OPERATIONAL AND SCIENTIFIC NOTES

A PORTABLE SYSTEM FOR AERIAL APPLICATIONS OF VERY LOW VOLUMES OF TECHNICAL GRADE CONCENTRATES OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS*

MATTHEW M. YATES, Director

Chambers County Mosquito Control District, P. O. Box 1109, Anahuac, TX 77514.

In 1965 Dearman, Powell and Thompson described a portable spray system for aerial low volume applications of insecticidal concentrates. There have been numerous modifications of this system since the original description and some of the modifications have been documented in a Chevron Chemical Company publication (Anonymous, date unknown). The primary uses of these systems have been in application of materials to control numerous species of adult mosquitoes. This article describes our modifications of the system illustrated in the Chevron publication and discusses a new application for the modified system; i.e., larviciding with undiluted liquid formulations of *Bacillus thuringiensis* var. *israelensis (B.t.i.)*.

In 1973, Chambers County Mosquito Control personnel substituted a model 360A Beecomist® spray unit for the flat fan spray tips illustrated in the Chevron publication. Since 1974, several different modifications of this Beecomist system have been used in Chambers County to apply technical grade naled and malathion from helicopters and small fixed-wing aircraft. The system is not very different from those used by some other mosquito abatement districts in their adulticiding operations.

In 1982, we substituted an 80–100μ perforated stainless steel sleeve assembly for the smaller 20–40μ porous sleeve assemblies we use for adulticiding operations and used the system to apply undiluted *B.t.i.* liquid formulations. We believe this to be a new use for this type of system. The March, 1983 issue of *Pest Control Technology* featured our use of this system but did not describe it (Anonymous 1983). The following description and Fig. 1 detail the system as it is used for *B.t.i.* applications.

The system consists of a compressed air source and pressure regulator, a 37.85 liter (10 gal) beverage container, electric solenoid valve, two diaphragm check valves, two bushings, Beecomist spray nozzle and 80–100μ perforated stainless steel sleeve assembly, two push-pull switches (electrical), plastic tubing (chemical), wires (electrical) and fittings (chemical).

The compressed CO_2 provides pressure to the beverage container via the pressure regulator and tubing and acts as a propellent for the *B.t.i.* The gas flows from the bottle via the regulator into one of the two openings at the top of the metal beverage container and forces the *B.t.i.* out through the other opening. The *B.t.i.* flows through the ½-inch plastic tubing to the solenoid (electrical).

The solenoid is activated by a switch located in the cockpit of the aircraft. There are two wires (electrical) on the solenoid. One is a ground wire and is secured to the airframe of the aircraft. The second wire is attached to one of two terminals on the switch located inside the aircraft. The second terminal on the switch connects to a single wire in the switch box that is connected to the power supply of the aircraft: one wire (positive) supplies current to both the Beecomist switch and solenoid switch. Both switches are located on the switch panel and both switches are equipped with in-line fuses to protect the aircraft's electrical system and prevent damage to the spray components in the event of a malfunction in the system.

When the solenoid is activated, *B.t.i.* flows through the solenoid body to the diaphragm check valves located in line between the solenoid and Beecomist dispenser body. The diaphragm check valves are necessary to provide a positive shut-off of the system between spray runs. The diaphragms close and stop flow of *B.t.i.* between the solenoid and Beecomist whenever the inline pressure drops below 15 pounds per square inch. This is necessary to insure that the insecticide line between the solenoid, which is located inside the hopper of the aircraft, and the Beecomist spray unit, which is located below the outer one-third of the aircraft wing, remains fully charged with *B.t.i.* at all times.

The diaphragm check valves connect to bushings which attach directly to the Beecomist dispenser body. Since the diaphragm check valves close when the pressure drops below 15 pounds/inch^2, small diameter orifice plates must be inserted between the diaphragm check valves and bushings to restrict the flow of *B.t.i.* and permit higher operating pressures when applying very low volumes of material.

