# DISTRIBUTION OF *BACILLUS THURINGIENSIS* (H-14) BY WATER DURING FLOODING OF RICE FIELDS

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ABSTRACT. Application of *Bacillus thuringiensis* (H-14) to rice fields by aerial broadcast spraying of entire fields is not economically feasible for most mosquito abatement districts. This paper presents the results of tests based upon the hypothesis that water movement during flooding of rice fields would distribute *B. thuringiensis* (H-14) from a point-source or from a narrow band applied at the water entrance to a much larger area. Progressive development of the concept employed decreasing the amount and increasing the time of introduction at the point of water entry. The series of experiments demonstrated distribution of toxic concentrations throughout the rice pan and into pans downfield. Application by airplane along a single flight path over the point of water entrance to the field also resulted in distribution of toxic amounts of *B. thuringiensis* (H-14) over an expanded area.

## INTRODUCTION

*Psorophora columbiae* (Dyar and Knab), a major pest mosquito produced from rice fields in the Gulf Coast states, is presently controlled by adulticidal spraying. Cost-effective larval control methods are not available. Use of pesticides such as organophosphates or other chemical pesticides on rice fields is prohibited by environmental or economic constraints. The development of *Bacillus thuringiensis* (H-14) (hereinafter referred to as *B.t.* H-14) as a mosquito larvicide has provided a potential alternative. This agent was effective against larval *Ps. columbiae* in small field tests (Hembree et al. 1980, Mulla et al. 1982, McLaughlin and Billodeaux 1983). The presently recommended volume of diluted formulations of *B.t.* H-14 ranges from ca.19 to 93 liters/ha. The acreage of rice that must be treated to effectively prevent adult infestation is not determined. However, the acreage must be quite large. Aerial application of such volumes to several thousand acres is too costly. Another factor prohibiting broadcast treatment of entire fields is the spatiotemporal diversity of flooding of portions of rice fields requiring larvicidal treatment. Flooding of a field often requires from 4 to 7 days, and *Ps. columbiae* can develop from egg to pupal stage in 4–5 days. Multiple control actions may be needed for a field. Because farmers flood independently, further diversity occurs in the location and timing of required applications of control agents. These factors combine to prevent widespread adoption of *B.t.* H-14 as a larvicide for *Ps. columbiae* in rice fields.

An alternative method of distribution of *B.t.* H-14 was hypothesized. Introduction of *B.t.* H-14 during flooding might distribute toxic concentrations throughout a large portion of a pan, and perhaps to several pans within a field. Two methods were investigated. One method introduced *B.t.* H-14 directly into the flow of water as it entered. This method introduced the material over a time period ranging from minutes to several hours. The amount of *B.t.* H-14 dispensed in relation to the potential area to be covered was reduced as testing progressed. The second method applied *B.t.* H-14 by airplane along 1 or 2 flight paths over the edge of the field containing the point of water entry. The concentration of *B.t.* H-14 introduced over the flight path area was expected to be diluted as the water displacement occurred. Therefore, an end point would be detectable by assay of the water for toxic concentrations of *B.t.* H-14. This paper presents the results of the stepwise development of these methods.

## MATERIALS AND METHODS

TREATMENT SITE. The discreet 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 whose own elevation does not vary more than ca. 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 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 out of one pan into the next across plastic covered overflows, through pipes with adjustable gates, or even by shovel cuts in the levees. A pan is usually flooded in less than 1 day, but usually no more than 3 pans flood/day. Pans vary in size from less than 1 ha to ca. 10 ha. The fields used as treatment sites in this test are depicted in Figs. 1–4. The solid curving lines show the levees bounding the cultivated areas, or pans.

