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FACTORS AFFECTING DISTRIBUTION OF *BACILLUS THURINGIENSIS* SEROTYPE H-14 DURING FLOODING OF RICE FIELDS

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Bacillus thuringiensis serotype H-14 has been recognized as a highly effective mosquito larvicide. Formulations and methods of application have been developed for use in a wide range of habitats against a variety of mosquito species (Lacey 1985). One method of application developed for use in irrigated croplands was the introduction of a diluted suspension of a flowable concentrate formulation at a slow rate over a period of several hours for control of *Psorophora columbiae* (Dyar and Knab). A constant flow rate device (McLaughlin 1983) was placed at the entry point of irrigation water into a rice field. The initial development of concept and testing of operational efficacy were reported earlier (McLaughlin and Vidrine 1984a, 1984b). The optimum amount of formulation to place in 20.8 liter (5.5 gal) containers, the rate of addition at the irrigation water inlet, and a comparison of three flowable concentrate formulations have also been reported (McLaughlin and Vidrine 1984c, 1984d). Development of this system for treatment of entire fields required determination of the major hydrological factors controlling distribution of larvicide. That information was used to establish procedures from timing and placement of containers of larvicide as flooding progressed downfield. The purposes of the note are: 1) identify the major hydrological factors influencing distribution of the larvicide; 2) and establish procedures for treatment of rice fields based upon these factors. The procedures are guidelines and permit flexibility for adjustment to the individuality of each field.

TEST SITE AND DESIGN. Fifteen rice fields in Jefferson Davis Parish, La. were used as they were flooded in the spring of 1982. Three dosages (0.63, 1.89 or 5.67 liters) of a flowable concentrate formulation of *Bacillus thuringiensis* H-14 were diluted in water to 20.8 liters and dispensed via the constant flow device at 80 ml/min. Data were collected on the variables influencing distribution as follows: 1) the number of levee overflows, 2) their relative location in the earthen levees subdividing each field into "pans" or "paddies" that flooded in sequence from the upper to the lower end of the field, 3) the number of these levees in the flooded portion of a field, 4) wind direction and speed during introduction of the material, 5) number of pans flooded initially, 6) numbers of pans flooded 24 hr later, 7) soil moisture at those two times, 8) the rate of water flow at the water entry source (where the material was added to the water at the start of introduction) and, 9) at 24 hr later.

Detailed scale maps (1.0 cm = 50 m) were prepared of each field from US Geological Survey and Agricultural Stabilization and Conservation Service aerial photographs. Field topographic features such as levee contours, overflows in levees, compass direction and areas of each pan were placed on the maps. Areas were determined with dot-chart overlays. Water flow rates at each overflow at the start of the test and 24 hr later were calculated by measurement of width, depth and velocity (corrected by a factor 0.9 of the observed surface velocity). Wind speed was determined with a hand-held anemometer. Soil moisture was graded as dry, moist (forms a crumbly ball when surveyed) or wet (formed a mud ball or water dripped when squeezed).

DETERMINATION OF AREA OF DISTRIBUTION. Larval mosquito populations were inadequate in the field for assays of *B. thuringiensis* H-14 activity *in situ*. Thus, a bioassay system was used to detect location of toxic concentrations of *B. thuringiensis* H-14. This system was reported in the initial description of this method of application (McLaughlin and Vidrine 1984a) and with the results of the data comparing amounts of formulation (McLaughlin and Vidrine 1984c). Water samples were collected at regularly-spaced intervals around the periphery of each pan at the end of the treatment application and again 24 hr after the start of the test. Twenty 3rd or 4th instar larvae of laboratory reared *Aedes aegypti* (Linnaeus) were exposed to each of the water samples (5 larvae each in 4 cups with 20 ml of the field water sampled). No mortality occurred among the checks during the 48 hr observation period. The percentage of larvae killed in each sample

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was noted on the map in the position of the water sample. All points greater than 5% mortality on the map were connected by a line. Mortality greater than 5% was chosen to indicate the presence of the larvicide at some level of activity. No inference was intended regarding relative concentrations. The area bounded by the line was then measured by means of dot-chart overlays to estimate the area of distribution of *B. thuringiensis* H-14.

DATA ANALYSIS. Mean effective area of distribution at the end of the introduction (ca 4.5 hr) and at 24 hr after start of the test were evaluated for effects of the variables listed above. Evaluation was done by stepwise regression of residuals after removal of replicate (5) and treatment (3 amounts of formulation) effects. Least square means, standard errors and linear contrasts among means were calculated for maximum improvement of the correlation coefficient (r-square) by stepwise linear regression.