The *B.t.i.* concentrate flows through the diaphragm check valves, orifice plates, bushings and body of the Beecomist into the rapidly rotating perforated stainless steel sleeve assembly (10,000 rpm). The very small droplets produced as the *B.t.i.* is forced through the sleeve

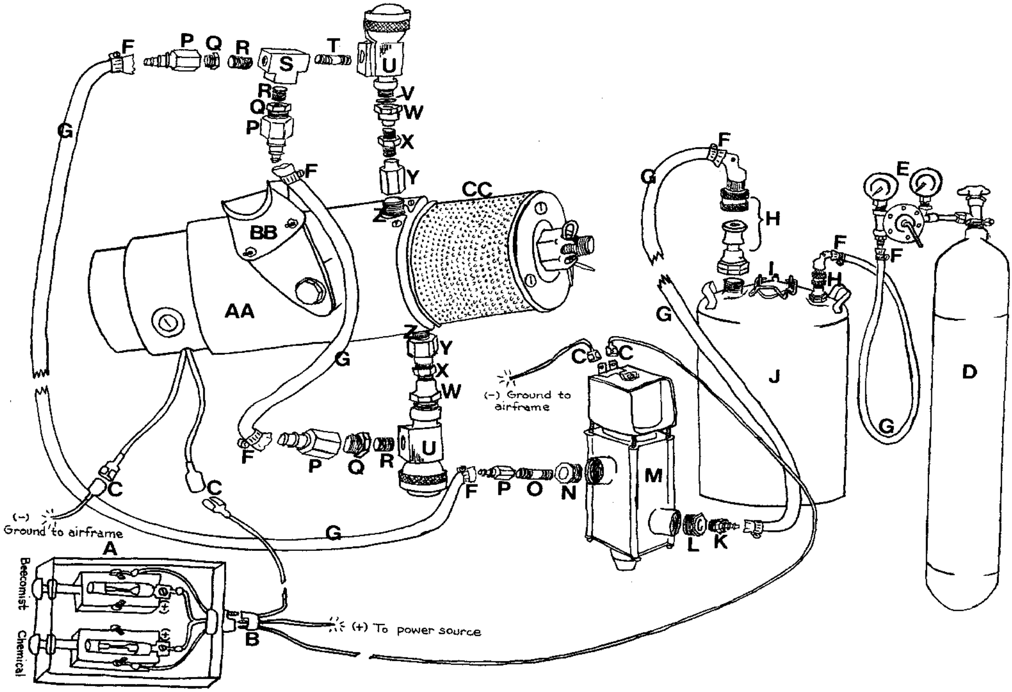

Fig. 1. A portable ULV larviciding unit for aerial applications of technical grade liquid formulations of *Bacillus thuringiensis* var. *israelensis*.

Parts are not drawn to scale and some are enlarged to show detail. A. control panel with cover removed to show wiring scheme and push-pull type Beecomist and solenoid (or chemical) switches; B. quick-connect electrical plug, boat trailer type; C. push-on electrical connectors; D. CO_2 cylinder; E. pressure regulator and gauges; F. ½″ (1.3 cm) hose clamps; G. ½″ (1.3 cm) flexible high-pressure hose; H. quick couplings; I. filler cap; J. 10 gallon (37.9 liter) stainless steel beverage container; K. ⅜″ to ½″ (1.0 cm to 1.3 cm) hose fittings; L. ⅜″ to ¾″ (1.0 cm to 1.9 cm) bushings; M. 12-volt DC solenoid (Directo® valve model #144); N. ¾″ to ¼″ (1.9 cm to 0.6 cm) bushing; O. ¼″ (0.6 cm) nipple; P. ½″ to ¼″ (1.3 cm to 0.6 cm) hose fitting; Q. ¼″ to ⅛″ (0.6 cm to 0.3 cm) bushing; R. ⅛″ (0.3 cm) all-thread nipple; S. ⅛″ (0.3 cm) tee; T. ⅛″ (0.3 cm) nipple; U. Diaphragm check valve; V. D-4 spraying system orifice plate; W. ⅛″ to ¼″ (0.3 cm to 0.6 cm) bushing; X. ⅛″ (0.3 cm) union, male threads; Y. ¼″ to ⅛″ (0.6 cm to 0.3 cm) reducer; Z. ¼″ (0.6 cm) all-thread nipple; AA. Beecomist spray body, 12-volt model 360A; BB. boom mounting brackets; CC. 80–100μ perforated stainless steel sleeve assembly.

