EFFECTIVENESS OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* AGAINST *PSOROPHORA COLUMBIAE* BREEDING IN RICE FIELDS

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ABSTRACT. *Bacillus thuringiensis* var. *israelensis (B.t.i.),* was applied for control of *Psorophora columbiae* larvae to rice fields being flooded for a second rice crop in August and September 1980. Rates of 2 commercial wettable powder formulations applied in a water suspension ranged from 0.25 kg/ha to 0.6 kg/ha. Active ingredients, expressed as the potency value (International Toxic Units), ranged from 8.1×10^8 to 1.9×10^9/ha. Three applications by airplane resulted in 91, 96 and 99% reductions of very dense (4.5 to 18.6 larvae/dip) larval counts as compared to counts taken 24 hr prior to treatment. The *B.t.i.* was also adhered to rice hulls and applied prior to flooding the rice fields or at the time of flooding, and at the same rates as applied in water suspension. The rice hull tests resulted in inadequate control.

Increased restrictions upon chemical adulticide usage have magnified the need for alternative methods of mosquito control. *Bacillus thuringiensis* var. *israelensis* Serotype H-14 (*B.t.i.),* has recently been developed as an efficacious agent for many mosquito species (Goldberg and Margalit 1977, deBarjac 1978a, 1978b, 1978c; Garcia et al. 1980, Mulla et al. 1982a, 1982b). This agent has produced satisfactory *Psorophora columbiae* Dyar and Knab larval mortality in small experimental plots of rice (Hembree et al. 1980). Flooding of cut-over fields for regrowth of stubble for a second crop is the source of most *Ps. columbiae* populations in the fall in southwestern Louisiana. Pupae or adults often occur in the first levee area of a field before eggs hatch in the last levee area because flooding often lasts 4 to 7 days. These conditions make it extremely difficult to apply larvicidal control agents by broadcast aerial spraying as a routine procedure. The purpose of this study was to develop a feasible larvicidal program using *B.t.i.* The first tests were to determine the efficacy of wettable powder formulations. A second objective was to test the efficacy of a rice hull carrier for *B.t.i.* as a preflood application as one possible solution to the intermittent and slow flooding problem.

MATERIALS AND METHODS

The *B.t.i.* formulations tested were wettable powders provided by Biochem Products[3] (Bactimos™)[4] and by Abbott Laboratories[5] (ABG 6108D)[4]. Bactimos, bioassayed in our laboratory, had a relative potency of 3000 I.T.U./mg as compared to the International Standard IPS-78 assigned a potency of 1000 I.T.U./mg. ABG 6108D assayed at 500 I.T.U./mg.

The test sites were harvested rice fields reflooded for second crop production in Jefferson Davis Parish in southwestern Louisiana. During July, August and early September, *Ps. columbiae* completed development to the pupal stage in 4–5 days. These fields contained from about 5 to 20 ha. Our tests were conducted on portions of the fields (0.5 to 5.0 ha) which were bounded by the levees. The fields were completely covered with water at the time of application but water continued to be added during the test.

AERIAL APPLICATION OF WATER SUSPENSIONS. The conventional method of spraying water suspensions of wettable powders by airplane was tested on 3 fields: 1) Fontenot farm, 0.5 kg on about 0.8 ha area (ca. 1.9×10^9 I.T.U./ha) (Bactimos B-676) on August 20, 1980; 2) Compton farm, 1.0 kg on about 2.0 ha (ca. 1.5×10^9 I.T.U./ha (Bactimos B-676) on August 22, 1980; 3) Bowers farm 3.6 kg on about 1.6 ha (ABG 6108D) (ca. 1.1×10^9 I.T.U./ha) on September 17, 1980. The aerial sprays were applied at the rate of 93.1 liters of water per ha.

APPLICATION WITH RICE HULLS AS A CARRIER. The second method of application used rice hulls as a carrier for *B.t.i.* Bactimos was adhered to rice hulls by mixing with gelatin. Thirty-six g of gelatin were dissolved in 1 liter of water at 60° C, cooled to 45° C and blended with 113.5 g of Bactimos. Two liters of this liquid gelatin

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[4] Mention of a trademark, proprietary product or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

[5] Abbott Laboratories, N. Chicago, IL (Permit No. 27523–EUP–1).

were thoroughly mixed by hand in a large garbage can with 4.5 kg of rice hulls. The hulls were then spread in large metal trays for drying.

The rice hulls plus Bactimos were first applied to an area at one end of the Compton field on August 22, 1980. The hulls were applied with a "cyclone-spreader" (a device used to manually broadcast seeds). At the time of this application water had entered one end of the levee area and brought many second and third instar larvae from the previous levee area. One end of the plot was dry and the hulls were spread on the ground before the water was available for hatching larvae. Water started entering the rice pan through the levee as we applied 0.4 kg of rice hulls (10 kg/ha of hulls, 1.7×10^9 I.T.U./ha).

