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THE USE OF STICKY OVITRAPS TO ESTIMATE DISPERSAL OF *AEDES AEGYPTI* IN NORTHEASTERN MEXICO

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ABSTRACT. A newly designed sticky ovitrap was used to determine the dispersal flight of the dengue vector *Aedes aegypti* in northeastern Mexico. Unfed marked females were released in the field where 100 sticky ovitraps had been positioned within a circular area 300 m in diameter. Success of this method was represented by a 7.7% (31 of 401) recapture rate during a 19-day sampling period. The maximum dispersal distance was 120 m with a mean of 30.5 m ± 4.5 SD. Drastic hot and dry climatic conditions at the study site may have restricted mosquito dispersal. As expected, mostly gravid female mosquitoes were captured in the ovitraps. The method of sticky ovitraps proved to be useful and inexpensive for *Ae. aegypti* dispersal studies in Latin American countries. In addition to the marked mosquito populations, unmarked *Ae. aegypti, Culex* sp., roaches, and even small vertebrates such as lizards and sparrows were found glued to the sticky ovitraps. Based on these studies, the use of sticky ovitraps is recommended for *Ae. aegypti* dispersal studies, specially for dengue control programs in developing countries.

KEY WORDS Dengue, oviposition, sticky ovitraps, *Aedes aegypti,* dispersal

INTRODUCTION

Dengue fever currently represents the most important mosquitoborne disease in Mexico. Reinfestation of the Americas by the primary vector, *Aedes aegypti* (L.), increased during the 1980s and continued in the 1990s (Gubler and Kuno 1997). Three states located in northeastern Mexico (Tamaulipas, Nuevo Leon, and Coahuila) accounted for nearly 70% of dengue prevalence in 1999 (Boletin de Epidemiología 1999).

Dispersal flights of *Ae. aegypti* are of paramount importance for epidemiologic and control programs. If a dengue case is reported from 1 specific dwelling in a neighborhood, the flight range of infective female *Ae. aegypti* determines the size of a barrier control area surrounding the case, that is, space spraying and breeding site elimination (PAHO 1994). Traditionally, this type of focal control has assumed a dispersal distance of less than 100–150 m for *Ae. aegypti* females. However, findings of Reiter et al. (1995) in Puerto Rico showed that, when areas are cleared of suitable breeding sites, infected mosquitoes may travel distances up to 840 m. This is explained by the need for gravid *Ae. aegypti* females to extend flight distances in the search of available oviposition sites. Obviously, possibilities of new dengue cases in untreated or unsuspected areas increase the epidemiologic importance of such flight behavior. Thus, comparative flight dispersal research in ecologically diverse habitats is needed to determine if *Ae. aegypti* fly beyond generally recognized distances.

To monitor dispersal distances in mosquitoes, methods using fluorescent dusts are the 1st choice (Service 1993). Although most of the time these techniques yield acceptable results, they are time-consuming and demand intensive and prolonged efforts. We proposed a new and inexpensive method to capture marked *Ae. aegypti* females based on sticky ovitraps. This technique was used to determine flight dispersal of this species in northeastern Mexico. Our study had 2 objectives: to evaluate the effectiveness of sticky ovitraps as a new method to assist in flight behavior studies of container-breeding mosquitoes, and to determine the flight dispersal range of *Ae. aegypti* in a temperate dengue-endemic area in Mexico.

MATERIALS AND METHODS

Study area: The city of Guadalupe is located in Nuevo Leon state in northeastern Mexico. Together with 6 other municipalities it makes up metropolitan Monterrey, the 3rd largest city in Mexico (population: 4 million.) Average elevation is 540 m, but a dominant flattened topography is interrupted at its margins by the Sierra Madre Oriental range, specifically Cerro de la Silla, 1,100 m in elevation. Herein, at the mountain outskirts, lies Colonia 5 de Mayo, our study site. It has slopes of 25–30° and coordinates of 23°N, 98°17′E. Hundreds of dwellings built mostly with wooden roofs and walls allow *Ae. aegypti* populations to thrive in 200-liter drums used to store domestic water. Annual rainfall averages 600 mm, with a mean temperature of 28°C. Natural vegetation consists of bushes but trees 3–15 m in height are found in the backyards of most houses (Secretaria de Programación y Presupuesto 1981). The State Vector Control Program has reported dengue outbreaks yearly at this location since 1995.

