Efficacy and Duration of Three Residual Insecticides on Cotton Duck and Vinyl Tent Surfaces for Control of the Sand Fly *Phlebotomus papatasi* (Diptera: Psychodidae)

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ABSTRACT

This study evaluated the toxicity and duration of 3 residual insecticides against the Old World sand fly, *Phlebotomus papatasi*, an important vector of cutaneous leishmaniasis, on 2 types of tent material used by the US military in Afghanistan and the Middle East. Vinyl and cotton duck tent surfaces were treated at maximum labeled rates of lambda-cyhalothrin (Demand CS, Zeneca Inc, Wilmington, DE), bifenthrin (Talstar P Professional, FMC Corporation, Philadelphia, PA) and permethrin (Insect Repellent, Clothing Application, 40%), then subsequently stored in indoor, shaded spaces at room temperature (60%-70% relative humidity (RH), 22°C-25°C), and under sunlight and ambient air temperatures outdoors (20%-30% RH, 29°C-44°C). Insecticide susceptible colony flies (F110) obtained from the insectary of US Naval Medical Research Unit No. 3, Cairo, Egypt, were exposed to treated tent surfaces for 30 minute periods twice monthly for up to 5 months, then once monthly thereafter, using the World Health Organization cone assay. Lambda-cyhalothrin treated cotton duck tent material stored indoors killed *P. papatasi* for 8 months, while the complementary sun-exposed cotton duck material killed adult flies for 1 month before the efficacy dropped to less than 80%. Sand fly mortality on permethrin- and bifenthrin-treated cotton duck decreased below 80% after 2 weeks' exposure to sunlight. Shade-stored permethrin and bifenthrin cotton duck material killed more than 80% of test flies through 5 months before mortality rates decreased substantially. Vinyl tent material provided limited control (less than 50% mortality) for less than 1 month with all treatment and storage regimes. Lambda-cyhalothrin-treated cotton duck tent material provided the longest control and produced the highest overall mortalities (100% for 8 months (shaded), more than 90% for 1 month (sunlight-exposed)) of both cotton duck and vinyl tents.

Sand fly control is essential for reducing the risk of human infection with *Leishmania* parasites, the etiological agents of visceral and cutaneous leishmaniasis (VL, CL, respectively). Across the Middle East and north Africa, phlebotomine sand flies occur in large numbers and with such a widespread distribution that transmitted *Leishmania* parasites cause an average of 350,000 cases of CL annually, representing 12% of the global burden.[1] Despite this sizeable number, CL in that region is believed to be largely underreported and thus expected to be even more prevalent. Even in the absence of CL, large sand fly populations have produced biting rates in excess of 1,000 bites per night on military bases in Iraq. The impact of this quantity of bites significantly degrades the quality of life and operational readiness of deployed personnel.[2]

Conflicts with Soviet invaders, Taliban, and tribal groups have resulted in the destruction of the Afghan public health infrastructure, and mass migration of displaced persons, some with prior infection, from rural to urban areas where living conditions are inadequate. As a result, the incidence of CL in Afghanistan has been increasing for several decades and the increased presence of sand flies is conducive to greater numbers of CL cases.[3] Kabul's population is presently estimated at 3.9 million people, up from about one million in 1980 just after the Soviet invasion.[4] As of 2002, there were an estimated 200,000 CL cases in Kabul alone.[5] Such large numbers of CL cases put deployed service members at increased risk of infection.

Integrated methods of sand fly control in desert environments include insecticide applications, habitat modification (destruction of mammal reservoirs and their burrows), use of personal protective measures, such as insecticide-treated bed nets (ITNs), and topical- and clothing-applied arthropod repellents.[6] Ultra low volume

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(ULV) space sprays, thermal fogging, and residual insecticide treatments comprise the chemical control methodologies for sand fly control, and are used by military preventive medicine personnel to provide quick relief from biting insects.[7,8] Insecticide treatment of immature sand flies is not practical, as large areas must be treated with backpack sprayers, and larval habitat is in many cases unknown. Rapid control of biting, adult female sand flies is more practical and critical to both reduction of nuisance biting and disease risk. Adult control is partly reliant upon space sprays and residual insecticide treatment of surfaces on which the flies rest. Habitat modification, useful in some circumstances for long-term sand fly control, requires continuous monitoring and environmental manipulation, is expensive and time consuming, and in general is only applicable for bases which will be in place for many years. Habitat modification is not useful for decreasing biting insect pressure on more temporary bases, most often cost prohibitive, and in some cases illegal if protected wildlife is present or the host-nation will not allow it.[9]


Figure 1. Vinyl extendable modular personnel tents used for transitional billeting, Kuwait, August, 2009.