The results of the analysis are presented in Table 1. Inclusion of additional variables of the model neither substantially improved the r-value nor decreased the error mean square. Two periods during the tests were considered; distribution at the end of application of *B. thuringiensis* H-14 and distribution at 24 hr postapplication. The results 24 hr after application are the most important of the 2 periods from an operational standpoint. The earlier observation period provided more highly correlated data, probably because of a shorter time for variation to develop. The maximum regression correlations from consideration of all possible variables are also presented in Table 1. The regression equation based upon only those independent variables of practical use by a control operation under field conditions are presented in Table 1. All r-values showed that a high percentage of the variation in the data was accounted for by these variables.

A discussion of each variable in relation to employment of this system follows. The number of outlet overflows in the levees was highly correlated in every model. Two outlets were optimum. When there was a single outlet, the best location was at the far end of the pan. The least effective location was directly across from the inlet overflow. A logical explanation exists, based upon previous studies on flooding patterns (McLaughlin and Vidrine 1984a, and unpublished data). An outlet opposite the inlet established a flow of water directly across the pan and contributed to formation of a "dead pool" of water with little or no circulation at the far end of the pan. An outlet at the far end established a flow and contributed to increased distribution in the subsequent pan downfield.

Several "variables" are closely related observations of the same phenomenon: 1) the number of levees through which water was flowing at the start of the application, 2) at the end of the application, and 3) the number of pans flooded 24 hr after application. These factors

Table 1. Regression equations for optimum r-square improvement. Regression of maximum effective area (ha) covered by distribution of *Bacillus thuringiensis* H-14 by water during flooding of rice fields.

	All significant variables		Variables of practical use	
Variable	End of application 1] r = 0.98	24 hr postapplication 2] r = 0.96	End of application 3] r = 0.97	24 hr postapplication 4] r = 0.85
No. of outlets	10.3160	15.0105	9.9350	15.5131
No. of levees	3.4698		3.3172	
No. of pans flooded at 24 hr postapplication	−2.0253		−1.4691	
Wind speed	−0.1865	−1.8713		
Area flooded during 24 hr		1.3318		0.6684
Water flow rate at 24 hr at source		−0.0021		
Wind direction		0.0537		

Regression equations: y = maximum area of effective distribution.

1] y = 10.316 × no. outlets per levee + 3.4698 × no. of levees through which water flowed during application − 2.0253 × no. of pans flooded at 24 hr after application − 0.1865 × mean wind speed during application.

2] y = 15.0105 × no. of outlets per levee + 1.3318 × area flooded during 24 hr + 0.0537 × wind direction in degrees (North = 0) −1.8713 × mean wind speed during application −0.0021 × water flow rate (liters/sec) at source at 24 hr.

3] y = 9.9350 × no. of outlets per levee + 3.3172 × no. of levees through which water has flowed during application − 1.4691 × no. of pans flooded 24 hr after application.

4] y = 15.5131 × no. of outlets per levee + 0.6684 × area (ha) flooded during the 24 hr period.

are closely related to each other as observations of a specific flooding condition. The area flooded by water at 24 hr also was closely related to a specific flooding condition. The practical size limit for a single pan appeared to be ca. 12 acres. A total area of 18 acres (7.3 ha) in no more than 4 pans was the overall limit for acceptable distribution. These factors are measures of one hydrological phenomenon, and are logically related as borne out by the regression analysis. The flow rate of water at the application inlet 24 hr after start of treatment was also included in the optimum model (Table 1) for maximum area of distribution at 24 hr. This was also a logically related factor influencing distribution.

Wind speed (knots) and direction (0 = north, 180 = south, etc.) were included in the optimum model at 24 hr posttreatment. These tests occurred during the early season when the water covered the young rice. Therefore, the wind had an unrestricted effect upon the water surface. Spring winds are often quite strong and quite persistent. However, during late summer when harvested fields are flooded for production of a second crop from regrowth of stubble, winds are light and variable and the harvested stubble breaks wave action. Wind is not considered to be a factor influencing *B. thuringiensis* H-14 distribution in late season.

Although the factors described in Table 1 for the optimum model account for maximum fit of the model to the data, all of these factors are not practical in deciding where and when to place the next constant flow device. Table 1 also presents the best models based upon practically observable variables. This also occurred in the stepwise regression of all factors. The variables listed in Table 1 were used as the major determinants for developing practical procedures for optimum placement of dispensing devices. Those recommendations are presented in Appendix A.

The major hydrological and topographical factor determining the dispersion of flowable concentrate formulations of *B. thuringiensis* H-14 were: 1) the number of overflows in earthen levees bounding the pans (paddies) where rice grows within a field, 2) the number of levees through which water is flowing at the start of treatment, and 3) the number of pans and the area flooded in 24 hr. Recommendations based upon these factors are presented for practical use of the system. The guidelines permit flexibility in the treatment regimen as dictated by the flooding conditions of a particular field. This information comprises the concluding component of the point-source introduction system for use of *B. thuringiensis* H-14 to control larval *P. columbiae* populations in rice fields. New formulations or changes in rice field management may require additional study and further modifications in the system and its rules for application.