Effectiveness of sticky ovitraps: Sticky ovitraps were built based on the reasoning that egg-laying females must look for oviposition sites. *Aedes aegypti* lays eggs individually upon surfaces of inner

Table 1. Frequency distribution of dispersal distances for marked–recaptured *Aedes aegypti* females using sticky ovitraps in Guadalupe, Nuevo Leon, Mexico.

Distance (m)	8	18	24	26	36	40	44	63	80	120
Recaptured females	6	6	6	4	1	3	1	1	2	1
% recaptured	19.4	19.4	19.4	13.0	3.2	9.7	3.2	3.2	6.5	3.2

walls slightly above the water surface (Clements 1999). Plastic 3.8-liter black containers were filled with water and a 6-cm strip of thin black cardboard was added as an oviposition substrate. Glue used to catch rodents by pest control operators (Trapper™, Bell Laboratories Inc., Madison, WI) was heated for softening and smeared onto the cardboard strip. Paper clips held the strip inside the container to avoid water reaching the glue, and thus desolving deterrent chemical substances from the glue. The glue is formulated to tolerate long periods of high heat and humidity while maintaining its sticky properties. Traps were placed in an area exposed to marked and released *Aedes* mosquitoes. Hypothetically, gravid females would become stuck at the moment of attempting to lay eggs. Effectiveness of sticky ovitraps was calculated based on the percent of ovitraps catching at least 1 marked *Ae. aegypti* female after the study period, and the recapture rate of the marked–released *Aedes* population. Additionally, to support the usefulness of sticky ovitraps for gravid females of the dengue vector, we described the age-structure of captured females through their abdominal appearance (empty or gravid), by examination of tracheolar skeins (Clements 1992), and by examination of spermatheca for sperm (WHO 1975). During each sampling visit other species found stuck in the ovitraps were identified and registered.

Dispersal flight range assessment with marked mosquitoes: To obtain high enough numbers of field-collected female mosquitoes to compensate for mortality and migration, larvae were obtained from several breeding sites. Collected larvae were allowed to develop to adult stage in a 200-liter metal drum located in the backyard of 1 of the dwellings. Emerging nonfed adult females were transferred to 3.8-liter cardboard containers and dusted with fluorescent dyes (BASF, Holland, MI) using a Pasteur pipette. Males were discarded after marking. Females were counted and released at a central point where sticky ovitraps had been placed previously. Release time was approximately 1800 h. Marking and releasing was done on 6 consecutive days, using 6 different colored dyes. The number of mosquitoes marked each day, with their colors, were: 145 green, 63 red, 30 yellow, 30 orange, 72 green-light, and 61 green-yellow, for a total of 401. The recapture period started 1-day after release and continued for 19 days. One hundred sticky ovitraps were distributed in an area 300 m in diameter. Groups of 25 traps were placed in 4 linear transects following cardinal orientations north, south, east, and west. Ovitraps were placed in shaded areas of backyards of most dwellings. Therefore, the distributions of recapture distances by ovitraps were influenced by size of backyards. Ovitraps were sampled daily, when ovitraps were inspected for female mosquitoes and other insects. Recapture distances and orientation were also recorded. At the same time, marked *Ae. aegypti* were carefully removed from ovitraps and taken to the laboratory for age-structure determination. Daily temperature, rain, and wind speed and orientation data were similarly recorded with field weather devices.

Data analysis: Descriptive statistics were calculated for dispersed distances according to the location of ovitraps catching marked *Ae. aegypti.* A

Table 2. Age-structure, abdominal appearance, and insemination stage according to recapture day for marked–recaptured *Aedes aegypti* females sticky ovitraps in Guadalupe, Nuevo Leon, Mexico.[1]

Mosquito no.	Abdominal appearance	Spermatheca	Parity	Recapture day
1	Empty	+	N	1
2	ND	ND	ND	3
3	Gravid	+	N	3
4	Gravid	+	N	3
5	Gravid	+	N	3
6	Gravid	+	N	4
7	ND	ND	ND	4
8	Gravid	+	N	5
9	Gravid	+	N	5
10	Empty	+	P	5
11	Empty	+	N	5
12	ND	ND	ND	5
13	Gravid	+	N	6
14	Gravid	+	N	6
15	Gravid	+	P	7
16	Gravid	+	P	8
17	Gravid	+	ND	8
18	Gravid	+	ND	8
19	ND	ND	ND	9
20	ND	ND	ND	9
21	Gravid	+	P	10
22	Gravid	+	P	10
23	ND	ND	ND	10
24	Empty	+	N	11
25	Empty	+	P	11
26	Gravid	+	P	11
27	Gravid	+	ND	12
28	ND	ND	ND	13
29	ND	ND	ND	17
30	ND	ND	ND	19
31	ND	ND	ND	19

[1] N, Nulliparous; ND, not determined; P, parous.

