CONTROL OF ANOPHELINES IN CANAL ZONE JUNGLES WITH ULV AERIAL APPLICATIONS OF FENTHION [1, 2]

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Abstract. A 20 square mile plot of jungle terrain in the Canal Zone was treated twice with fenthion applied by aircraft at the rate of 0.075 pound per acre. A swath width of 0.4 mile was used. The second application was made 9 days after the first in order to kill new larvae before they pupated, and new adults before they oviposited. The predominant anopheline species in the test were *Anopheles albimanus* Wiedemann and *Anopheles triannulatus* (Neiva and Pinto). Initial control in excess of 95 percent with each application of fenthion resulted in overall population reduction greater than 81 percent for 31 days following the second application, 1.0 to 1.3 miles from the breeding source, and in excess of 75 percent for 24 days 0.1 to 0.5 mile from the breeding source. *Anopheles* migration from the breeding source to a point 1.3 miles away required only 3 days, but extensive migration for distances in excess of 0.5 mile was not indicated.

Control of anopheline mosquitoes in jungles is uniquely difficult because the insecticide must somehow be directed into the protected habitats. Therefore, during the past several years, we have been investigating the problem in the jungle of the Panama Canal Zone. Mount *et al.* (1970b), in 1969, found that ultralow volume (ULV) aerial sprays of malathion and fenthion applied at rates of 0.456 and 0.1 pound per acre, respectively, gave fair to excellent control. However, applications were made as drift sprays over relatively small areas so reinfestation was rapid; also only about 50 percent of the insecticide penetrated the jungle canopy. (No droplets over 68 microns were collected under the canopy; and droplets as large as 152 microns were found in open areas.) Thus, the evidence of the 1969 tests suggested that good control of anophelines might be attainable if a much larger portion of the jungle, including the major breeding areas, was treated, so that reinfestation from outside the treated portion would be minimum. We therefore conducted another test in 1970 in the same general area of the Canal Zone to evaluate this hypothesis. Fenthion was chosen as the insecticide because the effective dose against adult mosquitoes was about one-fifth the effective dose of malathion and because fenthion is an effective larvicide. Two applications were made. We theorized that since fenthion would not kill any pupae or eggs, one application would not give control for any length of time because adults and larvae emerging from the untouched stages would rapidly reinfest the area. A second application, if it was made within 8 to 9 days, would kill new larvae before they pupated and most adults before they oviposited. Finally, we felt that the test area should be treated rapidly and just before dusk so the concentration of insecticide would be highest at the time the mosquitoes were leaving their resting sites. Thus, we decided to use the drift spray technique to obtain wide swaths (0.4 mile).

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and the Environmental Health Laboratory, U. S. Army Forces Southern Command, Ft. Clayton, Canal Zone. The Special Aerial Spray Flight of the 4500th Air Base Wing (TAC), U. S. Air Force, Langley Air Force Base, Virginia, supported the test by supplying the aircraft and crew that made the applications of insecticide. Edward R. Ladd and Ronald E. Gumtow, Bureau of Sport Fisheries and Wildlife, U. S. Department of Interior, made independent studies of the effects of the ULV sprays on mammals, birds, bats, fish, and other aquatic organisms. They noted cholinesterase depressions in some animals but observed no obvious gross effects. Their detailed findings will be reported elsewhere.

DESCRIPTION OF TEST AREA

The site selected for the test was shaped like a parallelogram, 4 miles wide and 5 miles long (see Fig. 1); thus the total area was about 20 square miles or 12,800 acres. All or parts of several bays and basins of Gatun Lake were included. Most of the margins of the bodies of water were lined with aquatic vegetation, primarily *Elodea,* and these dense mats of floating foliage served as excellent breeding places for anopheline and other types of mosquito larvae because the vegetation sheltered them from fish predators and prevented excessive wave action; also, the water over the vegetation was warm, which promoted rapid larval growth.

