PN-ABF-892

AGENCY FOR INTERNATIONAL DEVELOPMENT
PPC/CDIE/DI REPORT PROCESSING FORM

ENTER INFORMATION ONLY IF NOT INCLUDED ON COVER OR TITLE PAGE OF DOCUMENT

1. Project/Subproject Number: 936 5948

2. Contract/Grant Number: DPE-5948-C-00-5044

3. Publication Date: 10/20/89

4. Document Title/Translated Title:

5. Author(s)
1.
2.
3.

6. Contributing Organization(s):

7. Pagination: 18 pp.

8. Report Number: AR-120-4

9. Sponsoring A.I.D. Office: S&T/H

10. Abstract (optional - 250 word limit)

11. Subject Keywords (optional)
1.
2.
3.
4.
5.
6.

12. Supplementary Notes

13. Submitting Official: Robert W. Lennox

14. Telephone Number: 527-6500

15. Today's Date: 10/31/89

DO NOT write below this line

16. DOCID

17. Document Disposition: DOCRD [ ] INV [ ] DUPLICATE [ ]

AID 590-7 (10/88)

VECTOR BIOLOGY & CONTROL

Vector Biology & Control Project
1611 North Kent Street, Suite 503
Arlington, Virginia 22209
(703) 527-6500

Telex 248812 (MSCI UR)
Cable MSCI Washington, D.C.

PN-ABD-292
ISN 63838

Honduras: Field Evaluations of *Bacillus Thuringiensis* var. *Israelensis* (*Bti*) Against *Anopheles* Larvae

by

Edward D. Walker, Ph.D.

and

Lawrence A. Lacey, Ph.D.

AR-120-4

Managed by Medical Service Corporation International
under contract to the U.S. Agency for International Development

Authors

Edward D. Walker is a visiting assistant professor in the Department of Entomology, Michigan State University.

Lawrence A. Lacey is vector biologist for the Vector Biology and Control Project.

Acknowledgement

The authors gratefully acknowledge the support, assistance and cooperation of the following individuals: Engineer Jose Ruben Gomez, Chief of Operations, Division of Vector Control, Honduran Ministry of Public Health and Social Assistance; Rose Hicks and Robert Haladay, USAID/Tegucigalpa; and Julian Andino, Gerardo Alvarado, Manual Snadoval, Santos Rosales, Luis Reyes, Miguel Argueta, Efrein Mejia and Victor Verde of the Division of Vector Control/Honduras.

Preparation of this document was sponsored by the Vector Biology and Control Project under contract (No. DPE-5948-C-00-5044) to Medical Service Corporation International, Arlington, Virginia, USA, for the Agency for International Development, Office of Health, Bureau of Science and Technology.

Executive Summary

Concerns about environmental contamination, insect resistance, cost and other problems associated with conventional chemical insecticides has intensified the search for alternative interventions for mosquito control. The discovery and commercial development of the mosquito larvicidal bacterium Bacillus thuringiensis var. israelensis (Bti) have provided a highly selective and safe biological control agent that will rapidly kill mosquito larvae while leaving mosquito predators and other non-target organisms unharmed.

During the development of the Health Sector II project in Honduras, Bti was proposed as the biological control component of the malaria control program. Protocols that had been developed for implementation of Bti use were reviewed last December by VBC staff members Drs. Andrew Arata and Lawrence Lacey. Their recommendations included small plot evaluation of Bti against Anopheles albimanus prior to implementation of a pilot study to determine the feasibility of using this intervention for malaria control. USAID/Tegucigalpa requested that VBC provide technical assistance to the Division of Vector Control (DCV) for initial product evaluation, on-the-job-training and for the design and implementation of a pilot study. VBC provided two consultants, Dr. Lacey and Dr. Edward D. Walker to address the product evaluation and some aspects of training.

Drs. Lacey and Walker went to Honduras in May 1989 to conduct field evaluations of Bti as a larvicide of Anopheles vectors of malaria in Honduras. A team of Honduran workers of the Division of Public Health and Social Assistance, Republic of Honduras, was assembled through the auspices of Engineer Jose Ruben Gomez to participate in the project and to learn about the application and evaluation of Bti in the field. Five tests were successfully conducted in lagoons, ricefields, canals, and flooded pastures in Zamora, Catacamas, Portrillos, and Zamorano. Efficacy of Bti, calculated as percentage reduction in numbers of Anopheles, albimanus and Anopheles pseudopunctipennis larvae in comparisons before and after treatment, was very high. Both granular and liquid formulations of Bti were used in tests and a motorized backpack sprayer was evaluated as a tool for broadcasting both types of formulations into the larval habitats.

