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Residual Mosquito Barrier Treatments on U.S. Military Camouflage Netting in a Southern California Desert Environment

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ABSTRACT Treating perimeters of vegetation with residual insecticides for protection from mosquito vectors has potential for U.S. military force health protection. However, for current U.S. military operations in hot-arid environments with little or no vegetation, residual applications on portable artificial materials may be a viable alternative. We evaluated bifenthrin residual treatments of U.S. military camouflage netting under hot-arid field conditions in a desert area in southern California exposed to abundant wild *Culex tarsalis* mosquitoes. We assessed the ability of the treatment to reduce the numbers of mosquitoes penetrating perimeters of netting and reaching CO_2-baited mosquito traps. Treated camouflage netting barriers reduced mosquitoes by ≥50% for 7–14 days and by 20–35% for 21–28 days compared to untreated barriers. Although reductions may be translated into reductions in risk of exposure to mosquito-borne diseases, we emphasize that barrier treatments should be a component in a suite of insect control measures to be effective.

INTRODUCTION

Barrier treatments reduce the numbers of biting insects such as mosquitoes or sand flies moving into a protected area. Typically, a band of residual insecticide is sprayed onto ambient vegetation, forming a perimeter around the protected area. The toxic barrier is designed to intercept the movement of target insects into the protected area, either repelling them, or exposing the insects to lethal or crippling doses as they rest during movement toward detected hosts.[1] Barrier treatments for protection from mosquito and sand fly disease vectors and nuisance insects have been the subject of research over the last 60 years,[2–8] using a range of natural substrates from open grasslands to jungles, forests, and hedges.[9–11] Although interior and exterior residual sprays on structures and treated bed nets have their own rich history and may be considered a barrier treatment,[12–14] pioneering work with other treated artificial substrates such as suspended or spread sheets,[15,16] livestock fencing,[17,18] attractant-baited, insecticide-impregnated targets dispersed along a perimeter,[19–21] and tents or shade cloth[22,23] (R. E. Coleman, unpublished data) have opened up new possibilities for military operational use of barrier treatments. In the last 5 years, barrier treatment studies have been increasing in frequency,[22–30] and a recent symposium at the 2009 Annual Meeting of the American Mosquito Control Association and a 2009 Florida Mosquito Control Association Dodd Short Course dedicated to barrier treatment research and implementation, show that this approach to protecting humans from biting insects warrants serious attention.

Although barrier treatment research has examined efficacy against insects of high military importance such as mosquitoes[31,32] and sand flies,[1,33,34] one major shortfall of barrier treatment research, given the current theaters of U.S. military operations, is that few studies have been conducted either to investigate the impact of deposition of residuals on natural or artificial substrates in desert environments or to identify the short- or long-term efficacy of such barriers against mosquitoes or sand flies. Hot-dry and dusty conditions in Iraq and Afghanistan coupled with persistent threats from disease-transmitting arthropods in those regions make it imperative to evaluate the suitability of barrier treatment technology to protect deployed military personnel stationed in temporary shelters in desert terrain.[35–37] Additionally, hot-arid climates generate problems for spray applications of pesticides because of rapid evaporation of solvents, as well as problems with persistence of applied chemicals caused by excessive UV and heat exposure and dust deposition.

A recent study by Britch et al.[30] investigated the feasibility and efficacy of barrier treatments on vegetation in a desert area of southern California. They found that single applications of bifenthrin residual sprays on sparse, low, xeric vegetation highly permeable to biting insects could be effective at substantially reducing mosquitoes in protected areas when compared to untreated areas of equal size for up to a month. Although this approach could potentially provide rapid long-lasting protection in nearly any location having regular or irregular perimeters of vegetation, there are desert areas that do not have vegetation in sufficient abundance or density to create treated barriers. However, military units could carry portable pretreated or treatable artificial barriers

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with them and set them up as needed, regardless of vegetation. Camouflage screening systems are already organic to many units in Operation Iraqi Freedom/Operation Enduring Freedom (OIF/OEF) theaters of operation, and we hypothesized that this material could be treated with residual insecticide and formed into a barrier with the potential to reduce mosquito abundance in a desert environment.

