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Insecticide-treated vertical mesh barriers reduce the number of biting mosquitoes

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Abstract. Mosquitoes foraging for blood sources normally fly relatively close to the ground where wind velocities do not exceed their flight speed. An experiment designed to block foraging mosquitoes from reaching inhabited houses was conducted in a rural settlement flanked by agricultural fields. Mosquitoes were collected during 9 nights using 30 carbon dioxide-baited traps deployed along the external walls of six houses in the row closest to the settlement's perimeter fence. Thereafter, a deltamethrin-impregnated mesh was draped along 400 m of the perimeter fence to a height of 2 m opposite three of the monitored houses. Mosquitoes were trapped for a further 11 nights. A significant difference in the numbers of mosquitoes caught before and after the intervention was demonstrated near protected houses, whereas no significant difference was observed in catches near control houses. The percentage of *Culex perexiguus* (Diptera: Culicidae), an important vector of West Nile virus, was significantly lower near protected houses (13%) than around control houses (45%). By contrast, the percentage of *Culex pipiens* was not significantly affected (16% at experimental and 18% at control houses). Although the results presented here are preliminary, the data demonstrate the potential efficacy of vertical insecticidal barriers for mosquito control.

Key words. *Culex perexiguus*, culicine mosquitoes, insecticide-impregnated nets, mosquito control, vertical barrier, West Nile virus.

Introduction

West Nile virus (WNV) (Flaviviridae: *Flavivirus*) is a zoonotic arboviral pathogen of birds transmitted by culicine mosquitoes. The virus is indigenous to Africa, Asia, Europe and Australia (Kramer *et al.*, 2008). Most WNV infections are asymptomatic, but some acute cases may progress to encephalitis, coma and death (Hubalek, 2001). West Nile fever (WNF) epidemics have raged in many African, Middle Eastern and, more recently, European countries (Hubalek & Halouzka, 1999). In North America, WNV was first detected during an epidemic of meningoencephalitis in New York City in 1999, from which it spread to the lower 48 states (Campbell *et al.*, 2002; Hayes *et al.*, 2005).

Small outbreaks of WNF have been documented in Israel since the early 1950s, peaking with an epidemic involving 419 hospitalized cases and four deaths (Bernkopf *et al.*, 1953; Marburg *et al.*, 1956; Goldblum *et al.*, 1957). In the following decades WNV has remained both enzootic and endemic in the country (Goldblum *et al.*, 1957; Katz *et al.*, 1989; Malkinson & Banet, 2002; Cohen *et al.*, 1999). In the summer of 2000, a large-scale epidemic of WNF occurred in Israel; 417 human cases were confirmed, including 326 hospitalizations and 33 deaths (Chowers *et al.*, 2001). Densely populated urban areas in the coastal plains of Israel were the most heavily affected (Weinberger *et al.*, 2001).

Mosquito surveys conducted in Israel showed that WNV vectors belonged to three mosquito (Diptera: Culicidae)

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species: *Culex pipiens* Linnaeus, 1758 (52% of the catch, WNV infection rate 0.5); *Culex perexiguus* Theobald, 1903 (20% of the catch, WNV infection rate 2.7) and *Aedes caspius* Pallas, 1771 (15% of the catch, WNV infection rate of 0.6). Based on these findings, *Cx. perexiguus* was considered to represent the major vector of WNF in Israel (Orshan *et al.*, 2008).

The results reported here represent offshoots of a study on the control of phlebotomine sandfly vectors of cutaneous leishmaniasis in a newly emerged focus at Kibbutz Sde Eliyahu in the Bet She'an Valley in Israel (R. Faiman, unpublished). The leishmaniases are vector-borne diseases transmitted by phlebotomine sandflies (Diptera: Psychodidae) in tropical, subtropical and temperate regions in some 88 countries (Desjeux, 2004). Cutaneous leishmaniasis is prevalent in large parts of Israel and neighbouring countries (Jaffe *et al.*, 2004). Adult sandflies normally arrive at houses from uninhabited areas and travel in short flights close to the ground (Killick-Kendrick, 1999; Faiman *et al.*, 2009a). Therefore, we postulated that a vertical barrier separating agricultural from residential areas should reduce the number of sandflies arriving at houses.

To begin with, we did not consider a 2-m high vertical mesh barrier an effective obstruction against mosquitoes as these insects fly higher and for longer distances than sandflies (Service, 1997). Despite this doubt, mosquitoes were collected and their numbers recorded to enable us to draw conclusions about the potential efficacy of vertical barriers against mosquito adults.