Introduction

Malaria progressively declined in the Republic of Honduras over the ten year period from 1978 to 1987. Passive case detection (thick blood films) with a volunteer collaboratory system revealed 34,000 cases in 1985, 29,000 cases in 1986, and 19,000 cases in 1987 (Arata and Lacey 1988). However, the incidence of malaria per 100,000 inhabitants in all 7 health regions of the republic in 1988 was 619.3 (Ministry of Public Health and Social Assistance, Department of Epidemiology, compiled data); conservatively estimating a national population of 4.65 million, this indicates an increase in 1988 to nearly 29,000 malaria cases. Apparently, malaria in Honduras continues to be dynamic and resilient, despite the control efforts of the DCV (i.e., Division of Vector Control, Ministry of Public Health and Social Assistance).

Table 1 shows the incidence of several different transmissible illnesses among ambulatory patients who presented for health care in 1985, 1986, and 1988. Although the ranking of such incidence data is suspect because of the lack of objective criteria, the relative incidence of several different transmissible illnesses among ambulatory patients who presented for health care in 1985, 1986, and 1988. Although the ranking of such incidence data is suspect because of the lack of objective criteria, the relative incidences do support the notion that malaria continues to be a major health concern in Honduras when compared with other prevalent, transmissible illnesses.

Table 1. Incidence* of transmissible illnesses in ambulatory patients seeking medical care, Honduras, 1985, 1987, 1988

	Year		
Illness	**1985**	**1987**	**1988**
Polio	0.1	0.2	0.1
Whooping Cough	7.7	11.1	4.9
Measles	148.1	30.9	24.1
Tuberculosis	49.2	84.7	77.1
Syphilis	98.7	125.5	85.7
Gonorrhea	161.8	183.4	142.4
Malaria	773.7	410.1	619.3
Dengue	7.0	4.3	20.0
Diarrhea	4,462.1	6,210.8	5,473.8
Respiratory Infections	7,530.5	13,839.4	14,425.0

*Expressed per 100,000 inhabitants in 7 health regions.

Local variations in malaria incidence occur in Honduras as a function of control efforts and successes. As a result, malaria remains highest in incidence where control is difficult for a variety of reasons such as limited resources, difficult access, reduced community compliance, insecticide resistance, lack of general sanitation, multiple breeding sites, and other factors. For example, data on variation in incidence of malaria in three geographically disparate areas of Honduras from 1984 to 1988 (Table 2) show decreases in two areas (Sonaguera and Catacamas) where Division of Vector Control activities have been high and relatively successful, and an increase where activities have been sporadic (Potrerillos). These areas happen to be sites of field trials reported here.

Table 2. Incidence of Malaria in Three Localities in Honduras, 1984–1988

Locality	Year 1984	1985	1986	1987	1988
Sonaguera					
Population	26,049	27,098	28,188	29,322	30,502
No. cases	791	1,329	988	359	278
Incidence*	30.4	49.0	35.4	12.2	9.1
Catacamas					
Population	59,474	62,753	66,214	69,866	73,721
No. cases	2,475	2,357	2,416	1,748	1,402
Incidence	41.6	37.6	36.5	25.0	19.0
Potrerillos					
Population	11,037	11,252	11,472	11,696	11,924
No. cases	66	112	105	144	374
Incidence	6.0	9.9	9.1	12.3	31.4

*Per 1,000 individuals

The concept and practice of larval mosquito reduction for malaria control is not new (WHO 1973). The development of environmentally safe, highly specific microbial insecticides to kill mosquito larvae (Lacey and Undeen 1986) has added to the battery of larvicides and physical larval control methods available to reduce larval population densities. One of these new agents, Bacillus, thuringiensis var.

israelensis serotype H-14, or Bti, is commercially available in granular, flowable concentrate (aqueous suspension), and sustained-release formulations. Field evaluations of Bti against Anopheles larvae in the United States have generally shown this material to be highly efficacious, even when low volumes have been applied aerially (Yates 1984, Lacey and Inman 1985, Sandoski et al. 1985, 1986). Although laboratory evaluations of Bti against Anopheles, albimanus have been conducted (e.g., Perich et al. 1987), no field evaluations have been done in areas endemic for malaria in Latin America.

