

DISTRIBUTION OF A FLOWABLE CONCENTRATE FORMULATION OF *BACILLUS THURINGIENSIS* SEROTYPE H-14 DURING IRRIGATION OF RICE FIELDS AS A FUNCTION OF THE QUANTITY OF FORMULATION¹

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ABSTRACT. *Bacillus thuringiensis* serotype H-14 can be added to irrigation water for control of *Psorophora columbiana* in rice fields. The optimum quantity of a flowable concentrate formulation to achieve the most efficient usage was determined. Tests of three quantities (0.68, 1.89 and 5.68 liters diluted to 20.8 liters with water and dispensed at 80 ml/min into irrigation water) were replicated five times. The optimum amount was 1.89 liters with an average coverage of 5.34 ha. or 0.35 liters/ha. Use of the smaller amount (0.68 liters) resulted in a reduced coverage of available flooded area. The largest amount (5.68 liters) resulted in decreased efficiency and did not disperse over a larger area than did the 1.89 liter amount. The treatment of a field with the optimum amount of 1.89 liters at each introduction point for maximum coverage would require dispensing at successive but not necessarily adjacent levee overflows as flooding progresses downfield.

INTRODUCTION

The discovery by Goldberg and Margalit (1977) of a highly lethal *Bacillus thuringiensis* isolate specific for larvae of nematocerous Diptera and its subsequent designation as serotype H-14 by de Barjac (1978) were followed by rapid commercial development. Flowable concentrate formulations of *B. thuringiensis* H-14 (hereinafter referred to as *B. t.* H-14) were successfully introduced into rapidly flowing rivers and streams for black fly control (Gaugler and Finney 1982). Broadcast aerial applications of *B. t.* H-14 to flooded rice fields for *Psorophora columbiana* (Dyar and Knab) larval control have been effective (Hembree et al. 1980, Mulla et al. 1982, McLaughlin and Billodeaux 1983). Aerial application of high volumes to large areas of rice has been cost-prohibitive and logistically difficult for mosquito abatement districts. A new methodology was developed utilizing flowing water to distribute the *B. t.* H-14 during irrigation of the rice field (McLaughlin and Vidrine 1984a, 1984b). The formulation is diluted to a larger volume and introduced into the water inlet for several hours. The purpose of this paper is to present the results of a test designed to determine which of three amounts

of a flowable concentrate formulation would be optimal for use in this system.

MATERIALS AND METHODS

TEST DESIGN. The test was conducted as a randomized complete block, with three treatments consisting of the amount of formulation (Teknar[®] 04.5) and with each treatment replicated five times. The amounts were 0.68, 1.89 and 5.68 liters, and each was diluted to a final volume of 20.8 liters with water from the irrigation inlet of the field to be treated. The diluted formulation was dispensed via a constant flow device (McLaughlin 1983) fitted with an adjustable orifice. The mixtures were contained in the plastic containers used to ship Teknar and dispensed at 80 ml/min (66–100 ml/min) with an average dispensing time of 4.5 hr. Treatment commenced when the first pan to be treated was at least one-third covered with water or overflowing at the outlet (= inlet to the next pan). Each replicate consisted of a portion of a separate field being flooded on a different day. Assignment of replicates to fields was made by random drawing of lots prior to starting the test. Testing started on March 22 and ended on May 15, 1982.

TEST SITE DESCRIPTION. Applications of *B. t.* H-14 were made to discrete portions within fields. The discrete treatment area is referred to as a "pan." Farmers subdivide a field by construction of earthen levees. These are placed so that, ideally, each pan covers an area lower than the previous pan, and within-pan elevation does not vary more than 15 cm. This land leveling and terracing procedure ensures that each pan floods rather evenly, and the final water depth is controllable within the limits desired for rice production. 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

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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. The water flows from one pan into the next across plastic-covered overflows, through pipes with adjustable gates, or even through shovel cuts in the levees. A pan is usually flooded in less than one day, but usually no more than three pans flood per day. The pans vary in size from less than 1.0 to ca. 15 ha. The fields were located in Jefferson Davis Parish in southwest Louisiana. Rice had been planted, and reflooding was conducted to provide a temporary watering of the barely emergent rice.