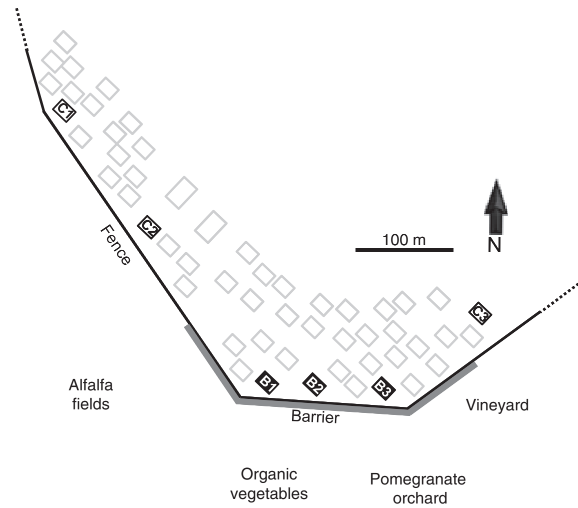

Fig. 1. Schematic map of the southern part of Kibbutz Sde Eliyahu, showing control (C1, C2, C3) and barrier (B1, B2, B3) monitoring stations. The fence is shown as a narrow black line. The net barrier is marked by a thick grey line along the southern margin of the fence. Service roads run parallel to the fence and adjacent to it on both sides. An additional monitoring station was located in the vineyard outside the perimeter fence.

Materials and methods

Study site

All experiments were conducted in Kibbutz Sde Eliyahu, a collective farming community in the northern Jordan Valley (32°26′17″ N, 35°30′46″ E; 185 m a.s.l.; population 700). The terrain in this area consists of flat, cultivated farmland. The climate is hot and dry; the mean maximum daily temperature is 30–33 °C in summer and 15–20 °C in winter. Peripheral residential areas of the village comprising single-storey, two-family houses surrounded by trees, lawns and irrigated gardens were selected for the study.

Trapping mosquitoes

Mosquitoes were trapped using miniature Center for Disease Control (CDC)-type traps assembled in our laboratory using parts identical to those used by commercial manufacturers and modified to operate on 4.5-V DC adaptors connected to the electricity supply. Traps were suspended diagonally from iron pegs, with their opening ∼30 cm above the ground in the down-draft position (traps were placed close to the ground as the study was primarily designed to collect sandflies) (Faiman *et al.*, 2009b). Carbon dioxide (CO_2) was supplied from 27-kg compressed gas cylinders via pressure regulators set at 12 p.s.i. The CO_2 flowed through bifurcating rubber hoses (inner diameter: 6 mm) to each trap. The openings of the tubes were fitted with precision orifices supplying a constant flow of CO_2 at 500 mL/min (orifice: barbed yellow 0.007; Airtrol Components, Inc., New Berlin, WI, U.S.A.).

Mosquito exclusion experiment

Six houses in the row closest to the perimeter fence of the village were selected as monitoring stations and five CO_2-baited traps were deployed adjacent to the external wall of each house. An additional monitoring station was established external to the fence using five CO_2-baited traps set up along a service road by the vineyard shed (Fig. 1). Mosquitoes were trapped during 9 nights in July and August before the erection of the barrier. Thereafter, a 400-m segment of the security fence was draped with a deltamethrin-impregnated barrier to a height of 2 m (Polyester Net®, 450 holes/inch^2; Vestergaard Frandsen A/S, Kolding, Denmark). The lower fringe of the net was covered with soil to anchor it to the ground (Fig. 2). The three monitoring stations located centrally behind the barrier are hereafter referred to as the 'barrier group' and the three flanking monitoring stations (one to the northeast and two to the northwest of the barrier) are designated the 'control group' (Fig. 1).

Insecticide decay assay

To evaluate the rate of decay of deltamethrin from the net, a separate net from the same batch was placed on the peripheral fence and 30 × 30-cm samples were removed from it on days 1, 14, 32 and 59 (three on each day for a total of 12 samples) following deployment. Samples were kept in air-tight bags in darkness at 4 °C. Levels of insecticide were assayed