Ultra low volume space spraying is the standard Department of Defense (DoD) response for control of biting sand flies. Some measure of control has been achieved with space spraying in the Middle East, however, ULV applications are not successful in providing long-term biting relief and effective disease control.[10,11] Adult populations rebound quickly despite a lack of evidence that indicates resistance development to adulticides in Egypt. No documented instances of sand fly resistance exists in the Middle East or Afghanistan.[2,12]

Residual insecticide treatment of sand fly resting areas, especially on surfaces that shelter humans, offers longer lasting protection from biting sand flies than does ULV space spraying or application of topical and clothing repellents. Further, the residual insecticide treatments are simple to implement, due to the presence of insecticides and application equipment typically available to deployed preventive medicine personnel. As with ITNs, a surface treated with insecticide can deliver a lethal dose to host-seeking insects that rest on those surfaces. Advantages of residual insecticide applications over space spray measures (ULV and thermal fog) include long-term protection from a single treatment, serendipitous use of human odors as a means to draw sand flies toward treated surfaces, and savings in manpower and insecticide costs associated with not needing to perform repeated, nonresidual space spraying.[7]

Presently, over 180,000 US and NATO personnel are now serving in *Leishmania*-endemic areas in Afghanistan and the Middle East. Many of these troops are quartered in temporary shelters, often in tents (Figure 1). Recent model tents provide better protection from the environment than older tents because they are equipped with better flooring, screened windows and doors, and are often air conditioned. Cotton duck material was used for military tents several decades ago, but modern tents are constructed of vinyl due to improved protection from sunlight, wind, dust, and rain. Greater numbers of deployed personnel are now living in modular or hardened quarters than were at the beginning of Operations Enduring Freedom and Iraqi Freedom, when troops were originally quartered in tents lacking air conditioning or flooring, or simply slept under open sky. This may explain, in part, the increase of CL cases in Iraq and Afghanistan at the start of those wars, with a subsequent drop in the number of cases through the present.[13] In situations where tents are used in place of buildings or containerized modules, "rubber" (vinyl) tents are most often used by deployed forces as command posts, dining facilities, administrative spaces, medical clinics, and for billeting in both permanent bases and smaller forward observation bases. Although cotton duck tenting is seldom used currently, it was the DoD tent mainstay prior to the introduction of vinyl tents and is still commonly used by rural populations and in refugee centers. Cotton duck liners are still present in the supply system along with general purpose, Tent, Extendable Modular Personnel (TEMPER), and Arctic tents that do not have vinyl surfaces.[14]

This study was designed to evaluate the efficacy and longevity of 3 insecticides, stocked by DoD and available to deployed forces, used as residual treatments on cotton duck and water-repellent vinyl tent material. Colonized (susceptible) *Phlebotomus papatasi* (Scopoli) sand flies were exposed for 30 minutes to treated tent material every second week for 20 weeks, then once monthly, encompassing an 8-month period using the World Health Organization (WHO) cone bioassay in an attempt to determine which of 3 DoD-stocked pyrethroid insecticides was most useful for killing these flies and how long they lasted on cotton and vinyl tent surfaces before retreatment became necessary. This research is intended to benefit people living or working in tents who are concerned about protecting themselves from biting sand flies and *Leishmania* infection in desert environments common to much of the Middle East and Afghanistan.