DATA COLLECTION. Maps of each field were prepared to a scale of 1.0 cm = 50.0 m from aerial photographs obtained from the USDA Soil Conservation Service. All field topographic features, such as levees, water entrances, overflows or cuts in levees for flooding of successive pans, and area, were included. The area was measured by use of dot chart overlays with a rated accuracy of ± 5 to 12% depending on the area measured. The most common error range for the size of the pans measured was ca. 8%.

Dispersion of *B. t. H-14* was determined by detection of toxic concentrations in water samples. Water samples were collected in sealable plastic bags and labeled to permit plotting of the location on the maps. Samples were collected at 25 or 50 m intervals along transects radiating from the water inlet to the opposite levee and along the entire periphery of each pan at its bounding levees. Samples were collected when the formulation had been completely dispensed, and again at 24 and 48 hr thereafter. The water samples were assayed by exposing 10 or 20 laboratory reared 2nd instar *Aedes aegypti* (Linn.) larvae to three 100 ml subsamples of each sample for 24 hr. No food was added. Appropriate untreated samples and untreated laboratory water accompanied each assay; no mortality occurred among control larvae. Mortalities of less than 5% from the three treated subsamples were not considered as toxic water samples in order to be conservative regarding the area covered. The mortality within each sample was recorded and plotted on the scaled maps of the pans, and then the area in which toxic amounts of *B. t. H-14* were detected was calculated by connecting a line from the locations of the farthest downfield toxic samples and the use of dot chart overlays. The maximum distance of dispersion of *B. t. H-14* was measured on the maps by linear scale from the source to the farthest toxic sample, using a curved scale as necessary and following the levees from the inlet source through cuts in levees and downfield to the farthest toxic sample. Data were evaluated from each time pe-

riod, and a final composite was prepared for each test.

RESULTS

At the start of the test, water inlet flow rates at the point of introduction of *B. t. H-14* averaged 2,937 liters/min (range 1,383–8,198, CV = 68%); at the end of the introduction the average flow rate was 3,255 liters/min (range 1,314 – 8,517 liters/min, CV = 70%). The estimated concentration of Teknar in ppm at the water inlet during dispensing of the formulations was 1.0 ppm (0.68 liter quantity), 3.2 ppm (1.89 liters quantity) and 11.0 ppm (5.68 liters quantity). Water sample mortality data on the untreated internal checks at 24 hr had zero mortality in all cases. No mortality occurred in any water samples collected at 48 hr post-treatment. Mortality ranged from 0 to 100% in water samples taken at the end of introduction and at 24 hr.

The area covered by water in each test at 24 hr is shown in Fig. 1. This represents the maximum coverage possible by the treatment applied to any one field. Figure 2 shows the area covered by *B. t. H-14* as drawn from the composite of the water sample mortality data taken at the end of the introduction period and at 24 hr. The variety of levee configurations encountered in the tests are seen in the figures. Hydrological phenomena affecting water movement during flooding generally prevent 100% coverage of a pan (McLaughlin and Vidrine, unpublished data). The hectares covered by *B. t. H-14* are indicated in Fig. 2 as Arabic numerals on each map. A comparison of Figures 1 and 2 provides a visual portrayal of the efficiency of each test in distribution of *B. t. H-14*. All the maps are not of the same scale, so comparisons can be made between pans in a field but not between different fields.

Table 1 presents the mean hectare coverage for each of the quantities. The means were significantly different at the 0.01 level ($F_{.01 df 2, 12} = 6.93$; $F_{calc} = 8.3$). Duncan's new multiple range test showed that the 0.68 liter quantity covered significantly ($\alpha = 0.05$) less area than the two higher amounts. The two higher amounts did not differ from each other. The greatest area covered (13.7 ha) occurred with 1.89 liters in Rep. IV (see Fig. 2).