This report summarizes results of field trials conducted with Bti, against Anopheles in Honduras during May 1989. Dr. Lacey participated int he activities from May 1 to May 11, while Dr. Walker participated (as a consultant to VBC) throughout the month. The purpose of the activities was not merely to test the efficacy of Bti, but more broadly to train DCV workers in the use, formulation, application, and follow-up evaluation of the material, with the idea that Bti may ultimately become a component of an integrated malaria control program. Through the cooperation of Engineer Jose Ruben Gomez, Chief of Operations of DCV, a team of enthusiastic DCV workers was assembled to work and learn with Drs. Lacey and Walker during the month-long project.

Field Trials: Materials and Methods

Bti

The formulations of Bti used were as follows: (1) Vectobac-G (Abbott Laboratories), a 4-8 mesh corncob granule formulation with 0.2% active ingredient (A1) absorbed onto the granules, giving 200 international Toxic Units (ITU) per milligram; (2) Vectobac-12AS (Abbott Laboratories), an aqueous suspension formulation containing 1.2% A1 in suspension with 1200 ITU per milligram; and (3) Teknar HP-D (Zoecon Corporation), an aqueous suspension formulation containing 1.6% AI in suspension, with 3000 ITU per milligram.

Zamora Field Trial

This trial was conducted on May 6-7, 1989, at a lagoon surrounded by an African oil palm planting, near the town of Zamora (vicinity of Tocoa, Department of Colon). The vicinity is heavily agricultural consisting of pastured cattle and banana and African oil palm plantations.

A motorized backpack sprayer was calibrated for rate of output of the corncob granules as follows. Several 1-m^2 squares of black plastic were cut and laid on the ground in a 10 m x 20 m area near the lagoon. The sprayer was loaded with granules and started, and an applicator (Walker) passed over the area, spraying granules as in an actual application onto water. Several passes were made and the granules were collected and weighed. The rate of

output for granules from the sprayer was 10 Kg/ha, determined after converting mass of granules collected per m^2 to mass per ha.

The team measured four 10 m x 20 m plots in the vegetated margins of the test lagoon. One plot was designated to receive a rate of 5 kg of granules per ha, another plot was designated to receive 10 kg/ha, while a third plot was designated to receive 20 kg/ha. The fourth plot, distant from the first three, served as a check (i.e., untreated reference) to treated plots. Pre-treatment dips (50 per plot, standard pint dipper) were taken, and the number of culex and Anopheles larvae per dip were counted and recorded. Then the applications over each plot were conducted by Walker. Tests with liquid Bti formulations were not made at this site.

Post-treatment dips (at least 50 per plot) were taken on May 7, 24 hours after application. The efficacy of each rate of application was calculated as follows:

$$\text{Percentage reduction} = 100 \times \left[1 - \frac{T_a \times C_b}{T_b \times C_a}\right],$$

Where T_a is the average of the dip counts post-treatment in the treated plot, T_b is the average of the dip counts pre-treatment in the treated plot, C_a is the average of the dip counts post-treatment in the check plot, and C_b is the average of the dip counts pre-treatment in the check plot. This simple formula corrects for changes in the check plot that presumably were not due to any insecticide effects and would have also occurred in the treated plot.

Catacamas Field Trial

Catacamas (Department of Olancho) lies in a river valley surrounded by mountains, and the city's environs are heavily agricultural. Cattle and cattle tanks are common, as is production of rice, beans and cane. Lumbering and wood processing also takes place in the area. New, primitive housing (unfired mud bricks) is being constructed on the outskirts of the city, but some of the new neighborhoods appeared unoccupied or abandoned. The local DCV activities include residual house spraying, surveillance and mapping of larval sites, and physical larval control measures. Even though it was very hot and dry during this visit, physical larval control projects were underway.