The next 2 tests used Bactimos adhered to rice hulls and application was by airplane. Distribution patterns of hulls were monitored by placement of pans in the fields. Application to the Strohe #12 field was to the lower (farthest from water source) half of a rectangular 3.2 ha area. Seven kg of hulls (0.35 kg Bactimos)/ha were applied. Only the lower half was treated and the upper half was used as an untreated check. However, the water continued to enter the upper end of the field although the pump had been stopped. Continued water movement carried some larvae into the treated end, particularly along the levee edge.

The last application (Bowers) was to a rice pan that was dry when the hulls were applied. We intended to test pre-flood applications to the upper (near to water inlet) portion, with the advantage of easier timing of application on reduced area to be covered by utilizing the flow of water to carry the hulls to the rest of the field. About 0.4 ha of a 1.0 ha area received 4.5 kg of hulls (1.7×10^9 I.T.U./ha). We then cut the levee. Water flow was much more rapid than anticipated from past experience, and covered the field to a depth of ca 50 cm in 6 hr. In all tests of rice hulls plus *B.t.i.*, rice hulls collected from the water after application and from the pans were assayed. One hull was placed in 100 ml of water with 20 4th instar *Aedes aegypti* (Linn.) larvae. This always produced 100% mortality, indicating that active *B.t.i.* was still present.

EVALUATION OF TESTS. Data collection included pre- and posttreatment counts of larvae in the field with the standard metal dipper method; 1 dip was collected every 20 steps for the length of both the treated and untreated portion of the field along 4 paths in each area. Water from the rice field was collected within 1 hr prior to the application and tested in the laboratory as a control against *Ps. columbiae* larvae also collected about an hour prior to application. This water sample was also used to add a known dose of *B.t.i.* in the laboratory to determine any possible adverse effect of the field water on *B.t.i.* The larvae collected prior to treatment were placed in the water collected before spraying *B.t.i.* Posttreatment water was collected one day after application and assayed in the laboratory. These tests provided a means of assessing the effectiveness of *B.t.i.* applied in the field. This procedure was repeated 4 to 6 times for each levee area. Instar composition of the population was also determined by examining 100 larvae.

Water samples were collected from the nozzle tips of the airplane sprayer prior to adding the *B.t.i.* This material was assayed to determine the absence of toxic residue by placing larvae in the water samples and observing them for 24 hr. The *B.t.i.* was added to pretreatment water and mortality of larvae in this water was compared to mortality in known doses in laboratory water.

RESULTS AND DISCUSSION

Some general results regarding the tests are presented first. The laboratory assays showed: 1) there was no chemical residue in the plane, 2) the *B.t.i.* suspension collected at the nozzle was as potent as expected, 3) the water in the rice fields did not alter the potency observed by laboratory assays, 4) *Ps. columbiae* larvae were susceptible to *B.t.i.* added to water at known doses, and were also killed by water collected immediately after treatment, 5) there was no residual activity in the field water collected one day after application, and 6) another point, present in all tests with *B.t.i.*, other aquatic insects were still alive. There were numerous Coleoptera, Hemiptera and even a few *Ps. ciliata* (Fabr.), none of which seemed to be affected by *B.t.i.*

AERIAL APPLICATION OF WATER SUSPENSIONS. Results of aerial application of water suspensions of *B.t.i.* are presented in Table 1. The Fontenot field treated on August 20 had an area that curved to one side and narrowed to almost a point at the far end where trees prevented the pilot from applying *B.t.i.* to the far end. This narrow end had the only larvae found 1 day posttreatment.

The Compton field treated on August 22 was a long field with no obstructions and aerial application resulted in good coverage. Larvae were 3rd–4th instars. Counts were reduced from almost 20 larvae per dip uniformly over the field to only 14 larvae in 114 dips.

The last application of the water suspension was on a field at the Bowers farm on September

Table 1. Results of aerial application of wettable powder formulations of *Bacillus thuringiensis* var. *israelensis* against *Psorophora columbiae* larvae in rice fields being flooded for second crop production. Data are average number of larvae per dipper for 2nd, 3rd and 4th instars.

	Pretreatment				One day posttreatment				
Field	2nd	3rd	4th	All stages	2nd	3rd	4th	All stages	Overall % reduction
Fontenot	0.5	0.8	3.2	4.5	0.0	0.0	0.4	0.4	91
Compton	0.0	8.9	10.7	19.6	0.0	0.0	0.1	0.1	99
Bowers	5.6	0.0	0.0	5.6	0.0	0.0	0.2	0.2	96

17. Flooding had started September 15 and larvae were 2nd instar at the time of application. The larvae surviving treatment were found randomly scattered throughout the field. They were large late 3rd or 4th instars. *Psorophora columbiae* larvae develop from late 2nd instar to 4th instar in one day at this time of year. Comparison of counts taken 1 hr prior to treatment to counts taken 1 day after treatment could raise a question regarding natural mortality in 1 day. Therefore, larval counts from fields not treated are relevant. A field sampled on September 15 had 121 larvae in 32 dips (3.8 larvae/dip). The next day the counts were 131 larvae in 32 dips (4.1 larvae/dip). Another field had 194 larvae/28 dips (6.9 larvae/dip) on September 15, and on September 16 the counts were 198 larvae/32 dips (6.2 larvae/dip). A third had 358/62 dips (5.8 larvae/dip) vs. 387/62 (6.2 larvae/dip). Therefore, no decrease in larval counts in only one day can be reasonably expected. Comparisons of pretreatment counts vs. counts taken 1 day after treatment should be more readily accepted as a true indicator of the effects of the treatment. Larval counts varied tremendously (less than 1 to over 10 or 20/dip) in different fields. Thus, a comparison of numbers from treated or untreated fields introduces more variability than comparison of pretreatment to posttreatment counts over a one day time span. The only alternative is to conduct a large number of tests and allow randomization to account for variation.

