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PERMETHRIN AS A TENT TREATMENT FOR PROTECTION AGAINST FIELD POPULATIONS OF *AEDES* MOSQUITOES

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ABSTRACT. A tent treated with an oil-based 1% permethrin formulation (0.936 g AI/m²) was evaluated for protection against *Aedes* spp. Mosquito biting assessment was conducted in the field using 8 human subjects positioned inside and outside tents. Subjects inside tents received 66.8% fewer bites than subjects outside tents prior to treatment. Following treatment, subjects inside the treated tent had significant ($P \leq 0.05$) protection (84–94% bite reduction) for 42 days. Subjects outside the treated tent experienced 43–82% fewer biting mosquitoes than subjects outside the untreated tent. The treated tent remained lethal ≥42 days to mosquitoes exposed to the tent fabric in field bioassays.

INTRODUCTION

Traditional methods of personal protection from biting insects consist of application of repellent to skin and clothing. Recent work has demonstrated that the application of permethrin, an insecticide having low mammalian toxicity, to clothing or nearby substrates can provide protection from mosquito biting activity. Permethrin provided greater than 90% protection from mosquitoes when impregnated into military uniforms (Schreck et al. 1984, Lillie et al. 1988). Mosquito bednetting treated with permethrin protects people in tropical countries from mosquitoes that transmit disease (Rozendaal and Curtis 1989, Curtis 1992). When exposed to accelerated weathering, cloth fibers treated with permethrin were effective for several weeks as a toxicant and repellent (Gupta et al. 1989, 1990). Permethrin applied as a residual lawn treatment provided several days of protection against spring *Aedes* spp. mosquitoes (Helson and Surgeoner 1983). Schreck (1991) found that permethrin impregnated into tent fabric provided greater than 96% protection for more than 9 months against biting of *Aedes aegypti* (Linn.) mosquitoes released inside the treated tent.

The purpose of this study was to determine if a tent sprayed with a commercially available 1% permethrin formulation would protect people, both inside and outside the tent, against spring *Aedes* spp. mosquitoes.

MATERIALS AND METHODS

Two Canadian Armed Forces tents were used in the evaluation. The tents were floorless, made of canvas and had a sloping roof 3 m high at the apex with a ground floor area of 2.5 × 6.0 m. Door flaps were centered on the 6-m side. Tents were erected on May 25 in Guelph, Ontario (43°35′N, 80°20′W) in a large deciduous woodlot with secondary growth under the canopy. Tents were positioned 25 m apart on flat dry ground. On either side of the experimental area were snowmelt pools (>1 ha) where large numbers of immature *Aedes* spp. develop.

Each night 8 subjects were used, with an additional person timing biting counts and recording weather conditions. Single subject substitutions occurred on 5 occasions when subjects were unable to participate. Subjects wore green military uniforms with sleeves rolled up to the elbows. Subjects did not wear repellent but could use headnets on nights of severe biting activity. The subjects were divided into 2 groups of 4. Position for counts and group were determined randomly each night by drawn lot. Four people were assigned to each tent: 2 inside at opposite corners and 2 outside at opposite corners. Each tent had one door flap open throughout the study providing an approximately 1.5-m² area for entry into the tent. A Coleman® lantern hung inside each tent 2 m off the ground during biting counts to provide light and to simulate camping conditions.

Bite counts were made on 4 days pretreatment and were initiated each day at approximately 1900 h, when 4 sets of 20-min counts were recorded. Each set included 4 5-min bite counts and after each 5-min count, subjects rotated positions at their respective tents. After each 20-min set, subject groups would switch tents. Thus each night, a subject made 16, 5-min bite counts, 8 at the treated tent and 8 at the untreated tent, rotating twice through all positions. During each 5-min count, subjects aspirated all mosquitoes biting exposed hands and forearms. Mosquitoes were aspirated into 150-ml clear plastic vials with an X cut into the plastic lid that prevented escape. After 5 min the subjects sealed the plastic lid with tape, then recorded the number of mosquitoes captured.

Before bite counts were initiated each night, the total number of mosquitoes resting on all inner surfaces of both tents was recorded. During

bite counts, temperature, relative humidity, and wind speed were recorded. A rain gauge positioned between the tents measured rainfall throughout the study. Overnight temperatures were obtained from the University of Guelph Arboretum climatological station located ≈1 km from the study site. Battery operated CDC light traps were placed in the tents after bite counts each night. Traps were baited with carbon dioxide released from a 1-liter thermos full of crushed dry ice placed approximately 0.5 m above the traps and were operated from *ca.* 2100 h to 0800 h. Mosquitoes collected were pooled each night and 30/night were randomly selected and identified using keys of Wood et al. (1979).

