STUDIES ON FLIGHT RANGE AND SURVIVAL OF *ANOPHELES ALBIMANUS* WIEDEMANN IN EL SALVADOR. II. COMPARISONS OF RELEASE METHODS WITH STERILE AND NORMAL ADULTS IN WET AND DRY SEASONS[1]

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ABSTRACT. A series of studies to investigate the flight range and survival of *Anopheles albimanus* Wiedemann was conducted near Zapotitan, El Salvador. Recaptures of released adult mosquitoes, marked with fluorescent powders, were made up to 11 days after release and at an average distance of 500 m in the dry season, and up to 14 days at an average distance of 1000 m in the wet season. Comparison of chemosterilized adults with untreated adults indicated no observable differences in survival or flight range and the dispersal distances of both sexes were almost equal. When small and large containers were compared for transporting adults to the field, almost twice as many of the mosquitoes held in the larger containers were recaptured.

One test was completed in the wet season to compare dispersal into a 12-km^2 area when mosquitoes were released daily for 10 days from release stations in 1- or 2-km grid patterns. The recapture data indicated there was uniform dispersal within both grids and that the percentage of recaptures was much higher with the multiple release test method than with individual releases.

INTRODUCTION

The increasing interest in the use of integrated methods of mosquito control has made it necessary to gain a broader knowledge of population dynamics. If we are to manage mosquito populations, we must be able to understand and predict their habits and population fluctuations over time, and we must be able to relate this knowledge to environmental pressures, whether they occur naturally or are imposed by man. Numerous investigators throughout the world have sought information on the flight range and survival of vectors of malaria. Most of these studies were made when mosquito densities were high, and they consisted of observations of the distances mosquitoes migrated from breeding sites that were presumed to be isolated. For example, Zetek (1915) studied the flight range of *Anopheles albimanus* Wiedemann in Panama and was probably the first to release and recapture mosquitoes stained with dyes. He recaptured stained mosquitoes at various distances from the release point but was able to find only one marked *A. albimanus* that dispersed as far as 1800 meters. Fisher (1923) reported that anopheline species invaded habitations 3.2 km or more from breeding places, and Curry (1934) concluded that migration flights of *A. albimanus* could exceed 19 km in Central America; however, neither of the last investigators utilized marked mosquitoes.

We have found in research published previously (Hobbs et al., 1974) that *A. albimanus* females could be recaptured up to 3 km from the release point during the dry season in an irrigated agricultural area but the average distance at which individual mosquitoes were recovered was approximately 0.5 km. During these releases, females were recaptured 10 days after release, that is, when they were 11 days old. This observation was of epidemiological significance since the minimum duration of the developmental cycle of the malaria parasite within female *A. albimanus* has been considered to be 8 days. Thus, under optimum conditions, it may be possible that malaria transmission occurs during the dry season.

A series of studies to investigate the feasibility of the sterile male release tech-

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nique to control *A. albimanus* was conducted in 1972 by the Insects Affecting Man Research Laboratory, ARS, USDA, Gainesville, Florida, in cooperation with the Central America Research Station (CARS), Tropical Disease Program, CDC, USPHS. Lofgren et al. (1974), in one of a series of publications resulting from this research, showed that the release of chemosterilized males controlled an isolated natural population of this species. Subsequently, Weidhaas et al. (1974) described the dynamics of this test population; they determined the absolute density of mosquitoes in the release area, the rates of growth of the population, the competitiveness of released males, the survival of the various stages of the mosquito, and the ability of the mosquitoes to transmit malaria. They constructed models of population dynamics and vector capacity to estimate the effects of control methods and demonstrated the validity of the concepts of the sterile male technique with *A. albimanus.*

In the present paper, we report the results of biological studies with *A. albimanus* in which we (1) compared the flight ranges and survival rates of chemosterilized and normal adults during the rainy and dry seasons, (2) compared the dispersal of chemosterilized mosquitoes released from 2 grid patterns, and (3) evaluated 2 methods of transporting marked adults to the release sites.

DESCRIPTION OF TEST SITE

The study area was a large valley between the volcanoes of Santa Ana and San Salvador in the Department of La Libertad, El Salvador, C.A. In the center of the basin surrounding the locality of Zapotitan, there is a large government-sponsored drainage and irrigation project. The project area is approximately 54 km^2 and averages 475 m above sea level. Numerous irrigation canals furnish water throughout the year to grow a diversity of agricultural crops and extensive pasture for beef and dairy cattle. The area has a good network of all-weather roads and is flat, so there are no natural barriers to mosquito dispersal.