The land area was heavily forested and was classified as modified rain or tropical monsoon forest. Several layers of vegetation are present within the forest, though they are not always readily apparent because of the hilly terrain (elevation ranges from 90 to 450 feet above sea level), and

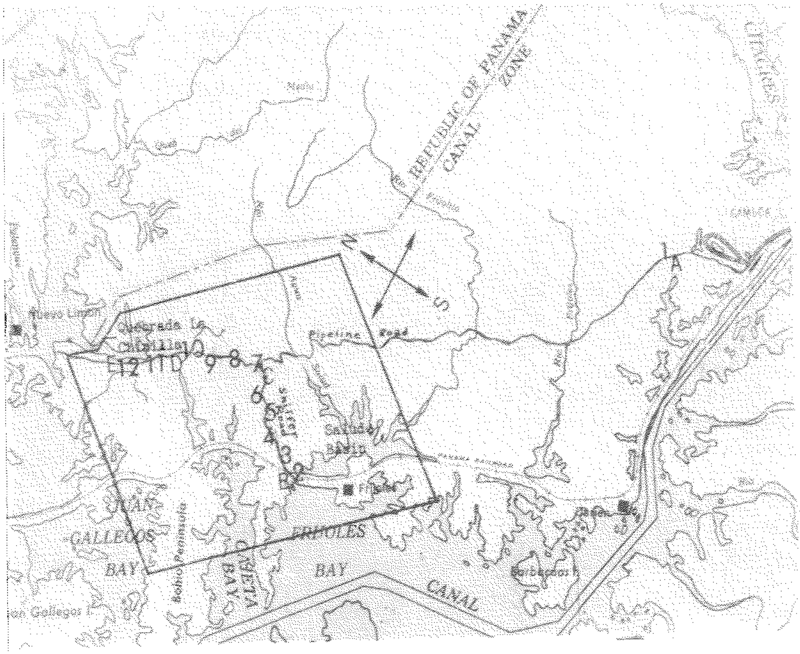

FIG. 1.—Map of central portion of Canal Zone showing area treated with fenthion and locations of man-biting stations (numbers) and horse-baited traps (letters). (Scale 1:100,000)

a great variety of plant species is represented. The large trees forming the upper canopy range from about 90 to 150 feet tall, and below them is a layer of palms and smaller trees, from 60 to 90 tall. Occasionally, a third layer of small palms, trees, and shrubs is evident.

The Gamboa Pipeline Road (a paved, all-weather road) crosses the plot from southeast to northwest on a line approximately 1 to 1½ miles from the northeast border; it is the only access road. The Panama Canal Railroad also traverses the plot on a line approximately parallel to Pipeline Road but 1 to 2 miles from the southwest border. An unpaved road (Sniffer Road) runs between Pipeline Road and the railroad in about the center of the plot.

The site used as the check (collections of adult mosquitoes) was located on Pipeline Road near Gamboa about 5 miles from the southeast border of the test area.

EVALUATION TECHNIQUES

Because of the extremely limited access to the test area, the mosquito populations could not be randomly assayed over the entire test plot. Therefore, to compensate and to enhance our evaluation, we used five techniques of evaluation in the areas that were accessible: man-biting collections, horse-baited traps, larval collections, gonotrophic aging, and bioassays. This utilization of manifold assays also permitted us to derive some information concerning the biology of the mosquitoes from the data.

MAN-BITING COLLECTIONS. Man-biting collections of female mosquitoes were made between 1800 and 1900 hours at one station in the control area and at 11 stations along Pipeline and Sniffer Roads. The stations in the test area were about ½ mile apart and were located at the edge of the jungle or just under the jungle canopy at distances of 300 to 6500 feet from the breeding areas (see Fig. 1). A single individual at each station collected the mosquitoes with a battery-operated aspirator modified from a 3-cell electric vacuum cleaner as they landed on his bare legs. Then the collections were returned to the laboratory where the specimens were identified. Three such collections were made before the first application of insecticide and 13 during the 40-day posttreatment evaluation period (Oct. 21 to Dec. 1, 1970).