The treated tent was selected randomly by coin toss and treated on June 14. A 1% permethrin oil-based formulation, Coopers Delice Pour-on (PCP #22681), was applied to the tent using a 7-liter-capacity Landmark® garden hand-pump sprayer. This formulation was selected because preliminary laboratory studies indicated that wettable powder and emulsifiable concentrate formulations of permethrin dripped off the water-repellent fabric leaving little residue. All inside and outside surfaces were treated, except the outside roof. The total volume applied equalled 10.87 liters, which represented 0.936 g AI/m^2.

A bioassay was used to determine if the permethrin-treated tent effectively killed mosquitoes during the study. Each day of testing, 5 mosquitoes collected on-site were aspirated into Petri plates that were taped to the inner walls of each tent. Every 5 min the number of live mosquitoes was recorded until all mosquitoes were dead in the treated tent. A different spot was chosen at random each day for placement of Petri plates.

Bite counts were made on days 0, 1, 2, 3, 7, 14, 21, 28, 35, and 42 posttreatment (June 14–July 26). The duration of the study coincided with the yearly peak of mosquito biting activity. Previous studies at the same woodlot have shown that numbers of mosquitoes decline after mid-July (Surgeoner and Heal 1991[1]). To calculate percent reduction in biting activity, mosquito counts for the 2 positions inside each tent were combined and averaged, as were the numbers for the 2 positions outside each tent. Percent reduction inside or outside the tents was calculated as: ([no. biting in untreated tent − no. biting in treated tent]/[no. biting in untreated tent]) × 100. This was repeated with "outside" data. A modified Abbott's formula (Neal 1976) was used also to calculate percent reduction taking into consideration natural temporal changes in the mosquito population. This was calculated as: 100% − ([no. biting in treated tent posttreatment/no. biting in treated tent pretreatment] × [no. biting in untreated tent pretreatment/no. biting in untreated tent posttreatment]). The efficacy of treatment was analyzed with a 3-factor linear model with subject, location (positions inside or outside tents), and day as factors. All of the factors were treated as random variables. The number of mosquitoes collected from the 2 positions inside and outside the tents from both rotations each night were combined for final analysis. Therefore, each location count for each individual was based on 4 rotations. The square root of the counts was used in the final analysis to reduce the inequality in variance between counts on different days at different locations. For pretreatment counts, data for all 4 days were combined and means were compared between the 2 tents using Student's *t*-test. The analyses were completed using Statistical Analysis Systems version 6.04 (SAS Institute Inc., Cary, NC).

RESULTS

Counts inside tents were not significantly different ($P \leq 0.05$) between tents on 2 of 4 pretreatment sampling dates. On one pretreatment date, the tent that would be treated had significantly more biting mosquitoes inside, whereas on another date the tent that remained untreated had significantly more biting mosquitoes inside. When all pretreatment counts were combined ($n = 128$) for the 4 days pretreatment, 7.4% fewer mosquitoes were collected inside the tent that would be treated. This was not significantly different from the tent that remained untreated ($P \leq 0.05$). Bite counts outside tents were not significantly different ($P \leq 0.05$) between tents on 2 of 4 pretreatment sampling dates; however, there were significant differences in counts between tents on the other 2 sampling dates. The tent that would be treated had 19.2% fewer mosquitoes biting outside for the pretreatment period overall and this was significant ($P \leq 0.05$). On pretreatment sampling dates, bite counts inside versus outside of the untreated tents were significantly different. People inside tents experienced 66.8% fewer mosquitoes than people outside tents. Mosquito resting counts, performed before subjects took their positions, demonstrated the presence of mosquitoes in both tents prior to treatment. On 2 pretreatment days CDC traps caught similar numbers of mosquitoes in each tent but numbers were low (range 14–27),

[1] Surgeoner, G. A. and J. D. Heal. 1991. Percent repellency of various products against spring *Aedes* spp. mosquitoes. Directorate of Preventive Medicine, National Defence Headquarters, Ottawa, Ontario, Canada K1A 0K2 (unpublished report).

probably due to overnight temperatures that reached a low of ≤5°C on both nights. On the 2 remaining pretreatment dates the CDC trap bulb malfunctioned in the tent that would be treated. In that tent the CDC trap caught an average of 87 mosquitoes, whereas the trap in the other tent caught an average of 235 mosquitoes.

Ambient air temperatures ranged from 15 to 25°C during biting count evaluations. Wind was always less than 10 kph and typically less than 5 kph. During the study there were 4 major rain events with a total of 70 mm of rain recorded. These were 8 mm on June 19, 21 mm on June 21, 24 mm on July 19, and 27 mm on July 26. After rain events, water pooled on the roof of the tent was not observed to permeate the fabric. There was no indication that rainfall reduced protection associated with the permethrin treatment.