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LIMITED AREA AERIAL APPLICATION. Test conditions are summarized in Table 1. A wettable powder formulation (Bactimos[3, 4], 50% a.i. with a potency of 3500 IU/mg) was applied in 1 or 2 flight paths, ca. 20 m wide, along the edge of the field where water entered. The diluted volume was 14.4 liters/ha, containing the amount of Bactimos indicated in Table 1. The Koll field received 1.13 kg/ha over the flight path (1.36 kg Bactimos over 1.2 ha). Water covered ca. 8.0 ha over 6 to 8 pans at time of application. The Frey field received 1.0 kg/ha over the flight path (2.4 kg over 2.4 ha). The Frey field had two water sources, flooding the field from opposite ends towards the center. Thus, twice the recommended high rate of 0.5 kg/ha was applied over the flight path area. Application occurred 29 hours after flooding had started, and water covered almost all of the first pan.

POINT-SOURCE INTRODUCTION. A flowable concentrate (liquid) of *B.t.* H-14 (TEKNAR[3, 5], 0.8% a.i., 1500 IU/mg potency) was used. Three tests were conducted. The first two were on farms of Koll and Frey (designated as Frey-1). TEKNAR was diluted to 30% of its initial concentration in the first two tests. The first test (Koll, pan 12) dispensed 9.46 liters of TEKNAR in 54 min (175 ml TEKNAR/min). This is an unrealistic rate in relation to label recommendations but was used to initially determine whether distribution would occur. Water entered the pan 30 min before starting the test. The second test (Frey 1) introduced 6.62 liters of TEKNAR during 215 min (31 ml TEKNAR/min) to determine whether a reduced rate of introduction during a longer time period would result in distribution. Water had covered ca 3.6 ha of the 4.5 ha in the first pan at the start of the test. These two tests introduced *B.t.* H-14 at only one location in a field and were evaluated for the acreage of detectable toxic concentrations.

A final test over a large acreage was conducted to determine whether moving the point of introduction to successive levees downfield as the field flooded would also result in the potential for adequate treatment of an entire field. This test was conducted on the Strohe farm in a 35.5 ha field divided into 12 pans. Each pan was ca 400 m long and 75 m wide with water overflows at the levee along the end of the field where the underground water supply pipe entered the first pan. The diluted formulation was dispensed from a 95 liter metal drum fitted with a brass stopcock type valve. The flow rate was 145 ± 15 ml/min of the diluted TEKNAR suspension. The TEKNAR formulation was diluted to the final volume of 95 liters in the drum each time it was recharged. The amount of TEKNAR added to the drum is given in Table 1. The rate of introduction of TEKNAR in the Strohe test varied from 8 to 13 ml/min. Treatment of the Strohe field occurred at three locations during five days of flooding. The diluted formulation was dispensed during 66 hr of this time. The second and third locations for introduction of *B.t.* H-14 were determined by the presence of large numbers of *Ps. columbiae* larvae/dip in a pan as compared to larval counts up to that point in the field. This indicated that maximum movement of *B.t.* H-14 had probably occurred, and continued introduction at the same location might not result in further distribution of toxic concentrations of *B.t.* H-14.

EVALUATION OF THE TEST. Two methods were employed. Toxic concentrations of *B.t.* H-14 were monitored by assay of water samples. Water samples were collected in 20 or 40 ml plastic sealable vials. Collections were made every 20 m along transects radiating from the water inlet to the opposite levee and also along the entire perimeter of the bounding levees. Bioassay of the water samples was conducted in the laboratory. Five 3rd or 4th instar *Aedes aegypti* (Linn.) larvae were exposed to each water sample. Internal controls were conducted with each assay to determine larval health, susceptibility to a known $LC_{90}$ concentration of *B.t.* H-14. and for absence of toxic materials in the field water prior to addition of *B.t.* H-14. Mortality was determined 1 day after larvae were exposed to the field water samples. In all cases, the internal checks were satisfactory for acceptance of mortality in the field water samples as being caused by the applied *B.t.* H-14.