On May 11 and 12, a team of Honduran DCV workers and Walker explored the Catacamas area (including the village of Siguate) for potential larval sites. Because of the prevailing dry weather, many mapped sites were completely dry or the water was low and very organic and no Anopheles larvae were found. Twenty-two cattle tanks (of a potential 82 on DCV maps)

were sampled with no positive tanks found. On May 12, two positive tanks (breeding sites 42, DCV Region VII, Area 2 codes) were found, containing low populations of Culex, Uranotaenia, and Anopheles larvae amongst a narrow corona of vegetation. We also explored the campesino agricultural cooperative called Villa Linda on the northeastern side of the city. At this cooperative we found 41.9 ha of irrigated rive fields (breeding site 11, DCV Region VII, Area 2 codes). The fields were carefully laid out into rectangular parcels of 0.23 ha each, and were tiered to receive irrigation water. The irrigation system consisted of a series of channels, dikes and sluiceways carrying water from a nearby river. The levees between the individual rice parcels were intercropped with cane, corn, beans and peppers. We learned that three of the ricefields were permanent wet during the irrigation process because of their position relative to the irrigation system and the other fields. We sampled these fields and found numerous Anopheles and Psorophora larvae.

We obtained permission to work from the president of Villa Linda cooperative, Sr. Esteban Ramirez, and began to design a Bti test in the Villa Linda ricefields on May 16. Each ricefield (measuring 17.7 m x 128.1 m) was divided into three plots of dimensions 17.m x 42.7 m, yielding a total of 9 plots. The 3 plots of each field were randomly assigned to be treated either with granular formulation of Bti (Vectobac-G), a liquid formulation (Vectobac-12AS) or to be left untreated as checks. Two teams of workers (3 persons per team) collected and recorded pre-treatment dip counts. For each plot, one person from each team recorded data, another person collected 25 dips from the levee, and the third person walked in the ricefield and collected 25 dips. Fig. 1 shows the sampling scheme. Teams worked on opposite sides of each plot, so that 100 dips per plot were taken, for a total of 900 pre-treatment dips.

Applications were conducted on May 17. The backpack sprayer continued to work well for the granular formulation, and only a single rate of application (10 kg/ha) was chosen for the test. Three Honduran workers practiced application of granules by backpack sprayer first on dry ground and then in plots designated for granular treatment. We lacked essential components to convert the machine for liquid application, therefore we used a Hudson can for liquid application. Because each plot was 0.07 ha (0.20 acre) and we desired a rate of 1 liter of formulated liquid per acre, we mixed 200 milliliters with sufficient water (ca. 10 liters in the Hudson can to cover completely a plot (0.2 liter/0.20 acre = 1 liter/1.0 acre). For each plot designated to receive a liquid treatment, the Bti/water mixture was applied with a flat-fan nozzle at the level of the rice heads until the can was empty. Walker and an older, more experienced DCV worker (Julian Andino) acted as guides during the applications.

Post-treatment dip counts were taken identically as pre-treatment dip

counts (total, 900 dips) 24 hours after application on May 18. Efficacy of application of the different formulations was calculated as before, except that the plots (rather than the dips) became the basic unit of replication.

We returned to lagoons 42 and 43 on May 18 to conduct more tests in the Catacamas area. but because these lagoons had dried considerably and there were no larvae in dip samples, no further tests in Catacamas were conducted.

Potrerillos Field Trials

The team that had conducted the Catacamas work, with the addition of DCV worker Luis Reyes, initiated field trials in Potrerillos (Department of Cortes) on May 23. Potrerillos is a heavy agricultural area, with local production of cattle, cane, bananas and pineapple. We found a canal in the Pedrera vicinity of Potrerillos that came from nearby Rio Blanco. This canal (ca. 30 m wide) was largely open water, with shorelines patchily bordered with floating vegetation. Among these patches of vegetation were high numbers of Anopheles and Culex larvae. A narrow (7-8 m) perpendicular branch of the canal, choked with Pistia, led to an extensively flooded pasture (>50 ha). The branch and the pasture also had Anopheles and Culex larvae, but these were scattered and in low numbers.

We initiated a field trial in the canal on May 23 and 24. The vegetated patches were divided into parcels of 16 m in length with an arbitrary 5 m width into the canal. Actually, most of the patches were less than 5 m in width, ranging from 2 to 5 overall.