MATERIALS AND METHODS

Study Site

We used a desert area in the Coachella Valley, California, to evaluate barrier treatments of bifenthrin on two styles of Department of Defense (DoD) woodland pattern camouflage netting under hot-dry and dusty field conditions (Fig. 1). This natural study site is situated to the west of a cluster of fish ponds and marshy areas in a region cut by irrigation canals and 0.5 km from the northwest shore of the Salton Sea (33.46° N, 116.06° W; –64 m). The effluent from commercial fish ponds and seasonal flooding of marshy areas along the Salton Sea create a large productive habitat for the development of wild *Culex tarsalis* Coquillett mosquitoes. The study was timed in March 2008 to take advantage of the annual February through May rise of the Salton Sea that triggers development of abundant wild *Cx. tarsalis* populations and a consequent high biting pressure.[38] Salton Sea surface elevation data, an index of water level, from the Coachella Valley Water District (CVWD, P. O. Box 1058, Coachella, CA, 92236) confirmed this expectation (Fig. 2). The dominant plant species at the study site included *Tamarix chinensis* Lourteig (salt cedar), *Pluchea sericea* (Nuttall) Coville (arrow weed), *Atriplex canescens* (Pursh) Nuttall (salt bush), and *Salicornia virginica* Linnaeus (pickle weed).

Camouflage Material Systems

We constructed four 3 m × 3 m frames each 2 m in height using 3-in steel fence pipes and fence hardware (Fig. 3A). Current-issue DoD woodland pattern radar transparent camouflage netting (Saab Barracuda LLC, Lillington, North Carolina) 2 m in height and 12 m long was suspended tightly around each frame to form open-topped square enclosure systems (Fig. 3B). The netting consists of a rubberized 3 mm × 3 mm screen backing, upon which is stitched rubberized woven fabric resembling leafy material; we attached the netting with the leafy side facing out (Fig. 4A). We also deployed two older-issue 9 m × 10 m woodland pattern radar scattering hexagon dome systems (obtained from DRMO surplus) consisting of rubberized leafy fabric (Fig. 4B) tacked to 5 cm × 5 cm

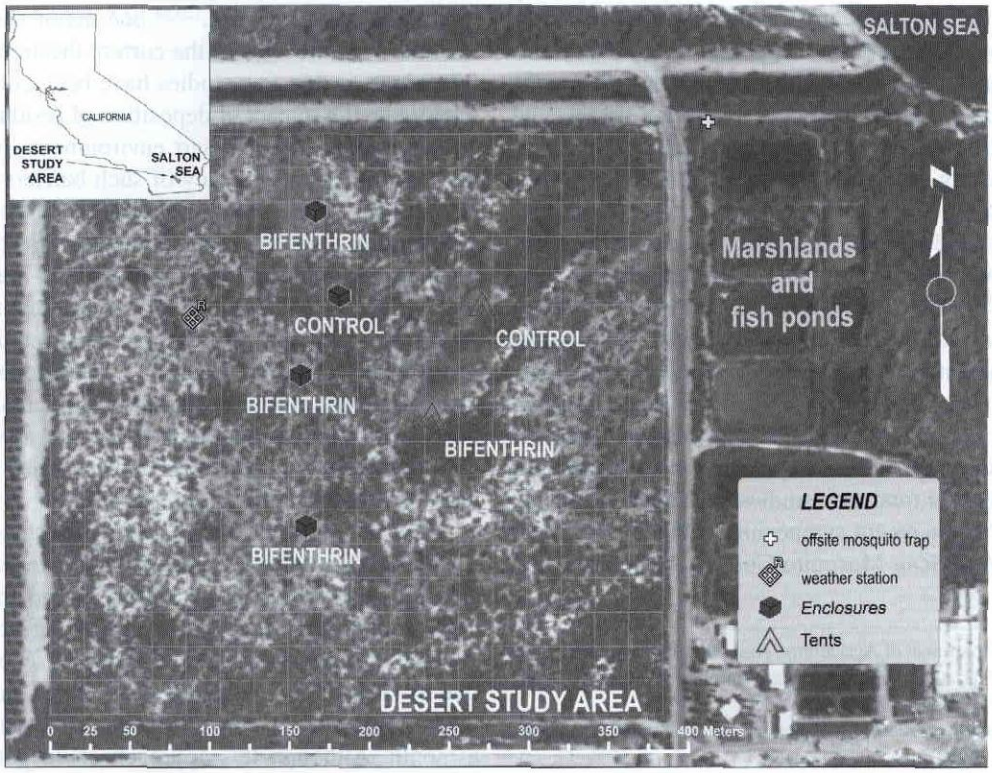

FIGURE 1. *Desert study area in the Coachella Valley, southern California. The background image is a 1-m resolution natural color Digital Orthophoto* Quadrangle (NAD 1983, UTM Zone 11N) available from the USGS at http://seamless.usgs.gov/. The shore of the Salton Sea is visible in the northeast corner of the image, with the fish hatchery ponds and marshlands to the east of the study area. Cube icons on the image show the location of the four enclosure systems, and tent symbols show the location of the two dome systems, and labels indicate bifenthrin treatments or untreated controls. The single white cross symbol at the northwest corner of the fish ponds shows the offsite control mosquito trap. The "R" on the weather station symbol marks that a weather data recorder was present.