During bite counts, approximately 1,000–1,200 mosquitoes were collected nightly. Twelve mosquito species were identified from 450 individuals subsampled throughout the study. These species and percent composition were: *Aedes canadensis* (Theobald) (34.4%), *Aedes stimulans* (Walker) (25.8%), *Aedes euedes* Howard, Dyar and Knab (18.0%), *Aedes punctor* (Kirby) (9.5%), *Aedes excrucians* (Walker) (4.0%), *Aedes fitchii* (Felt and Young) (4.0%), *Aedes trivittatus* (Coq.) (2.2%), *Aedes provocans* (Walker) (0.9%), *Aedes vexans* (Meigen) (0.4%), *Aedes cinereus* (Meigen) (0.2%), *Aedes implicatus* Vockeroth (0.2%), and *Coquillettidia perturbans* (Walker) (0.2%).

Following treatment, mosquitoes were never observed resting in the permethrin-sprayed tent, whereas in the untreated tent an average of 24 mosquitoes was seen during sampling dates. Throughout the posttreatment study period the CDC trap in the untreated tent caught an average of 195.7 ± 155.7 mosquitoes per night, whereas the CDC trap in the treated tent caught an average of 56.9 ± 51.3 mosquitoes per night. After application of permethrin there was a significant ($P \leq 0.05$) reduction in counts inside the treated tent compared to the untreated tent. A 94% reduction in biting activity was seen on the first posttreatment sampling date (day 0), 4 hours after treatment (Fig. 1). Biting activity remained above 84% for 42 days thereafter, with the exception of day 14 when it was 59%. Outside, there was significantly less biting activity at the treated tent throughout the study. Reduction ranged from 82 to 43% on day 42 (Fig. 1). Using the modified Abbott's formula, percent reduction inside the treated tent ranged from 93 to 82% with the exception of day 14 (56%). Outside the treated tent, percent reduction ranged from 77 to 29% on day 42. Throughout the study, all mosquitoes bioassayed on the walls of the treated

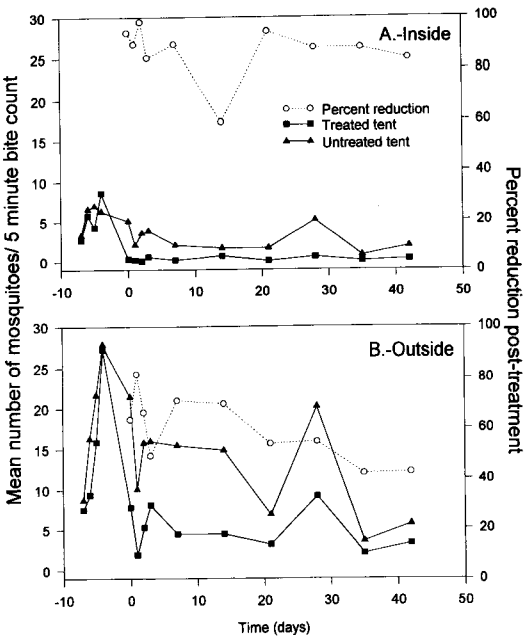

Fig. 1. Comparison of mosquito bite counts and percent reduction inside (A) and outside (B) a tent treated with a 1% permethrin solution, and a tent left untreated.

tent died within 20 min, whereas no mortality was observed with the untreated tent.

Analysis of variance revealed significant differences ($P < 0.001$) between location (positions inside and outside), day, and subject. Location was the greatest source of variation ($F = 64.61$), followed by day ($F = 10.20$) and subject ($F = 8.46$). There were significant interactions between day × location ($P < 0.001$) and subject × location ($P < 0.005$). Therefore, the reduction in mosquitoes collected in and around the treated tent posttreatment was due to the application of permethrin and was independent of other variables tested.

DISCUSSION

A tent sprayed with an oil-based solution of 1% permethrin at 0.936 g AI/m² provided 84% protection from mosquitoes inside the tent for 6 wk. Exposure of field-caught mosquitoes to the treated tent fabric confirmed that the permethrin remained lethal to mosquitoes for the duration of the study. Resting counts showed that mosquitoes were not present in the treated tent. Mosquitoes collected on people in the treated tent likely entered minutes before or during bite counts, perhaps attracted by the people.

Tent treatment is a simple and easily accomplished procedure that is appropriate for military

uses for protection from disease-carrying mosquitoes, and also may be suitable for some recreational uses. However, a tent treated with an oil-based formulation poses a potential fire hazard. Throughout the study the treated tent fabric felt greasy. A strong oily smell lasted for about 3 days, becoming faint, but detectable, for the duration of the study. A water-soluble permethrin solution would be more appropriate to obtain federal regulatory and consumer acceptance; however, our preliminary findings showed that water-soluble formulations had minimal adhesion to the fabric. Perhaps a water-soluble permethrin formulation with a higher viscosity than those tested would adhere to water-repellent tent fabric.

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