search being carried out also needs to be augmented by introducing modern techniques and concepts. Scientists from advanced laboratories should be encouraged to spend sabbatical leave to work in collaboration with Chinese scientists.

On the other hand medical entomologists and vector control specialists from the developing countries should visit China to gain first hand experience of primary health care and individual and community participation at its best and especially the mass patriotic health movements. This cross fertilization at Technical Cooperation between Developing Countries (TCDC) level should greatly assist WHO in fulfilling its goal for "Health for All by the year 2000" based on Primary Health Care.

EFFECTIVENESS OF *BACILLUS THURINGIENSIS* SEROTYPE H-14 AGAINST *CULEX QUINQUEFASCIATUS* IN SMALL DITCHES

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ABSTRACT. *Culex quinquefasciatus* larvae were effectively controlled by applications of *Bacillus thuringiensis* serotype H-14. Two roadside ditches were treated every 1–4 days from August 16 through October 14, 1979 with ca. 0.6 gm/m^3 of a preparation assayed at 491 International Toxic Units per mg. A large, continuously ovipositing adult population maintained a newly hatching larval population throughout the test. A population of 4th instar and pupae developed in the untreated ditch as normally expected, but these stages were not produced in the treated ditches.

Culex quinquefasciatus Say is a serious pest in the southern United States both as an annoyance and as a potential carrier of disease (Chamberlain et al. 1959, Villavaso and Steelman 1968). Populations of *Cx. quinquefasciatus* in urban areas develop in drainage ditches polluted with various types of household and commercial organic waste products. Storm sewer construction also provides breeding habitats in cities. Higher costs of diesel oil as well as commercial larvicides and undesirable side effects have increased the need for alternative control agents. Williams and Palmisano (1981) reported satisfactory results in roadside ditches with methoprene and indicated costs would be lower than for diesel oil. Use of manure disposal lagoons from farm animals provides a breeding habitat that has increased in recent years (Axtell et al. 1975, 1980; Ruiz and Axtell 1978). The high organic content of these lagoons was suggested as a contributing factor for low efficacy of the chemicals at recommended label rates.

Bacillus thuringiensis var. *israelensis* (flagellar antigen serotype 14) (deBarjac 1978, Goldberg and Margalit 1977) was applied to two small ditches containing household effluent and *Culex quinquefasciatus* larvae. The test was conducted from August 16 through October 11, 1979 at Lake Charles, Louisiana. An adult population of mosquitoes provided large numbers of continuously hatching egg rafts throughout the test. The purpose of the test was to observe the effect of *B.t.* H-14 when applied on a regular schedule against a continuously hatching population of mosquitoes.

MATERIALS AND METHODS

The *B.t.* H-14 was prepared by H. T. Dulmage, ARS, USDA, Brownsville, Texas as the total solids from the fermentation batch and was designated as Batch HD500/R179. The material was bioassayed and compared to the material prepared by H. deBarjac, Institut Pasteur, designated IPS-78 (1000 I.T.U./mg). HD500/R179 was found to assay at a relative potency of 491 ± 57 I.T.U./mg against 2nd instar *Cx. quinquefasciatus* and had an estimated ED_{50} value of 0.29 ± 0.005 μg/ml.

Application was made by suspension of the powder in well water with a small amount of a non-ionic surfactant in a hand-carried (ca. 18 liter) tank. The tank was fitted to a CO_2 cylinder with a pressure regulator. The spray nozzle was a flat-spray type normally used in agricultural spray systems. Calibration of the system provided highly repeatable volumes delivered per time unit. The operator could measure the length of the ditch, practice walking the distance, and very accurately deliver the material in a uniform pattern along the length of the ditch.