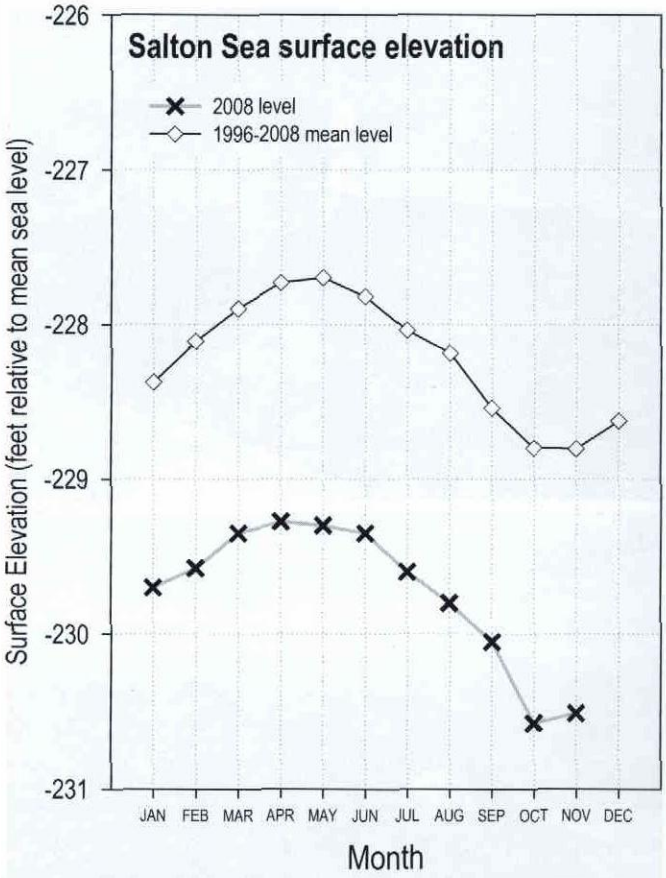

FIGURE 2. Monthly mean Salton Sea surface elevation, an indication of water level, in feet relative to mean sea level. The annual February to May rise in water level triggers development of *Culex tarsalis* in shore habitat. The overall low water level in 2008 compared to the long-term mean reflects an ongoing and gradual annual decline over the last decade. Nevertheless, the trend of increasing water level in spring 2008 indicates the presence of natural breeding habitat for wild *Cx. tarsalis* throughout the 28-day population sampling period in the desert study area.

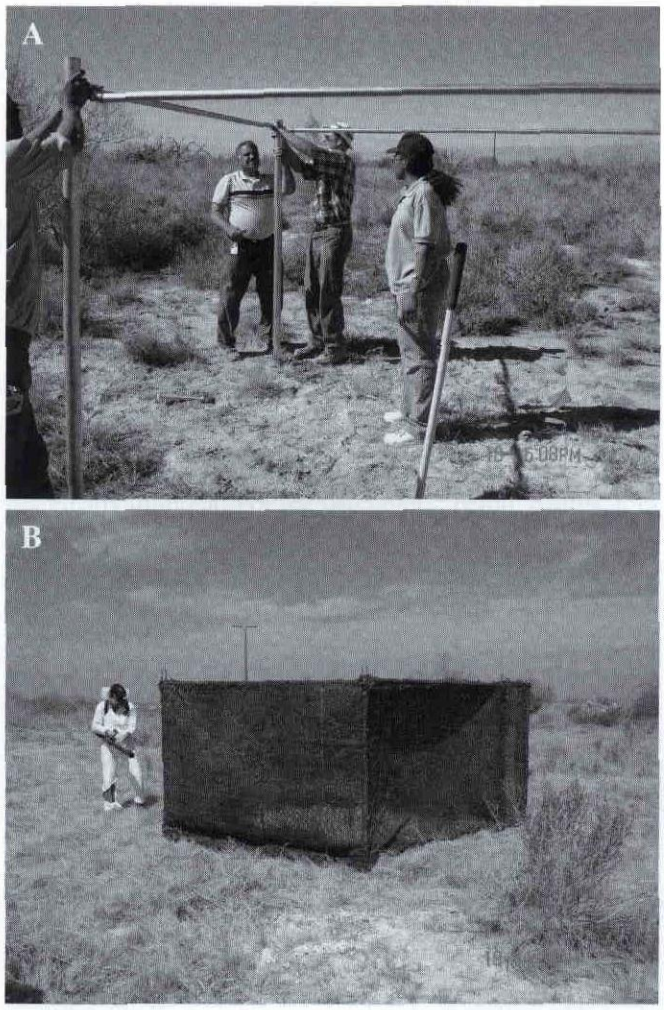

FIGURE 3. Constructing (A) and treating (B) the woodland pattern enclosure systems.