The climate of Zapotitan has marked alternating wet and dry seasons. The annual rainfall of the valley averages 1627 mm, but an average of only 19 mm of this total occurs during the months of December through March. The average monthly relative humidity ranges from 67 percent during the dry season to 78 percent during the wet season. The irrigation canals not only provide wet- and dry-season mosquito breeding, but also provide areas with humid microclimates suitable for mosquito survival during the dry season.

An estimated 3200 to 3500 people live in the Zapotitan agricultural district. Although the majority of the people are concentrated in 6 small villages, many live on small farms scattered throughout the area. Malaria is endemic in the district, and the incidence of the disease was relatively high in 1973. Voluntary collaborators procured 2119 slides from inhabitants for the year; 604 were identified as positive for *Plasmodium vivax* (Grassi and Feletti), and 28 were positive for *Plasmodium falciparum* Welch.

METHODS AND MATERIALS

In 1972, a laboratory colony of *A. albimanus* was established at the CARS with mosquitoes collected from the Zapotitan Valley. The colony was increased to produce ca. 50,000 pupae per day by using the mass rearing methods described by Ford and Green (1972). Mosquitoes that were to be chemosterilized were treated by placing them as pupae in a 1.0% aqueous solution of *P,P*-bis(1-aziridinyl)-*N*-methylphosphinothioic amide for 1 hour, rinsing them in clean water, and transferring them to clean water in plastic cups for adult emergence. Both treated and untreated mosquitoes emerged in separate 64 x 64 x 42-cm cages containing cotton pads saturated with 2.5% sugar water for food.

Adults less than 24 hours old were removed from the cages with battery-powered aspirators in groups of ca. 1000 mosquitoes. Each group was immobilized quickly in a freezer at 10° C, transferred to wide-mouth plastic bottles coated inside with a thin layer of Day-Glo® fluorescent dye powder, and gently rotated to thoroughly mark all the adults. The marked mosquitoes were then placed in the transportation cages which consisted of plastic foam chests (50.8 x 30.5 x 30.55 cm) that had the top opening covered with a Tubegauze® sleeve. After 12,000 to 15,000 adults were placed in the chests, damp toweling was used to cover the gauze to increase humidity, and the plastic foam top was taped on to protect the mosquitoes from heat and low humidity during transport. Seven different colors of powder were used so it was possible to color code serial releases or combinations of chemosterilized and untreated mosquitoes released in the same or different locations.

All releases were made at dusk (about 6:30 p.m.). Human bait recaptures were made the hour following each release. The collection sites were on the cardinal compass points at either 100 or 250 m from the release point. At the end of the 1-hour collection period, the dead adult mosquitoes in each chest were counted, and the estimate of the number of mosquitoes released was adjusted accordingly. Thereafter, daily collections of *A. albimanus* adults were made for 1 hour (9:30 to 10:30 a.m.) at predetermined collection sites in stables and cattle corrals and at natural resting places such as bridges, irrigation culverts, and buttressed tree trunks. Collection sites were the same for each release to maintain continuity of distances and directions from the various release points. Collections continued until no marked adults were recaptured from any of the sites for 2 successive days. The maximum distance from a release to a recapture point for any single test was 5.5 km. All releases were arranged with a male to female ratio of 1:1. All field collected mosquitoes were returned to the laboratory individually in vials; species other than *A. albimanus* were discarded, and the mosquitoes were examined with a UV light to detect those marked with fluorescent powder. Both marked and unmarked adults were enumerated, and data from marked individuals recorded as to their release date, treatment, and sex.

EXPERIMENTAL DESIGN

STUDY I. Releases were made from October to December 1972 during the end of the rainy season, to evaluate methods of marking adult mosquitoes and transporting them to the release points, surveying for collection recapture sites, and developing procedures for recognizing marked mosquitoes after recapture. From 5,400 to 57,000 adults were released every 5th day, and each group of adults was marked with a different color. All mosquitoes in these tests were treated as pupae with chemosterilant.

STUDY II. Marked releases were made from March to April 1973 to determine the ability of *A. albimanus* to disperse and survive in the dry season. We expanded the study area used in Study I and more thorough surveys were conducted to find the best collection sites for both male and female mosquitoes. From 23,000 to 51,000 adults were released every 5th day, in 5 separate releases. However, to determine whether differences existed in dispersal and longevity between normal adults and those treated with chemosterilant, 50% of the adults were sterilized and marked with 1 color, and the remainder were untreated and marked with another color. Releases were made at different locations within the study area, but the numbers and locations of collection sites remained constant, thereby permitting comparisons of the distances and directions the mosquitoes moved in each test.

