COMPARISON OF THREE *BACILLUS THURINGIENSIS* SEROTYPE H-14 FORMULATIONS AGAINST *PSOROPHORA COLUMBIAE*[1]

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ABSTRACT. The dispersibility of three liquid formulations of *Bacillus thuringiensis* serotype H-14 was compared in field tests against *Psorophora columbiae* larvae. The materials (1.89 liters diluted to 20.8 liters) were dispensed at 80 ml/min into irrigation water entering rice fields. The area covered was assessed 48 hours post-treatment by larval sampling of the periphery of the treated pan and all downfield pans flooded at that time. Area affected by dispersed *B. thuringiensis* (H-14) was determined by delineation of the counts of zero to 1.0 larva/dip with many zero counts versus areas of 1.1 or more larva/dip and few samples of zero larvae. No significant differences occurred in area of dispersal or in the percentage of the total flooded area into which they were dispersed. The study also resulted in establishment of a new method of testing biological control agents applied to rice field irrigation water.

INTRODUCTION

The discovery by Goldberg and Margalit (1977) of a new strain of *Bacillus thuringiensis*, highly virulent for mosquito larvae and subsequently designated as serovar H-14 by de Barjac (1978), provided a new potential larvicidal control agent. Small plot field tests of *B. thuringiensis* serotype H-14 (hereinafter referred to as *B. t.* H-14) by Dame et al. (1981), Hembree et al. (1980), Mulla et al. (1982) and McLaughlin and Billodeaux (1983) produced excellent larval mortality and indicated the potential for use of *B. t.* H-14 as a control agent for larvae of *Psorophora columbiae* (Dyar and Knab). These and other reports have established the efficacy of *B. t.* H-14 when applied by conventional methods. McLaughlin and Vidrine (1984a) showed that a diluted suspension of a flowable concentrate formulation of *B. t.* H-14 could be dispensed into the irrigation water of rice fields for several hours and would reduce larval populations to 0.1/dip or less. An 86% larval reduction was achieved from treatment of 700 ha during an operational-scale test (McLaughlin and Vidrine 1984b).

Although initial development of the point-source application method used Teknar[4,5], (the only flowable concentrate formulation on the market at the time), other formulations are now available. Formulation technology is rapidly expanding, with impetus from at least three commercial firms. There will undoubtedly be improvements upon some of these, and perhaps altogether new formulations, in the near future. The tests reported were designed to provide comparative data on the dispersibility of the three formulations available in 1983.

MATERIALS AND METHODS

TEST DESIGN. The test was conducted as a randomized complete block consisting of 3 commercial formulations each replicated 3 times. The three flowable concentrate formulations were Teknar, Bactimos[4,6] and Vectobac[4,7]. Although these formulations have label statements indicating different IU/mg potency, Dame et al. (1981) pointed out that equivalent IU/mg potencies do not necessarily translate into comparable doses or rates of application between formulations in field tests. McLaughlin and Vidrine (1984c) found that 4 pints (1.89 liters) of Teknar diluted to 44 pints (20.8 liters) was optimal. The optimal rate of introduction was found to be 80 ml/min. The placement of the constant flow rate dispensers is shown in Fig. 1. The dispensers were 5-gal. plastic containers fitted at the pour-spout with an external device that permitted the formulation to flow by gravity through a hole in a plastic cap. A constant flow rate is achieved by formation of a liquid column of a constant height in a vertical tube, open to the atmosphere and external to the large container. (See McLaughlin (1983) for details.)

Fields were selected by assessing their potential for a large *Ps. columbiae* population from pretreatment sampling. Larvae were sampled with a 400 ml dipper. Fields with 1.5 larva/dip