assembly drift perpendicular to the wind direction and are dispensed over a rather large swath. The Beecomist motor, which spins the sleeve assembly, is activated by a switch located inside the cockpit of the aircraft. One of the two wires on the Beecomist spray unit is grounded directly to the airframe of the aircraft. The other wire goes directly to the switch panel and is wired in the same manner as the solenoid switch discussed above. Electrical quick-connect plugs are used in line at several locations within the wiring system to facilitate quick installation or removal of the system from the aircraft.

We use the Beecomist system on a Cessna Ag Wagon, which is an agricultural type of aircraft. The CO_2 bottle, beverage container and solenoid valve are secured inside the insecticide hopper of the aircraft. The Beecomist switch and solenoid switch panel is secured inside the cockpit. The Beecomist spray unit is attached to the outer one-third of the right wing of the aircraft's spray boom with hose clamps. The Beecomist spray system is angled 20° down from the horizontal plane of the wing. The electrical wires going from the Beecomist to the spray switch panel, which is located in the cockpit, are secured to the spray boom with plastic quick-ties. The electrical wires connecting the solenoid valve and spray switch are routed from the hopper through the cockpit window. The tubing transporting the *B.t.i.* from the solenoid, located inside the insecticide hopper, to the Beecomist is secured to the spray boom with plastic quick-ties. The system is versatile and portable enough that it could be easily adapted to other types of aircraft or rotorcraft.

This system has been successfully used in a number of aerial applications of undiluted technical grade *B.t.i.* against natural populations of larval mosquitoes. Field trials on August 19, 1982 against first instar *Psorophora columbiae* (Dyar and Knab) larvae in a second crop rice-field gave 100% control using Teknar Aqueous Concentrate® (AC) at 1.16 liter/ha. A trial against *Anopheles quadrimaculatus* Say and *Culex salinarius* Coq. larvae in a second crop rice-field on September 9, 1982 gave better than 95% control of both species using Teknar AC at 1.16 liter/ha. Both of the above applications were made using a Cessna Ag Wagon at 161 km/hr, 7.6 m altitude and flying a marked 9.1 m swath.

Trials on May 26, 1983 against first through third instar *Culex quinquefasciatus* Say larvae, retained in 473 ml plastic tubs containing 355 ml of tap water, gave 71% control with Bactimos Flowable Concentrate® and 97% control with Teknar AC. Both formulations were applied at 0.58 liter/ha at an airspeed of 177 km/hr, an altitude of 7.6 m and a swath width of 30.5 m.

This system was used to dispense Teknar AC at 0.58 liter/ha and 1.16 liter/ha against *Anopheles quadrimaculatus* larvae in two separate trials in Arkansas rice-fields in July, 1983. The 1.16 liter/ha application was made using a marked 9.1 m swath and the 0.58 liter/ha rate was flown using a marked 18.3 m swath width. Larval mortality was in excess of 95% in both trials (Sandoski, Yates, Meisch and Olson, personal communication).

The following discussion and Tables 1 and 2 detail our most recent work using the Beecomist system for aerial applications of *B.t.i.* in Chambers County, Texas. The primary purpose of these trials was to determine the relative effectiveness of Teknar AC and Vectobac Aqueous Suspension® (AS) using the Beecomist system. A secondary purpose was to obtain further data on the effective swath width using the Beecomist system and this application technique.