STUDY III. In the third series of tests (September to October 1973), comparisons were made during the wet season of 2 methods of transporting mosquitoes to the field and of dispersal from multiple release points in 1- and 2-km grids. In the transportation tests, 2 single point releases of 43,000 and 34,000 marked, chemosterilized adults were made 19 days apart, at 2 different locations, and 14 collection sites were monitored daily until no marked adults were recaptured for 2 successive days. Half of the adults in each release were held and transported in the plastic foam picnic chests, the other half were held and transported in 3-oz. plastic foam cups (1000 adults/cup); each group of mosquitoes was marked with a different color. The method of holding mosquitoes for transportation to the field has been reported by Smittle and Patterson (1974) as an efficient method for transporting large numbers of treated mosquitoes of other species in field release studies.

Sterile-male release programs require a system of releasing the males that insures uniform distribution in the test area. Therefore, we conducted a study in which daily releases of adult mosquitoes were made on 2 grid patterns of different size for 10 consecutive days. One pattern contained 9 release points (3 x 3) with each point 2 km from the next point (16 km^2). The second pattern had 20 release points (4 x 5) with the points 1 km apart (12 km^2). The grids were superimposed over each other. Both grids surrounded the recapture points used in single releases described previously. The adults for each grid were marked with separate colors so that they could be identified in collections. There were 20,000 adults released in each pattern daily, split equally between the points (1000 per site for the 1-km grid; 2225 per site for the 2-km grid), except for the 9th day when the adult stock was low, and only 12,500 were released in each pattern. All adults released during this study were transported in the small cups.

RESULTS AND DISCUSSION

Preliminary tests in Study I showed that dispersal was omnidirectional and rapid. Recaptures were made in various directions 100 m from the release point within a few minutes after release, at stables 250 m away within 1 hour, and 1 km away the following morning. Marked mosquitoes were recaptured for 12 days after release and at distances up to 3 km from the release point. Most importantly, these tests showed that the marking techniques were excellent, and the fluorescent colors were discernable with UV light even after the mosquitoes had been in the field for 12 days. The transportation cages contained the adults satisfactorily, and mortality due to the combined effects of marking and transporting was less than 5%.

Data from Study II again confirmed that the adults dispersed rapidly, being collected at human bait stations 100 m from the release point within 1 to 3 minutes and at 250 m within 7 minutes; large numbers were collected at 250 m at all cardinal points of the compass within 1 hour. Adults 11 days old were recaptured at distances up to 3 km. Hobbs et al. (1974) reported the results obtained in this test on dispersal of normal, untreated *A. albimanus;* a summary of these results is given in Table 1, along with those for chemosterilized adults. The data show no meaningful difference in the recovery rates of treated or untreated males or females. On the basis of recapture rate and distance, there was no indication that the sterilizing treatment had an adverse effect upon longevity. The larger percentages of females recaptured were expected since many of the collections were made in stables, which atracted female *A. albimanus* mosquitoes in search of a blood meal. A few males were recaptured in the stables and a few others were collected during daily searches of numerous other natural resting sites.

The distance of dispersal was also tabulated by sex and treatment. The average distance between the points of release and recapture was approximately 0.5 km for each category (Table 1). There was no pattern that showed a difference in distance flown by treated or untreated adults of either sex.

It is realistic to suspect that some adults exceeded the observed limits of longevity and dispersal but were not detected due to the limits of the study. However, it was

Table 1. Recovery of data on marked *A. albimanus* recaptured during the dry season in El Salvador, March to April 1973.