nonrubberized netting and supported with telescoping poles, guy lines, and pegs (Fig. 5B). To the extent possible we situated the enclosures and tents at least 50 m apart from each other in areas with similar vegetation densities along two north–south axes in the desert field (Fig. 1). The enclosures were approximately 250 m from the fish ponds, and the tents were approximately 200 m from the fish ponds. We first mapped the desert study area using a 1-m resolution 3-band (RGB) natural color Digital Orthophoto Quadrangle (DOQ; available from the USGS, http://seamless.usgs.gov/) in ArcGIS 9.2 (ESRI, Redlands, California) geographic information system (GIS), extracted coordinates of candidate locations for enclosures and domes using the GIS, and finally marked the final locations of all systems on the ground using a GPS and pin flags. We surveyed and recorded points in the field using a GeoXT (Trimble, Sunnyvale, California) handheld GPS, which operated at approximately 3-m precision (uncorrected) and was set at the NAD 1983 datum for spatial reference to UTM Zone 11N to match the USGS DOQ.

Sprayer

The Stihl SR 420 (Andreas Stihl, Waiblingen, Germany) is a backpack cold-mist blower powered by a 3.4-hp single-cylinder 2-cycle Stihl engine, with an airflow rate of up to 625 cfm and an air velocity of 180 mph. The sprayer uses an air-shear atomization head with screens to alter the spray release pattern. The flow rate can be set from a control knob placed near the head that has six metering nozzle settings ranging from 4.7–100 oz/min. Setting 2 was used for this study, which produced 93.8 oz/min. The Stihl SR 420 has a net weight of 24 lbs, a pesticide tank capacity of 3.7 gallons, and the bystander noise level is 75 dBA.

Barrier Treatments

The barrier treatments consisted of applications of bifenthrin on camouflage netting using a Stihl SR-420 backpack sprayer and untreated controls. We applied Talstar bifenthrin (FMC Corp., Philadelphia, Pennsylvania) in water at the label rate of 1.0 oz per 1,000 ft^2, adjusting walking speed to 1.39 mph

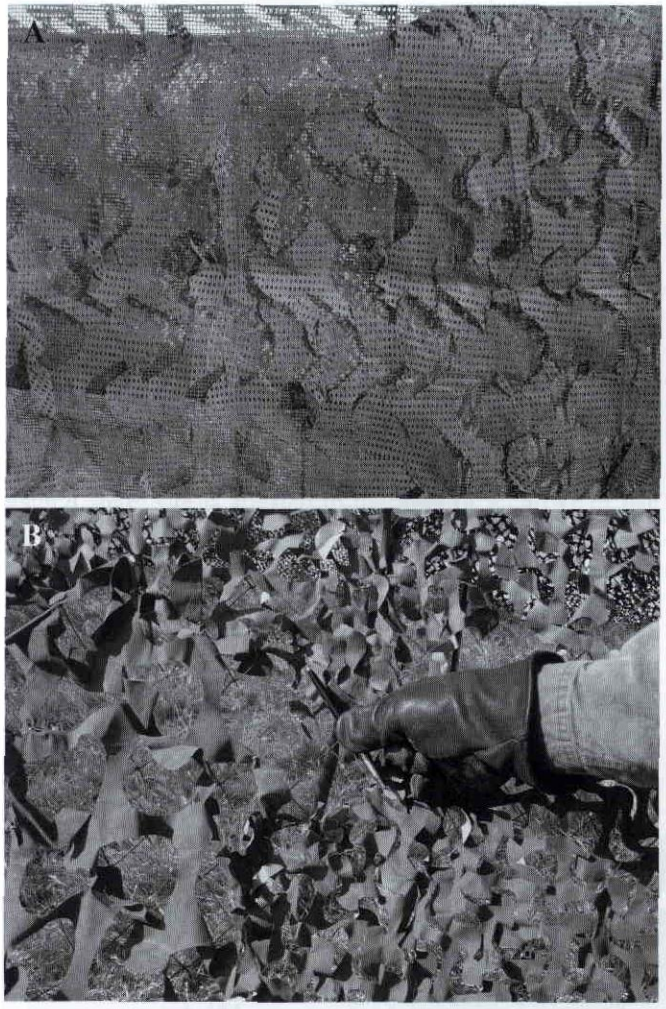

FIGURE 4. *Detail view of the current issue DoD woodland pattern radar* transparent camouflage netting (A) and of the older issue woodland pattern radar scattering camouflage netting (B) used in this study for enclosure and dome systems, respectively.

