® 12AS AND TEKNAR® HP-D LARVICIDES AGAINST 3RD-INSTAR *OCHLEROTATUS TAENIORHYNCHUS* AND *CULEX QUINQUEFASCIATUS* IN SMALL PLOT FIELD STUDIES

T. G. FLOORE, J. L. PETERSEN AND K. R. SHAFFER

John A. Mulrennan, Sr., Public Health Entomology Research & Education Center, College of Engineering Sciences, Technology and Agriculture, Florida Agriculture & Mechanical University, 4000 Frankford Avenue, Panama City, FL 32405-1933

ABSTRACT. Efficacy studies were conducted with VectoBac® 12AS and Teknar® HP-D larvicides against 3rd-instar *Ochlerotatus taeniorhynchus* and *Culex quinquefasciatus* in small field test plots. The products were obtained off the shelf from distributors and had different lot numbers. They were evaluated over a 2-year period in spring 2002 and 2003. Application rates were 0.29, 0.58, and 1.10 liter/ha and evaluations were made 24 and 48 h after treatment. Both products performed well in these studies, with VectoBac 12AS being more effective at the 0.29 liter/ha rate.

KEY WORDS Larvicide, *Culex quinquefasciatus, Ochlerotatus taeniorhynchus,* Valent VectoBac® 12AS, Certis Teknar® HP-D, small plot tests

INTRODUCTION

Efficacy studies were conducted in small field test plots against colony-reared 3rd instars of *Ochlerotatus taeniorhynchus* (Wiedemann) and *Culex quinquefasciatus* Say. Both VectoBac® 12AS (Valent BioSciences Corp., Libertyville, IL) and Teknar® HP-D (Certis USA, Columbia, MO) contained 1,200 International Toxic Units of *Bacillus thuringiensis* var. *israelensis* de Barjac (*Bti*). Heimpel (1967) reported that the crystalliferous bacterium *Bacillus thuringiensis* was successfully tested against 137 insect species, including some in the order Diptera. *Bacillus thuringiensis* var. *israelensis* was 1st recognized in the mid-1970s (Margalit 1990) as exhibiting mosquito larvicidal properties. During the 1980s, gene analysis and biochemical identifications of *Bti* crystal endotoxin and cloning of the *Bti* genes (DNA) in many combinations led to the identification of the mosquiticidal properties of the *Bti* crystals (Boyle and Dean 1990). Several strains and commercial products of *Bti* have been developed and used and some have gone out of production. Skeetal® by Novo Nordisk (Danbury, CT) and Certis Teknar are 2 that are no longer produced. Powell and Jutsum (1993) stated that for a biocontrol agent to be commercially effective, it needed to occupy a niche where chemicals did not work, were unavailable, or were politically unacceptable. In addition, such agents needed to be cost effective, easy to apply, and reliable. *Bacillus thuringiensis* var. *israelensis* marketed as Valent VectoBac 12AS and Certis Teknar HP-D met those criteria.

The John A. Mulrennan, Sr., Public Health Entomology Research & Education Center (Center) has evaluated larvicides that mosquito control districts apply under normal application conditions for many years (Rathburn and Boike 1973). Boike et al. (1990) evaluated VectoBac 12AS and Teknar HP-D against laboratory-reared and wild strains at the Center. In 1987, Bacitmos® (Valent) and Skeetal were evaluated with VectoBac AS and Teknar in laboratory beaker tests, with little difference observed between the products (Boike et al. 1987). Colony-reared larvae of *Cx. quinquefasciatus, Cx. nigripalpus* Theobald, and *Oc. taeniorhynchus* were used in this study. In 1990, VectoBac 12AS and Teknar HP-D were evaluated against both 3rd-instar colony-reared and malathion-resistant wild *Cx. quinquefasciatus* and *Oc. taeniorhynchus* in laboratory beaker tests. No differences were observed between either colony or wild strains or larvicides. In 1987, the effect of water pH on VectoBac AS was evaluated against larval *Cx. quinquefasciatus* in laboratory beaker tests (Floore et al. 1987). Water pH values between 6.3 and 8.6 did not affect the efficacy of VectoBac AS on 3rd-instar *Cx. quinquefasciatus.* In 1991, efficacy studies with a VectoBac 12AS–vegetable oil formulation (JAXOIL) and VectoBac 12AS were conducted in laboratory pan tests and small field test plots against larval *Cx. nigripalpus, Cx. quinquefasciatus,* and *Oc. taeniorhynchus* (Floore et al. 1991). Slight differences were observed between the formulations. Because it had been several years since we had evaluated the efficacy of these products, we obtained Valent VectoBac 12AS and Certis Teknar HP-D from distributors to reevaluate in our small

Fig. 1. Small field test plots used in the efficacy study.

test plots. These studies were conducted during May 2002 and June 2003.