MATERIALS AND METHODS

Sand Flies

A laboratory population of *P. papatasi* sand flies was used to study the effectiveness of insecticide-treated tent material. The flies (F110) were reared in the Vector Biology Research Program insectary, US Naval Medical Research Unit No. 3 (NAMRU-3), Cairo, Egypt. Flies were colonized by the procedure of Modi and Tesh,[15] at 27°C±2°C, 80%±10% RH, and a light:dark photo period of 12:12 throughout their development. Adults were offered 30% sugar solution daily, and provided blood meals on hamster, *Pachyuromys duprasi* Lataste for egg production. Three to five-day old sand fly females were used in this study.

Insecticides

Three insecticides were applied to tent materials at their highest labeled rates for residual fly control: Demand CS (9.7% lambda-cyhalothrin, National Stock Number (NSN) 6840-01-428-6646) (Zeneca Inc, Wilmington, DE), Talstar P Professional (7.9% bifenthrin, NSN 6840-01-525-6888) (FMC Corporation, Philadelphia, PA) and Insect Repellent, Clothing Application (40% permethrin, NSN 6840-01-334-2666). Although classified and used as a repellent in the DoD supply system, permethrin is also an insecticide and is formulated for adult mosquito control as a ULV spray. The insecticides were chosen based on recommendations from the Armed Forces Pest Management Board's "Standard Pesticides Available to DoD Components" list* and are used by preventive medicine personnel and pest control contractors.

Insecticide Stock Solution Preparation

Demand CS was mixed at the rate of 12 mL per 3.79 L (one gal) of water to produce a 0.03% solution for application to 92.9 m^2 (1,000 sq ft) of surface area. The resultant applied surface concentration was 1.2 μg/cm^2 lambda-cyhalothrin. Talstar P Professional was mixed at the rate of 30 mL per 3.79 L of water to produce a 0.06% solution for application to 92.9 m^2 of surface area and produced a surface concentration of 1.9 μg/cm^2 bifenthrin. Insect Repellent, Clothing Application was mixed at the rate of 75 mL active ingredient per 3.79 L of water to produce a 0.5% solution, designed specifically for thorough treatment of 4 combat uniforms (half the rate of the 150.8 mL bottle developed for use with a 7.57 L (2-gal) sprayer). Cotton duck tent material was sprayed 3 times, producing a moist and darkened surface without runoff, as recommended by the label. This treatment produced a surface concentration of approximately 94 μg/cm^2, or ¾ of the recommended cotton battle dress uniform finished concentration of 125 μg/cm^2.

Tent Material and Applications

Vinyl and cotton duck tent material were used for this study. Vinyl tent material was obtained from a desert TEMPER tent (NSN 8340-01-185-2628). These tents are comprised of 13.5 oz polyester duck and are standard use tenting for deployed US forces. The material is naturally water-repellent and is factory treated with a flame retardant. Due to the flame retardant finish, insecticide application on vinyl tenting is not recommended.[14] However, we chose to treat vinyl tenting with insecticide to determine if any residual protection could be obtained. The cotton duck tent material was collected from a Tent, General Purpose, Medium (NSN 8340-543-7788) made from 9.85 oz cotton duck. In preparation for testing, the vinyl tent material was washed with soap and water. The cotton duck tent material was dry cleaned twice at a commercial cleaner using perchloroethylene to remove any insecticide residues from prior treatment.

Environmental treatments included shade-stored and sunlight-exposed treated tent. Shade-stored (sun protected) cotton duck and vinyl tent was kept on shelves inside the Vector Biology Research Program's storage locker. Air conditioning resulted in consistent temperatures ranging from 22°C-25°C for the duration of the study. Sunlight-exposed test material was set on the roof of the Vector Biology Research Program building from June 2, 2009, through February 25, 2010, and exposed to temperatures ranging from 9°C-44°C.