HORSE-BAITED TRAPS. Four horse-baited traps were operated in the test area and one in the check area (see Fig. 1). These traps were similar to those described by Bates (1944). A horse, which was kept in a pen near each trap, was placed in the traps late in the afternoon when an assay was to be made and returned to the pen the following morning. All the mosquitoes in the trap were then collected with an aspirator and returned to the laboratory for identification. The assays were made on about the same schedule as the man-biting collections.

LARVAL COLLECTIONS. The bays and basins of Gatun Lake are extremely difficult to reach because of the lack of access roads; however, a boat was kept at a dock at a site on the lake near Frijoles on the railroad. On the days assays were to be made, personnel traveled by train to Frijoles and then used the boat to make larval collections at 10 stations in Salud Basin. A total of 20 dips per station was made, and all anopheline larvae taken were counted. The bays or basins in the southern portion of the test area were inaccessible because of the difficulty of moving the boat from one area to another, but similar collections were made by boat in the bay in the northwest corner of the plot (Quebrada La Chinilla); however, this latter area was near the treatment border, and few larvae were found so the data were not used. We also made larval collections outside the area near Nuevo Limon in sites intended as untreated checks, but winds during both applications were from the south so some insecticide drifted into this area. These data were also discarded.

BIOASSAYS. Caged adults and larvae of

Anopheles albimanus Wiedemann were used for the bioassays. The adults (3- to 5-days-old) were held in 16-mesh screen wire cages (20 or 25/cage) which were suspended on stakes about 3 feet above ground. The larvae were exposed at ground level in 1-pint paper cups (20 or 25/cup). One assay unit (one cage of adults and one cup of larvae) was placed at the edge of the jungle along the open road and the other 75 to 100 feet into the jungle at each of the man-biting collection stations (Fig. 1) except the station near the railroad. The insects were exposed from just before treatment until 0700 to 0900 the following morning. Then the adults were transferred to clean cages, and the adults and larvae (in the exposure cups) were returned to the laboratory. Mortality counts were made after 24 hours.

Gonotrophic Aging. Female *A. albimanus* from two of the horse-baited traps in the test area and from the control trap were collected with an aspirator and returned alive to the laboratory. After they were identified, they were grouped into lots of 10 per vial and held at 50 to 55 ° F until the ovaries could be dissected to provide insight concerning the reproductive and migrational behavior of the population. Thus, from each collection, about 30 females were dissected in saline solution, and the ovaries were examined for tracheole extension and/or ovarian dilations as follows: One ovary from each female was observed intact and classified as nulliparous if tight coils or tracheoles were visible; if the tracheoles could not be distinguished as tightly coiled, the second ovary was dissected with fine needles to separate the individual ovarioles. Loss of the preparation through desiccation was prevented by adding a drop of glycerin before the cover slips were placed over the ovary. The slide was subsequently observed by phase microscopy to determine whether the mosquito was parous or nulliparous. When parous, the number of gonotrophic cycles (evidenced by ovarian relics) was recorded.

The collections of these females were made before and after the first application from the control trap (A), the horse-baited trap in the treated zone nearest the breeding area and the railroad (B), and the horse-baited trap at the junction of Pipeline and Sniffer Roads (C) (see Fig. 1). However, few mosquitoes were collected in trap C after the second application, so females were taken only from trap B. Also, we did not sample females from the control trap after the second application because we had insufficient time to process them.

INSECTICIDE APPLICATIONS

The fenthion (93 percent technical) was applied from a UC-123B aircraft equipped with a conventional insecticide spraying system. Since the aircraft flew at a speed of 150 mph and the swath spacing was 0.4 mile, a delivery rate of 5 gallons per minute was required to obtain the desired dose, 1 fl. oz. (0.075 lb.) per acre. This rate was obtained by using 14 No. 8004 flat fan tips and a pump pressure of 29 psi. No system of marking the swaths at ground level was feasible so we relied on natural topographical features and compass headings from maps to obtain the proper spacing. However, the wide swaths and the reliance on drift made any minor errors in spacing less significant. All swaths were flown in the same direction, that is, starting from the northwest side of the plot, since the lake margin provided the most easily recognizable landmarks. The length of the swaths was determined by operating the spray system for the time necessary to fly the length of the plot (5 miles ÷ 2½ miles/min.=2 min).