MATERIALS AND METHODS

The small field test plots consisted of 36 uncovered concrete cattle troughs (0.6 m wide × 1.7 m long × 0.6 m deep; J. B. Hill Contractors, Inc., Leesburg, FL) in screen enclosures (Fig. 1). The troughs were filled with well water 2 days before introduction of larvae. The water surface area in the plots measured approximately 0.7 m^2, with a depth of approximately 0.15 m. The salt concentration (3–5∂) for the tests with *Oc. taeniorhynchus* was made by adding 150 ml of sodium chloride (Fisher Scientific Products, Atlanta, GA) to the well water. Approximately 800 3rd-instar laboratory-reared susceptible *Oc. taeniorhynchus* or *Cx. quinquefasciatus* were added to each plot. A mixture of powered liver and brewer's yeast (3:2; 50 ml) was added to each trough twice daily. Emergent grasses were present in all the troughs. The water temperature was recorded by using an Onset HOBO ProSeries Temperature data recorder (Onset, Pocasset, MA) and the salinity was measured with a salinity refractometer (SR-1) (Onset).

Two Valent VectoBac 12AS larvicides (lot 91-631-N9 [replicate 1] and 80-809-N9 [replicate 2]) and 2 Certis Teknar HP-D larvicides (lot 4831522 [replicate 1] and 2830842 [replicate 2]) were obtained from distributors in 2.5-gal containers. Application rates were 0.29, 0.58, and 1.1 liters/ha. Appropriate dosages were calculated and pipetted from the 2.5-gal container into 1,000-ml volumetric flasks, then mixed with water to form a stock solution and distributed by pipette evenly in the troughs at the required application rates. Troughs were dipped by using a standard larval dipper 24 and 48 h after treatment at preset dipping stations, 1 at each corner of the plot (n = 4), 2 along the long axis of the trough wall, and 2 located along the center axis of each trough approximately 0.3 m from each end. Tests were replicated twice each year.

Data analysis: Only live larvae dipped at each station were counted. Data for each posttreatment period were recorded, and corrected for control mortality by using Mulla's formula (Mulla et al. 1971):

$$\% \text{ mortality} = \frac{\text{no. control larvae} - \text{no. treatment larvae}}{\text{no. control larvae}}$$

Data were subjected to analysis of variance (PROC GLM; SAS Institute 2002) and a Student–Newman–Keuls multiple range test on the means after an arcsine transformation on the percent data. Differences were considered significant at P = 0.05.

RESULTS AND DISCUSSION:

Results for the 2-year study are shown in Tables 1 and 2. No significant differences (P = 0.8701, F = 0.03, df = 1) were found between years but significant differences (P = 0.0002, F = 14.33, df = 1) were found between species and between species and treatment (P < 0.0001, F = 24.70, df = 4). Both products were more effective against larval *Oc. taeniorhynchus* than against larval *Cx. quin-*

Table 1. Comparison of VectoBac® 12AS and Teknar® HP-D against 3rd-instar *Culex quinquefasciatus* in small test plots.

Product[1]	Rate (liter/ha)[2]	Replicate (lot)	Time after treatment (h)	2002		2003	
				No. larvae	% mortality	No. larvae	% mortality
Control			24	383	0.00	560	0.00
Teknar A	0.29 a	1	24	71	81.46	103	81.61
Teknar A	0.29 a	2	24	69	81.98	11	98.04
Teknar A	0.58 b	1	24	14	96.34	24	95.71
Teknar A	0.58 b	2	24	25	93.47	6	98.93
VectoBac B	0.29 a	1	24	15	96.08	4	99.29
VectoBac B	0.29 a	2	24	33	91.38	6	98.93
VectoBac B	0.58 b	1	24	6	98.43	6	98.93
VectoBac B	0.58 b	2	24	4	98.96	3	99.46
Control			48	286	0.00	502	0.00
Teknar A	0.29 a	1	48	4	98.60	69	86.25
Teknar A	0.29 a	2	48	16	94.41	0	100.00
Teknar A	0.58 b	1	48	7	97.55	1	99.80
Teknar A	0.58 b	2	48	5	98.25	1	99.80
VectoBac B	0.29 a	1	48	0	100.00	0	100.00
VectoBac B	0.29 a	2	48	5	98.25	0	100.00
VectoBac B	0.58 b	1	48	0	100.00	0	100.00
VectoBac B	0.58 b	2	48	0	100.00	0	100.00

[1] Product followed by different uppercase letter represents significant differences ($P = 0.05$) between products as determined by Student–Newman–Keuls multiple comparison tests.

[2] Rate followed by different lowercase letter represents significant differences ($P = 0.05$) between application rates as determined by Student–Newman–Keuls multiple comparison tests.

quefasciatus at 24 and 48 h after treatment at the 0.58 liter/ha rate. In 2002, no comparison was made between species at the 0.29 liter/ha rate. However, VectoBac 12AS was more effective than Teknar HP-D against *Cx. quinquefasciatus* (96.5% vs. 81.7%) at the 0.58 liter/ha and 1.1 liter/ha rates. In 2003, VectoBac 12AS at the 0.29 liter/ha rate was slightly more effective (±3%) against *Oc. taeniorhynchus* than against *Cx. quinquefasciatus* both 24 and 48 h after treatment (Tables 1 and 2). The 0.58 liter/ha rate of VectoBac 12AS was slightly more effective (±2%) against *Oc. taeniorhynchus* than Teknar HP-D in both years.