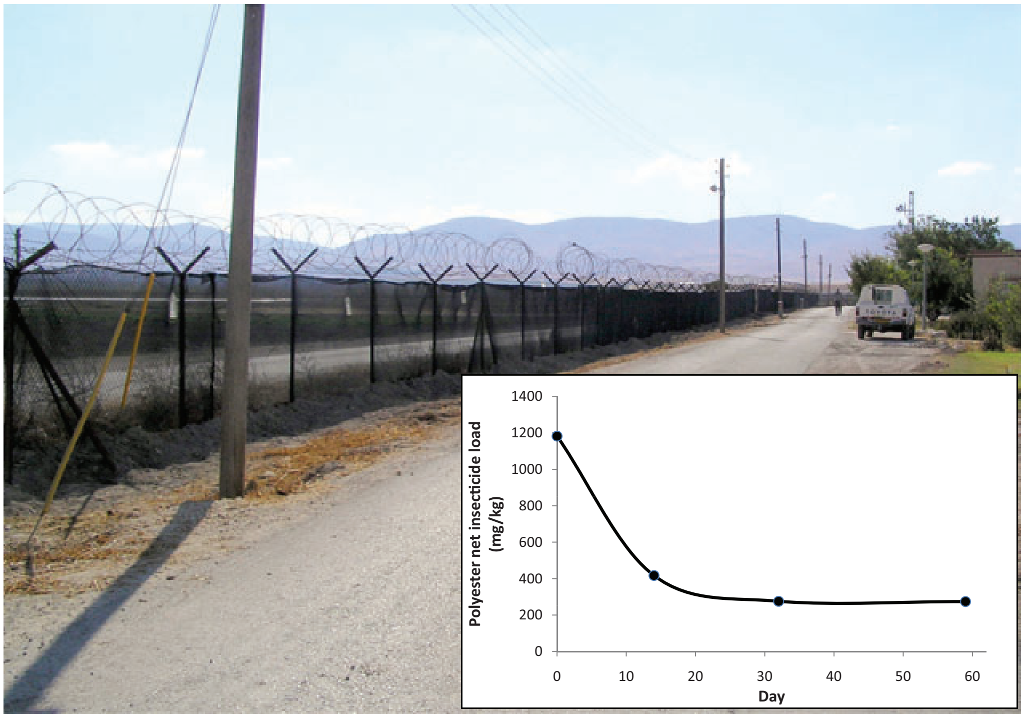

Fig. 2. Photograph taken from within the kibbutz of the perimeter fence around Sde Eliyahu. The southern section of the perimeter fence is draped with a Polyester Net® barrier. Inset: the curve shows the decay in the mean content of deltamethrin per kg of Polyester Net® fabric exposed to ambient conditions in Sde Eliyahu over 60 days. Decay followed a polynomial curve, reaching close to the minimum within 3 weeks, after which it stabilized.

by an independent laboratory (BactoChem Ltd, Ness-Ziona, Israel) using gas chromatography and mass spectrometry (U.S. Food & Drug Administration, 1995). Decay analysis was performed by plotting the mean quantity of insecticide found in samples of identical sizes removed at different time-points (Fig. 2, inset).

Mosquito species identification

Trapped mosquitoes were transported to the laboratory where they were counted and preserved in 100% ethanol or frozen dry (−20°C). Identification was based on morphological characterization of the palps, thoracic and wing scales, wing veins (Comstock-Needham system) and leg colouration, as well as several biometric ratios using the key to the mosquitoes of Israel developed by the Israeli Ministry of Health, Entomological Laboratory and based on Gutsevitch *et al.* (1970).

Statistical tests

Trap yield data were tested for the normality of their distributions and degrees of variance to assess the type of distribution the data conformed to and the appropriate analysis. Two normality tests were used; a one-sample Kolmogorov–Smirnov Z-test was used for absolute mosquito numbers, and the Shapiro–Wilks test was used for log-transformed data and group means.

Mosquito numbers were log-transformed [$\log(n+1)$] to normalize the distribution and control the variance in cases of non-normal distribution, caused chiefly by aggregated distribution. This allowed the derivation of the geometric 'Williams mean' (Mw), which represents a more accurate and sensitive mean than an arithmetic mean. Geometric means are amenable to parametric statistical analysis in most cases (Williams, 1937; Bidlingmayer, 1969).

Mann–Whitney U-tests and Wilcoxon signed-rank tests were applied to compare mean trap yields between or within the test groups, before and after the barrier net was erected. Two-way analysis of variance (ANOVA or Kruskal-Wallis test) was utilized to assess the effect of the trap locations and treatment type on mean mosquito yields.

All statistical analyses were performed using SPSS for Windows® Version 17.0 (SPSS, Inc., Chicago, IL, U.S.A.) and Microsoft® Office Excel® 2007.