Mount *et al.* (1970a and 1970b) showed that accurate information about wind velocity and direction was essential for proper utilization of the drift spraying technique. Thus, we obtained the services of a meteorological team from the U. S. Army, Ft. Clayton, Canal Zone; the group determined wind conditions from theodolite sightings on helium-filled balloons released along Pipeline Road about 2 miles from the northwest side of the plot. The readings were made at about one-half hour intervals from 1530 to 1800 concurrent with the applications.

At 1700 hours the day of the first application (Oct. 21, 1970), the winds were from the southwest (205°) at a speed of 5.4 mph at 300 feet altitude. The aircraft began spraying at 1710 from a height of 350 feet. At 1730 the winds died completely; however, the plot was more than half treated, so the application was continued even though drift spraying requires winds. Treatment of the plot was finished at 1745. No rain was noted for at least 24 hours after treatment.

The second application was made Oct. 30, 1970. Rain fell on the test area from 1600 to 1720. However, at 1720 the winds were 7 mph from the south (180°) at 150 feet, the sky had cleared although the jungle foliage was very wet, and conditions appeared suitable for the application. The flight pattern used during the first treatment was flown for the second treatment, but the aircraft flew at 150–200 feet. During the treatment, spraying conditions improved generally, and when the spraying was completed (1815), the winds at 200 feet were 5 mph at a direction of 190°. There were no winds at ground level during the period.

A total of 206 gallons of fenthion was applied in the two treatments; theoretically, 200 gallons should have been applied.

RESULTS AND DISCUSSION

The predominant anophelines in the test area were *A. albimanus* and *Anopheles triannulatus* (Neiva and Pinto); *Anopheles punctimacula*, Dyer and Knab and *Anopheles apicimacula* Dyer and Knab were also found but usually represented less than 1 percent of the total anophelines collected. (*A. apicimacula* were collected most often in the horse-baited traps). A few *Chagasia bathana* (Dyar) were caught and were included in the total count of anophelines since they belong to the subfamily *Anophelinae*.

The data from the man-biting collections (Table 1) and from the horse-baited traps (Table 2) have been summarized in the tables according to the distance of the collection sites from the breeding area. Biting activity was greatest from about 1815 to 1845 and did not appear to be affected greatly by light rainfalls or minor fluctuations in temperature (72–75 ° F). Immediately after the first application of fenthion, the reduction in numbers of anophelines ranged from 95 to 100 percent at all stations more than 0.1 mile from the breeding area and also at the horse-baited traps at the lake. However, the man-biting collections taken 0 to 0.1 mile from the breeding area showed only a 69 percent reduction. Thereafter, control decreased rapidly except that some increase, especially in the man-biting collections, occurred after 5 to 7 days. This pattern may have resulted from the dispersal into the jungle of adults that survived the treatment as pupae. The initial influx of these emerging mosquitoes caused control to decrease after 3 days, but the continued dispersal and death of these adults apparently caused control to increase again. Thus, the data imply that some anophelines were able to migrate as much as 1.3 miles from the breeding site within 3 days.

After the second application, the percentage control (based on average number of females caught before the first treatment) ranged from 91 to 100 percent in the horse-baited traps and the man-biting collections. Two days later, the man-biting stations near the lake showed a reduction of 76 percent which was maintained during the next week; similarly, the horse-baited traps at the lake showed a 92 percent reduction that then ranged from 82 to 89 percent the next week.

Also, at the man-biting stations and horse-baited traps in the jungle, control remained generally high for 10 days after the second application and correlated well between 17 and 21 days posttreatment with distance of the stations from the breeding source. All traps 1.3 miles into

TABLE 1.—Control of adult *Anopheles* spp. resulting from ULV aerial applications of fenthion indicated by collections from humans.

