Acta Tropica 124 (2012) 48–53

Contents lists available at SciVerse ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

# Diurnal resting behavior of adult *Culex pipiens* in an arid habitat in Israel and possible control measurements with toxic sugar baits

Yosef Schlein, Günter C. Müller*

*Hebrew University, Hadassah Medical School, Kuvin Center for the Study of Tropical and Infectious Diseases, Department of Parasitology, Jerusalem 91120, Israel*

ARTICLE INFO

*Article history:*
Received 27 February 2012
Received in revised form 11 June 2012
Accepted 23 June 2012
Available online 29 June 2012

*Keywords:*
*Cx. pipiens*
Resting sites
Sugar feeding
Control
ATSB
Attractive toxic sugar baits
Arid habitat
Israel

ABSTRACT

The distribution of resting *Culex pipiens s.l.*, L. in vegetation at the margins of breeding sites and the effects of a narrow, surrounding, sprayed belt of sugar, food dye and toxin on adult mosquitoes were studied near two pairs of control and experimental sewage ponds close to human habitation in the Judean hills. Control belts were without toxin. A sprayed belt of sugar and toxin 0.5 m from the water gradually reduced the population to an average of 38.3 mosquitoes per trap, 7.6% of the highest catch, which was 504.6 mosquitoes per trap in the control site. In the second experiment, in which bait belts were 5 m from the water, the toxic bait spraying was followed by a rise in catches from 207.9 to 274.9 mosquitoes. This was 41% of the 670.2 mosquitoes per trap in the parallel control site. In areas without toxin treatment, diurnal catches by net amounted to 20,705 mosquitoes. Of these, 86.1% (17,825) were caught within 1 m of the water while only 8.2% (1701) were caught at a distance of 3 m. The remainders were caught up to 20 m away. Parity status was determined for female samples caught by net. In areas without toxin, parous females accounted for 37% of the catch and 13.2% were young, meconium containing specimens. The population diminished following spraying of toxic bait 1 m from the water and included 13% parous females and 17.6% had meconium in the gut.



## 1. Introduction

Observations indicate that different mosquitoes choose various types of habitats for diurnal rest, however it is extremely difficult to find the resting mosquitoes of many species (Clements, 1999). Outdoor resting sites are divided into two general types, either having a solid background such as in caves or the shelter of bridges, or within vegetation (Gillies, 1988). The variations in resting habitats of different species are indicated in a brief summary by Clements (1999) and also in a recent broad study that was carried out in Tuskegee National Forest, Macon County, Alabama (Burkett-Cadena et al., 2008). There is evidence that a high proportion of adult *Culex pipiens s.l.*, L. (Nudelman et al., 1988) are ready to feed on sugar when they are on the vegetation at the margins of their breeding site (Schlein and Pener, 1990; Müller et al., 2010a). We therefore thought that this may be a way to target newly emerged mosquitoes for control and carried out this study to test this hypothesis. The main tool of investigation was the use of sugar and oral toxin in suitable areas to demonstrate their importance by the depletion of the mosquito population. Unlike other similar studies with attractive toxic baits (Müller et al., 2007; Müller and Schlein, 2008; Gu et al., 2011; Beier et al., 2012), the baits in this study did not include attractants. Thus without olfactory influence of attractive baits, the movement of mosquitoes was their normal behavior (Müller and Schlein, 2006; Schlein and Müller, 2008). Indeed, the results demonstrated that in the dry climate of the region, a narrow belt of vegetation near the water serves as a resting place for emerging young adults but also includes the older adult mosquito population. This avenue of investigation showed that mosquitoes gathering in a limited area can be a target for control.

## 2. Materials and methods

### 2.1. Study sites

The study was carried out in the spring and summer of 2008 in the Judean hills that separate the Mediterranean zone from the desert. The centers of the selected sites were sewage ponds in two villages that are 6–9 km south of Jerusalem. All the ponds were located in small shallow valleys that drain to the eastern slopes.

One experiment (A) was carried out in the areas surrounding two ponds situated at the eastern outskirts of the first village. The area around the pond that was treated with toxin was termed Experimental area A and the area around the other untreated pond was termed Control area A. The ponds at village A were ~20 × 15 m and ~20 × 10 m respectively. A second experiment (B), was carried

* Corresponding author. Tel.: +972 525257521.
*E-mail address:* GunterCMuller@hotmail.com (G.C. Müller).

0001-706X/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.actatropica.2012.06.007

out in a separate village at areas surrounding two ponds, measuring ~15 × 10 m and ~10 × 8 m. The treated site was termed Experimental B and the untreated site was termed Control B. The distance of the ponds from the nearest houses was between one and two km in each of the villages. Village B is approximately 5 km north of Village A.

In this region, the mean annual rainfall is 250–350 mm and the vegetation, which includes Mediterranean and Desert elements is mostly concentrated in the valleys where it remains dense during the dry season, particularly four to eight meter around the ponds. Generally, the summer vegetation on the hills is sparse for the scarcity of water and overgrazing.