Insecticides were applied with a 3.79 L compressed air sprayer (NSN 3740-00-191-3677) at a pressure of 50 psi to 55 psi while wearing appropriate personal protective equipment (vented goggles, half-face respirator with organic vapor cartridges, long sleeve shirt, neoprene gloves), to simulate insecticide application under field conditions using equipment and insecticides readily available to military personnel. Tent material was cut into 30.5 cm by 61 cm strips, representing 1/500 of a 92.9 m^2 treatment area (per label directions) (Figure 2). These two 1,860.5 cm^2 strips were sprayed with 1/500 of 3.79 L of solution (7.4 mL of finished product). To calibrate, 3.79 L of tap water was added to the 3.79 L sprayer, pressure brought to 50 to 55 psi with the nozzle inserted in a 100 mL graduated cylinder, and water sprayed until 7.4 mL of water was collected. Three replications averaged one second to obtain the required 7.4 mL sample. During tent treatment, the nozzle was swept the length of 61 cm in one second to produce the desired concentration. At an application distance of 30.5 cm to 38.1 cm, the fan nozzle covered a width of slightly more than 30.5 cm, the width of test strips. Both vinyl and

*http://www.afpmb.org/sites/ default/files/pubs/standardlists/dod_pesticides_list.pdf cotton tent material were treated on horizontal concrete surfaces and allowed sufficient time to air dry before packaging and shipping. Samples were treated at the US Department of Agriculture facility in Gainesville, FL, individually bagged in laminated cambric sample bags (Hubco, Hutchinson, KS), wrapped in duck 5 inch W stretch wrap, film extensible (Lowe's Home Improvement), and shipped to NAMRU-3. Testing commenced about a month after tent treatment, the time necessary to clear samples from airport inspectors and prepare the laboratory for sand fly testing.

Bioassays

These assays followed the method described by WHO,[16] in which 4 WHO plastic cones were fastened to the treated tent liner. A batch of 60 flies per tent type/insecticide combination was tested, in 4 replicates of 15 non–blood-fed female sand flies per cone. All flies were held in the cones for 30 minutes. After exposure, flies were placed in 150-ml plastic cups (15 individuals per cup), with a 30% sucrose solution provided, and maintained in a climatic chamber for 24 hours at 27°C±2°C and 80%±10% RH. Percentage mortality was scored after 24 hours, according to WHO cone assay criteria.[16] All tests were performed with the same tent material/insecticide combination tent samples. Cone test sites were randomly selected on the same treated tent samples throughout the study.

RESULTS

Cotton Duck, Sunlight-Exposed

Lambda-cyhalothrin applied at maximum fly control rates produced the highest level of mortality in sand flies. Sand fly mortality remained above 80% for 4 weeks (93.3% mortality at week 4) before dropping off quickly to 65% at week 6, and declining to 41.6% at week 8 when testing was stopped. Permethrin-treated cotton duck killed 78.3% of test flies on week 2, declined to

Figure 2. Vinyl tent strips (61 cm by 30.5 cm) used for residual insecticide efficacy studies against the sand fly *Phlebotomus papatasi*, a vector of cutaneous leishmaniasis in Afghanistan and the Middle East.

65% mortality on week 4, and slowly receded to 33.3% morality on week 10 when testing was stopped. Bifenthrin-treated cotton duck killed 91.6% of test flies on day 0, 66% on week 2, and fell to 40% on week 6 at which time testing ended (Figure 3).

Cotton Duck, Shade-Stored

Shade-stored (sun-protected) cotton duck was kept on shelves inside the Vector Biology Research Program's storage locker. Air conditioning was kept between 22°C-25°C for the duration of the study. Lack of high temperatures with no sun exposure greatly extended the life of insecticides. Lambda-cyhalothrin treated cotton duck continued killing 100% of all test flies through the end of week 38, providing complete protection well into 8 months. Permethrin-treated cotton duck provided 100% mortality for test flies through week 8, killed 86.6%, 91.6%, and 90% of flies on weeks 10, 12, and 14 respectively, and killed all flies between weeks 16 and 24. Mortality then rapidly dropped off to 10% on week 28 and finally 5% on week 32, when testing stopped. Bifenthrin provided 100% control of test flies through 6 weeks, killed between 90% and 96.6% of flies between weeks 8 and 16, and killed all test flies between weeks 18 and 22 before mortality rates dropped off quickly to 18.3%, 13.3%, and 15% on weeks 26, 30, and 36, respectively. Time-mortality rates of sand flies exposed to shade-stored, insecticide-treated cotton duck tenting is shown in Figure 4.