Results

Mosquito populations

Baseline trapping was conducted during April to August 2009 in the vineyard just outside Sde Eliyahu in order to characterize the mosquito population in the study area. During this period, 11 008 mosquitoes (Diptera: Culicidae) were collected. Species comprised: *Cx. pipiens* (36%); *Cx. perexiguus*

(34%); *Ae. caspius* (20%); *Anopheles sergenti* Theobald, 1903 (7%), and *Uranotaenia unguiculata* Edwards, 1913 (2%). Several *Culex theileri* Theobald, 1903, *Anopheles tenebrosus* Dönitz, 1902, *Anopheles claviger* (Meigen) 1804 and *Aedes detritus* Haliday, 1833 were also trapped, constituting together 1% of the total catch. The insignificant constituents of the mosquito fauna (including *U. unguiculata*) were excluded from the statistical analysis.

Vertical net barrier

Six trapping stations, each of which comprised five CO_2-baited traps placed around a two-family, single-storey house, were monitored during the subsequent experiment. These were divided into two groups: three houses represented the barrier group (B1, B2, B3) and were located along the section of the fence draped with the net barrier, and three houses represented the control group (C1, C2, C3) and were positioned along the fence on either side of the barrier (Fig. 1). Mosquitoes were trapped during weekly pre-treatment nights ($n = 9$) and during an additional 11 nights (1 night per week). Species composition was markedly different from that monitored in the vineyard; *Cx. perexiguus* accounted for >45% and *Cx. pipiens* for <18% of the catch in the control group (Table 1).

Spatial and temporal distributions were neither normal nor uniform (Kolmogorov–Smirnov normality test, $P < 0.01$). Derivation of the geometric Mw by log transformation reduced the variation and normalized the distribution of the means (Shapiro–Wilks normality test, $P > 0.05$). No significant difference was observed in control group catches before and after the intervention (ANOVA, $F = 0.44$, d.f. = 18, $P > 0.5$). However, a significant decrease in trap yield means was apparent in the barrier monitoring stations (ANOVA, $F = 2.16$, d.f. = 18, $P < 0.05$) (Fig. 3). In separate analyses of trap yields for the different monitoring stations, all barrier monitoring stations exhibited a decrease. However, only one of these (B1) exhibited a significant decrease in mean trap yields ($P = 0.03$) and one was only marginally significant (B3, $P = 0.056$). A notable, albeit non-significant, decrease was also observed in the third barrier monitoring station (B2, $P = 0.095$) (Fig. 4).

A comparison of the numbers of mosquitoes belonging to different species captured near treatment houses with those captured near control houses showed the most striking difference in relative numbers of *Cx. perexiguus.* A significantly lower percentage of *Cx. perexiguus* was noted near treatment houses. By contrast, only marginal insignificant differences were noted for *Cx. pipiens*, *An. sergenti* and *Ae. caspius* (Table 1, Fig. 5).

Table 1. Predominant mosquito species collected near houses along the southern perimeter fence of Sde Eliyahu.

Mosquito species	Control traps, %	Barrier traps, %
Culex perexiguus	13.16	45.06
Culex pipiens	15.74	17.88
Anopheles sergenti	1.29	2.58
Aedes caspius	2.86	1.43

Control traps denote those in unprotected houses; barrier traps denote those in houses situated directly behind the barrier; figures represent percentages of the entire catch at those traps.

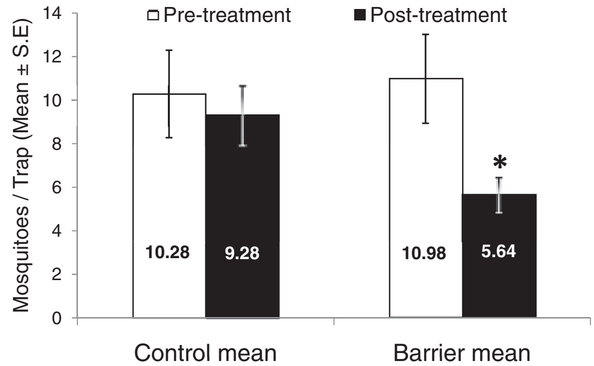

Fig. 3. The effect of the net barrier on numbers of mosquitoes. Mean ± standard error (Mw ± SE) mosquito catches per trap before (white bars) and after (black bars) construction of the barrier. Only the barrier group showed a significant difference post-treatment (51% reduction). *, Mann–Whitney U-test, $Z = -5.7$, $P < 0.01$.