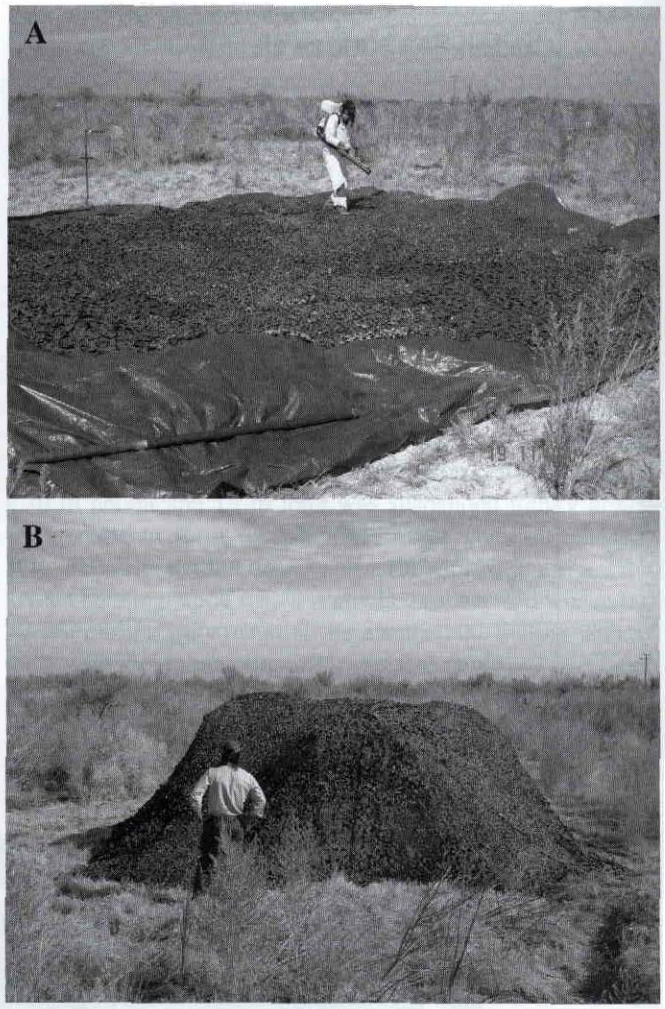

FIGURE 5. *Treatment of woodland pattern hexagonal dome system* (A) and dome system pitched on support poles (B).

to account for the 93.8 oz/min calibrated flow rate of the Stihl to finish with near identical dispersal of active ingredient on all treated enclosures and the dome. The spray operator applied bifenthrin within the allotted spray times to the outside surface of the three standing enclosures (Fig. 3B) and to one of the domes while it was positioned on a plastic sheet on the ground before pitching (Fig. 5A). We assigned numbers to each system and drew numbers from a hat to designate treated and control systems. A portable Integrated Sensor Suite weather station with a Vantage Pro2 data recorder (Davis Instruments, Hayward, California) was erected at 2 m on a pole within the field (Fig. 1). Figure 6 shows weather patterns for March through May 2008 from this weather station. The conditions at the time of the spray were ~75 °F, ~25% relative humidity (RH), and 4–6 mph wind speed (data taken from the Weather Underground, http://www.wunderground.com, for Thermal Airport, Thermal, California, approximately 17 km north of the study area, because the on-site weather station recorder malfunctioned on this day). We measured the temporal pattern of bioactivity of the bifenthrin barrier treatments on the camouflage material under desert conditions with field surveillance of mosquitoes within treatment and control areas over a 1-month period.

Mosquito Collections

For mosquito population sampling in the field we set modified encephalitis virus surveillance (EVS) mosquito traps[39] baited with dry ice (CO_2) without light at the centroid of each enclosure and dome system on a permanent stanchion to simulate a human presence and ran them overnight (approximately 5:00 p.m.–8:00 a.m.) the night before treatment (day −1), the night immediately following the treatment (day 0), the night after the first day post-treatment (day +1), and overnight once a week thereafter for a month (days +7, +14, +21, and +28). A fourth offsite control trap was set ~250 m away on the east side of the field close to immature mosquito development sites (Fig. 1). Traps were collected the next morning and mosquitoes transported to the lab for identification and counting and then archived in labeled Petri dishes in a refrigerator. We assessed efficacy of