The mean percentage coverage of available area is also shown in Table 1. The means were significantly different ($F_{calc} = 34.4$). The 0.68 liter quantity covered the least percentage of the area, and the two highest amounts were not different from each other; ($\alpha = 0.05$, Duncan's new multiple range test). Expression of the ratio between the quantity of Teknar

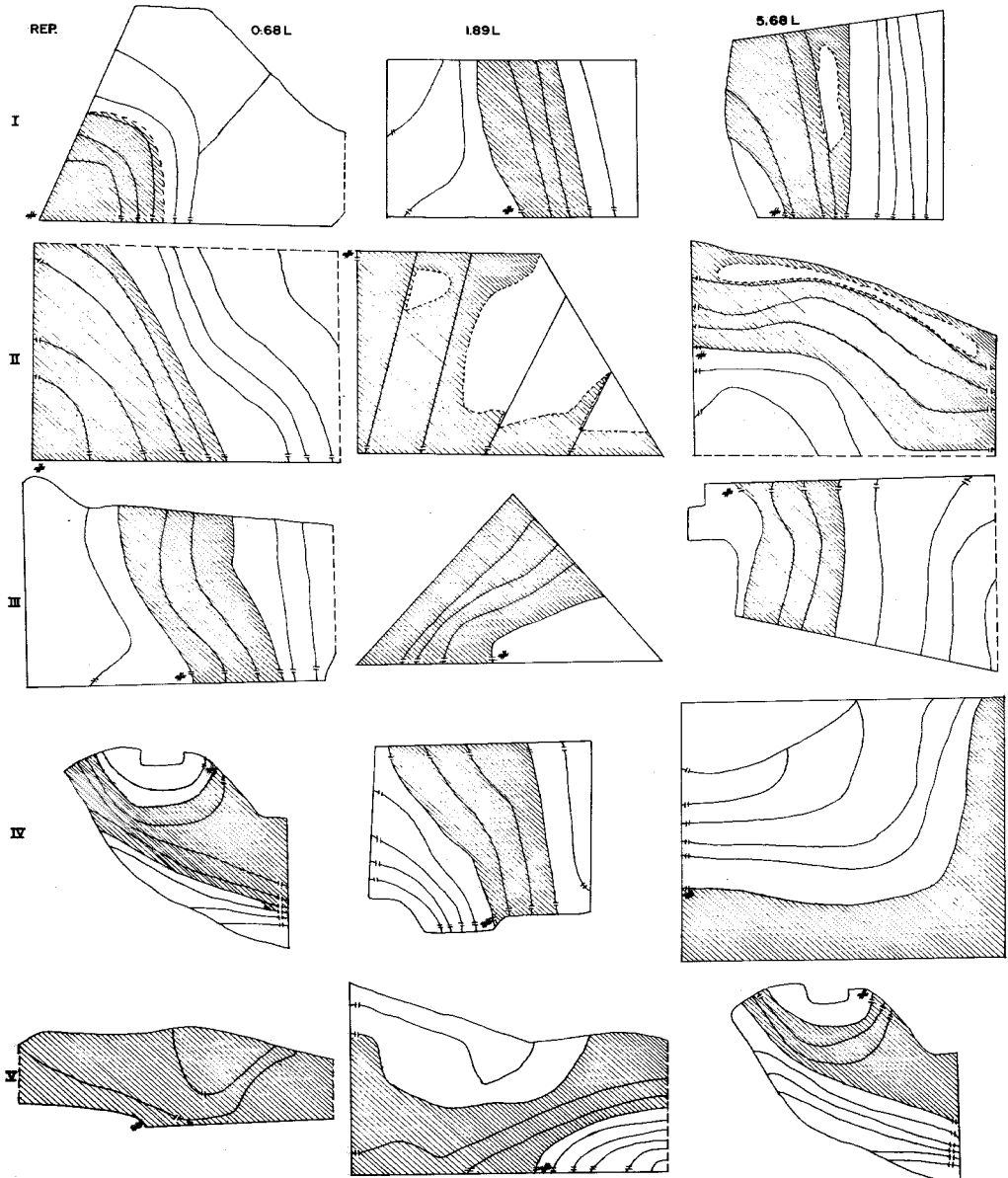


Fig. 1. Maps of fields that were used to evaluate 3 amounts of flowable concentrate *Bacillus thuringiensis* H-14. Area covered by slashed bars represent area flooded at 24 hours after introduction of *B. t.* H-14. Replicates are represented by Roman numerals. Linear and curvilinear lines represent the earthen levees that separate pans, while the slashes in the lines (//) represent the overflows. Maps are not drawn to the same scale.