Table 3. Percentages of daily recaptures for marked–recaptured *Aedes aegypti* females with sticky ovitraps during a 19-day sampling period in Guadalupe, Nuevo Leon, Mexico.

Recapture day	Recaptured females	% recapture
1	1	3.2
2	0	0.0
3	4	13.0
4	2	6.5
5	5	16.1
6	2	6.5
7	1	3.2
8	3	9.7
9	2	6.5
10	3	9.7
11	3	9.7
12	1	3.2
13	1	3.2
14	0	0.0
15	0	0.0
16	0	0.0
17	1	3.2
18	0	0.0
19	2	6.5
Total	31	100.0

nonparametric Kruskal–Wallis test for related variables analysis (Zar 1999) was used to estimate if recapture rates were significantly different according to orientation of ovitraps.

RESULTS

Effectiveness of sticky ovitraps

Ovitraps produced acceptable recapture rates of marked and released mosquitoes. A group of 31 (7.7%) out of 401 females were found glued to the oviposition carboard strip of the sticky ovitraps. Additionally, from these 100 ovitraps positioned in a diameter of 300 m, 10 (10%) effectively caught at least 1 individual female *Ae. aegypti.* Three traps closer to the central release point were more successful, catching 58.2% of the total 31 recaptured marked mosquitoes (Table 1).

As expected, most unfed-released females captured by the sticky ovitraps were gravid (Table 2). Specifically, 16 (76.1%) individuals showed advanced ovarian development from the 3rd to 12th day after release. We captured 5 (23.8%) empty or unfed marked females at days 1, 5, and 11. This group did not oviposit without coming in contact with the sticky paper, because no larvae were found in the ovitrap. Filled spermathecae occurred in mosquito females captured from days 1 to 12. Thirteen out of the 31 (41.9%) captured females were damaged at the time of removal from the sticky cardboard, and ovaries were difficult to observe for age-structure determination. However, the 1st parous females showing tracheole endings permanently stretched were on day 5 after release. In total, 22.6% of females were identified as parous, 35.5% as nulliparous, and 41.9% as not determined.

Dispersal flight range assessment with marked mosquitoes

The sticky ovitrap in which 6 (19.4%) marked *Aedes* females were captured was 8 m from the release point. The farthest recapture was at 120 m (Table 1). A distance limit of <26 m produced 58.2% (22) of total recaptures, <80 m yielded 96.8% (30), but only 3.2% (1) reached the longest distance of 120 m. Mean dispersal was 30.5 m ± 4.5 SD. Daily recaptures occurred consistently during the 19-day study period. All caught undamaged *Aedes* females were gravid. This observation was confirmed for each distance where sticky positive ovitraps were positioned, and during the entire recapture period. About 38.8% of total recaptures were obtained at <5 d, 74.4% at <10 d, and 90.5% at <15 d (Table 3).

Ovitraps of the east linear transect recaptured 38.7% (n = 12), the highest mosquito number (Table 4). North and south transects registered similar recapture figures, 25.8% (8) and 25.8% (8), respectively. The west orientation yielded the lowest num-

Table 4. Orientation and dispersal distances of marked–recaptured *Aedes aegypti* females in sticky ovitraps in Guadalupe, Nueovo Leon, Mexico.

