EVALUATION OF BEECOMIST®-APPLIED *BACILLUS THURINGIENSIS* (H-14) AGAINST *ANOPHELES QUADRIMACULATUS* LARVAE IN RICE FIELDS[1, 2]

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ABSTRACT. The Beecomist® spray head was evaluated for aerial application of *Bacillus thuringiensis* var. *israelensis* (serotype H-14; *Bti*) at various ultra low volume (ULV) rates against natural populations of *Anopheles quadrimaculatus* larvae in rice fields. Deposits on Kromekote® cards indicated that 0.54 liter/ha of neat *Bti* penetrated the dense canopy of the rice field. Mean number of droplets 65 cm below canopy level was 4.9 ± 5.0/100 cm^2. At 1 day posttreatment, applications of 0.54, 0.27, 0.11, 0.07 and 0.04 liter of *Bti*/ha resulted in reductions of 97.9, 94.4, 93.0, 71.1 and 21.8%, respectively, of *An. quadrimaculatus* larvae/dip. Calculated lethal field dosages (LFD_{50} and LFD_{90}) were 0.05 and 0.16 liter/ha, respectively.

INTRODUCTION

Ultra low volume (ULV) application of insecticides has effectively been used to control mosquito adults (Glancey et al. 1965, Knapp and Roberts 1965) and larvae (Stevens and Stroud 1966). Mount et al. (1968) reviewed the advantages of ULV application. The Beecomist® spray head represents one method of applying insecticides at ULV rates. The Beecomist system has the advantage of being quickly adaptable to a wide variety of aircraft for ULV application and is capable of dispensing formulated materials at extremely low volumes. Less than 2 liters of material is needed to charge the spray system, and unlike conventional spray systems which require large volumes of water as a carrier, application of ULV rates by Beecomist allows for treatment of large areas without reloading the system.

Bacillus thuringiensis var. *israelensis* (serotype H-14; *Bti*) has proven to be an efficacious control agent against rice field mosquitoes (Hembree et al. 1980, Dame et al. 1981, Stark and Meisch 1983), but cost limitations of conventional application techniques have limited its use. The Beecomist spray head was successfully used to apply malathion for adult mosquito control as early as 1974 (Rupp and Sutherland 1976). Only recently has the Beecomist spray head been proposed for use in larval mosquito control (Anonymous 1983). In 1984, Yates described a system based on the Beecomist spray head, elaborated the adaptability of this application technique for agricultural aircraft and reported excellent control of first and second instar *Culex quinquefasciatus* Say larvae using Beecomist-applied *Bti* at a swath width of 91.4 m. If liquid-formulated *Bti* could be used in the Beecomist system at less than label recommendations and still achieve effective control, then this environmentally compatible microbial agent would be a more economically viable option for mosquito control.

To further evaluate Beecomist-applied *Bti* for control of natural populations of *Anopheles quadrimaculatus* Say larvae in rice fields, a cooperative research program involving researchers from Chambers County Mosquito Control District (TX), Texas A&M University and the University of Arkansas was initiated in the summer of 1983. This paper presents the results of that study.

MATERIALS AND METHODS

Investigations were conducted in rice fields near Stuttgart, AR (Arkansas County) during the summers of 1983 and 1984. Fields were selected on the basis of adequate numbers of *An. quadrimaculatus* larvae in 100 random dips (near or greater than 0.5 larvae/dip) and uniformity of rice stand. At treatment, rice in the fields was approximately 0.7-1.0 m high and in the pre-panicle stage.

The 1983 investigations were conducted in 17 and 18 ha rice fields. Three plots, 76 × 152 m, were located in the fields, two in the 17 ha field and one in the 18 ha field. Plots within the same field were separated by a minimum of 300 m. Treatments were randomly assigned to the plots and consisted of an untreated check and two *Bti* treatments. Each treatment was conducted only once due to time and manpower constraints. Treatments were conducted with Teknar® AC (*Bacillus thuringiensis* var. *israelensis*

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(serotype H-14), 1500 AA Units/mg (*Aedes aegypti* (L.)).

Applications were made using a Piper Pawnee 260 with a Beecomist Model 360A spray head mounted on the spray boom of the starboard wing approximately 0.7 m from the outer edge of the wing. The system employed was similar to that described by Yates (1984). The spray head was equipped with an 80–100 u perforated stainless steel sleeve. The 95 liter stainless steel tank holding the neat Teknar and the 11 kg CO_2 cylinder used to pressurize the system were placed in the hopper of the aircraft. The system was pressurized at 276 kPs which resulted in a flow rate of 1.31 liter/min. A switch box mounted in the cockpit activated the system. Air speed was 161 km/h and altitude during application was ca. 15 m.

Applications were made near sunset. Winds were 4–8 km/h during application. The check plot was upwind from the treatment plots at initiation of application. Flight paths were perpendicular to the wind and along the length of the plots with the Beecomist spray head on the upwind wing of the aircraft. Dosages of 0.54 and 0.27 liter *Bti*/ha were achieved by flying 9.1 and 18.3 m swaths over the respective plots.