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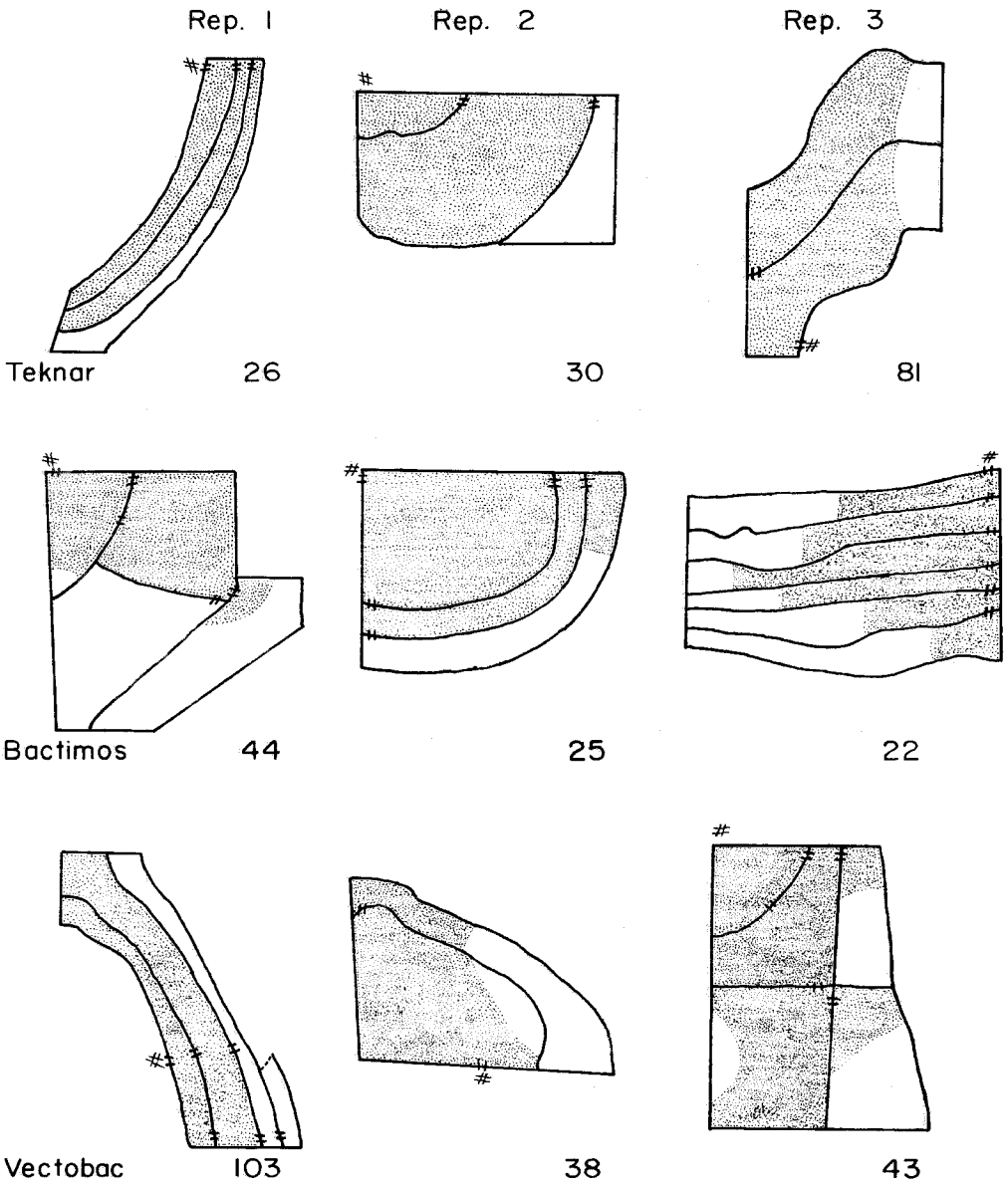

Fig. 1. Maps of areas flooded at 48 hr sampling in fields that were treated with three formulations of *B. t.* H-14. Three replicates of each formulation are designated with each field identified by an Arabic number. The stippled area represents the area affected by the treatments. The maps are not all to the same scale. The number symbol (#) indicates the point of introduction of the treatment, while the levees are represented by linear or curvilinear lines. The (//) marks intersecting the lines represent the overflows through which water was observed moving from one pan to another.

(determined by pretreatment counts of second instar larvae by a sequential counting plan to determine the mean and CV of the mean, as developed by McLaughlin et al., unpublished data) were used in the test.

Application of the formulation commenced as soon as the pan(s) was sampled and pretreatment counts determined to be suitable for testing. Thus, water always covered nearly all of one pan and occasionally parts of several pans; larvae were in the second or early third instar. Testing started on August 2 and ended on September 3, 1983.

TEST SITE DESCRIPTION. The discrete treatment area, referred to as a "pan," was the area between the earthen levees subdividing each field along similar elevation contours to ensure proper water management. Flooding occurs from one source or several, depending upon the type of irrigation system available, field size and topography. Successive flooding from the upper to the lower end of the field is achieved by allowing the upper pan to fill to a desired depth prior to release of the water to the next pan. A pan is usually flooded in less than 1 day; usually, no more than three pans flood per day. Pans vary from less than 1 ha to ca. 10 ha. Fields in Jefferson Davis Parish in southwest Louisiana were used in these tests.