The test sites were located about 3.2 km (2 miles) apart. Both were roadside ditches in residential areas, contained liquid matter from residential homes, and had a culvert covered by a driveway through which the water was carried by a concrete drain pipe approximately 50 cm in diameter. Continuous egg raft deposition by a large adult population was enhanced by these covered drain pipes as well as by the vegetation on the ditch borders. The Catalina Street ditch, 41 m long, 0.75 m wide at the water surface, and approximately 0.1 m deep at midwidth, contained an estimated 3.075 m³ of water, to which 1.7 gm of HD500/R179 was applied. The desired doage was 0.6 gm/m³, which was 20 × the laboratory assay ED_{50} value and about 9 × the ED_{90}. The intent was to apply 10 times the ED_{90} rate to avoid the possibility of using too low a dosage, thus preventing an adequate evaluation of the effect upon a continuously breeding population for the remainder of the season. Applications were made on Monday and Thursday during most of the test period. Actual dates are indicated on Fig. 1. The test site on Pear Street was 36 m long, 0.9 m wide, averaged about 0.15 m deep at midwidth, and contained an estimated volume which required 3.06 gm of HD500/R179 to achieve the 0.6 gm/m³ concentration. Thirteen applications were made at the Catalina Street site (22.1 gm total material). The normal expression of rate of application is in kg/ha of surface area. Water depth differences in this test resulted in a treatment rate of 0.55 kg/ha of estimated surface area at Catalina, and 0.94 kg/ha at Pear, even though the concentration per unit volume was almost identical.

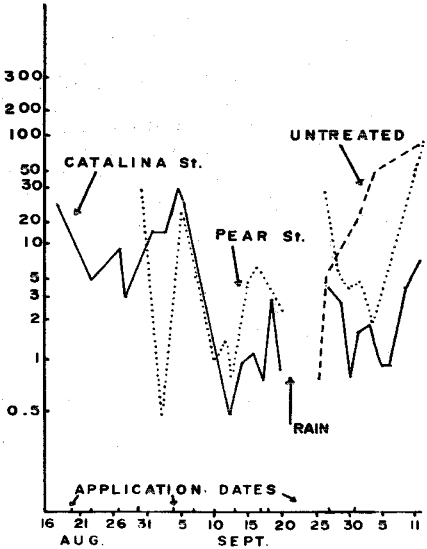

Fig. 1. Field test of *Bacillus thuringiensis* (serotype H-14) against *Culex quinquefasciatus* in small ditches at Lake Charles, LA (Vertical axis represents average number of 4th instar larvae and pupae per dipper).

An untreated control observation in a third ditch was added September 24, when a comparable population was found. This ditch was located across the road on Catalina Street and was caused by a 32 cm rainfall on September 19. That rain flushed all ditches so the test may be viewed in 2 time periods, (August 16–September 20 and September 24–October 11). The untreated ditch (Catalina-West) was about one-half the size of the Catalina Street treatment ditch. Daily collections were made from the ditches. Larval density per m^3 was calculated from the average number of larvae collected per dip with a long-handled, metal dipper that held about 330 ml when filled to the level commonly used when sampling water for mosquito larvae. Samples were taken at about 1-m intervals along each ditch. The instar composition of the populations was determined each time by examination of the larvae.

The effectiveness of the treatments and the possible deleterious effect of the ditch water on larvae and/or on the *B.t.* H-14 were assayed in the laboratory. All laboratory tests were conducted in wax-coated paper cups filled with 100 ml water and 20 larvae.[1,2] Three cups were used for each condition being assayed. They were maintained in a room at 25–28°C. Mortality was determined 1 day after initiation of the test. The persistence of the toxic action of *B.t.* H-14 was tested by assay of non-diluted ditch water collected at 1, 2 and 3 days post-treatment.

Samples of water and larvae were collected prior to application of the *B.t.* H-14 material. The effect of ditch water on larvae was monitored with laboratory well water and pre-treatment ditch water (0.0 dose) against both laboratory-reared and ditch-collected 2nd instar *Cx. quinquefasciatus* larvae. Interference by ditch water upon the effectiveness of *B.t.* H-14 was monitored by addition of a known dose to both well water and pre-treatment ditch water and assayed against 2nd instar larvae from both sources. The effectiveness of the applied *B.t.* H-14 was monitored by collection of ditch water immediately after the application and assaying against 2nd instar larvae collected prior to treatment. Treated field water was tested without dilution. These tests provided evaluation of any effects due to the ditch water, its effect upon *B.t.* H-14 or the larvae, and of the potency of the *B.t.* H-14 prior to application.