Kromekote® water sensitive cards, 10 × 10 cm, were attached to stakes placed between drills with the sensitive surface 65 cm below the canopy and 5 cm above and parallel to the water surface. Cards were spaced ca. 3 m apart across the width of the 0.54 liter/ha plot and perpendicular to the flight path in the center of the plot. Cards were visually examined after application and the total number of droplets/100 cm^2 was determined with a dissecting microscope. Mean number of droplets/100 cm^2 card and standard deviation were calculated.

Prior to treatment larval density in each plot was estimated using a standardized procedure. Two transects (1 and 2) were established along the length of the plot, each ca. 25 m from the respective sides of the plot. Two transects (3 and 4) were established along the width of the plot, each ca. 50 m from the respective ends of the plot. An additional transect (5) was established diagonally across each plot. The sampling procedure was as follows: dip samples were taken at 1.5 m intervals along transects 1 and 2, at 0.75 m intervals of transects 3 and 4, and 0.7 m intervals of transect 5. Total number of larvae/100 dips was recorded. Total number of samples/plot was 500. Samples were made using 450 ml dippers fitted with wooden handles. Dips were made at the surface of the water and in close association with vegetation to assure maximum encounter of *An. quadrimaculatus* larvae. All plots were sampled at 12 hr pretreatment and 1 day posttreatment. Mean reduction of *An. quadrimaculatus* larvae/dip for all transects was calculated for each sampling period.

Investigations in 1984 were conducted in 14, 16 and 31 ha rice fields. Plot form and size were modified in 1984 to better accommodate changes in wind direction at time of treatment. Five plots, 152 × 152 m, were located in the fields, one in the 14 ha field, two each in the 16 and 31 ha fields. Plot separation and treatment assignment were consistent with the 1983 tests. Treatments consisted of untreated checks and three *Bti* applications. When two *Bti* applications were made during the same day, one check plot was used for both treatments. Treatments were conducted with freshly formulated Teknar AC and applications were consistent with those of 1983.

Application aircraft, equipment and flight specifications were the same as those used in 1983. Swath width was 18.3 m in all 1984 tests. Dosages were achieved by varying system pressure to result in the flow rate corresponding with the desired dosage. Pressures of 124, 97 and 76 kPs resulted in flow rates of 0.54, 0.36 and 0.18 liter/min, corresponding with dosages of 0.11, 0.07 and 0.04 liter/ha, respectively. Orifice plates, D–8, were placed between the diaphragm check valves and bushings of the bottom port of the spray head during application of the 0.07 and 0.04 liter/ha rates (Yates 1984). The top port of the spray head was closed during these applications. This was done to insure that the line between the solenoid and the spray head remained fully charged with *Bti*. Winds were 1–5 km/h during the 0.11 liter/ha application and 8–11 km/h during the 0.07 and 0.04 liter/ha applications. Check plots were upwind from treatment plots during applications.

Prior to treatment larval density in each plot was estimated using a standardized procedure but location of transect differed from that used in 1983. One transect was established which connected opposite corners of the plot. Four sampling stations were established along the transect with 43 m between each station and the corners. The sampling procedure was as follows: 25 dip samples were taken at 1 m intervals in each cardinal direction at the sampling station. Total number of larvae/100 dips was recorded. To facilitate rapid evaluation of larval populations, total number of samples/plot was reduced to 400. Sampling periodicity, apparatus and technique were consistent with those of 1983. Mean of reduction in *An. quadrimaculatus* larvae/dip for all stations was calculated for each sampling period.

Larval reduction data were corrected for control mortality using Abbott's formula and subjected to probit analysis (Finney 1971).

Fiducial limits (95%) were calculated for the lethal field dosages, LFD_{50} and LFD_{90}.

RESULTS AND DISCUSSION

Examination of Kromekote cards indicated that 0.54 liter *Bti*/ha penetrated the dense canopy of foliage and contacted the water surface. The number of droplets on the 26 cards ranged from 0 to 19 and averaged 4.9 ± 5.0 droplets/100 cm^2. In contrast, Yates (1984) reported a range of 0–82.7 droplets/99 cm^2 on Kromekote cards following an application of Teknar at 0.59 liter/ha. Kromekote cards in that study were placed in canopy free areas and the swath width was 91.4 m.

In 1983, excellent control of indigenous *An. quadrimaculatus* larvae was achieved with *Bti* treatments at rates of 0.54 and 0.27 liter/ha (Table 1). At 1 day posttreatment, number of larvae/dip in the 0.54 liter/ha plot was reduced by 97.9%. The 0.27 liter/ha treatment provided 94.4% reduction while number of larvae/dip in the check plot was reduced by 32.5%. Natural mortality could account for the reduction of larvae in the check plot. Drift from the 0.54 liter/ha treatment could also have been involved as the wind shifted from southeast to southwest during application. Applicator error may have been involved as the pilot failed to promptly "switch-off" the flow after completion of some passes.