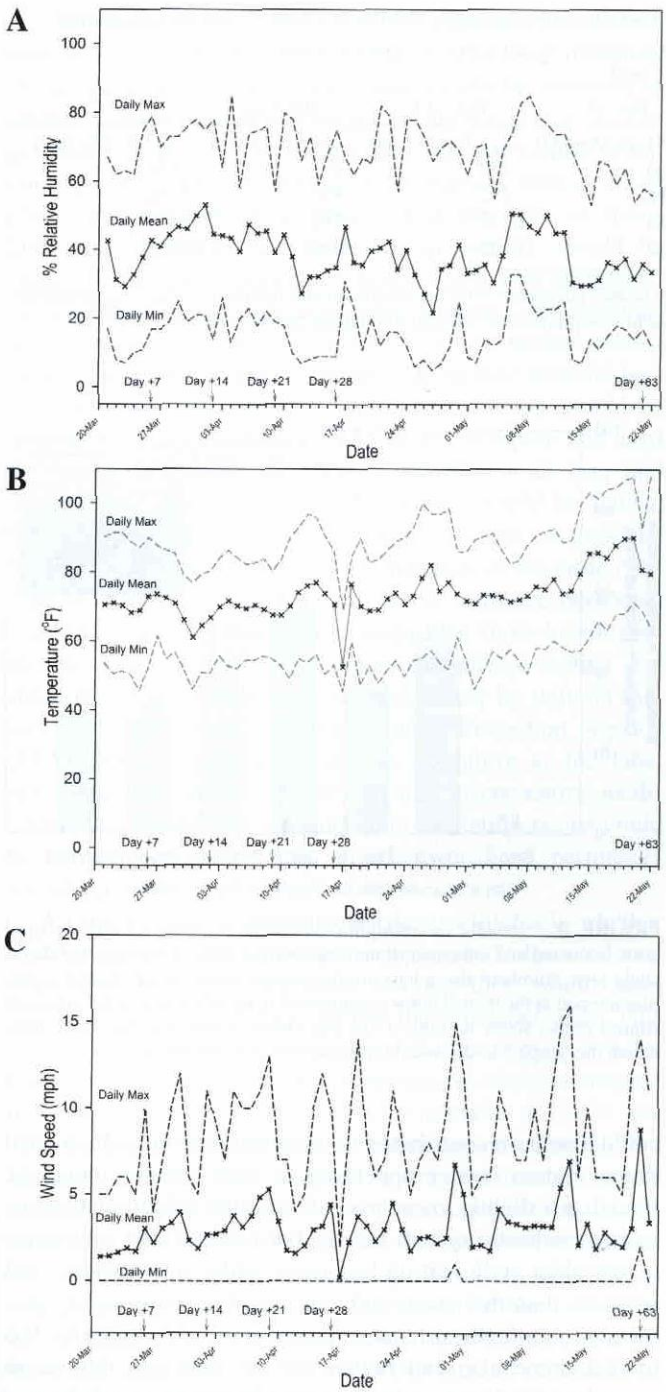

FIGURE 6. Weather station data for March 21–May 22, 2008. Sample days relative to day of treatment are marked above the *x*-axis. No precipitation was detected in this sample period. Data are missing from day −1 (March 18), day 0 (March 19), and day +1 (March 20) due to a malfunction of the weather data recorder.

barrier applications by differences in trap counts of local wild female mosquitoes between treated and untreated systems.

RESULTS

For the 28-day duration of the study, treated enclosure and dome camouflage netting systems consistently reduced mosquito populations reaching sentinel EVS traps set within the systems. Trap count data are shown in Table I, and Figures 7 and 8 show these data converted into percent reductions in trap counts in treated versus untreated systems. Biting pressure as indicated by trap counts of female mosquitoes (Table I) was high throughout the study at enclosure and dome control trap locations and at the offsite trap in the northeast of the desert area (Fig. 1), providing evidence that low trap numbers observed in treated enclosures and the treated dome resulted from the repellent or toxic effect of the chemical barrier and not local population fluctuations. The vast majority (i.e., >95%) of mosquitoes in the trap samples were *Cx. tarsalis*.

As expected, trap counts on day −1 (i.e., the day before the treatments were carried out) in enclosures and domes were not greatly different. For the two domes, before treatment of one of them, trap counts were nearly identical on day −1. For the four enclosures, the one enclosure destined to be left as an untreated control happened to have a count 28% higher than the mean catch of the three enclosures destined to be treated the next day, possibly due to unforeseen variables such as an unusually high CO_2 flow from a cracked dry ice reservoir, or a larger movement of mosquitoes through that region of the plot. However, subsequent post-treatment overnight trap samples with much greater differences suggest that the treated enclosures had a reducing effect on arrival of mosquitoes at traps, and that the untreated enclosure was not inherently more attractive to mosquitoes, or situated in a zone with greater densities of mosquitoes.

Mosquito reductions in treated enclosures and the treated dome were not uniform over the 28-day study period. For the treated enclosures, reductions declined gradually and linearly over the first week, though remaining above 50%, and then fell to a more flat but varying pattern of 20–35% reduction in mosquitoes for the remainder of the study. At the treated dome, reduction in mosquito counts as compared to the untreated dome were overall lower than for the enclosures, hovering around 40–50%, but despite some heterogeneity did not decline until after the second week of the study, falling to about 30% reduction in mosquitoes for the remainder of the study.