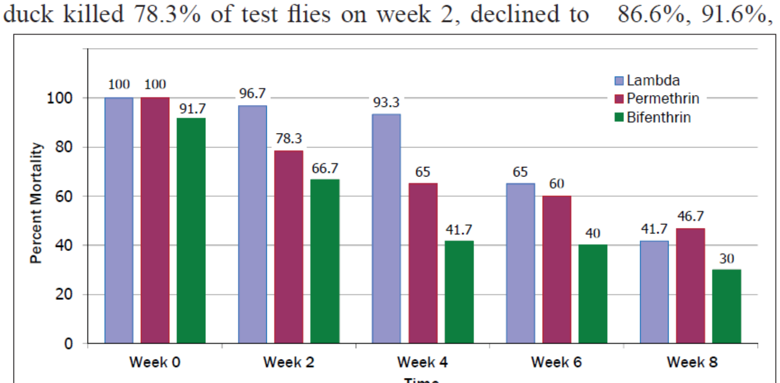

Figure 3. Mortality rates of *Phlebotomus papatasi* to lambda-cyhalothrin-, permethrin-, and bifenthrin-treated, sunlight-exposed cotton duck tenting after initial treatment, May-July 2009.

Vinyl, Sunlight-Exposed

TEMPER (vinyl) tenting treated with candidate insecticides did not produce high mortality rates beyond the initial test day (day 0), and treatment efficacy wore off quickly. Sunlight-exposed, Lambda-cyhalothrin-treated vinyl killed 94% of test flies on day 0, 18.4% on week 2, and 17.5% on week 4 when testing ended. Permethrin-treated vinyl killed 12.2% of test flies on day 0, 2.4% on week 2, and none on week 4, effectively ending that test. Bifenthrin-treated vinyl killed 10.2% of test flies on day 0, 37.5% on week 2, and 11.1% on week 4 at which time testing ended. Time-mortality rates of sand flies tested on sunlight-exposed, insecticide-treated vinyl tenting is shown in Figure 5.

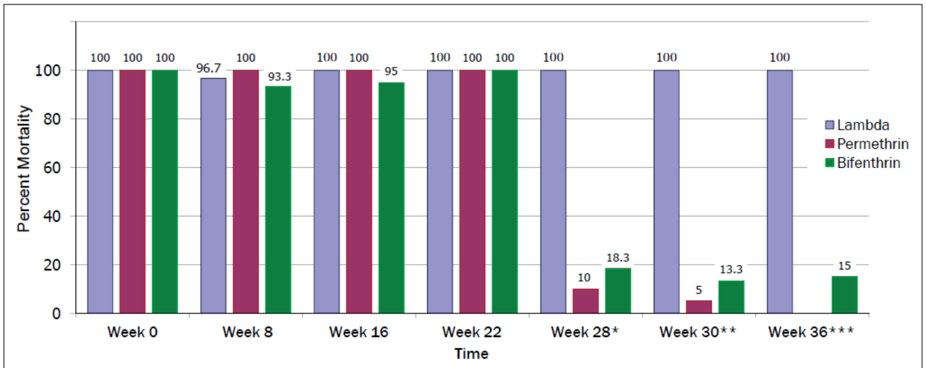

Figure 4. Mortality rates of *Phlebotomus papatasi* to lambda-cyhalothrin-, permethrin-, and bifenthrin-treated, shade-stored cotton duck tenting after initial treatment, May 2009-June, 2010.
Notes:
*Week 28 result for permethrin only. Week 26 results of lambda-cyhalothrin and bifenthrin included in week 28 chart (no data on week 28).
**Week 30 results for lambda-cyhalothrin and bifenthrin. Permethrin result for week 32 included in week 30 graph (no data on week 30).
***No week 36 data for permethrin.