Release	Treatment[a]	No. released[b]	No. recaptured		Percent recaptured		Avg. distance (m) between release and recapture	
			Males	Females	Males	Females	Males	Females
A	Treated	17,250	1	95	0.01	1.10	225	466
	Untreated	17,250	7	65	0.08	0.75	546	445
B	Treated	11,500	12	101	0.21	1.76	342	409
	Untreated	11,500	15	168	0.26	2.92	452	360
C	Treated	17,750	16	139	0.18	1.57	463	318
	Untreated	17,750	6	119	0.07	1.34	270	357
D	Treated	25,500	7	265	0.05	2.08	475	955
	Untreated	25,500	16	359	0.12	2.82	311	1125
E	Treated	13,750	10	122	0.14	1.77	760	581
	Untreated	13,750	8	161	0.12	2.34	800	616
Avg.	Treated	....	..	..	0.12	1.66	453	546
	Untreated	....	..	..	0.13	2.03	476	581

[a] Treated adults were exposed to *P,P*-bis(1-aziridinyl)-*N*-methylphosphionothioic amide as pupae.
[b] Consists of 50 percent males, 50 percent females.

evident that adult *A. albimanus* have the ability to survive and disperse under the adverse conditions of the dry season in El Salvador, in areas offering the proper environment. This survival time may be of epidemiological significance, since it appears that some female mosquitoes survive long enough during the dry season to transmit malaria.

In Study III a total of 126 females was recollected in the single releases, and the average dispersal distance was about 950 m (Tables 2 and 3). Fifteen males that had been carried to the field in chests were recaptured as compared to only 3 of the males carried in cups. Average dispersal distance of the former was 621 m and of the latter 1300 m; for all adults it was 942 m. Adults were recaptured 13 days after release and the maximum distance at which a mosquito was recovered was 3.2 km. A total of 512 females and 49 males was recaptured from the multiple release test. It was not possible to obtain maximum dispersal and longevity data from the multiple releases. However, the last

Table 2. Recovery of marked *A. albimanus* during the wet season in El Salvador, September to October 1973

Release	Method	Total no. released	Male/female	No. recaptured		Percent recaptured	
				Males	Females	Males	Females
			Single Release				
A	Chest	21,000	10,500	12	52	0.114	0.495
	Cup	22,000	11,000	0	21	0.0	0.190
B	Chest	17,000	8,500	3	34	0.035	0.400
	Cup	17,000	8,500	3	19	0.035	0.223
Mean	Chest	....	....	..	..	0.075	0.448
	Cup	....	....	..	..	0.018	0.207
			Multiple Release (10 days)				
C	1-km grid	191,750	95,875	31	366	0.032	0.380
	2-km grid	191,900	95,950	18	206	0.018	0.211

Table 3. Distance from release point at which marked *A. albimanus* adults were recaptured during the wet season.

	Average distance of adult recaptures (m)				
	Males		Females		
Release	Chests	Cups	Chests	Cups	All marked adults
A	742	0	889	755	835
B	500	1300	1021	1147	1049
Mean	621	....	955	951	942

marked adult was recaptured 12 days after the last release, making it at least 13 days old.

Comparison of the 2 transport methods in Study III indicated that when the adults were crowded in small containers and unable to move about naturally, there was a decrease in their biological activity in the field as measured by recapture rates. There was adequate surface area within a foam ice chest to permit 15,000 adult mosquitoes to rest normally without disturbing the surrounding adults. The 1000 adults that were confined in the small area between stacked cups had no room for movement and were actually piled on top of each other. When the cups were unstacked in the field, the mosquitoes dispersed readily and practically no immediate mortality was observed. However, there were always many wing and body scales left coating the bottom of the cups, and occasionally the adults lost legs in the cramped space. Since there was minimal transport mortality, and the dispersal data showed no differences between the 2 methods, the reduction in percentage of recapture probably resulted from a decrease in daily survival rates. The human bait collections made on the evening of release provided an additional indication of an adverse effect from the confinement in the cups. In the hour immediately following both releases, the collectors recaptured marked females from foam chests (48 and 59, respectively) but did not collect a single female from the cups. This might indicate that their feeding behavior may have been affected, which in turn could contribute to a lower survival rate. It is felt by the authors that the transport problem should not present a major obstacle to future releases since the size and shape of insulated containers can be modified to hold preselected numbers of adults with minimal adverse effects.

A first analysis of the results of the study on the dispersal of mosquitoes from the 1- and 2-km grids suggests that dispersal in the smaller grid was more uniform than that in the larger grid. However, further analysis indicates that this is not true if one assumes that dispersal is omnidirectional, and recapture rates of mosquitoes are based only on mosquitoes that would have remained within the recapture areas. The 1-km grid (Fig. 1) contained 4 rows with 5 stations in each while the 2-km grid had 3 rows with 3 stations each. The 1st and 3rd rows of the 1-km grid coincided with the 1st and 2nd rows of the 2-km grid. A 5th row to coincide with the 3rd row of the larger grid was not used because of difficulty in carrying mosquitoes into this area. If we assume, as indicated previously, that mosquitoes dispersed equally in all directions, then of the mosquitoes released at the 4 corners, only 25% dispersed into the test area while 75% migrated in other directions. Likewise, only 50% of those released at each of the 4 points along the perimeter of the square would have flown in directions that would have allowed them to be recaptured. Random dispersal from the center point should have permitted recaptures of these adults in any direction except for a few that might have migrated out of the plot. With 2,225 adults released at each of the 9 points of