RESULTS

The field treatments were very effective in reducing the number of 4th instar larvae and pupae and thus effectively prevented adult emergence. The number of 4th instar and pupal stages per dipper are shown in Fig. 1 for the 3 ditches (Catalina Street, Pear Street and the untreated ditch). The continuous presence of egg rafts assured a large population of new larvae. Application of *B.t.* H-14 killed the 1st and 2nd instar larvae the first day but some 2nd and 3rd instar larvae survived and developed into 4th instar larvae and pupae before the next application. Counts of 1st instar larvae showed a dramatic decrease the day after treatment, and a dramatic increase each ensuing day prior to the next application. Against this background of a continuously developing large population of new larvae, the number of 4th instar larvae and pupae shows the excellent control attained with *B.t.* H-14. Large numbers of 4th instar and pupae would normally result from such a population, as is borne out by the data for the untreated ditch on September 24. One week later and after 2 treatments, the treated ditches had 1–2 4th instar larvae and pupae per dip, whereas the untreated ditch had an average of 35 4th instar larvae and pupae per dip. Therefore, the tests showed that *B.t.* H-14 has the potential to drastically reduce

[1] Southeast Cup Division, Maryland Cup Corporation, Cup S-303, 3-oz wax coated cup, holding 100 ml to the ridge formed for lid retention.

[2] Mention of a proprietary product does not constitute a recommendation or endorsement by the USDA.

emerging adult populations even with the presence of extremely large adult oviposition pressure. The oviposition rate, rate of larval development, and other population factors would influence the timing and frequency of *B.t.* H-14 application required to prevent emergence of a significant adult population in any specific situation. This test demonstrated the efficacy of *B.t.* H-14 when modest amounts of it are applied twice a week against very large and continuously breeding mosquito populations. The rates used in these tests (ca. 0.5 to 1.0 kg/ha) are in the midrange suggested by the manufacturers for their more potent formulations. Therefore, more potent commercial formulations would be expected to be effective at rates lower than those we used.

The laboratory assay data, summarized in Table 1, further support the efficacy of *B.t.* H-14, and also indicate a possible area of concern. The known dose in well water generally produced high mortality. However, a known dose in pre-treatment ditch water killed fewer larvae than it did in well water. This shows a reduced effect but does not reveal whether there was a direct action upon the toxic crystals or if ingestion by larvae was reduced, e.g., crystals were removed from the feeding zone, or by competition for feeding, i.e. selection of organic food and decreased intake of crystals. The variability of the mortality achieved in the pre-treatment ditch water to which a known dose was added further emphasizes the possible effect of water quality upon effectiveness of *B.t.* H-14. These data point out the advisability of conducting an assay with water from the test site.

Nearly all 2nd instar larvae in the treated ditch water were killed except upon 2 occasions when mortality was 84% and 87%. In the latter, Pear Street on August 31, the pre-treatment ditch water with a known amount of *B.t.* H-14 added had the least effect of any subsequently tested pre-treatment water. Thus, the amount applied to the ditch water was adequate to kill almost all 1st and 2nd instar larvae at the time of treatment. Dilution of the treated ditch water by ½ or 1/20 indicated some reduction in effec-

Table 1. Percentage mortality of *Cx. quinquefasciatus* exposed to *Bacillus thuringiensis* serotype H-14 formulation HD500/179 in water from ditches in laboratory assay at 24 hours post-treatment.

Date	Untreated field and well water	Well water[a] known dose added	Pre-treatment field water[a] known dose added		Post-treatment field water[b]	
			Catalina	Pear	Catalina	Pear
8/16	7	97	62	—	95	—
8/20	0	53	30	—	100	—
8/23	0	5	98	—	100	—
8/27	0	—	—	—	87	—
8/31	0	65	—	23	—	84
9/4	0	75	38	62	98	97
9/7	0	100	23	50	94	97
9/10	0	95	55	57	99	99
9/13	0	45	24	35	100	98
9/17	0	100	0	62	97	98
9/18	2	97	63	51	100	100
9/27	0	82	67	58	99	99
10/1	0	85	70	66	100	100

[a] Dose of 0.0469 μg/ml 8/16–8/27; 0.0938 μg/ml remainder of tests.

[b] Treatment applied at $\simeq$ 0.59 μg/ml. Estimate of water volume in ditch may have varied by at least 2x.

tiveness. Generally, the 1/20 dilution rate showed much less toxicity than the ½, indicating that the optimum effective dose was somewhere between 1/10 and ½ the rate applied in this test. Assay of the ditch water at full strength at 1, 2 and 3 days post-treatment failed to produce mortality. Observations of newly hatched larvae at 1 day post-treatment further indicated that the *B.t.* H-14 was in the effective feeding zone of the larvae less than 1 day and probably less than 12 hours.

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