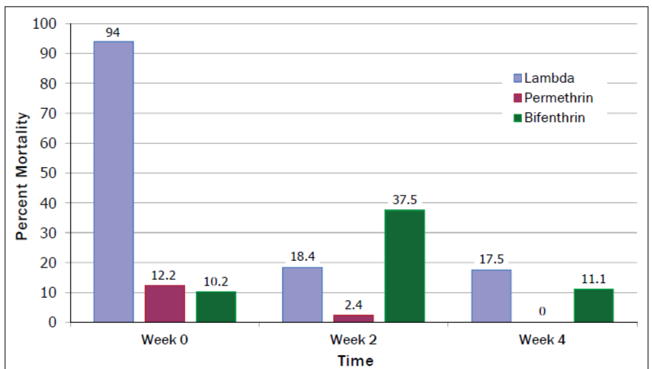

Figure 5. Mortality rates of *Phlebotomus papatasi* to lambda-cyhalothrin-, permethrin-, and bifenthrin-treated, sunlight-exposed vinyl tenting after initial insecticide application, May-June, 2009.

Vinyl, Shade-Stored

Vinyl tenting treated with insecticide and stored on shelves in work space conditions (no sunlight, 22°C-25°C ambient temperatures) provided higher mortality in sand flies than did sunlight-exposed vinyl tenting (Figure 5). Lambda-cyhalothrin-treated vinyl killed all test flies on day 0 and 59% on week 2 before falling off to 37.5% on week 4, the final test date. Permethrin-treated vinyl killed 29.3% of test flies on day 0, 11.1% on week 2, and 10% on week 4, when testing ended. Bifen-thrin-treated vinyl killed 11.1% of test flies on day 0, 26.1% on week 2, and 6.5% on week 4, the final test date. Time-mortality rates of sand flies tested on shade-stored, insecticide-treated vinyl tenting is shown in Figure 6.

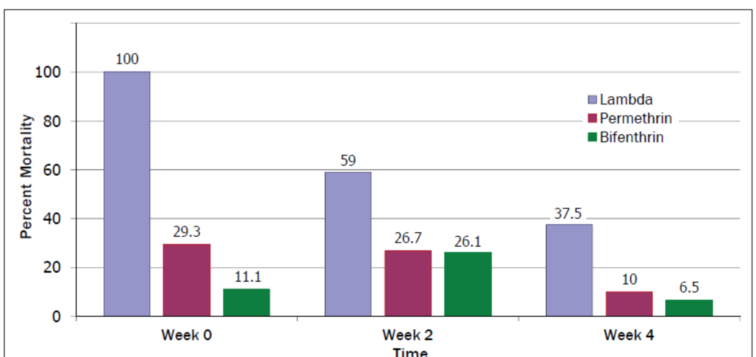

Figure 6. Mortality rates of *Phlebotomus papatasi* to lambda-cyhalothrin-, permethrin-, and bifenthrin-treated, shade-stored vinyl tenting after initial insecticide application, May-June, 2009.

COMMENT

The application of residual insecticides on insect resting surfaces is an important component of programs designed to protect humans from disease-carrying arthropods. In troop deployment situations, displaced persons, or refugee camps, or on short-term contingencies or emergencies in which minimal preparation is afforded those tasked to respond, residual insecticide application to berthing and work spaces takes on increased importance. It has been found that in high temperature, low humidity desert climates common to North Africa, the Middle East, and much of Afghanistan, DoD-stocked residual insecticides can provide long-term protection from sand flies if applied to shaded, indoor surfaces.[2,6]

Lambda-cyhalothrin, permethrin, and bifenthrin are the active ingredients in many commonly used insecticides in the United States and overseas, and are often used as residual sprays for controlling arthropod pests. Our results indicated that cotton duck tent material unquestionably provides a better substrate for insecticide retention than does vinyl tent material, and will provide much longer protection against biting sand flies with both indoor and outdoor (sunlight-exposed) treatments. Lambda-cyhalothrin, a second generation pyrethroid insecticide, has an alpha-cyano group side chain that provides additional stability to the compound against ultraviolet degradation and hydrolysis,[17] whereas permethrin and bifenthrin lack this side chain and are more susceptible to environmental photodegradation.[18] Also, lambda-cyhalothrin formulated as Demand CS is microencapsulated, which provides further protection against environmental degradation. The permethrin and bifenthrin test products were not microencapsulated. Residual treatment of shade-stored, cotton duck tenting with lambda-cyhalothrin, (which is representative of a scenario involving the interior walls of a tent in a field setting), provided the longest duration of control of *P. papatasi*, a common sand fly vector of CL throughout most of the aforementioned region. Under this regimen, all exposed sand flies were killed throughout 38 weeks of bioassay testing, although the end point was set at 6 months (26 weeks).