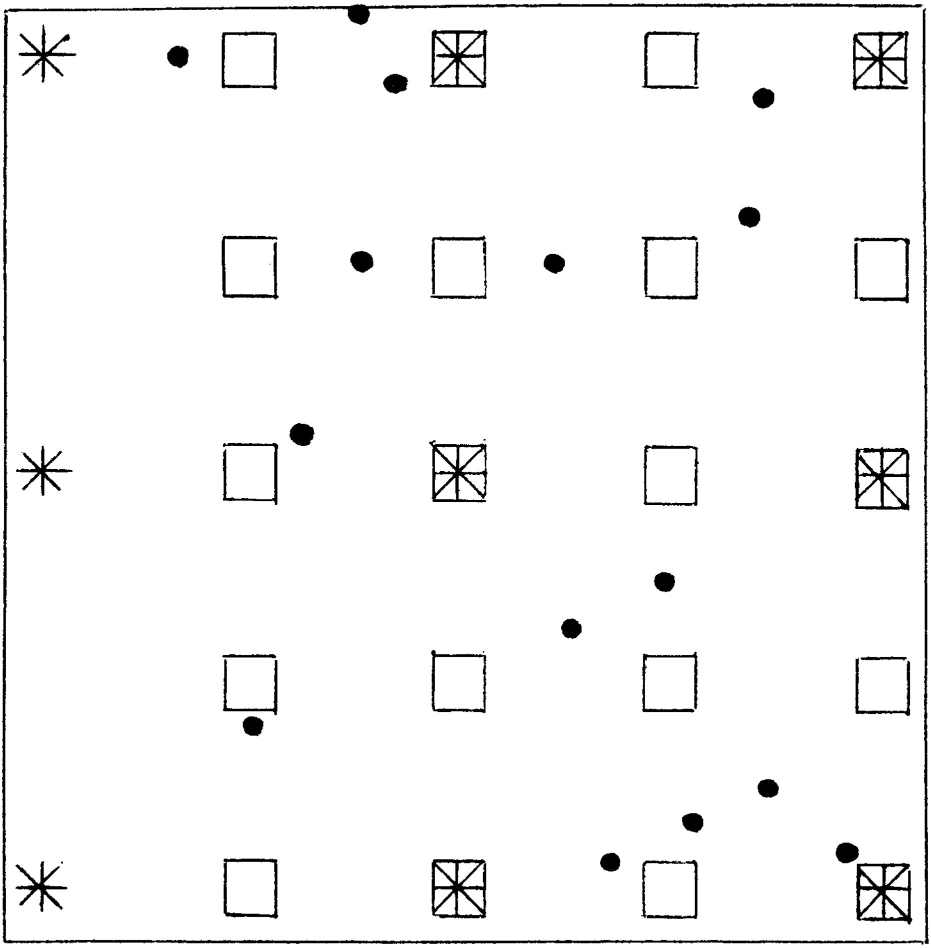

Fig. 1. Relationship of release points for 1-km (□) and 2-km (⊠) grid patterns and recapture sites used (●) in 10-day releases of *A. albimanus* in El Salvador, September to October 1973.

the 2-km grid, theoretical calculations indicate that only 8,900 would have randomly flown into the recapture area. Using the same rationale for the 1-km grid, releases were made at 4 corner, 10 side, and 6 inside stations. With 1,000 adults released at each point, a total of 12,000 would have flown into the area. This daily release ratio of 12,000 to 8,900 would have by itself reduced the expected recapture rate from the larger grid by 26 percent. If the smaller grid had been completed by a 5th row of release points, 4,000 additional mosquitoes would have been released and the expected ratio would have been 16,000 to 8,900 or 1.80:1.