In 1984, excellent control was achieved with *Bti* at 0.11 liter/ha (Table 1). At 1 day posttreatment, number of larvae/dip in the 0.11 liter/ha plot was reduced by 93.0% while number of larvae/dip in the check plot increased 17.5%. At 0.07 liter/ha, number of larvae/dip was reduced by 71.1%. Activity was further reduced at 0.04 liter/ha with a 21.8% reduction, while number of larvae/dip in the check plot decreased 10.5%.

Mortality of *An. quadrimaculatus* reported here was comparable to that reported by Stark and Meisch (1983) with a conventional application of 2×10^9 ITU/ha in 16 liter/ha. We obtained equivalent mortality at lower dosages and reduced total volume (7.99×10^8 AA Units/ha in 0.54 liter/ha, 3.99×10^8 AA Units/ha in 0.27 liter/ha and 1.62×10^8 AA Units/ha in 0.11 liter/ha). Stark and Meisch (1983) tested a wettable powder *Bti* formulation (ABG–6108 II WP (8278–77)). We used a liquid formulation in this study, Teknar AC.

Sun et al. (1980) and Dame et al. (1981) suggested that *Anopheles* spp. were less sensitive to *Bti* than culicine larvae, perhaps due to their behavior, i.e., association with floatage and aquatic vegetation (Horsfall 1955). Application of ULV *Bti* via Beecomist spray head may result in greater contact with the larvae than other methods of application because of the finer suspension which may remain in the feeding zone longer.

The data were subjected to probit analysis (Fig. 1). The LFD_{50} and LFD_{90} values were 0.05 and 0.16 liter/ha, respectively. Fiducial limits (95%) were 0.02–0.17 liter/ha for the LFD_{50} value and 0.10–0.26 liter/ha for the LFD_{90} value.

The low volume of formulated material dispensed and the adaptability of the system to various aircraft make the Beecomist appealing to mosquito control operations. Current cost of Teknar ($3.96/liter) at 0.16 liter/ha rate is $0.63/ha. Established application cost of *Bti* at $0.80/acre ($1.98/ha) using the Beecomist spray head is much less than the estimated $3.00/acre ($7.41/ha) using conventional application equipment (Anonymous 1983). These cost estimates are for comparative purposes only as application costs vary considerably depending on aircraft, number and area of sites to be treated. Accurate estimates for these application costs are unavailable.

Table 1. Mean number and reduction (%) of *Anopheles quadrimaculatus* larvae/dip prior to and following treatment with Teknar® via Beecomist© spray head.

	Mean larvae/dip				
	Pretreatment		Posttreatment		Corrected percentage reduction
(liter/ha)	Untreated	Treatment	Untreated	Treatment	
1983					
0.54	0.77[1]	1.43	0.52	0.02	97.9
0.27	0.77[1]	0.52	0.52	0.02	94.4
1984					
0.11	0.97	1.14	1.14	0.08	93.0
0.07	1.14[2]	0.54	1.02	0.14	71.1
0.04	1.14[2]	0.40	1.02	0.28	21.8

[1, 2] One check plot was used for treatments during the same day.

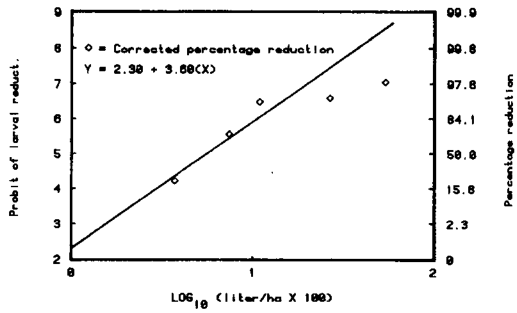

Fig. 1. Dosage mortality response (liter *Bti*/ha: % reduction in number of larvae/dip) of *Anopheles quadrimaculatus* larvae to Teknar® applied via Beecomist® spray head.

This study and others (Anonymous 1983 and Yates 1984) affirm the potential of the Beecomist system as an effective and economical means of treating large areas with *Bti*, an environmentally compatible larvicide. Additional research is needed to determine the effect of increased swath width. Costs will be further reduced as swath width increases. Theoretically, a Beecomist-applied *Bti* program would reduce the need for control of adult mosquito populations as larvae could be economically controlled over a large area. More research on an operational basis is needed.

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ERRATUM

Mosquito News, vol. 44, no. 4

Chadee, D. D., N. K. Connell, A. Le Maitre and S. B. Ferreira. Surveillance for *Aedes aegypti* in Tobago, West Indies (1980–82), pp. 490–492.

On page 490, column 1, INTRODUCTION, lines 10–17 should read:

The Insect Vector Control Division (IVCD) immediately reinstituted an island-wide survey but *Ae. aegypti* was not encountered (Ministry of Health 1977). In 1977, *Ae. aegypti* larvae were found in an abandoned tire at the Works Department at Scarborough Wharf, and an island-wide survey found three other foci in Mason Hall and in Crown Point, 6 km and 3 km respectively, from Scarborough.