The interval between time of collection of sets of water samples was dictated by the progression of flooding of the field. The three limited area sites (Koll, Frey 2 and Frey 3) were sampled 15 to 30 min after the airplane had sprayed the material and again 24 hr after treatment. The point-source introduction tests were more intensively sampled. The first test (Koll) was sampled 30 min, 1, 2, 5 and 29 hr after ending *B.t.* H-14 introduction. Water moved rapidly down long, narrow pans in this field. The second test (Frey 1) was sampled 1, 4 and 24 hr after ending the introduction. Water samples were taken in only pan 1 at the Strohe

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Table 1. Field tests of *Bacillus thuringiensis* serotype H-14 for control of *Psorophora columbiae* larvae in reflooded rice fields in Jefferson Davis Parish, Louisiana, August 1981. (A wettable powder formulation was applied by airplane in the small area tests and a flowable concentrate formulation was used in all point-source introductions.)

| Date | Application method | Total *B.t.* H-14 applied |  | Location name | Time period of application (hr) |
|---|---|---|---|---|---|
|  |  | Formulation | I. U. |  |  |
| Aug. 6 | Small area | 1.36 kg | $3.38 \times 10^9$ | Koll, N | 2 flight paths (40 m wide) |
| Aug. 13 |  | 1.20 kg | $2.10 \times 10^9$ | Frey 2 | 1 flight path (20 m wide) |
| Aug. 13 |  | 1.20 kg | $2.10 \times 10^9$ | Frey 3 | 1 flight path (20 m wide) |
| Aug. 6 | Point-source | 9.46 liters | $1.14 \times 10^8$ | Koll, SE | 0.9 |
| Aug. 13 |  | 6.62 liters | $7.94 \times 10^7$ | Frey 1 | 3.6 |
| Aug. 24 |  | 11.36 liters | $1.36 \times 10^8$ | Strohe 1 | 23.0 |
| Aug. 25 |  | 11.36 liters | $1.36 \times 10^8$ | Strohe 1 | 17.0 |
| Aug. 26 |  | 8.04 liters | $9.65 \times 10^7$ | Strohe 5 | 12.0 |
| Aug. 27 |  | 3.79 liters | $4.55 \times 10^7$ | Strohe 5 | 5.0 |
| Aug. 29 |  | 3.79 liters | $4.55 \times 10^7$ | Strohe 10 | 9.0 |

field because of insufficient labor availability for proper bioassay of this large area. However, the necessity for bioassay was greatly diminished in this field because a very large number of larvae/dip now occurred, whereas the earlier tests were conducted in the first part of the second-crop season during traditionally lower population densities.

The second method of evaluation of the tests utilized counts of larvae collected in a dipper 9 cm wide × 5 cm deep (ca 350 ml capacity). Dipper samples were taken every 20 m along transects and levee contours in the same pattern used for water sample collections, and usually concurrently with the water sample collection. Pretreatment samples were taken in portions of the test pan already flooded as well as from pans upstream from the treatment site in the Koll and Frey fields. Larval counts were taken in these test areas 24 hr and again at 48 hr postapplication. A sample consisted of the total number of larvae in two dipper samples. Larval counts were obtained from nearby fields during conduct of the Strohe test because continuous downfield treatment precluded pretreatment counts in any area except the initial pan.

Maps were drawn to scale of each test field with the assist of aerial photographs from the County ASCS office. The location of each sample and the resultant mortality were used to draw lines of furthest transport of toxic concentrations of *B.t.* H-14 at each sampling period. This was done for each of the two methods of evaluation, and the maps were compared. The charts from samples prior to the final sample were used to verify logical progression of the wave of toxic water. The area of effective coverage was calculated by standard cartographic techniques.

## RESULTS

LIMITED AREA AIRPLANE FLIGHT PATH METHOD. Pretreatment counts averaged 1.6 third instar larvae/dip (42 larvae in 26 dips) in the Koll field. Figure 1 presents the schematic of the field and the results. Posttreatment counts averaged less than 0.1 larvae/dip (16 larvae in 176 dips) in the entire unshaded area in Fig. 1. Water samples collected 24 hr after application produced 100% mortality among larvae in samples from the first 4 pans and in portions of the 5th, 6th and 7th pans. This zone paralleled the treated edge of the field, extended about 120 m into the field and is shown in Fig. 1 as a dashed line across those levees. Water samples taken 48 hr post-application did not kill any larvae. The furthest effect observed in this test is shown by the counts of 1.1 larvae/dip (79 larvae in 73 dips) represented by the shaded area commencing in the 9th pan. The 10th and 11th pans had 122 larvae in 64 dips ($\bar{x} = 1.9$). Table 2 presents these results and a comparison of the area over which toxic concentrations of *B.t.* H-14 were moved.