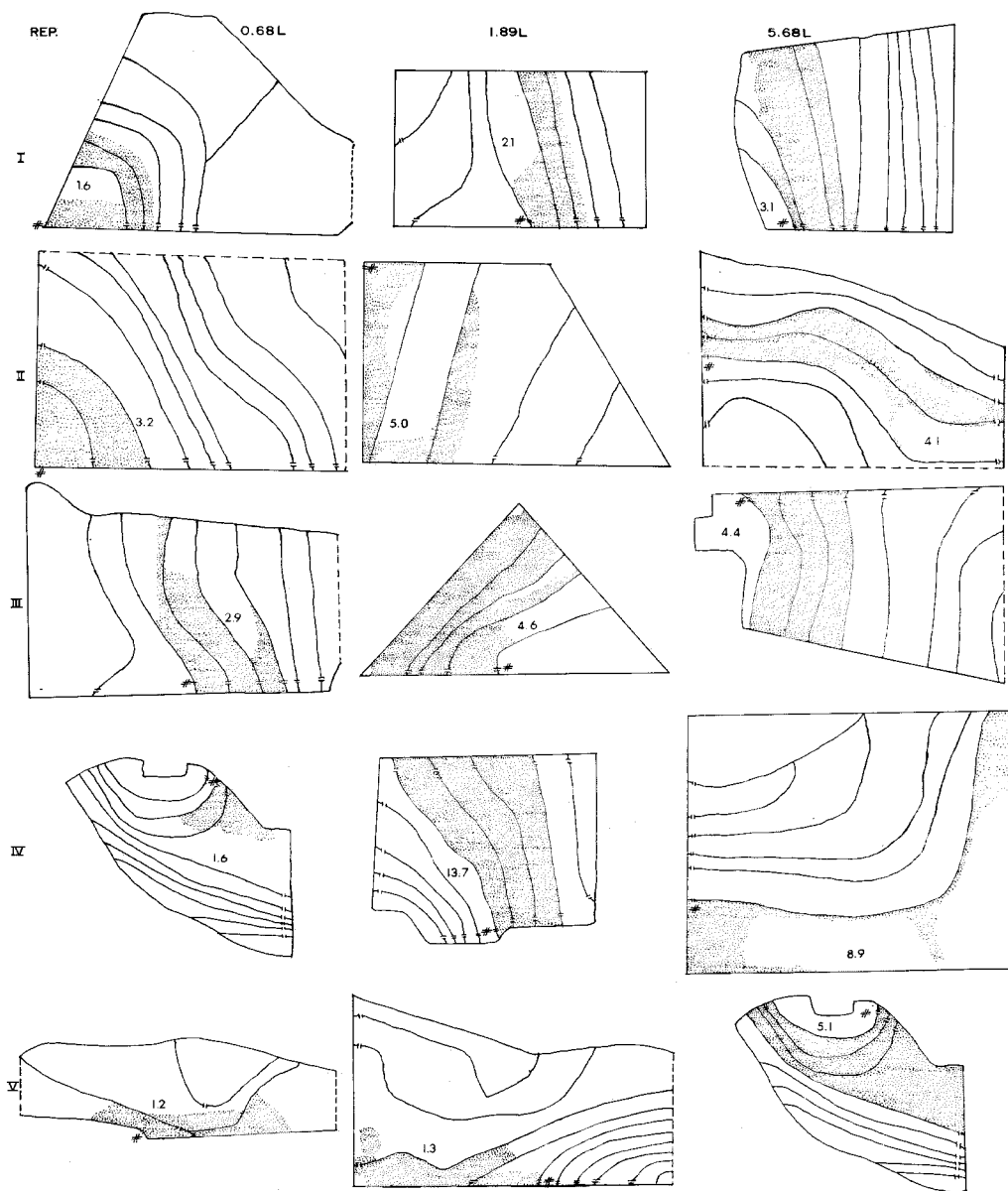


Fig. 2. Maps of fields that were used to evaluate three amounts of flowable concentrate *Bacillus thuringiensis* H-14. Stippled areas represent the area coverage by *B. t.* H-14 at 24 hr. as determined by larval mortality in the water sample assays. Replicates are represented by Roman numerals, while area covered is represented by Arabic numerals in each map. The typographic "number" sign (#) represents the placement of the container from which *B. t.* H-14 was dispensed to the field. Linear and curvilinear lines represent the earthen levees that separate pans, while the slashes in the lines (//) represent the overflows. Maps are not drawn to the same scale.

Table 1. Results of dispensing 3 amounts of a flowable concentrate formulation of *Bacillus thuringiensis* (H-14) into irrigation water during flooding of rice fields.*

Averages	Rates		
	0.68 liter	1.89 liter	5.68
Area covered (ha)	2.09 ^a	5.34 ^b	5.10 ^b
Maximum distance (m)	347 ^a	582 ^a	741 ^a
Mean percent covered	34 ^a	61 ^b	63 ^b
Overall treatment rate (liter/ha)	0.33	0.35	1.11

* Means with the same letters indicate non-significant differences at $\alpha = 0.05$ from Duncan's New Multiple Range Test.

applied and the final area covered as the mean treatment rate (liter/ha) in Table 1 provides another way of assessing the efficiency of the three quantities. The 5.68 liter amount was much less efficient than the other two amounts.

The maximum distance of transport of *B. t.* H-14 is shown in Table 1. The mean distances of the three quantities were not significantly different at the $\alpha = 0.05$ level of probability. ($F_{\text{calc}} = 0.8$). However, the 0.68 liter amount was never found to be transported to the farthest edge of the flood water, while both of the higher levels were found at the farthest edge of the water (Fig. 2, 1.68 liter in Rep. IV, 5.68 liter in Reps. III, V).

DISCUSSION

The results as expressed in Table 1 clearly suggest that the intermediate level (1.89 liters) is the optimum of the three tested quantities. This quantity outperformed the lowest level in hectares treated and mean percentage of available flooded area covered, and performed equally as well as the higher level in these tests. The intermediate level performed at a mean treatment rate (liters/ha) equal to that of the lower rate and much less than the higher level. However, the highest level tended to be more consistent as reflected in the smaller standard deviation as compared to the intermediate level (Table 1).