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Appendix 1. Recommendation for Optimum Placement of Containers

Initial treatment in a field:

1. Single overflow in outlet levee: Place container at water entry overflow into field when water covers 1/2 to 2/3 of 1st pan in the field.
2. Two overflows in outlet levee: Place container at water entry overflow into field, but water may be flowing through the downfield overflows to 2nd pan prior to treatment.

Subsequent placement of downfield containers:

1. If 2 overflows exist in levee chosen for container, always use the one with the greatest water flow.
2. Placement interval must be less than or equal to 24 hr after first treatment. New container may be required sooner if area flooded exceeds limits listed below.

3. Place container at overflow in furthest downfield levee through which water has again covered at least 50% of the pan, with adequate flow to indicate a reasonably rapid flooding of that pan. Selection of this levee must consider limitations of area and number of pans downfield from prior container, as specified below.

Limitations imposed by area and number of flooded pans:

1. If the pans flooded from the last container have 1 overflow, no more than 12 ac (4.9 ha) limit, or at the entry to the 4th pan if the previous 3 pans do not contain 12 ac.
2. If the pans flooded downfield from the last container have 2 overflows, the area limit is increased to 18 ac (7.3 ha) and the pan limit is increased to 4.

ACTIVITY OF AN AVERMECTIN INSECTICIDE, ABAMECTIN (MK-936), AGAINST MOSQUITOES AND CHIRONOMID MIDGES IN THE LABORATORY[1]

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Recently, an avermectin microbial pesticide, Abamectin® (MK-936 or avermectin B_1: a mixture containing ca. 80% avermectin B_1a and 20% avermectin B_1b) was discovered (Putter et al. 1981). This compound is a macrocyclic lactone glycoside isolated from the actinomycete soil microorganism *Streptomyces avermitilis* and has shown superior activity against a wide variety of terrestrial arthropods of agronomic importance (Price 1983, Schuster and Everett 1983, Trumble and Nakakihara 1984). The insecticide was also highly active against the red imported fire ant (Lofgren and Williams 1982, Glancey et al. 1982). This note reports the activity of Abamectin in the laboratory against medically and economically important aquatic insects, mosquitoes and chironomid midges. Since avermectins exhibit delayed toxicological effects (Wright 1984), the activity of Abamectin was compared to that of diflubenzuron, an insect growth regulator, tested simultaneously as a standard.

Technical grade formulations of Abamectin (91%) and diflubenzuron (90%) in 6–7 serial dilutions were made in acetone. For mosquito bioassays, 4th instars of *Aedes aegypti* (Linn.), *Ae. taeniorhynchus* (Wiedemann), *Anopheles albimanus* Wiedemann, *An. quadrimaculatus* Say, *Culex nigripalpus* Theobald, *Cx. quinquefasciatus* Say, *Cx. salinarius* Coquillett, and *Wyeomyia mitchelli* (Theobald) were utilized. These species were maintained at the Florida Medical Entomology Laboratory at Vero Beach, Florida. For midge bioassays, 4th instars of *Chironomus crassicaudatus* Malloch and *Glyptotendipes paripes* Edwards were obtained by collecting and hatching eggs from adults captured in the vicinity of Lakes Monroe and Jessup in Sanford, central Florida. The captured adults were released for oviposition in midge rearing chambers maintained in the laboratory.

Mosquito bioassay methods were generally the same as described by Mulla et al. (1974). Twenty mosquito larvae were placed in a 120-ml disposable cup containing 100 ml of tap water. Five or six different concentrations of Abamectin and diflubenzuron were tested against each mosquito species each time. Each concentration was replicated three times and three untreated checks were maintained in each test which lasted for 3–7 days. The cups were examined daily and the larval or pupal mortality or adult emergence in each treated cup was recorded at the time of complete adult emergence in the checks. One ml of 1% hog liver + yeast (3:2) was added to each cup at 2-day intervals. The midge bioassays were conducted in 1200-ml clear plastic rearing units previously described by Ali and Lord (1980). Each unit received twenty 4th instar larvae, 150 g of sterilized fine sand and 500 ml tap water and was continuously aerated to maintain an air flow rate of 40 ± 10 ml/min. The experimental design for chemical treatments of midge larvae was the same as used for mosquito bioassays. For midge food, 0.1 g of ground dog food (Dog Kisses®, Hartz Mountain Products Corp.) was added to each unit at 2-day intervals. Dead larvae, pupae and living or dead adults in each unit were counted and removed daily. The experiment was followed usually for 5–7 days until no living larvae or pupae remained in the checks. Each mosquito or midge bioassay was repeated at least three times; evaluation against chironomid species had to be repeated 5–6 times because of the high mortality encoun-

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