Figure 2 shows the schematic results of the limited area Frey 2 and 3 tests. The area of zero larvae/dip at 24 hr postapplication sampling is again shown by the clear area. Representative counts are shown in the shaded area, indicating the limit of distribution of toxic concentrations away from the application zones. Again, the furthest point of collection of toxic water samples as shown from the laboratory assay is de-

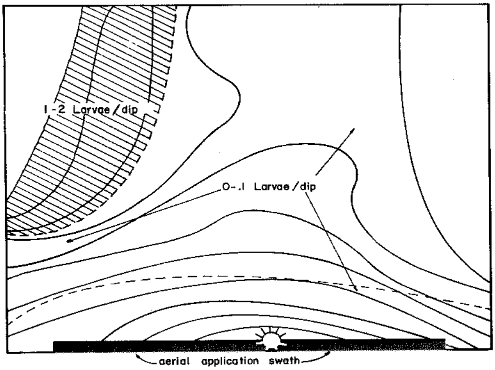

Fig. 1. Limited area application test on Koll field.

～ Levee boundaries

—/⁄— Cuts in levees for water flow

▬ Aerial flight path (Figs. 1 and 2)

☼ Water entry point to field

---- Boundary of area containing toxic water samples

✳ Point-source of *B.t.* H-14 introduction (Figs. 3 and 4)

▨ Larval averages of more than zero but less than 3 per dip

▨ Larval averages as shown in inserts, Fig. 4

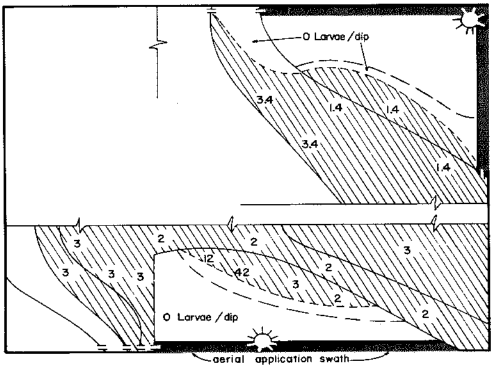

Fig. 2. Limited area application tests on Frey 2 (bottom) and Frey 3 (top). Representative mean counts of groups of dipper samples in an area are shown within the shaded area.

Table 2. Results of application of *Bacillus thuringiensis* serotype H-14 to rice fields over a limited area or by point-source for dispersal by water movement.

| Application method and date | Available area covered by water at 24 hr | Area (ha) with toxic water samples/ % of available area | Area (ha) of 0 larvae/dip/ % of available area | Pretreatment count/dip in test fields | Posttreatment count/dip in nearby fields | Posttreatment count/dip in areas >0.1/dip |
|---|---|---|---|---|---|---|
| Limited area | | | | | | |
| Koll (Aug. 6) | 12.5 | 3.6/29 | 11.0/88 | 1.6 | 4.6 | 1.1 |
| Frey 2 (Aug. 13) | 3.7 | 3.1/84 | 3.1/84 | 4.9 | 2.8* | 8.4 |
| Frey 3 (Aug. 13) | 3.6 | 3.3/92 | 3.3/92 | 1.8 | 2.8* | 1.4 |
| Point-source | | | | | | |
| Koll (Aug. 6) | 5.0 | 3.2/64 | 2.7/54 | 1.3 | 1.7 | 0.2 |
| Frey 1 (Aug. 13) | 5.0 | 4.7/94 | 4.6/92 | 0.9 | 2.8* | 2.2 |

* Average from 3 fields on 3 dates before, during and after the tests.

lineated by the dashed lines. The nearly coincident position of the effective distribution lines from the two methods of sampling is notable. Larval counts in the first pan of Frey 2 (bottom of Fig. 2) averaged 8.4/dipper in the small area near the levee 24 hr posttreatment. The area covered by water 24 hr posttreatment was calculated from the charts and is presented in Table 2.