Nine patches were identified. Three pateches were designed to receive a treatment with liquid Bti (Vectobac-12AS), three were designed to receive a treatment with another liquid Bti formulation (Teknar HP-D) and three were to serve as controls. The treatments were randomized among the positions of the patches. Teams of samplers and data recorders took 50 dips within each patch and recorded the number of Anopheles and Culex larvae.

We had to test and calibrate the motorized backpack sprayer for liquid application prior to actually applying the Bti to the patches. As mentioned above, we lacked essential components to convert the hopper of the sprayer for liquid, but we successfully "jury-rigged" the hopper with styrofoam parts cut from packing stock. After this conversion, we calibrated the sprayer with water by determining the volume sprayed at full power in one minute (result, one liter). Because each patch was 16m x5m (0.008 ha or 0.02 acre) and we wished to apply 1 liter of mix over each plot for one minute, we mixed 20 ml of formulation with 1 liter of water (0.02 liter/0.02 acre = 1 liter/acre).

Applications were made on May 24 by four different Honduran members of the team after each had taken the opportunity to apply water through the backpack sprayer in practice runs over land. The sprayer created a fine mist

which emitted from the wand tip (equipped with a wind-powered fan) to a maximum distance of about 5 meters. We did not test the sprayer without the fan, but the plume distance possibly could have been increased if the fan had been removed.

Post-treatment dips (50 per plot) were taken on May 25 in the same manner as pre-treatment dips, and the number of Anopheles and Culex larvae counted. Percentage reduction was calculated as before.

A second field trial in Potrerillos was initiated on May 25 in a portion of the flooded pasture. Six small, contiguous 10 m x 10 m plots were measured in an area with mosquito larvae and floating vegetation (including Pistia) in knee-deep water (schematic in Fig. 3). The team took 25 pre-treatment dips within each plot (May 25), and conducted the number of Anopheles larvae. Then three Honduran members of the team applied granular Bti at a rate of 10kg/ha to three of the plots, while the other three plots were left untreated. Post-treatment dips (25 per plot) were taken on May 26, and percentage reduction calculated as before.

Zamorano field trial. Zamorano (about 1 hour drive from Tegucigalpa) is in a high, dry, farmed valley surrounded by mountains. The team visited the Pan-American Agricultural School there on May 29 to inspect aquaculture ponds for Anopheles larvae for possible field trials. Of 8 ponds and one large lagoon inspected, one pond (20.3 m x 26.2 m) had a population of Anopheles and Culex larvae in the vegetated margin (a narrow band less than 1 meter in width). We took pre-treatment dips on May 29, returned on May 30, and applied liquid Bti (Vectobac-AS) at a rate of 1 liter/acre with a Hudson can to the vegetated margin. We calculated that 11 ml of liquid (pre-mix) was required, given that the area of the margin (assuming a width of 1 m) was 46.5 m^2 (0.0047 ha or 0.011 acre): 0.011 liter/0.011 acre = 1 liter/acre. Application was made with a Vectobac/water mixture until the Hudson can was empty.

Honduran members of the team returned to the site on May 31 and took post-treatment dips (24 hours after treatment), but Walker did not participate in this trip because of his return to the United States. At the time of this writing, the post-treatment data have not arrived for evaluation.

Results

Zamora

Although Anopheles darlingi has been collected in the northern coastal zone of Honduras, the Anopheles larvae we collected at Zamora were all An.albimanus; nor were An. pseudopunctipennis collected at this site.

Results of pre- and post-treatment collections of Anopheles and Culex larvae at Zamora are shown in Table 3. Percentage reduction was 100% for all three application rates for larvae of both genera (5, 10, and 20 kg/ha) with the exception the 10 kg/ha plot, where a single live Culex larva collected after treatment lowered the percentage reduction to 95%.

Table 3. Total numbers of Anopheles and Culex larvae collected and percentage reduction of these larvae in a Bti field trial (corncob granules) at Zamora, Department of Colon, Republic of Honduras, May 6-7, 1989.