Significant differences ($P = 0.0001$, $F = 20.37$, $df = 1$) were found between products in both years but no significant differences ($P = 0.6152$, $F = 0.49$, $df = 2$) were found between replications. Results of Student–Newman–Keuls means separation tests suggest differences in individual product lot numbers as well.

Differences were found in mortality between the Teknar HP-D formulations, with lot 2 exhibiting more control (Tables 1 and 2). In 2003, more variation in mortality was noted between lots and products against *Cx. quinquefasciatus* (Table 1) than against *Oc. taeniorhynchus* (Table 2). Teknar HP-D at the 0.29 liter/ha rate was 89% effective against *Cx. quinquefasciatus* at 24 h after treatment, whereas VectoBac 12AS was 99% effective. This difference also was noted at 48 h after treatment (Table 1). In 2002, little difference (±2%) was observed between the lots or products (Table 2). In the 2003 tests, Teknar HP-D was more effective (±3%) than VecoBac 12AS at 24 h after treatment at the 0.58 liter/ha rate in tests with *Oc. taeniorhynchus*. More variation in efficacy was observed in 2003 than in 2002. In these studies, Teknar HP-D applied at 0.29 liter/ha demonstrated less than 90% effectiveness against *Cx. quinquefasciatus* at 24 h after treatment. Although less than 90% control might be acceptable in some control situations, within mosquito control agencies we expect 90% effectiveness in our plot studies. At 48 h after treatment, greater than 90% efficacy was demonstrated. Skovmand et al. (1998) demonstrated differences between *Bti* products and mosquito strains, and Becker et al. (1992) showed that water temperature affected potency. Water temperature averaged 25.7°C in 2002 and 24.9°C in 2003 and appeared not to affect our study.

CONCLUSIONS

Studies conducted in 2002 and 2003 in small field test plots with larval *Oc. taeniorhynchus* and *Cx. quinquefasciatus* larvae compared VectoBac 12AS and Teknar HP-D *Bti* larvicides at 3 application rates. Two lots of each larvicide were obtained from distributors. In 2002, both products were nearly 100% effective against larval *Oc. taeniorhynchus* at application rates of 0.58 and 1.1 liter/ha at both 24 and 48 h after treatment (Table 2). VectoBac 12AS exhibited more control at 0.29 liter/ha and 0.58 liter/ha than did Teknar HP-D against *Cx. quinquefasciatus* (Table 1). Differences in effectiveness were observed between product lots,

Table 2. Comparison of VectoBac® 12AS and Teknar® HP-D against 3rd-instar *Ochlerotatus taeniorhynchus* in small test plots.

Product[1]	Rate (liter/ha)[2]	Replicate (lot)	Time after treatment (h)	2002		2003	
				No. larvae	% mortality	No. larvae	% mortality
Control			24	438	0.00	126	0.00
Teknar A	0.29 a	1	24			15	88.10
Teknar A	0.29 a	2	24			8	93.65
Teknar A	0.58 b	1	24	3	99.32	4	96.83
Teknar A	0.58 b	2	24	1	99.77	0	100.00
Teknar A	1.1 b	1	24	0	100.00		
Teknar A	1.1 b	2	24	0	100.00		
VectoBac B	0.29 a	1	24			1	99.21
VectoBac B	0.29 a	2	24			0	100.00
VectoBac B	0.58 b	1	24	3	99.32	6	95.24
VectoBac B	0.58 b	2	24	2	99.54	2	98.41
VectoBac B	1.1 b	1	24	0	100.00		
VectoBac B	1.1 b	2	24	0	100.00		
Control			48	148	0.00	66	0.00
Teknar A	0.29 a	1				4	93.40
Teknar A	0.29 a	2				0	100.00
Teknar A	0.58 b	1	48	0	100.00	1	98.48
Teknar A	0.58 b	2	48	0	100.00	0	100.00
Teknar A	1.1 b	1	48	0	100.00		
Teknar A	1.1 b	2	48	0	100.00		
VectoBac B	0.29 a	1				0	100.00
VectoBac B	0.29 a	2				0	100.00
VectoBac B	0.58 b	1	48	0	100.00	0	100.00
VectoBac B	0.58 b	2	48	0	100.00	0	100.00
VectoBac B	1.1 b	1	48	0	100.00		
VectoBac B	1.1 b	2	48	0	100.00		

[1] Product followed by different uppercase letter represents significant differences ($P = 0.05$) between products as determined by Student–Newman–Keuls multiple comparison tests.

[2] Rate followed by different lowercase letter represents significant differences ($P = 0.05$) between application rates as determined by Student–Newman–Keuls multiple comparison tests.

particularly for Teknar HP-D, and between years (2002 and 2003). In this study, VectoBac 12AS was more effective than Teknar HP-D at the 3 application rates evaluated both years.

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