Effective and extended control (greater than 80%) was produced by permethrin on shade-stored cotton duck through 24 weeks of testing. The efficacy dropped rapidly to 10% on week 28 and then to 5% on week 32, when testing was discontinued. Bifenthrin also provided greater than 80% test fly mortality through 22 weeks, then decreased suddenly on week 26, similar to results obtained with permethrin. We concluded that all 3 products are useful as long lasting residuals on cotton tenting, provided indoor surfaces were treated. However, while both permethrin and bifenthrin provided satisfactory control under such conditions, our results clearly indicate that when available for indoor use on cotton duck tenting, lambda-cyhalothrin is the best choice.

Cotton duck tent treated with all test products and exposed to sunlight provided high levels of mortality for only about a month, after which efficacy dropped quickly. Availability of cotton duck military tents is now the exception rather than the rule. Cotton duck tents are sometimes found in material storage buildings on bases and are being replaced with vinyl tents when they reach the end of their service life. However, there are nonvinyl TEMPER tents, arctic tents, and tent liners used by the armed forces. Once residual treatment has been applied, any of the three aforementioned nonvinyl products could provide useful protection against biting sand flies, especially in temperate climates with short sand fly seasons, such as is common in Afghanistan.

Insecticide-treated vinyl tenting exposed to direct sunlight provided no noteworthy protection against host seeking *P. papatasi*. The efficacy of lambda-cyhalothrin dropped from 94% on day 0 to 18.4% on week 2 and 17.5% on week 4 when testing was discontinued. Just over 30 days had elapsed from the time of insecticide application to the beginning of the test, day 0. The 94% kill rate on day 0 demonstrated that the insecticide application had worked, albeit for a very short while once lambda-cyhalothrin was exposed to sunlight. In contrast, sunlight-protected lambda-cyhalothrin-treated vinyl tent killed all test flies on day 0 and 59% on week 2 before falling off to 37.5% on week 4, the final test date. Although protection was less than desirable during weeks 2 and 4 (less than 80%), some short-term protection against biting sand flies was obtained and will supplement other personal protective measures in the field (N,N-Diethyl-3-methyl benzamide (deet) skin repellent, permethrin-treated bed nets and uniforms). Permethrin and bifenthrin-treated vinyl tenting failed to control more than 30% of test flies at any point in time (days 0, 14, 30), regardless of exposure to sunlight.

The Armed Forces Pest Management Board Technical Guide No. 36[14] does not recommend treating vinyl TEMPER tenting with insecticide. The fire retardant added to an already water-repellent vinyl surface impedes the uptake of insecticide, as clearly seen from these tests. However, some protection is gained for approximately a month if the interior surfaces of a TEMPER tent are treated with lambda-cyhalothrin. In areas where biting pressure is very high and bites can exceed several hundred per night, the added value of treating the inside of TEMPER tents for short deployments is obvious.[2]

Our results indicated that, at best, lambda-cyhalothrin-treated vinyl tent provided minimal control (from 100% control on day 0 to approximately 35% control on day 30) of host-seeking *P. papatasi* under shaded conditions (no direct sunlight exposure). Permethrin- and bifenthrin-treated vinyl surfaces offered no appreciable protection against host seeking sand flies. Nonvinyl tent material, if available as cotton duck tent liners or tent surfaces, can greatly aid in protection against sand flies under hot, sunlight-exposed and cooler, shaded conditions. All 3 insecticides gave excellent protection (greater than 90%) for at least 5 months when used indoors. Under short deployment conditions (less than 30 days), Demand CS will enhance proven personal protective measures (deet skin repellent, permethrin-treated bed nets) against biting sand flies when applied to the interior surfaces of vinyl tents.

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