The trials were conducted on the Chambers County Airport property on July 19 and July 26, 1984. Test stations were established by driving 0.46 m wooden survey stakes approximately 0.15 m into the ground at 6.1 m intervals. There were 16 stations in each row. There were three rows of stakes. The rows were 3.1 m apart. Each row of stakes was 91.4 m long. The upwind station in each row was designated '0A,' '0B' and '0C,' respectively. The stations most distant from the flight path of the airplane were 91.4 m downwind and were designated '91.4A,' '91.4B' and '91.4C,' respectively. Krome Kote® cards, 12 cm X 12 cm, were stapled to each wooden survey stake with the shiny side of each card facing up. Following treatment the cards were removed, labeled and the numbers of droplets within a 11.25 cm diameter circle on each card were counted and recorded.

One 473 ml plastic tub containing approximately 355 ml of tap water and approximately 15 live first and second instar larvae of *Cx. quinquefasciatus* were placed near each of the wooden survey stakes prior to treatment. Following treatment, the plastic tubs were covered, removed and transported to the insectary. Ten plastic tubs containing approximately 15 live first through third instar *Cx. quinquefasciatus* larvae were maintained away from the test area and handled in the same manner as the test tubs. These 10 tubs served as checks. Larval mortality in the treatment tubs and control tubs was checked 24 hours posttreatment. All the larvae used in the trial were reared from egg rafts collected from one site in Wallisville, TX.

In applying the *B.t.i.*, the airplane made one pass perpendicular to the prevailing wind direction so that the Beecomist was directly over the stations identified as '0A,' '0B' and '0C', in Tables 1 and 2. The airplane flew at an airspeed of 177 km/hr and an altitude of 10.7 m for both trials. Since previous data had indicated that a swath width of 30.5 m was possible, the system was calibrated to deliver 0.59 liter/ha over a 30.5 m swath. A mechanical problem resulted in an application rate of 0.62–0.74 liter/ha (30.5 m swath) for Vectobac AS in the July 16 trial. Teknar AC was applied at 0.59 liter/ha (30.5 m swath) in the July 26 trial.

Vectobac AS was applied as described above at 0914 hr on July 19, 1984. The skies were clear at the time of application and the temperature was 25°C. The wind was from the north-northwest at 3.2–4.8 km/hr. The number of droplets collected on the Krome Kote cards at each station is shown in Table 1. The percent mortality of the larvae in the cups at various stations is shown in Table 2.

Teknar AC was applied at 0815 hr on July 26, 1984 in the same manner as the Vectobac AS had been on July 19. The winds were from the north-northwest at 3.2–6.4 km/hr. The temperature was 25°C and the skies were clear. The results are shown in Tables 1 and 2.

Table 1 gives the number of droplets of *B.t.i.*

Table 1. Number of drops of *Bacillus thuringiensis* var. *israelensis* within an 11.25 cm circle of Krome Kote paper exposed to Beecomist® application at Anahuac, TX, July 1984.

VECTOBAC AQUEOUS SUSPENSION® at 0.62–0.74 l/ha*

Row	Distance downwind of flight line of airplane in meters: 0	6.1	12.2	18.3	24.4	30.5	36.6	42.7	48.8	54.9	61.0	67.1	73.2	79.3	85.3	91.4
A	7	0	4	58	64	128	105	93	62	60	29	50	72	82	22	2
B	0	0	1	10	16	189	64	66	134	80	94	20	1	29	2	0
C	2	0	2	3	84	86	50	97	128	57	39	9	6	3	0	4
Avg	3	0	2.3	23.6	55	134.3	73	85.3	104.7	65.7	54	26.3	26.3	38	8	2