Distance (m)	North	South	East	West	Total recaptured
8	0	6	0	0	6
18	0	0	6	0	6
24	6	0	0	0	6
26	0	0	4	0	4
36	0	1	0	0	1
40	1	0	0	2	3
44	0	1	0	0	1
63	0	0	0	1	1
80	0	0	2	0	2
120	1	0	0	0	1
Total	8 (25.8%)	8 (25.8%)	12 (38.7%)	3 (9.7%)	31 (100.0%)

bers, 9.7% (3). Nevertheless, recapture numbers for each of the 4 orientation transects did not differ significantly ($\chi^2 = 0.143$, df = 3, $P = 0.986$). Neither rainfall nor multidirectional winds of >10 km/h prevailed during the recapture time. Ambient daily temperatures averaged 27°C. In addition, the sticky ovitraps also captured the following groups of animals: unmarked *Ae. aegypti* females, *Culex quinquefasciatus* (Say) mosquitoes, roaches (*Periplaneta americana*), ants and wasps (Hymenoptera), beetles (Coleoptera), and small vertebrates such as lizards and sparrows.

DISCUSSION

After reaching the salivary glands of female mosquitoes after an extrinsic incubation period of 7–10 days, dengue viruses remain in this infected tissue for the life of the mosquito (Gubler and Kuno 1997). Subsequent bites on humans nearly ensure virus transmission. Therefore, vector variables such as flight dispersal and longevity become critical factors in understanding vectorial capacity. Most dengue–*Ae. aegypti* control programs in Latin America have based focal control measures on a maximum flight range of <150 m. However, Reiter et al. (1995) described dispersal distances flown by *Ae. aegypti* as much as 840 m. In northeastern Mexico, our results demonstrated a maximum dispersal distance of 120 m with a mean of 30.5 ± 4.5 m. We believe that our 7.7% recapture rate was enough to support the average flight range of *Ae. aegypti* under local hot and dry conditions. Such conditions have been shown to restrict the dispersal of *Ae. aegypti* in northern Australia (Muir and Kay 1998). Those authors found a mean dispersal distance of 56 m and 35 m for males and females, respectively, with a maximum flight range of 160 m. Dispersal differences between the present study and that of Reiter et al. could be explained by the more humid weather in Puerto Rico, the availability of abundant sugar sources such as flowers (Van Handel et al. 1994), or by the greater presence of domestic breeding sites in our study, inducing females to lay eggs in a smaller area. We agreed with the conclusions of Reiter et al. that availability of breeding sites modulates flight dispersal. However, the latter statement should be supplemented by Guillie (1961), who coined the term of "dispersal ambit" instead "dispersal range" to define it as the minimal needed distance for an *Anopheles* mosquito between breeding sites and blood-meal source. Partial or multiple blood meals could trigger egg development, as has been reported for *Ae. aegypti* elsewhere (Scott et al. 1993). This would increase dispersal distance during host-seeking behavior and oviposition activity. Although we did observe gravid females being caught almost consistently (Table 2), the collection of females before egg-laying by sticky ovitraps did not allow egg counts; therefore, counts of small numbers of eggs indicating partial oviposition behavior were not seen (Apostol et al. 1994).

Orientation of sticky ovitraps did not affect dispersal flight distances. We argued that abundant critical resources for the mosquito life in the study site such as shelter, food, and breeding sites favored an even distribution of egg-laying females. It is important to note that both strong winds and rainfall were absent in this study.

The use of sticky ovitraps was inexpensive and effective for determining dispersal flights of *Ae. aegypti* females. They captured marked and unmarked mosquitoes and fed and unfed mosquitoes, as well as different mosquito species. This recapture rate was attained even when abundant 200-liter water drums were present in most dwellings. Recaptures were almost made daily during the 19-day study. The traps were successful even in *Aedes*-endemic areas where abundant additional breeding sites are available. Muir and Kay (1998) also designed effective sticky lure traps to catch adult marked *Ae. aegypti* in Australia. Those authors coated black cardboard rectangles with polybutylene adhesive and reported recapture rates of 3.6–13.0%. Some traps showing multiple specimens were found in the Mexican and Australian studies. As Muir and Kay concluded, frequency of recaptures at certain trap locations suggested that shade, wind, and host availability affected the distribution of *Ae. aegypti*. Even the more expensive rubidium-marked eggs method (Reiter et al. 1995) has the advantage that larger areas can be sampled. The design of our sticky ovitraps presented some problems during the removal of marked samples, that is, damage to female ovaries. This hampered age-structure studies such as examination of tracheolar skeins and spermatheca. Finally, we concluded that sticky ovitraps may allow quick determinations for dispersal distances of the dengue fever vectors at very low costs. This technique is affordable, especially for dengue control programs in developing countries.

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