The area in which no larvae could be found ranged from 84 to 92% of the available area in the three limited area tests. Based upon the water sample data the spread was 29–92% of the available area.

Point-source introduction. In the Koll field, no larval mortality occurred in water samples taken the previous day in two pans upstream from the point of introduction of *B.t.* H-14. Larval counts in those pans averaged 1.0 3rd instar larva/dip. Figure 3 shows the terminal five pans of the Koll field used for this test. *B.t.* H-14 was introduced at the upper end of pan 12 (bottom center of Fig. 3). Water flowed down the length of the pan, ca 400 m, and then through cuts in the levee into the other pans, but pan 13 filled from both ends. The majority of the water that flooded pan 13 entered from the overflow directly across from the introduction point after all of the *B.t.* H-14 had been introduced. This prevented effective *B.t.* H-14 distribution in pan 13. Bioassay of water samples showed toxic water present in 3.2 ha of the available 5.0 ha (see Table 2). Counts of larvae from dipper samples taken at piles of harvested rice straw within 2 hr of flooding ranged from 20 to over 100 larvae/dip. No mortality would have occurred this soon after treatment, so these counts are an indication of the potential larval population. Results of larval sampling 24 hr posttreatment showed 0 larvae/dip, represented by clear area in Fig. 3 and 0.1–0.5 larvae/dip in the shaded areas.

In the Frey 1 field, bioassay of water samples collected hourly for the first 4 hr of the test produced 100% mortality in an expanding area whose shape approximated that of the boundary levee contours. Water samples at 24 hr produced 100% mortality in an extended area near the boundary levee except for a narrow band close to one area of the levee at the furthest point from the point of introduction of *B.t.* H-14. Larval samples taken within 2 hr of flooding of any location averaged 0.9 neonate larvae/dip. Larval samples taken at 24 hr were negative except for a narrow band near a levee at the furthest point from the water source where counts averaged 2.2/dip at 24 hr. The area where toxic water samples were collected was almost coincident with that of zero larval counts. The toxic water sample area and the area of zero larvae/dip calculated to be 4.7 ha of the available 5.0 ha in this pan (see Table 2).

The results of the Strohe field test are summarized in Table 3 and Fig. 4. Larval counts taken in a nearby untreated field averaged from 11.6 to 29.9 3rd or 4th instar larvae/dip during the time of *B.t.* H-14 introduction (see Table 3). Larval counts around harvested rice straw piles in the Strohe field 1 to 2 hr after flooding consistently ranged from 20 to over 100/dip. Delineation of the areas within pans into three categories is shown in Fig. 4. Areas of zero larvae/dip are represented as clear, 1.0 to 2.9/dip are lightly shaded and counts greater than or equal to 3.0/dip are shown as heavily shaded with the average count indicated. The treatments resulted in reduction of counts below 3.0/dip in most of the field. Small pockets of larvae were found in pans 3, 5, 6, 8 and 10. The area of less than 3.0/dip for each of the three introduction points ranged from 72.3 to 96.0% (Table 3). The overall coverage was 91.0% of the area with less than 3.0/dip. The

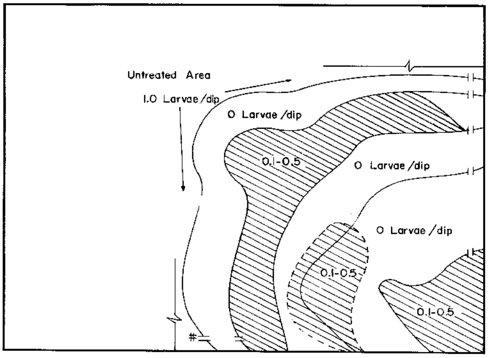

Fig. 3. Point-source introduction to the terminal 5 pans of the Koll field.