Rate of application	No. larvae in pre-treatment dips		No. larvae in post-treatment dips		Percentage Reduction	
	Anoph.	Cx.	Anoph.	Cx.	An.	Cx.
5 kg/ha	19	26	0	0	100	100
10 kg/ha	28	188	0	0	100	100
20 kg/ha	21	9	0	1	100	95
Check	3	14	11	31	----------	

Catacamas

Larvae at the Villa Linda ricefields in Catacamas consisted of a mixture of Anopheles pseudopunctipennis Anopheles albimanus, and Psorophora confinnis. Numbers of larvae in pre- and post-treatment collections, and results of Bti treatment (liquid and granular formulations) expressed as percentage reduction, are shown in Table 4. In general, larvae were abundant in these ricefields. Before application, Anopheles larvae averaged about 0.87 larvae per dip. After application, Anopheles larvae averaged 0.02 per dip in plots treated with granules and 0.03 per dip in plots treated with liquid. A greater degree of control (expressed as percentage reduction) occurred with Anopheles larvae (97.2% for granules, 94.4% for liquid) than with Psorophora confinnis larvae (72.8% for granules, 79.3% for liquid).

Table 4. Total numbers of Anopheles and Psorophora larvae collected in a Bti field trial (corncob granules and liquid formulations) at Catacamas, Department of Olancho, Republic of Honduras, May 16–18, 1989

Formulation	No. of larvae in 300 dips from pre-treatment plots		No. of larvae in 300 dips from post-treatment plots	
	Anoph.	Pso.	Anoph.	Pso.
Corncob	266	440	5	92
Liquid	259	495	10	79
Check	260	461	179	355

Potrerillos

Anopheles larvae at the canal in Pedrera, Potrerillos, were very abundant. Prior to treatment, there was an average of 4.2 Anopheles larvae collected per dip. Only An. albimanus larvae were collected; no An. pseudopunctipennis, An. darlingi or other Anopheles were found. Culex larvae were also very abundant, with an average of 5.28 larvae per dip before treatment.

Results of the first field trial at the canal in Potrerillos are shown in Table 5. These results were confounded by drastic reductions in numbers of Anopheles larvae, but not Culex larvae, in the check as well as treated plots. We believe this occurred owing to contamination of the reference plots, which were intermixed with the treated plots (see Fig 2 and Discussion). Consequently, results of percentage reduction calculations must be viewed with caution and interpreted conservatively. For Teknar HP-D solution, the Anopheles larvae were reduced by 79.3% and the Culex larvae were reduced by 82.1% For Vectobac-AS solution, the Anopheles larvae were reduced by 100% and the Culex larvae by 92.3%.

In the second field trial in the flooded pasture at Potrerillos, the total number of Anopheles larvae collected in pre-treatment dips in plots designated to receive granules was 117 (75 total dips in three plots) and in check plots was 97 larvae. After application, there was a total of two larvae collected in all three treated plots in 75 dips, and 23 larvae collected in the three check plots. Percentage reduction, calculated from data, was 92.5%.

Table 5. Total numbers of Anopheles and Culex larvae collected in a Bti field trial (two liquid formulations) at Potrerillos, Department of Cortes, Republic of Honduras, May 16–18, 1989

Formulation	No. of larvae in 150 dips from pre-treatment plots		No. of larvae in 150 dips from post-treatment plots	
	Anoph.	Cx.	Anoph.	Cx.
Teknar	629	641	4	154
Vectobac	605	999	0	104
Check	618	735	19	735

Zamorano

In the field trial Zamorano, 101 pre-treatment dips in the single test pond yielded 326 Anopheles larvae (3.23 larvae per dip). These larvae were a mixture of An. albimanus and An. pseudopunctipennis. Post-treatment data were not available at the time of this writing.

Discussion

The prevailing dry weather conditions during the month-long work period hampered the field trials because of the difficulty of locating suitable test sites. Consequently, a great deal of time had to be spent searching for larval breeding sites. However, we successfully conducted five field trails despite the dry conditions.

The field trials we report on here share in common the characteristics of being 24 hour tests designed to examine the acute effect of Bti application on Anopheles and other mosquito larval populations. We did not study, for example, the recovery of Anopheles at the different test sites for several days after treatment, to determine the duration of the treatment effect. However, each test definitively showed that Bti was very effective in reducing Anopheles (as well as other mosquito) larvae to low levels. The field tests were done in a sufficient variety of habitats (ricefields, lagoons, a canal, flooded pasture), indicating that Bti can have broad application to the diverse habitats in which Anopheles larvae are found in Honduras. In general, the tests indicate that both granular and liquid formulations of Bti worked equally well at high rates of application.