The maximum distance of transport of a toxic concentration was apparently not a valid criterion for evaluation of the performance of the rates in this study. The configuration of pans, existence of machinery ruts intentionally created to aid in water dispersion, and other variables greatly influence the pattern of flooding within a pan. The rate of water flow into a field, the size of pans, and levee overflow maintenance for depth management by the farmer greatly influence the flooding of a field. Extreme examples of these phenomena are obvious in Fig. 1 (see replicate II in 1.89 rate and

replicate IV in 5.68 rate). A fast flowing stream of water coursing down a rut the length of a pan or across a pan will carry toxic material for a long distance, but it will do little to adequately distribute the toxin over the adjacent flooding areas. Thus the formulation may be carried a long distance, but coverage of the pan may be limited. Our data show no difference in maximum distance carried, although the area covered is significantly different.

We concluded that the most efficient rate to use in this system of application to a rice field is 1.89 liters diluted to 20.8 liters and dispensed at a rate of 80 ml/min. The use of these results to develop practical control schemes must consider that these tests were designed to separate differences between these amounts of formulation, therefore resulting in less than maximum coverage of the flooded areas. Also, samples were taken at specific intervals of time, which means that the data represent a slice in time of a continuum of events. These tests were conducted to assist in establishing a rate for large scale treatment of entire rice fields against *Ps. columbae*. Maximum coverage of the field would require introduction of the toxic formulation at succeeding, but not necessarily adjacent, levees at approximately 24 hr intervals.

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18°–22°C, the threshold for transmission of *L. giganteum* (Jaronski and Axtell 1983).