Another, but similar, method for determining the numbers to be expected in the test area can be used. During the wet season, the average distance mosquitoes migrated from the release points was 942 m (Table 3). Thus, less than half of the adults released from the 3 bottom points of the large grid would have migrated into the test area. Also, those that did enter this area would have been compensated for by equal numbers from the middle row of points that would have migrated out of the plot. Then, in actuality, the bottom 3 points were insignificant as far as the numbers of adults they added to the recapture area. If this is true, and the same calculations as before are used, we then have 2 corner, 3 side, and 1 central point of the large grid in the same area as the 4 x 5 smaller grid. A total of 12,000 adults would have been released from the 1-km grid and 6,675 from the 2-km grid, and the ratio of recaptures again would have been 1.80:1.

A review of the actual recapture rates from the releases (Table 2) shows that for females, 0.380% were recaptured from the small grid and 0.211% from the large grid, or a ratio of 1.80:1. For males, the recapture rates were 0.032% and 0.018%, respectively, for a comparative ratio of 1.78:1. These ratios of recapture for both sexes compare almost exactly to the recapture ratios that should have been expected. This indicates that there was equal and uniform dispersal of the mosquitoes whether they were released at points 1 km or 2 km equidistant from each other.

The percentages of recapture shown in Table 2 were based on the total numbers of each category released and not on the numbers that we now accept as having dispersed into the test area. Based on the latter numbers, the recapture rates for females were 6.2% and 6.1% for the large and small grids, respectively, and 0.54% and 0.52% for males from the same release patterns. These recapture rates are then higher than those from any of the previous single releases, and are comparable to results of an earlier large-scale, multiple-release program completed at Lake Apastepeque (Weidhaas, et al., 1974). We might have obtained even higher recovery rates if the transport system other than the small cups had been used.

Analysis of our data shows there was an increase in dispersal and longevity of *A. albimanus* in the rainy as compared to the dry season. The average dispersal distance increased from approximately 500 to 942 m and longevity of adults was extended by 3 days. Also, while males were more difficult to recapture, they dispersed about as well as females in both seasons. However, the decreased percentage of recapture for all groups of mosquitoes in the wet season suggests that it is unnecessary for adults to seek sheltered resting sites during this part of the year since the increased humidity and vegetation offer sites not available during the dry season. For additional information on resting sites during the dry season, we placed numerous red and black cloth portable resting stations throughout the area. These devices had been reported as being attractive for anopheline adults to seclude themselves during the day (Breeland and Glasgow, 1967). In addition, on occasion we used CO_2 traps and potential male attractant chemicals near natural resting sites to increase their attraction. None of the attempts increased collections, and the cloth boxes were totally unattractive to *A. albimanus* adults, even when lined with damp towels to increase humidity.

The dry season releases with chemosterilized and untreated adults showed no significant differences between the recapture rates for males or females of either treatment, or for average distances of dispersal. Since there was also no apparent difference in longevity between the sterilized or unsterilized groups, it appears that treated adult males released into a natural population for sterile male release control programs should be biologically compatible with the indigenous males and function normally.

One of the most important implications of the tests was the indication that mosquitoes dispersed uniformly from points 2 km equidistant from each other. Future sterile male release projects will be made easier if releases can be made at points this far apart. However, decreased dispersal during the dry season may require closer release intervals at this time.

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PREDATION BY WATER MITES (ACARINA: HYDRACHNELLAE) ON THE IMMATURE STAGES OF MOSQUITOES [1]

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ABSTRACT. The post-larval stages of several water mites are reported for the first time as predators on the immature stages of mosquitoes. Nymphs and adults of *Thyas barbigera* and *T. stolli* were found to feed on *Aedes* eggs in the laboratory. Three types of feeding damage may result, each of which can be correlated with the age of the eggs at the time predation takes place. The nymphs and adult females of certain *Piona* spp. found in temporary woodland pools were observed to attack and kill *Aedes* larvae under both laboratory and field conditions. Predation by these mites appears to cause a progressive paralysis in the mosquito larvae, first affecting the head and associated structures and gradually extending posteriorly. A single case of a *Hydryphantes ruber* female preying on a IV-instar *Aedes stimulans* larva was observed in the laboratory.

Although mites are commonly found as ectoparasites on adult mosquitoes, few observations have been made regarding mites as predators on the immature stages of mosquitoes. Nymphs and adults of trombiculid mites, including the genera *Eutrombicula* and *Trombicula,* have been observed feeding on *Aedes aegypti* eggs in the laboratory (Jenkins, 1947). Hearle (1926) reported unidentified dark-red water mites preying voraciously on *Aedes vexans* larvae in a cottonwood swamp. To my knowledge, the only other published report of water mites preying on immature

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