TEKNAR AQUEOUS CONCENTRATE® at 0.59 l/ha**

Row	Distance downwind of flight line of airplane in meters: 0	6.1	12.2	18.3	24.4	30.5	36.6	42.7	48.8	54.9	61.0	67.1	73.2	79.3	85.3	91.4
A	0	2	3	29	73	20	36	29	51	33	33	18	29	48	4	5
B	0	2	34	48	175	90	10	30	32	61	96	35	21	20	21	36
C***	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Avg	0	2	12.3	25.7	82.7	36.7	15.3	19.7	22.7	31.3	43	17.7	16.7	22.7	8.3	10.3

* Prior to treatment the system was calibrated to deliver 0.59 liter/ha, based on a 30.5 m swath. A mechanical problem resulted in an actual application rate of 0.62–0.74 liter/ha based on the 30.5 m swath. If a 91.4 m swath is assumed, the application rate was 0.21–0.25 liter/ha.

** Prior to treatment the system was calibrated to deliver 0.59 liter/ha, based on a 30.5 m swath. If a 91.4 m swath is assumed, the actual application rate was 0.20 liter/ha.

*** The cards in this row were labeled incorrectly in the field, so this data was thrown out. Computation in this trial is based on data in the two rows 'A' and 'B' only.

Table 2. Percent mortality of first and second instar *Culex quinquefasciatus* larvae retained in plastic tubs and exposed to *Bacillus thuringiensis* var. *israelensis* as applied by the Beecomist® system at Anahuac, TX, July 1984.

VECTOBAC AQUEOUS SUSPENSION® at 0.62–0.74 liter/ha*

Row	Distance downwind of flight line of airplane in meters: 0	6.1	12.2	18.3	24.4	30.5	36.6	42.7	48.8	54.9	61.0	67.1	73.2	79.3	85.3	91.4
A	0	0	100	100	100	100	100	100	100	100	100	100	100	100	100	73
B	11	0	100	100	100	100	100	100	100	100	100	100	100	100	0	23
C	0	0	0	0	100	100	100	100	100	100	100	100	100	100	78	33
Avg	3.7	0	66.7	66.7	100	100	100	100	100	100	100	100	100	100	59.3	43

TEKNAR AQUEOUS CONCENTRATE® at 0.59 liter/ha**

Row	Distance downwind of flight line or airplane in meters: 0	6.1	12.2	18.3	24.4	30.5	36.6	42.7	48.8	54.9	61.0	67.1	73.2	79.3	85.3	91.4
A	0	7	93	100	100	100	100	100	100	100	100	33	100	64	100	100
B	7	100	100	100	100	100	100	100	93	100	100	100	100	100	100	100
C	0	25	100	100	100	100	100	100	100	100	100	100	100	100	75	100
Avg.	2.3	44	97.7	100	100	100	100	100	97.7	100	100	77.7	100	88	91.7	100

Percentages shown are uncorrected for control mortality:
Control mortality for VECTOBAC AS trial was 2.9%
Control mortality for TEKNAR AC trial was 7.3%

* Prior to treatment the system was calibrated to deliver 0.59 liter/ha, based on a 30.5 m swath. A mechanical problem resulted in an actual application rate of 0.62–0.74 l/ha based on the 30.5 m swath. If a 91.4 m swath is asumed, the application rate was 0.21–0.25 liter/ha.

** Prior to treatment the system was calibrated to deliver 0.59 liter/ha, based on a 30.5 m swath. If a 91.4 m swath is assumed, the actual application rate was 0.20 liter/ha.

collected inside an 11.25 cm circle drawn on each Krome Kote card; 11.25 cm is the size of the opening in each plastic tub. Droplets of Teknar AC, in general, stained the Krome Kote cards more darkly than droplets of Vectobac AS. A droplet size analysis has not been completed. However, in general, the larger droplets were deposited near the flight path of the airplane and droplet size seemed to be progressively smaller downwind for both formulations. The Krome Kote cards on row 'C' of the Teknar test were labeled incorrectly when they were collected. Therefore, these data were not included in Table 1. The averages given in Table 1 for Vectobac AS include the average of rows 'A,' 'B' and 'C.' The averages for Teknar AC include only rows 'A' and 'B.'