DATA COLLECTION AND ANALYSIS. Maps of each field were prepared to a scale of 1:20,000 from aerial photographs obtained from the USDA Soil Conservation Service. These maps contained levee contours. Area measurements were obtained by Soil Conservation Service scale overlays prepared for the aerial photographs.

Dispersion of each formulation was assessed by sampling for larval density with the standard 400 ml dipper. The sampling method consisted of counting the larvae in two dippers of water per sample at 35-step intervals while walking around the total periphery of the pan within 1 to 5 m of the levee. Pan size determined the total samples, but ranged from 20 to 142 per pan. Sampling was repeated 48 hr after the start of the test; each count was placed in its relative position on the map of the pan. Extent of dispersal was delineated on the maps for each pan downfield from the treatment site (see Fig. 1). Delineation of the dispersibility (extent of carry) was usually dramatically evidenced by large changes in larval counts, usually within a few samples of each other. Accordingly, we established that numerous contiguous counts of fewer than one larva/dip were consistent with area affected by *B. thuringiensis* H-14. Areas affected by *B. thuringiensis* H-14 routinely showed counts of less than one larva/dip, even though some areas, usually small blind spots in water flow, did have high counts. Affected areas were defined as having fewer than 1.0 larva/dip, except for one field, where 1.1 larvae/dip was considered as also affected.

Counts were taken in the flooded area at the 48-hr sampling. The mean larval count/dip of the areas affected by treatment and the areas not affected by treatment were calculated for each replicate of each formulation. Ratios of the area affected by treatment to the total area flooded at 48 hours were calculated. Analyses of variance and the Waller-Duncan k-ratio test for least significant differences were performed. Transformation of the data by arc-sine (for the percentage of area treated), square root, logarithmic and ranked methods did not alter the outcome in any instance.

RESULTS

The average larval counts are presented in Table 1. Analysis of variance (Table 2) of the pretreatment larval counts showed significant differences. The difference occurred between replicates ($p > F = 0.0079$), but not between formulations ($p > F = 0.2252$). A *t*-test for least significant differences (Table 1) confirmed that no differences occurred between formulations. The remaining analyses showed no significant differences between replicates; therefore, replicates were added into the error term, increasing the precision of the analyses. Delineation of the distribution area of *B. thuringiensis* (H-14) (as defined by having fewer than 1.1 larvae/dip) was found to be accurate by occurrence of nonsignificant differences between the mean post-treatment larval counts (Table 2). Thus, formulations can be compared on the basis of the area of distribution and the percentage of the total flooded area into which *B. thuringiensis* (H-14) was carried.

The area flooded and available for *B. thuringiensis* H-14 dispersion and the area affected by *B. thuringiensis* H-14 are presented in Fig. 1 and in Table 3 for the 48-hr post-treatment data. Figure 1 illustrates the varied levee configurations that occurred in the test. These various shaped pans provided a range of hydrologically different conditions affecting the dispersibility of the formulations. Each formulation was exposed through replication to similar conditions, including long, narrow pans and large, broadly rectangular or triangular pans (Fig. 1).

The area flooded at 48 hr was not significantly different (Table 2). Therefore, each formulation had similar areas available for dispersion. There were no differences between the areas covered by each formulation. Similarly,

Table 1. The average larvae/dip (number of larvae/number of dips) in fields treated with three flowable concentrate formulations of *B. thuringiensis* H-14.

Field no.	Pretreatments counts	48 hr post-treatment counts: Unaffected area	48 hr post-treatment counts: Affected area
	TEKNAR		
26	6.0 (360/60)	4.7 (178/38)	0.6 (85/138)
30	3.8 (275/152)	2.9 (154/54)	1.1 (144/130)
81	2.2 (112/50)	3.1 (52/17)	0.7 (29/40)
$\bar{x} \pm SD$	4.0 ± 1.9	3.5 ± 1.0	0.7 ± 0.3
	BACTIMOS		
44	6.7 (266/40)	2.4 (196/80)	0.4 (65/158)
25	4.0 (175/44)	2.2 (175/44)	0.7 (54/78)
22	2.9 (297/102)	6.1 (635/104)	0.6 (64/110)
$\bar{x} \pm SD$	4.5 ± 2.0	3.6 ± 2.2	0.6 ± 0.2
	VECTOBAC		
103	6.6 (329/50)	1.7 (89/52)	0.7 (189/284)
38	1.2 (94/76)	1.0 (88/90)	0.1 (11/80)
43	1.4 (153/110)	2.4 (114/48)	0.3 (55/196)
$\bar{x} \pm SD$	3.1 ± 3.1	1.7 ± 0.7	0.4 ± 0.3
lsd**	1.96	2.45	0.57

** Least significant differences (= 0.05) by the Waller-Duncan K-Ratio T-test (1sd).

there were no differences in the percentage of the flooded area into which the formulations were dispersed. We therefore conclude that these three formulations had similar performance.