Days post-treatment[a]	Percentage reduction at indicated miles from breeding area[b] 0–0.1 (327)	0.1–0.5 (414)	0.5–1.0 (87)	1.0–1.33 (59)	Check (25)
1	69	95	100	99	40
2	76	80	94	85	0
3	46	63	56	45	0
5	56	57	92	77	0
7	44	66	74	79	0
10 (1)	91	99	100	100	0
12 (3)	76	91	97	97	0
14 (5)	63	91	98	90	0
16 (7)	61	91	99	96	0
19 (10)	77	98	98	98	..
26 (17)	40	96[c]	91[d]	82	0
33 (24)	23	66	85	92	0
40 (31)	37	60	61	91	0

[a] Numbers in parentheses indicate days after second treatment.
[b] Mean pretreatment count in parentheses after distance.
[c] One station only.
[d] Two stations only.

the jungle showed good control at all collection intervals.

Control of larvae in Salud Basin was 99.5, 88, and 86 percent at 2, 5, and 7 days, respectively, after the first treatment. After the second application, 99.5 to 100 percent control was obtained for 10 days; and 31 days later, there were still only one-third as many larvae present as before the first treatment.

The bioassay data after the first treatment reflected the problem associated with the cessation of winds during the application. The stations at the upper end of Pipeline Road and those at the lower end of Sniffer Road all showed good kill (83 to 100 percent for larvae and 71 to 100 percent for adults), but the stations located near the center of the plot showed very erratic kill (40 to 100 percent for larvae

TABLE 2.—Control of adult *Anopheles* spp. resulting from ULV aerial applications of fenthion indicated by collections from horse-baited traps.

Days post-treatment[a]	Percentage reduction at indicated miles from breeding area[b] 0.01 (6917)	0.1–0.5 (2464)	1.3 (599)	Check (1109)
1	95	98	96	28
3	72	90	61	74
5	59	82	46	58
7	53	77	82	49
10 (1)	96	99.6	99.8	0
12 (3)	92	94	97	0
14 (5)	86	91	95	0
16 (7)	82	92	97	0
19 (10)	89	95	96	0
26 (17)	33	76	86	8
33 (24)	58	82	89	0
40 (31)	43	66	86	80

[a] Numbers in parentheses indicate days after second treatment.
[b] Mean pretreatment count in parentheses after distance.

and 4 to 88 percent for adults). The latter area was treated just before the winds stopped, therefore non-uniform distribution of the insecticide must have occurred. Thus major changes in meteorological conditions during insecticide applications can result in incomplete coverage. However, even with such conditions, good control of the native mosquito population was obtained. We had thought that the calm conditions would also affect insecticide distribution over the southern half of the plot, however, the bioassay data do not indicate this. Perhaps good distribution was obtained despite the calm conditions because the insecticide was dispersed into the wing tip vortices and the aircraft flew at a fairly high altitude (300–350 feet). This combination of conditions should have caused a wide distribution of the insecticide droplets.

During the second application, mortality of the caged adults was high at all stations (only one had less than 100 percent kill), and larval kill in open areas was excellent except at one station (40 percent). However, under the canopy, larval kill was very erratic (range of 0 to 100 percent and an average of 37 percent). The canopy was extremely wet so water dripping from the leaves into the cups may have interfered with the deposition of droplets in the water or caused water and insecticide to splash out of the cups.

The results of the ovarian dissections are shown in Table 3. Some parous females were obtained in the test area the day after the first treatment, but none were found again until day 5 at Station C and day 7 at Station B. Thus, the treatment severely reduced the original population of adult females, and it was only replenished when adult females emerged from the pupae that were not killed. This conclusion is supported by the biting counts for the first three posttreatment nights which indicated a rapid initial movement of newly-emerged mosquitoes away from the breeding area along Sniffer Road (see Table 1).