DISCUSSION

Barrier treatments of bifenthrin on two styles of military camouflage netting are effective at reducing mosquitoes at sentinel EVS traps when applied and implemented in a hot-arid desert environment. Although the DoD does not currently define a standard for efficacy of barrier treatments, we may echo Britch et al.[30] and tentatively define "effective" as a ≥50% reduction in mosquito counts in traps placed in the field for 7–14 days postspray in treated camouflage netting systems as compared to untreated control nets on the same day. Salton Sea water level throughout the mosquito surveillance period (Fig. 2), along with consistently high trap counts at the offsite trap, indicate that natural breeding sites for *Cx. tarsalis* were

TABLE I. *Culex tarsalis* collected in EVS mosquito traps in the four enclosure systems, two dome systems, and the offsite trap

Plot/Sample	Mosquito Count						
	Day −1	Day 0[a]	Day +1	Day +7	Day +14	Day +21	Day +28
Enclosures (Bifenthrin)	250.3 (217–272)	56.3 (49–61)	98.0 (96–102)	281.3 (208–400)	215.7 (189–269)	139.3 (125–154)	557.3 (496–640)
Enclosure (Control)	349	169	248	564	279	212	696
Dome (Bifenthrin)	318	199	347	588	592	392	1,168
Dome (Control)	320	385	554	1,328	1,128	552	1,672
Offsite Control	—	584	453	1,512	1,128	1,000	3,968

In the case of the enclosure systems, counts are presented as a mean of the three treated enclosures with the maximum and minimum values in parentheses. Trap data from the offsite control (not set on day −1) demonstrate that abundant wild mosquitoes were present throughout the study period.
[a]Day 0, the day of treatment.

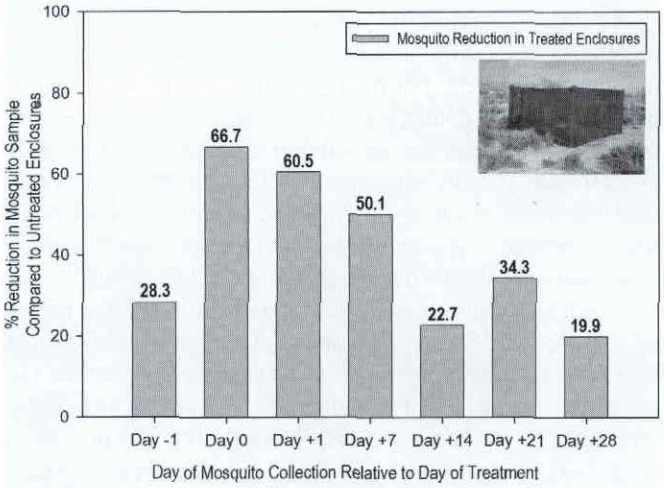

FIGURE 7. Results for mosquito surveillance conducted March–April 2008 in treated and untreated camouflage netting enclosure systems in the desert study area. (A) Numbers above bars are the percent reduction in mosquito samples trapped in the treated enclosures as compared to those trapped in the untreated control enclosure. For example, for day 0 (i.e., trapping conducted overnight immediately following the initial treatments) we trapped on average ~67% fewer mosquitoes in treated enclosures than in the untreated enclosure. Refer to Table 1 for trap counts throughout the study from which this graph was derived. Day 0, day of barrier treatment.

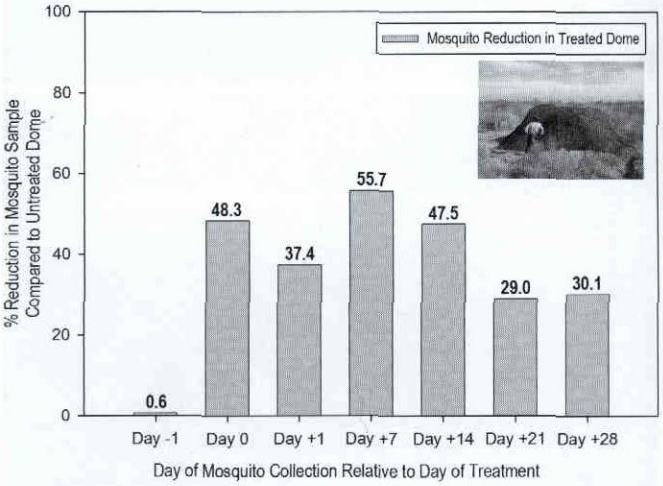

FIGURE 8. Results for mosquito surveillance conducted March–April 2008 in treated and untreated camouflage netting dome systems in the desert study area. Numbers above bars are the percent reduction in mosquito samples trapped in the treated dome as compared to those trapped in the untreated control dome. Refer to Table 1 for trap counts throughout the study from which this graph was derived. Day 0, day of barrier treatment.