The results of the test with liquid formulations in the canal at Potrerillos indicated that contamination of check plots had occurred. This contamination apparently only affected Anopheles larvae, as there was no concomitant reduction of Culex larvae in the check plots. We suspect that drift of Bti on the surface of the water, owing to gusts of wind during the test, may be the cause of the contamination. We do not think that air-borne contamination during spraying occurred because check and treatment plots were separated well enough to prevent this obvious problem.

The two malaria vector species that occurr in the test sites, An. albimanus and An. pseudopunctipennis, were both affected by Bti treatment. The previous lack of field trials of Bti against An. albimanus, the most important vector of malaria in Central America, demonstrates the uniqueness and importance of our results: An. albimanus is susceptible to Bti under field conditions, supporting the premise that Bti application, in particular whether lower rates of application produce an acceptable percentage reduction of larvae. We used high rates of application. Both granular and liquid rates could possibly be reduced by 50 to 75% with good results.

Conclusions and Recommendations

1. Bti was shown to be effective against larvae of Anopheles albimanus and An. pseudopunctipennis in natural habitats in areas of endemic malaria in Honduras. This information precedes any further, area-wide evaluations of Bti as a tool in malaria control.

2. A team of Honduran workers of the DCV successfully participated in the Bti trials, and learned about formulation, field application and follow-up evaluation of Bti during the field trial experiences.

3. We strongly recommend that this team remain intact and administratively dedicated to a Bti project. This team consists of Julian Andino (experienced driver and field worker), Gerardo Alvarado (auxiliary entomologist), Luis Reyes Saa (driver, field worker, and enthusiastic amateur entomologist), Manuel Sandoval (experienced, reliable field worker), and Santos Rosales (experienced, reliable field worker).

4. The team should continue to practice with backpack sprayer, and in particular should experiment with liquid Bti mixes can be used, with motorized sprayers, to achieve good larval control in typical Anopheles habitats such as cattle tanks and ricefields.

References cited

Arata, A.A. and L.A. Lacey. 1988. Review of protocol for the use of Bacillus thuringiensis var israelensis (Bti) for malaria control in Honduras in the Health Sector II project, December 12-16, 1988. Draft report, Vector Biology and Control Project, 17pp.

Lacey, L.A. and A. Inman. 1985. Efficacy of granular formulations of Bacillus thuringiensis (H-14) for the control of Anopheles larvae in rice fields. J. Amer. Mosq. Control Assoc. 1:38-42.

Lacey, L.A. and A.H. Undeen. 1986. Microbial control of black flies and mosquitoes. Annu. Rev. Entomol. 31:265-296.

Sandoski, C.A., M.W. Yates, J.K. Olson, and M.V. Meisch. 1985. Evaluation of Beecomist applied Bacillus thuringiensis (H-14) against Anopheles quadrimaculatus larvae in rice fields. J. Amer. Mosq. Control Assoc. 1:316-319.

Sandoski, C.A., W.C. Yearian, and M.V. Meisch. 1986. Swath width determination for Beecomist applied Bacillus thuringiensis (H-14) against Anopheles quadrimaculatus larvae in rice fields. J. Amer. Mosq. Control. Assoc. 2:461-468.
World Health Organization. 1973. Manual on larval control operations in malaria programmes. WHO, Geneva, 199pp.

Yates, M.M. 1984. A portable system for aerial applications of very low volumes of technical grade concentrates of Bacillus thuringiensis var israelensis. Mosq. News 44:583-587.

