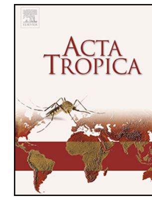
Acta Tropica 136 (2014) 58–67

Contents lists available at ScienceDirect

Acta Tropica

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



Measurement of landing mosquito density on humans

Donald R. Barnard[a,*], Catherine Z. Dickerson[a], Kadarkarai Murugan[b], Rui-De Xue[c], Daniel L. Kline[a], Ulrich R. Bernier[a]

[a] Center for Medical, Agricultural, and Veterinary Entomology, USDA-ARS, Gainesville, FL 32608, USA
[b] Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore 641-046, India
[c] Anastasia Mosquito Control District, 500 Old Beach Road, St. Augustine, FL 32085, USA

ARTICLE INFO

Article history:
Received 27 January 2014
Received in revised form 8 April 2014
Accepted 13 April 2014
Available online 22 April 2014

Keywords:
Mosquito
Vector
Density
Detection
Surveillance
Periodicity

ABSTRACT

In traditional vector surveillance systems, adult mosquito density and the rate of mosquito-human host contact are estimated from the mosquito numbers captured in mechanical traps. But the design of the traps, their placement in the habitat and operating time, microclimate, and other environmental factors bias mosquito responses such that trapped mosquito numbers may be at variance with the numbers actually making human contact. As an alternative to mechanical traps, direct measurement of landing mosquito density enables real-time estimation of the mosquito–human-host-contact parameter. Based on this paradigm, we studied methods to measure mosquito landing responses to a human host. Our results showed: (a) an 18% difference ($P < 0.0001$) in the mean number of female *Aedes albopictus* (Skuse) making initial contact with the skin ($9.11 \pm 0.74\ \text{min}^{-1}$) compared with the number remaining on the skin for 5 s ($7.42 \pm 0.69\ \text{min}^{-1}$); (b) an increase ($P < 0.05$) in the mean per minute (min^{-1}) landing responses of *Culex nigripalpus* Theobald and *Cx. quinquefasciatus* Say with increased sampling time; (c) no difference ($P > 0.55$) in the average number of *Ae. albopictus* landing on the arm ($8.6 \pm 1.6\ \text{min}^{-1}$) compared with the leg ($9.2 \pm 2.5\ \text{min}^{-1}$) of the same human subject; (d) differences among day-to-day landing patterns for the mosquito species we studied but measurable periodicity ($P < 0.05$) in each case when daily patterns were averaged for four or more diel periods; and (e) an effect on landing mosquito density from air temperature ($P < 0.0001$) for *Ae. albopictus* and *Cx. nigripalpus* and dew point temperature ($P < 0.0001$) for *Cx. quinquefasciatus*. Results from this study were used to develop a procedure for safely and accurately measuring mosquito landing density on a human subject.

Published by Elsevier B.V.

1. Introduction

Surveillance systems for adult mosquitoes rely on relative sampling methods (usually mechanical traps) for vector detection and to estimate density and other population parameters (Service, 1993; Silver, 2008; Southwood and Henderson, 2000). But the relationship between data from mechanical traps and the actual presence, abundance, and species composition of adult mosquitoes or the rate of mosquito–host contact is unknown (Barnard et al., 2011). Past failures to detect mosquito presence (Peyton et al., 1999; Sprenger and Wuithiranyagool, 1986) or mosquito-borne disease activity (Nash et al., 2001) thus may be a consequence of traditional reliance on mechanical trapping methods for vector surveillance.

In a chronology of adult mosquito sampling methods that rely on human bait, Silver (2008) writes that human bait catches or human landing catches are the single most useful method for collecting anthrophagic [mosquito] species. The technique is simple to perform, requires no expensive equipment, and when properly conducted precludes mosquito biting and disease agent transmission (Carroll, 2008; Schmidt, 1989; Service, 1969, 1971; Ulloa et al., 1997).

Direct catches of landing mosquitoes have been used to estimate the human biting mosquito population density (Corbet and Smith, 1974; Dia et al., 2005; Ho et al., 1973; Malaithong et al., 2010; Moreno et al., 2007; Roberts et al., 2002; Silver, 2008;). The direct catch method eliminates bias associated with mosquito sampling techniques that combine mechanical traps, semiochemicals, and visual stimuli to capture adult mosquitoes (Barnard et al., 2011; Davis et al., 1995; Sexton et al., 1986; Silver, 2008; Ulloa et al., 1997) or that intercept mosquitoes flying to a host (Achee et al., 2006; Costantini et al., 1998; Dia et al., 2005; Hiwat et al., 2011; Kweka and Mahande, 2009).

* Corresponding author at: USDA-ARS-CMAVE, 1600 SW 23rd Drive, Gainesville, FL 32608, USA. Tel.: +1 352 374 5930; fax: +1 352 374 5870.
E-mail address: don.barnard@ars.usda.gov (D.R. Barnard).

http://dx.doi.org/10.1016/j.actatropica.2014.04.019
0001-706X/Published by Elsevier B.V.

Elements of the experimental design in common to studies that use the direct catch method include (a) teams comprising one human subject (exposed as the bait) and a second (protected) individual who collects the mosquitoes landing on the bait (Garrett-Jones, 1964a,b; Haddow, 1954; Woke, 1962) and (b) a systematized approach to the selection of field sites for mosquito sampling and for scheduling observations (reviewed by Silver, 2008). Among the details lacking in these studies (and elsewhere) is a precise definition for the mosquito landing response and a constant technique for its measurement. Thus, for purposes of sampling the adult mosquito population, it is unclear what constitutes landing by a mosquito, where on the subject's body observation for landed mosquitoes should be made, the duration of such observations, and the area of skin to be examined.