The common plant species in the area are *Scolymus hispanicus* L. Compositae (Asteraceae), *Chrysanthemum coronarium* L. Compositae (Asteraceae), *Silybum marianum* (L.) Gaertn. Compositae (Asteraceae), *Echinops polyceras* Boiss. (Asteraceae), *Phlomis pungens* Willd. Labiatae (Lamiaceae), *Salvia syriaca* L. Labiatae (Lamiaceae), *Ballota undulata* (Sieber ex Fresen.) Benth. Labiatae (Lamiaceae), *Astomaea seselifolium* DC Umbelliferae (Apiaceae), *Ferula biverticellata*, Thieb. Umbelliferae (Apiaceae), *Noaea mucronata* (Forssk.) Asch. & Schweinf. Chenopodiaceae, *Alhagi graecorum* Boiss. Papilionaceae, *Capparis spinosa* L. Capparaceae and *Polygonum equisetiforme* Sm. Polygonaceae. In the vicinity of the water, there were some scattered *Nicotiana glauca* Graham Solanaceae an invasive species. At all four sites, even during the summer, there were always some plants flowering, especially *P. equisetiforme*.

### 2.2. Monitoring mosquito populations

The experiments were carried out from the end of April until the end of August in village A and from the end of April until the end of July in village B.

Mosquitoes were caught in traps overnight and additional mosquitoes were caught using the sweep net method. Post-treatment overnight trapping continued twice a week for the duration of the experiment.

### 2.3. Overnight trapping

Mosquitoes were caught overnight, in each site, with six lighted (incandescent) otherwise un-baited CDC miniature light traps (Model 512, John W. Hock, Gainesville, FL) without any other type of lure. They were hung on bamboo tripods approximately 1 m from the ground and were set in fixed positions around the pond at a distance of 15–20 m from the water. Traps were operated overnight from 1 h before sunset until 1 h after sunrise. Mosquitoes in trap nets were kept at 0–5 °C for 1–2 h, were anesthetized with $CO_2$, placed on a metal tray on crushed ice, and then processed or stored at −70 °C. For the observation of food dye in the gut, mosquitoes were immersed in saline solution with a few drops of detergent and examined under a dissection microscope.

### 2.4. Diurnal sampling

Mosquitoes were caught in the morning between 7:00 and 9:00 AM with a sweep net consisting of strong gauze net, fixed on a heavy iron frame, 50 cm in diameter, with a 1.5 m long handle. Catches were made for two consecutive days before treatment, then twice at the end of June, twice at July at sites A and B, and twice at the end of August in site B only. The pattern of catching at the four ponds was uniform.

Catching with the sweep net was carried out in fixed locations along transects beginning from the sewage pond and directed north, east, south and west. Eight catching-locations of 1 × 2 m were at distances of 1, 3, 6, 10 and 20 m from the water line, two locations on each of the four sides of the pond and two catches were made at each of these locations ($N = 40$ catches per pond per collection day). Each catching location was swept with 10 net strokes from right to left and vice versa. To anesthetize the insects, the net was introduced for 2 min into a plastic bag that had a piece of cotton wool soaked with about 2 ml ethyl acetate inside. Then the content of the net was emptied into a glass funnel of 50 cm diameter placed above a 500 ml beaker. Mosquitoes were collected from the beaker and treated as described above.

### 2.5. Bait solution: composition and application

Bait solution consisted of 20% (W/V) white sugar, 1.0% (W/V) food dye and 1.5% (W/V) boric acid. Control solution was the same but did not contain boric acid. The food dyes used were: E132, Indigotine and E102, Tartrazine 19140 (special green) in Experimental A area; E102, Tartrazine 19140 in Control A area; Indigo carmine 73015 (food blue no. 1) in Experimental B area and food blue no. 1 (Indigotine C.I.) in Control B area (food dyes producer, Stern, Natanya, Israel). Solutions were sprayed using a 7-liter hand sprayer (Killaspray, Model 4005, Hozelock-ASL, Birmingham, England). In experiment A, a belt of solution ~50 cm wide was sprayed directly along the margin of the water of the two sewage ponds; solution with toxin around Experimental A and solution without toxin around Control A. In experiment B, the solutions were sprayed as in experiment A but this time at a distance of 5 m from the ponds. Spraying at the four ponds was repeated on the first of May, first of June, first of July and at ponds in area A, sprayed again on the first of August. The ponds in area B were dried in August and therefore no treatment was given so mosquito monitoring continued only at Experimental A and Control A sites.

### 2.6. Age determination of mosquitoes

The physiological age was determined for *Cx. pipiens* females by the number of gonotrophic cycles as indicated by ovarian dilatations in dissected specimens (Polovodova's method in Detinova, 1962). Dissected samples consisted of 100 females samples caught as described by sweeping net on two consecutive days. Altogether ~10% of the females were excluded from the data since there were failures in the dissection of some females and gravid females at the last stages oogenesis were not dissected. If the regular sampling routine for estimation of the population size yielded less than 100 females, additional catching in the appropriate distance from water immediately complemented these samples. Young adult mosquitoes were identified by the presence of meconium in the gut.

### 2.7. Statistical analysis

Statistical analysis was carried out using the GraphPad Prism 4.0 statistical package. Significance was taken at $p < 0.05$. Comparisons between two samples were performed using Student's $t$ test while the Kruskal–Wallace with Tukey–Kramer HSD as post hoc test were used when the number of samples was greater than two.

## 3. Results

### 3.1. Effects of toxin spraying on the mosquito populations in Experiments A and B

In Experimental area A, which was sprayed with toxic sugar at a distance of 0.5 m from the water, the average CDC trap catch pre-treatment was 138.4 ± 58.3 male and 113.0 ± 42.2 female mosquitoes per trap. In the next month, post-treatment, the total number of mosquitoes decreased significantly ($p < 0.001$) and amounted to 35.5 ± 27.0 males and 43.0 ± 32.8 females per trap.