Table 2 shows the percent mortality of the first through second instar *Culex quinquefasciatus* larvae 24 hours posttreatment. The control mortality for the Vectobac AS test was 2.9%. The control mortality for the Teknar AC test was 7.3%.

Much work remains to be done to determine the limits of this system in applying *B.t.i.* in ultra low volumes. However, the effective swath width seems to be at least 76.2 m with one formulation and the effective dose rate against first and second instar *Culex quinquefasciatus* seems to be below 0.59 liter/ha with at least one formulation (Teknar AC). Trials in Arkansas in July, 1984 (Sandoski, Yates, Meisch and Olson, personal communication) also indicate that applications of *B.t.i.* below 0.59 liter/ha are effective against natural populations of *Anopheles quadrimaculatus* in ricefields. Additionally, the field trials previously mentioned in both Texas (August and September 1982) and Arkansas (July 1983 and July 1984) indicate that *B.t.i.* applied through this system can penetrate thick vegetative cover in ricefields.

The Beecomist ultra low volume larviciding system can be adapted to almost any type of aircraft. All electrical components are available in either 12- or 24-volt units and almost any type of compressed gas system can be used as a propellent. The insecticide reservoir (beverage container) can be secured to the passenger seat of a small aircraft, to the landing gear of a rotorcraft or inside the hopper of agricultural type aircraft. Additional beverage containers can be added in series to increase the payload. All component parts are readily available and relatively inexpensive.

Field trials in Chambers County, Texas (1982, 1983) and Arkansas County, Arkansas (1983, 1984) indicate that the Beecomist ultra low volume larviciding system is an effective, low-cost alternative to conventional larviciding techniques in rice-fields. Employment of the system reduces the application costs by reducing the amount of active ingredient necessary for control and by eliminating the need for large volumes of carrier material. While much work remains to be done in testing the effectiveness of this system against other species of mosquitoes in other habitats, the system offers a highly acceptable alternative to more expensive conventional methods of applying *B.t.i.* in some situations.

References Cited

Anonymous. Date unknown. Operating manual for ultra low volume application of Ortho Dibrom 14 concentrate. Chevron Chemical Co., Ortho Div., Res. and Dev. Dep., Richmond, CA.

Anonymous. 1983. Mosquito control with biological larvicides. Pest Control Technol. 11(3):20–28.

Dearman, A. V., H. F. Powell and R. K. Thompson. 1965. Portable sprayer for aerial LVC application. J. Econ. Entomol. 58:1050–1052.

INVERSIONS IN SPECIES A OF THE TAXON *ANOPHELES CULICIFACIES*

FARIDA MAHMOOD[1] AND R. K. SAKAI[1,2]

Three sibling species have been cytologically identified in the taxon *Anopheles culicifacies* Giles (Green and Miles 1980, Miles 1981, Subbarao et al. 1983). Two fixed paracentric inversions on the X-chromosome and 2 on arm 2R differentiate the members of this complex. Unlike many of the other anophelines which have been examined cytologically, no floating inversions have been reported in the members of this species complex. This paper describes a number of paracentric inversions observed in species A of this taxon.

All the mosquitoes used in this study came from field populations from various localities in Pakistan. The ovarian polytene chromosomes were prepared according to the method of Saifuddin et al. (1978). The Sattoki colony

[1] International Center for Medical Research and Training, 6, A.R. Chughtai Road, Lahore 3, Pakistan.

[2] International Health Program, University of Maryland School of Medicine, 10 S. Pine Street, Baltimore, MD 21201.

Reprint requests to R. K. Sakai, HIH, Bldg. 5, Rm. 114, Bethesda, MD 20205.