Insecticide decay analysis

The initial insecticide load on the net was ~1200 mg/kg. The decay curve was polynomial for 30 days (curve formula: $y = -0.0218x^3 + 2.466x^2 - 84.895x + 1182.2$) as the deltamethrin decayed, presumably as a result of ultraviolet light exposure. Insecticide load decreased significantly during the first 15 days, dropping to <400 mg/kg of mesh. During the following 40 days, the decay rate reduced until the curve was

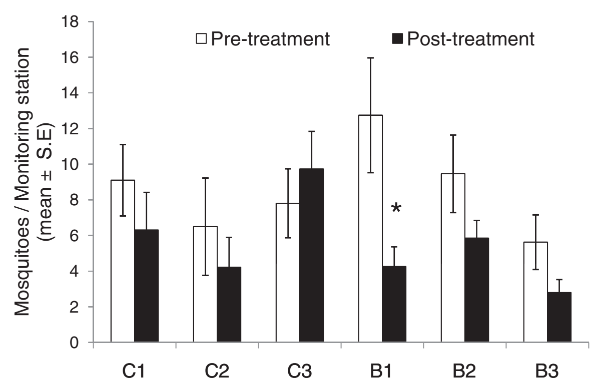

Fig. 4. The effect of the net barrier on numbers of mosquitoes caught in the individual monitoring stations. Mean ± standard error (Mw ± SE) mosquito catches per monitoring station before (white bars) and after (black bars) construction of the barrier. All three barrier monitoring stations exhibited a marked decrease in numbers caught, but this was statistically significant only in station B1. *, $P < 0.05$.

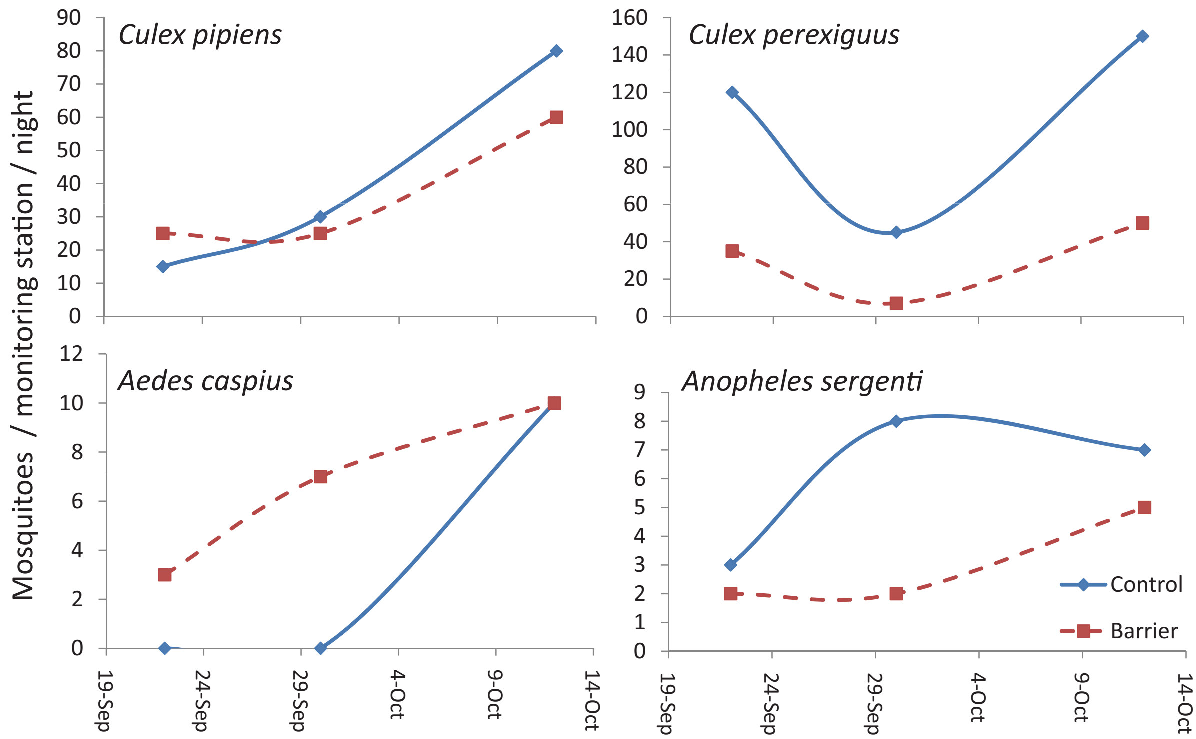

Fig. 5. Relative densities of mosquito species captured around houses (three monitoring stations per group with five CO_2-baited traps each) during the experiment (summer, 2009) [Williams mean (Mw)]. The most prevalent species was *Culex perexiguus*, numbers of which were significantly reduced in catches behind the barrier. *Culex pipiens* was also prevalent. *Aedes caspius* and *Anopheles sergenti* were less common by one order of magnitude, making it difficult to ascertain any significant effect of the barrier.

nearly linear as the load decreased by ~80 mg/kg of mesh in total (Fig. 2, inset).