DISCUSSION

Evaluation of biological larvicides distributed in irrigation water requires a different approach than when a broadcast application is made. Aerial spraying assumes a known distribution area and assessment is then made of the reduction of larval density within that area. Such tests usually are designed to determine the optimum rate of larvicide to be applied per area covered. Distribution of *B. thuringiensis* (H-14) by point-source introduction requires different assumptions and a different system for evaluation. The area covered varies due to hydrological conditions of flooding. The literature is replete with reports establishing its efficacy when adequate amounts are applied by conventional means. The development of the point-source method and demonstration of its efficacy has been documented by the authors and referred to earlier in this paper. The optimum amount of one liquid formulation (TEKNAR) and its rate of addition to the water has been determined by McLaughlin and Vidrine (1984c). Therefore, the test assumes that the active ingredient, *(B. thuringiensis* H-14), is effective and is not the subject of the test. The question to be resolved was whether the 3 formulations would be dispersed equally well when used at the same rate.

Table 2. Results of analyses of variance for comparison of three formulations of *B. thuringiensis* (H-14).

Data	Statistic: F	p > F	C.V.
Pretreatment counts	11.38	0.0185	22.85
Post-treatment counts, Unaffected area	1.05	0.4050	58.6
Post-treatment counts, Affected area	1.03	0.4134	52.7
Area flooded at 48 hr	1.36	0.3248	47.7
Area treated	1.57	0.2827	42.5
Percentage total flooded Area treated*	2.27	0.1850	13.4

* Arc-sine transformation of percentages.

The method of assessment must therefore be based upon definition of an area of effective coverage. The previous research by the authors established that larval density could be reduced to less than 1.0 larva per dip regardless of how high the initial count was, and most of the area would have no detectable larvae. Thus, a basis existed for defining the area of effective distribution of the formulated agent. The area was defined by visual examination of maps of the fields with the larval counts placed in their relative position in the pans. The results of these tests substantiated this method for assessment. Statistical analysis of the counts within the area defined as effectively treated

Table 3. Area flooded, area treated and percentage of treated area to available flooded area at 48 hours post-introduction of three flowable concentrate formulations of *B. thuringiensis* H-14.

Formulation	Field no.	Area-		
		Flooded	Treated	% treated
Teknar	26	6.2	4.8	77.0
	30	11.1	7.8	70.0
	81	6.6	5.7	87.0
$\bar{x} \pm SD$		8.0 ± 2.7	6.1 ± 1.5	78.5 ± 14.0
Bactimos	44	20.7	9.4	46.0
	25	4.3	3.3	77.0
	22	11.3	5.9	52.0
$\bar{x} \pm SD$		12.1 ± 8.2	6.2 ± 3.1	59.3 ± 21.0
Vectobac	103	15.4	9.8	64.0
	38	11.1	6.0	54.0
	43	20.3	14.6	72.0
$\bar{x} \pm SD$		15.6 ± 4.6	10.1 ± 4.3	63.6 ± 11.6

showed no difference existed in the mean count and the data were homogeneous.

No firm correlation exists for relating adult population levels in an area to larval reduction. This situation exists for other species. For instance, a standard control practice for *Culex quinquefasciatus* Say is to apply larvicides to breeding sites. No correlation is available between larval control and adult reduction because of those efforts, yet the drastic reduction of larval density is accepted as worthy of the effort. The same assumptions can be considered valid for *Ps. columbiae*.

The assessment of formulations for use by this system therefore depends upon the area effectively treated, which is assumed to be the area into which toxic concentrations or amounts of the active ingredient in the formulation were dispersed and remained available for ingestion by larvae within the time period chosen for assessment. We could not detect any differences in area of dispersal by any of the 3 formulations and under the conditions of this method of application all performed within the same range. New strains of *B. thuringiensis* (H-14) with substantially different potencies or even the use of new pathogens would change the basic assumptions and require evaluation to determine effective rates. New formulations with greatly different characteristics can be evaluated by this method because the formulations are what is different, not the agent.

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