Water samples drawn from the site on different days possessed variable amounts of infective material (Table 2). The samples from days 49, 52, 61 and 78 were notable because mosquito larvae were rare in the site when the water samples were collected.

Table 2. Rates of infection by *Lagenidium giganteum* (NC isolate) of *Culex quinquefasciatus* larvae exposed to water samples from the McAlpine Greenway site at intervals after fungus introduction, June 8, 1982.

No. days after introduction of fungus	% infection*
49	58
52	90
61	95, 30
66	14, 0
78	75, 25

* Twenty-five second-instar larvae placed in 2 liter water sample. Where two percentages are given, each value represents a separate sample.

In the bioassay of the fungus inoculum used in this trial there was 93% infection of larvae in deionized water, 92% in site water and no infection in the corresponding controls without fungus.

NEW HOPE RIVER SITE. Three days after *L.*

giganteum (LA isolate) was introduced into the site, 17% of collected larvae and 55% of sentinel larvae were infected (Fig. 2). Laboratory bioassay of the inoculum gave 82% infection at the field rate and 47% at ¼ the field rate with no infection among controls without the fungus. These infection rates were typical of laboratory bioassays. In subsequent days, prevalence and incidence levels remained high despite very low numbers of naturally present larvae. By day 28 the site had dried and larvae were absent. The prevalence and incidence levels also dropped. On day 37 rains flooded the site and caused a brood of *Ae. vexans* to hatch; 70% of this brood was infected by the fungus. During the next 11 days infection levels among sentinel larvae remained high even though larvae were rare or absent from the site. By day 57 of the trial the site dried out and remained dry for the next 50 days. In October the site was again flooded but no fungus was observed in either sentinel larvae or the larvae naturally present. Water temperature in the site had fallen to 16°C, which was below the threshold necessary for transmission of *Lagenidium*.

Culex quinquefasciatus larvae added to water samples from the site on 6 different days during the trial became infected after exposed for 48 hr (Table 3). The infection rate was 36% at 56 days after introduction of the fungus. The exceptionally high infection levels for larvae exposed to these samples indicated that large numbers of infective zoospores were present,

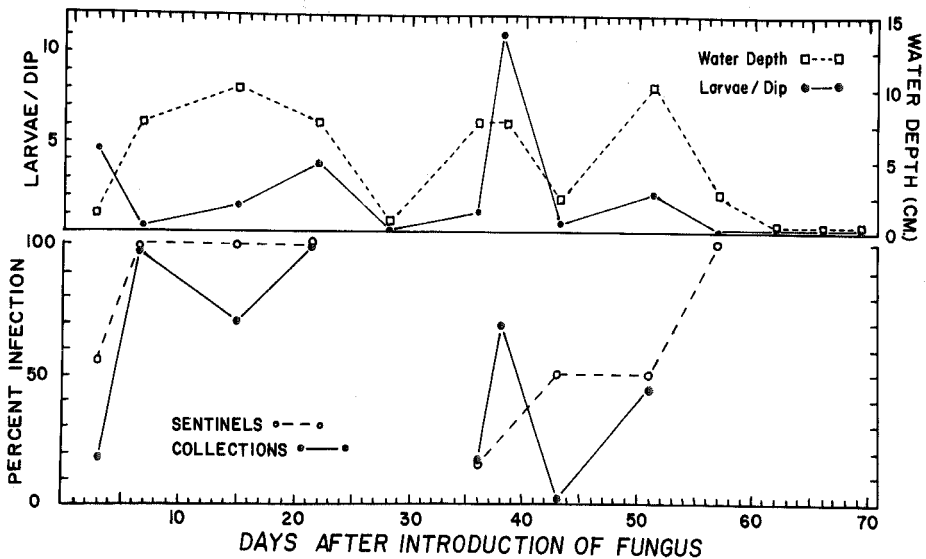


Fig. 2. Results of a field introduction of *Lagenidium giganteum* (LA isolate) into a 2.6 m² flooded depression in the New Hope River drainage area, Durham Co., NC. Upper graphs: Water depths and larval numbers during the trial. Lower graphs: Infection rates among sentinel larvae and larvae collected from the site.