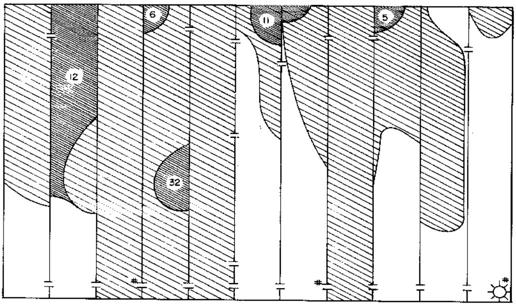

Fig. 4. Point-source introduction to the Strohe field.

Table 3. Results of application of *Bacillus thuringiensis* H-14 to Strohe rice field.

| Treated area | Area (ha) available | Area (ha) with larval counts/dip | | | % area less than 3.0/dip | Larvae/dip in untreated fields |
|---|---|---|---|---|---|---|
| | | 0.0 | >0–2.9 | ≥3.0 | | |
| Pans 1–4 | 13.0 | 7.0 | 5.3 | 0.7<(5.0/dip) | 94.6 | 29.2 |
| Pans 5–8 | 16.0 | 3.9 | 11.5 | 0.6 (29.0) | 96.0 | 19.4 |
| Pans 9–11 | 6.5 | 3.2 | 1.5 | 1.8 (11.0) | 72.3 | 11.6 |
| Field totals | 35.5 | 14.1 | 18.3 | 3.1 | 91.0 | |

area of zero larvae/dip was 39.7% of the total area.

## DISCUSSION

Successful implementation of biological control strategies must be based upon sound principles embodied in each of 3 major components—the pathogen, the host and the environment. Most biocontrol efforts have paid excellent attention to the pathogen, sufficient accord to the host for demonstration of reduction of host populations in limited field tests, but have neglected to fully exploit the remainder of the pathogen-host-environment triangle. The environment, in the context of pathogen-host interaction, includes the biophysicochemical factors permitting the necessary infection of host by the pathogen, but also those factors of a non-physicochemical and non-biological nature. The human aspect, including the social, economic and political factors that often play major roles in decisions regarding usage of the biorational agent, is a vital part of the broader concept of the environment. If the objective of use of a biological agent is to reduce pest insect populations to the non-pest status in relatively large areas and over lengthy time periods, then the end result is the same whether the agent was inactivated by a physical factor or simply was not employed adequately due to a human-related reason.

Application technology is the major roadblock in many cases. *Bacillus thuringiensis* H-14 is a case in point. Application over large areas of rice for control of *Ps. columbiae* by aerial spraying of large volumes of diluted formulation will kill nearly all larvae. However, it is not yet accepted as the method of choice and is not adopted as a standard practice. Most mosquito control districts in the rice belt are oriented to adulticidal programs. All fields do not produce larval populations large enough to be treated. The effect of a larvicidal program upon reduction of adult pest levels for the human and livestock populations is not known. Improved methods are needed for efficient detection and determination of meaningful population density levels for initiation of treatment actions. Cost-efficient technology is needed. The solution embodies most of these factors and must result in a favorable cost-benefit estimate. This is the missing aspect of the environmental side of the triangle. The information presented in this paper is one piece of information bearing on the single aspect of a more efficient application system.

This paper presents results of a series of developmental stages testing distribution of *B.t.* H-14 by water movement during flooding of rice fields. The initial test of introduction at the water entry point used a very large amount of *B.t.* H-14 and introduced it in 56 min. Successive development of the method reduced the amount and increased the time of dispensing the diluted formulation, culminating in successful coverage of a 36 ha field containing 12 separate pans. The aerial tests showed that a single flight path application along the edge of water entry to a pan will result in subsequent distribution to a much larger area.

The major impact of these results is that two methods of application of formulated *B.t.* H-14 to a small area or at a single point resulted in dispersion of lethal concentrations over a much larger area. The specific areas covered and rates used indicate potential efficacy and form the basis for further more definitive experimentation. As such, these results cannot be construed as firm evidence of the eventual effective rates. These results suggest that more efficient and less costly aerial application can be adopted for larval control of *Ps. columbiae* in rice fields, and that *B.t.* H-14 may also be effectively employed from the ground (a method not available by conventional techniques).

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