Categorizing the response of female mosquitoes that land and commence immediately to probe/penetrate host skin with their mouthparts is easy to do. However, female mosquitoes may alight on the skin, touch it with the proboscis, take flight, then repeat this behavior; land and move haphazardly across the skin; or remain stationary on the skin without initiating blood feeding (Grossman and Pappas, 1991; Service, 1971). The latter behaviors may comprise latent components of the mosquito landing response during the *exploratory* (foraging) *phase* of blood feeding (Clements, 1992). Their consideration as part of the landing response is important when seeking to estimate the rate of contact between humans and mosquitoes, particularly when landing mosquito density is substituted for the mosquito biting rate.

The main objective of this study was to devise a technique that enables reliable measurement of landing mosquito density on a human host. Our goal was the capacity to correctly classify and quickly count each female mosquito that lands on exposed host skin. Given achievement of this objective, we used the technique to study temporal patterns of landing for *Aedes albopictus* (Skuse), *Culex nigripalpus* Theobald, and *Cx. quinquefasciatus* Say and other facets of the mosquito landing response, including differences in the numbers of females that make coincidental contact with the skin compared with those that land and remain, differences in mosquito responses to the arm and the leg of a human subject, and the periodicity of landing activity.

A second objective was to devise a procedure for comparing the density of landing mosquitoes on a human subject with the mosquito capture rate in mechanical traps. This procedure is needed to evaluate estimates of mosquito population density based on data from mechanical traps for comparability with data from direct observation of landing mosquito density and to determine the usefulness of capture data from mechanical traps for depicting rates of mosquito-human host contact in a vector surveillance program.

2. Material and methods

2.1. Mosquito rearing and test venue

Landing responses were observed for *Ae. albopictus* (Gainesville strain [1992]), *Cx. nigripalpus* (Vero Beach strain [1999]), and *Cx. quinquefasciatus* (Gainesville strain [1995]). Cohorts of each species were reared from the egg stage (Gerberg et al., 1994) outdoors in two 6.4 m $L \times 4.3$ m $W \times 2.8$ m H (77.7 m^3) aluminum-framed enclosures each with fiberglass window screen sides, a sheet aluminum roof, and a single door for entry and exit. Adults emerged into 0.125 m^3 screened holding cages placed inside each enclosure. Twenty-four hours before mosquito landing observations began, we released 1000–5000 4–9 day-old nulliparous females from the holding cages into the enclosure (the number and ages of mosquitoes varied with time of year/environmental conditions). Adult mosquitoes in the holding cages and the enclosure had continuous access to 10% sucrose/water solution (via cotton wick).

2.2. Landing response defined: the exploratory phase of blood feeding

The classification of exploratory activity as part of the landing response is problematic. It requires a technique for differentiating mosquitoes that make coincidental contact with the skin from mosquitoes that land to blood feed. To develop this technique, we tested the hypothesis that the female mosquito population initially touching human skin in a defined period of time is not significantly different from the population remaining on the skin after initial contact. The test comprised a series of 1 min-long observations of mosquito landing in which the number of female mosquitoes that made any contact with exposed skin was compared with the number of females doing so that made mouthpart-skin contact *or* maintained continuous tarsal-skin contact for 5 s. *Ae. albopictus* was selected for these observations. This species lands readily and in high numbers on human hosts during daytime when exact counts of landing mosquitoes can be made without supplementary light. We used a 5 s time limit to differentiate incidental contact from exploratory (foraging) contact and classified the latter as the Land response (henceforth termed: Land, land, landed, or landing). The 5 s time limit reflects a 1 s increase from the average 4 s exploratory phase recorded for *Aedes aegypti* (L.) feeding on a human host with skin temperature of 36 °C (Grossman and Pappas, 1991).

To differentiate incidental contact from Land responses, we limited mosquito access to 75 cm^2 of skin on the left forearm. The skin was exposed through a 5 cm $W \times 15$ cm L window cut into a flexible clear vinyl sleeve tailored for fit to the forearm of the test subject. The sleeve was placed on the left arm with the window centered in the medial forearm area and held in position by fastening the ends together (in the lateral forearm area) with Velcro™. During each 1 min observation session, the forearm was extended forward from the body (with the window in an upright position) and the exposed skin observed for landing mosquitoes. Females that made incidental contact with the skin were counted and recorded. Landing females were captured using a mechanical aspirator (Hausherr's Machine Works, Toms River, NJ, USA) and their number recorded at the end of the test. None was allowed to bite. Observations were replicated 150 times each time using the same human subject (DRB).

2.3. Landing response defined: collection of mosquitoes from different body areas

In this study, we compared mosquito landing responses to the arm and the leg of the same human subject. A matched-pairs design was used. Each test required two outdoor screened enclosures, each with approximately the same number of female mosquitoes. To measure landing responses to the arm, we captured mosquitoes from 415 cm^2 of exposed skin for 15 min using a mechanical aspirator. The area of exposed skin was between two lines of circumference on the left forearm, one at 3 cm below the elbow, the other at 3 cm above the wrist, with the boundary in each case formed by protective clothing held in place with masking tape. To observe for landing mosquitoes, the forearm was extended in front of the seated test subject (approximately 45 cm above ground level) and rotated slowly in a counterclockwise then clockwise fashion.

The same method was used to measure landing responses to the leg, in which case the area of exposed skin formed a 415 cm^2 cylinder around the lower leg. Proximal and distal limits of the exposed skin were, respectively, 7 cm and 18 cm below the horizontal midline of the left kneecap. The circumference of each exposed skin area was bounded by protective clothing held in place with