Appendix A

Itinerary for EDW Trip to Honduras, May

May 1	Evening meeting with Humberto Cosenza, Department of Microbiology, National Autonomous University, about laboratory colonization of mosquitoes for DCV use
May 2	Briefing by USAID Tegucigalpa (Robert Haladay, Ross Hicks); meeting and introductions with Jorge Rubio (Director, DCV) and Jose Gomez (Chief of Operations, DCV).
May 3	Traveled to San Pedro Sula.
May 4	Traveled to La Ceiba; met with Luis Rivera (DCV entomologist); met with Joaquin Vargas (Chief of DCV Region VI); traveled to Olanchito.
May 5	Explored Sonaguera-Tocoa area for Bti test sites; established initial test site near Tocoa, at Zamora.
May 6	Calibrated motorized backpack sprayer for granular application at Zamora; measured plots, conducted pre-treatment sampling.
May 7	Conducted post-treatment sampling at Zamora; travelled to Trujillo.
May 8	Met with DCV workers in Trujillo. Travelled to La Ceiba, discussed results with Joaquin Vargas. Travelled to Tela and El Progreso in search of more sites; paid courtesy call to Enrique Hill at Rockefeller Foundation's Dengue project center at El Progreso; travelled to San Pedro Sula
May 9	Travelled to Potrerillos to examine potential test site; traveled to Tegucigalpa.
May 10	Toured university facilities of Humberto Cosenza and discussed establishment of laboratory colonies of mosquitoes.
May 11	Travelled to Olancho; discussed Bti project with Celso Gutierrez (Chief of DCV Region VII); travelled to Catacamas.
May 12	Searched Catacams and vicinity for potential test sites.
May 13	Travelled to Tegucigalpa.

May 15	Travelled to Catacamas; discussed Bti project with Rudy Ordonez (head of DCV Region VII, Area 2).
May 16	Set up field experiment in Catacamas rice fields.
May 17	Continued experiment in Catacamas rice fields.
May 18	Continued experiment in Catacamas in rice fields.
May 19	Travelled to Tegucigalpa.
May 20	Met with Jose Gomez, discussed Catacamas tests.
May 22	Travelled to Potrerillos, searched for test sites.
May 23	Initiated Bti test in Pedrera neighborhood of Potrerillos.
May 24	Continued test in Potrerillos.
May 25	Continued test in Potrerillos; initiated another test in Potrerillos.
May 26	Concluded second test in Potrerillos; travelled to Tegucigalpa.
May 27	Report writing.
May 28	Report writing.
May 29	Travelled to Zamorano, initiated test at Pan-American Agricultural school; returned to Tegucigalpa.
May 30	Travelled to Zamorano, continued test; returned to Tegucigalpa; briefing with USAID/Tegucigalpa (Ross Hicks).
May 31	Travelled to United States.
June 1	Briefing with VBC Project at Arlington.

Appendix B

List of persons contacted

Dr. Jorge Rubio	Chief, Division of Vector Control
Engineer Jose Gomez	Chief of Operations, DCV
Dr. Luis Rivera	Entomologist, DCV
Mr. Joaquin Vargas	DCV, La Ceiba
Mr. Pedro Medina	DCV, Trujillo
Mr. Enrique Hill	Johns Hopkins University Project, Dengue Control, El Progreso
Dr. Humberto Cosenza	Department of Microbiology National Autonomous University, Tegucigalpa
Mr. Ross Hicks	USAID, Health Sector II project, Tegucigalpa
Mr. Robert Haladay	USAID, Chief, Health and Population, Tegucigalpa
Dr. Ernesto Antonio Pinto	USAID, Health Sector II project officer
Celso Gutierrez	DCV, Juticalpa
Rudy Odonez	DCV, Catacamas
Servando Ortega	DCV, San Pedro Sula

Appendix C

Selection of Areas for Large-Scale Field Trials of Bti

After the most appropriate formulation and application techniques have been selected, large-scale field trials will be conducted in endemic malarious areas to determine the potential for using Bti for larval control as a means of suppressing malaria transmission Sites should be selected that permit easy access to all breeding sites and the sites should not be so numerous that a ground-applied formulation is not feasible. Selection of a treatment regime should be based on the need to threat (i.e. the appearance of first and second instar larvae). A sequential sampling system is recommended to determine when application should be made. Selected treated and untreated areas should also be used for entomological assessment of the effects of Bti treatments. In addition to larval sampling as proposed in the small plot section of the Arata-Lacey report (Ar-105-4), baited light traps could also be used.

As the area under control expands, careful collection malariometric information will help determine if this intervention is suitable as the main control method for the suppression of malaria transmission.