RICE HULL TESTS. Generally, the distribution pattern of the rice hulls seemed to be as uniform as when rice is seeded in the spring. The application at the Strohe field on September 3 had 73 ± 48 (range 6–150)/pan; and the Bowers test on September 15 had 63 ± 43 (range 4–144)/pan. Overall the average distribution was 68 ± 46 hulls/700 cm^2.

The Compton test (ground distribution of hulls) had larvae of all instars. The older larvae came from the previously flooded area along with the water. Dipper samples from water around piles of rice straw always had newly hatched larvae, often hundreds per dip. Counts of older larvae from the water flow, particularly close to the levee, averaged about 30 per dipper. Counts the following day resulted in 0.2 2nd instar/dipper, 0.7 3rd instar/dipper, and 0.2 4th instar/dipper, for a total of 1.1 larvae/dipper.

The Strohe #12 data show a reduction from 3.1 to 0.8 in larvae per dip in 24 hr. A continuing water flow carried many larvae along with it from the upper, untreated area of the field, and by 48 hr the larval count had increased to 1.6/dip. Even without the effect of the water current, the rice hull application was not nearly as effective as the aerial spray of a water suspension of *B.t.i.*

This experience led us to attempt to use water flow to an advantage in the last trial on a dry area of the Bowers field. After the hulls had been broadcast by the plane, the water was allowed to enter the field. We saw the hulls being carried along with the water as the leading edge swept by; hulls did not remain in one area. Total flooding occurred in 6 hr, and all detectable hulls were concentrated in a small area at the far end of the rice pan. Counts taken as soon as larvae hatched averaged 5.7 larvae/dip. The next day counts were 6.2/dip. The data show no change in larval counts over the entire field. However, dipper samples taken in the small area at the far end of the field where the hulls were seen to be concentrated had no larvae.

In conclusion, larval counts of *Ps. columbiae* were reduced 91 to 99% by *B.t.i.* applied as a wettable powder in water suspension from an airplane at the rate of 0.44 to 0.625 kg/ha (or 1.1×10^9 to 1.9×10^9 I.T.U./ha), in rice fields flooded for second crop production in August and September in southwestern Louisiana. Use of rice hulls to carry *B.t.i.* on fields being flooded or for a pre-flood application was not successful because flowing water moved the hulls away from the larvae before hatch.

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EFFECTS OF INDIGENOUS *TOXORHYNCHITES RUTILUS RUTILUS* ON *AEDES AEGYPTI* BREEDING IN TIRE DUMPS

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ABSTRACT. A study in Jacksonville, Florida showed that a dense natural population of the predator, *Toxorhynchites rutilus rutilus,* significantly reduced a natural population of *Aedes aegypti* in a tire dump, when compared with 2 other tire dumps with very low levels of *Toxorhynchites*. Production of prey pupae and adults was virtually eliminated by mean levels of 1 to 5 predator larvae per tire during a 10-week study. In these studies the predator was most effective in tires located under trees, and least effective in open areas.

INTRODUCTION

Much has been written recently on the possible use of *Toxorhynchites,* a predatory genus of mosquitoes, as biocontrol agents against mosquito species that breed in natural and artificial containers (Brown 1973, Steffan 1975, Lubega et al. 1975, D. A. Focks, personal communication). Some studies have been reported on the bionomics of laboratory-reared *Toxorhynchites* when released in the field (Focks et al. 1980, Gerberg and Visser 1978, Hu 1955), and their effect on container-breeding mosquitoes (Bonnet and Hu 1951, Gerberg and Visser 1978, Payne 1934, Peterson 1956). However, only limited observations have been reported on the effects that natural populations of *Toxorhynchites* have on other mosquito species in the field. Trpis (1972) found that in East Africa *Toxorhynchites brevipalpus* Theobald did not effectively control *Aedes aegypti* (Linn.) early in the rainy season due to its long developmental period, but that it was very effective in bringing *Ae. aegypti* under control later in the season. He suggested the release of laboratory-reared *Tx. brevipalpus* at the onset of the first rains to overcome this lag in biocontrol. Focks et al. (1980) found that natural populations of *Toxorhynchites rutilus rutilus* (Coq.) on an island near the west coast of Florida reduced the numbers of *Ae. aegypti* larvae (in automobile tires they had placed on the island) from more than 100 to less than 5/tire during late summer and early fall. Their data also indicated a definite time lag in effective control by the predator.

To characterize further the interaction of *Toxorhynchites* and *Ae. aegypti* and to determine the effect natural populations of this predator have on this important vector of disease, we conducted a cooperative study at selected tire dumps in Jacksonville, Florida.

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