still available and thus do not account for the reductions in population samples in treated enclosures or the treated dome. The structure of the camouflage nets themselves may have been responsible for the close tracking of female mosquito counts from the untreated dome with female mosquito counts from the offsite trap in the northeast (Table I): the older style open netting used in the dome systems is more permeable to mosquitoes and poses less of a physical barrier to mosquitoes reaching the trap when compared to the tight weaving of the new style netting, designed to snag less on equipment. On the other hand, unlike the dome systems, the enclosure systems were completely open on the top and the weaving was not small enough to exclude mosquitoes. In fact, mosquitoes forced to reach the interior of the enclosures by squeezing through the mesh may have contacted more bifenthrin, explaining the higher reduction in mosquito counts for the first week in treated enclosures when compared to the treated dome. The differences in structure between the two net types could also have contributed to the phenomenon of the treated dome system barrier appearing to have greater longevity, though at a slightly lower level of mosquito reduction, than the treated enclosure system barrier. For instance, the leafy layer of the older style netting had more folds, crenellations, and overlaps than the newer style netting (Fig. 4), possibly protecting the bifenthrin in more places from UV exposure. The treated dome also had higher surface area and thus more treated material potentially protected from direct sunlight than the smaller enclosure systems. As far as variation in reduction of mosquito numbers among the three treated enclosures (Table I), there could have been variability in the coverage owing to gusts of wind or slight folds in the material during the backpack sprayer application. Alternatively, given the spread of the enclosures across the plot, there could have been natural differences in the numbers of mosquitoes moving through the four zones of CO_2 bait.

Treated artificial barriers similar to those investigated in this study show great promise and are worthy of expanded study. Future work should aim to improve upon our initial

declaration of 7–14 days with ≥50% reduction in mosquitoes as a minimum standard for efficacy. The basic design of the artificial barriers described above should be subjected to further variations such as changes in the species of the target insect, pesticide type, formulation, and application protocol, type of camouflage netting or alternatives such as shade cloth, and environmental conditions at the time of spray. Different configurations of camouflage netting should be explored, such as variations in height, perimeter diameters, or placement of multiple concentric barriers. Investigations with these variations may identify the critical factors needed to achieve optimal control efficacy with treated artificial barriers in desert environments. Optimized systems should be assayed in regions containing Old World mosquito and sand fly vectors important in disease transmission in Iraq and Afghanistan, and the optimized systems should be fielded for trials with military units in theater as soon as possible. Even without further study, a dissemination of the basic concept of barrier treatments to preventive medicine personnel could have a rapid and positive impact on force health protection. Artificial materials such as camouflage netting and shade cloth are already implemented widely by units in theater, and application of Environmental Protection Agency (EPA)-approved pesticides such as bifenthrin at the label rate using hand-pump sprayers organic to preventive medicine detachments and company field sanitation teams could be carried out immediately anywhere artificial perimeters are set up.

A critical issue in assessing barrier treatments is whether females arrive at traps despite having contacted treated surfaces, in which case we must assume they would have attempted to bite a person within the protected area. This study was not designed to measure whether trapped females had been exposed to bifenthrin; however, we observed trapped mosquitoes from day 0 and day +1 for mortality at 12, 24, and 48 hours before freezing them for counting and archiving. We kept the containers that contained the trapped mosquitoes in a warm, humid mosquito rearing room and supplied cotton balls soaked in 10% sugar solution, but did not observe particularly excessive mortality in trapped females from treated plots versus control plots. We hypothesized that females in traps in treated enclosures or the treated dome had either not contacted the fabric sufficiently to obtain a lethal dose of bifenthrin or were resistant to the chemical. Information on resistance to pyrethroids in wild *Cx. tarsalis* is sparse, but data from Strong et al.[40] suggest that permethrin is still effective against populations of *Cx. tarsalis* in northern Colorado.

Nevertheless, the fact that even though their numbers may be reduced, female host seeking mosquitoes still penetrate treated perimeters. As with many mosquito control measures, we stress that barrier treatment technology should be implemented as part of a suite of integrated control measures and not relied upon solely. Companion measures should include: ULV or thermal fog adulticide operations, basic personal protection with products containing DEET or other EPA-approved compounds and clothing treated or impregnated with permethrin, removal trapping within the treated perimeter, source reduction outside of and within the treated perimeter, and supplemental barrier treatment of artificial surfaces within the treated perimeter. On the other hand, even in the absence of an integrated program of control, the results of the field mosquito population surveillance during the study allow us to hypothesize that a 40–60% reduction in mosquitoes crossing the treated barrier could translate into a 40–60% reduction in risk of exposure to mosquito-borne diseases for people within the protected area, compared to risk of exposure for people situated nearby in untreated areas.

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