No significant increase was observed in trap yields obtained immediately after the construction of the barrier, when the mesh contained high insecticide loads, and yields obtained 3 weeks later, when the net had lost most of its insecticide load (Fisher's exact test, $P > 0.5$).

Discussion

The findings reported here were obtained during a study designed primarily to investigate ways of preventing phlebotomine sandflies, vectors of cutaneous leishmaniasis, from reaching the peripheral houses of Sde Eliyahu (R. Faiman, unpublished). We did not expect the vertical barrier to be an effective strategy against mosquitoes as these insects are generally more tolerant of mild windy conditions than phlebotomines and are less inclined to fly as close to the ground (Gillies & Wilkes, 1978; Service, 1997). Mosquitoes captured in the CDC traps were counted, but unfortunately all those collected before the erection of the net barrier were discarded after counting and were not identified to species. Therefore, we were only able to determine the significant decline in the numbers of mosquitoes as a group (Figs 3 and 4).

To improve the quality of the results, we identified the species of all mosquitoes collected after the barrier had been erected and compared the numbers of each species between barrier group and control group collections (Table 1, Fig. 5). Thus, we were able to show that certain species were less prevalent around protected houses than around houses that were distal to the barrier (i.e. not protected) (Table 1). The species affected most significantly was *Cx. perexiguus*, the predominant mosquito around houses in Sde Eliyahu (Table 1). Significantly, *Cx. perexiguus* is considered the most important vector of WNV in Israel (Orshan *et al.*, 2008). The barrier had a far less significant effect on catches of *Cx. pipiens*, the second most prevalent mosquito near houses (Table 1, Fig. 5), which may reflect the existence of breeding sites within the settlement or, possibly, alternative foraging height behaviour (Bellini *et al.*, 1997).

Although malaria is not endemic to Israel, *An. sergenti* remains an important malaria vector in other countries of the Eastern Mediterranean region (Zahar, 1974). Numbers of *An. sergenti* near houses in Sde Eliyahu were significantly lower than numbers in the vineyard and thus it was difficult to determine whether the barrier had any effect on the ability of this species to reach houses (Fig. 5, Table 1). Numbers of *Ae. caspius* were generally low and appeared to be transiently affected after the erection of the barrier, but rose soon thereafter at both protected and unprotected houses (Fig. 5). *Aedes caspius* tends to fly at lower heights than other mosquito species, including *Cx. pipiens* (Bellini *et al.*, 1997). Therefore, we postulate that the transient nature of the reduction may reflect the steep curve in the decay of insecticide levels in the mesh (Fig. 2, inset). The strong impact on *Cx. perexiguus* and variable reductions in numbers of other mosquitoes indicate the need to evaluate the effects of such barriers for longer periods and to analyse these observations in relation to different wind conditions and other meteorological variables. Mosquitoes leave their breeding habitats for several reasons, but principally to forage for blood (Service, 1997). Although high-altitude, wind-assisted migration for long distances has been recorded for several mosquito species, during medium-range dispersal mosquitoes fly closer to the ground within what is referred to as the ‘barrier zone’, above which wind velocities exceed their flight speed (Bidlingmayer, 1985; Service, 1997; Chapman *et al.*, 2003). In experiments conducted in West Africa, flight barriers did not appear to have an effect on biting rates of *Anopheles gambiae* Giles, 1902 (Gillies & Wilkes, 1978). By contrast, insecticidal barriers have been shown to reduce the number of questing *Culex tarsalis* Coquillett, 1896 (Britch *et al.*, 2010). When flying over open, flat terrain, such as the farmland surrounding our study site, many mosquitoes tend to stay at around 2 m above the ground (Bellini *et al.*, 1997).

The results reported here clearly demonstrate the potential merit of vertical insecticide-treated barriers to intercept foraging mosquito females on their way to inhabited areas. This strategy warrants further investigation as a tool in mosquito control, perhaps using taller barriers (3.5 m), more frequent reapplication of insecticide and optimal trap set-ups.

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