The appearance of parous females at Station C the fifth night after treatment was consistent with the presumed preovipositional period, and the increase to 20 percent parous females after 7 days was consistent with the increase in apparent control after 5 to 7 days (Table 1). Thus, after the initial influx of young mosquitoes, that is, when the mean age of the population had increased, emergence or migration into the center of the plot was minimum. The pattern was the same after the second treatment, that is, the percentage

TABLE 3.—Percentage of parous females collected from horse-baited traps (29–37 females per collection).

Day	Percentage parous females collected in: Treatment zone: Near breeding (Station B)	Treatment zone: Away from breeding (Station C)	Control zone (Station A)
−1	10.8	9.4	13.3
0 (1st treatment)	..	..	..
+1	6.7 [a]	5.9 [a]	9.4
+3	0.0	0.0	13.3
+5	0.0	6.5	3.2
+7	13.8	20.0	13.3
+9 (2nd treatment)	..	..	..
+10	12.9	..	..
+12	6.7	..	..

[a] Seventeen females in sample.

of parous females was normal at one day and decreased to about 50 percent of normal at 3 days. Females were not examined subsequently, but any further decrease is doubtful. Presumably the low numbers of pupae in the breeding waters produced a low number of young females after the second treatment. Thus, the samples at 3 days represented only females that survived the second application and the small number of young females that had emerged within or migrated into the test area. We suspect that migrations may have been an important factor at Station B after the second application because the winds (5 mph at 190°) would have drifted the spray northward and little would have been deposited within 0.5 mile of the southern border of the plot (Mount *et al.*, 1970a); thus, mosquitoes migrating from untreated areas would have flown only 0.5 mile compared with 1 mile after the first application when winds were calm.

The first gonotrophic cycle of the female *A. albimanus* apparently occurred within 4 to 5 days after emergence; our delay in detecting it after the first treatment among females collected at Station B (near the breeding area) probably was a result of the preponderance of newly emerged females at that location. The examination of the spermathecae of females collected near the breeding area the first night after treatment revealed 100 percent insemination (25 females); on the second night, 25 females collected 330 yards from the breeding area were all inseminated, and the 10 dissected were all nulliparous and probably less than 2 days old (Christopher's stage 1b or 2a). Thus, females are probably inseminated before they leave the breeding area.

CONCLUSIONS

The results obtained in the present trial indicate that two properly-timed applications of an insecticide that is effective against both larval and adult mosquitoes can provide long term protection from anophelines provided a large area is treated (greater than 81 percent control was achieved 1.0–1.3 miles from the breeding areas for at least 31 days). Wind conditions at the time of spraying are important because they affect swath width and droplet distribution, however, apparently lack of wind can be compensated for by atomizing the droplets into the wing vortices and increasing height of insecticide release. Thus, good control was obtained with wide swaths (0.4 mile) with calm and windy (5 mph) conditions.

Anopheles migrated from the breeding source to a point 1.3 miles away in the jungle in only 3 days, but extensive migration for distances in excess of 0.5 mile was not indicated. Thus, prolonged protection of a location somewhat removed from a breeding source might be obtained by treating the surrounding area to a distance of as little as 1.5 miles. Since immigration of mosquitoes from outside the treated area is apparently restricted to the outer portion of the treated area, the transmission of malaria in the center of the treated zone should cease a few days after the first treatment when most adult survivors of the spray have died.

Literature Cited

Bates, Marston. 1944. Notes on the construction and use of stable traps for mosquito studies. J. Natl. Malaria Soc. 3:135–145.

Mount, G. A., Adams, C. T., Lofgren, C. S. and Weidhaas, D. E. 1970a. Altitude and drift of ultralow volume aerial sprays of technical malathion for mosquito control. Mosq. News 30(4): 580–584.

Mount, G. A., Adams, C. T., Pearson, W. G., Lofgren, C. S. and Weidhaas, D. E. 1970b. Ultralow volume aerial sprays of malathion and fenthion for anopheline mosquito control in Panama Canal Zone Jungle. Mosq. News 30(4): 604–610.