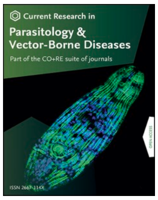
Current Research in Parasitology & Vector-Borne Diseases 5 (2024) 100156

Contents lists available at ScienceDirect

Current Research in Parasitology & Vector-Borne Diseases

journal homepage: www.sciencedirect.com/journal/current-research-in-parasitology-and-vector-borne-diseases



Transfluthrin diffusers do not protect two-person US military tents from mosquitoes in open field and canopy warm-temperate habitats

Barbara E. Bayer[a], Robert L. Aldridge[a], Bianca J. Moreno[a], Frances V. Golden[a], Seth Gibson[a,*], Jeffrey L. Wahl[b], Kenneth J. Linthicum[a]

[a] *United States Department of Agriculture, Agricultural Research Service, Center for Medical, Agricultural, and Veterinary Entomology, 1600 SW 23rd Drive, Gainesville, FL, 32608, USA*
[b] *Camp Blanding Joint Training Center, Environmental Safety and Health, Starke, FL, 32091, USA*

ARTICLE INFO

Keywords:
Integrated vector management
Military operational entomology
Passive control
Resistance management
Spatial repellent

ABSTRACT

Spatial repellents are volatile or volatilized chemicals that may repel arthropod vectors in free space, preventing bites and reducing the potential for pathogen transmission. In a 21-week field study, we investigated the efficacy of passive transfluthrin-impregnated diffusers placed in two-person United States (US) military tents located in canopy and open field habitats in north Florida to prevent mosquitoes from entering. Mosquito collections with US Centers for Disease Control and Prevention traps baited with light and carbon dioxide were conducted weekly for weeks 0–4, every two weeks for weeks 5–10, and monthly for weeks 11–21. Our results demonstrated that these transfluthrin-impregnated devices did not function as spatial repellents as expected and did not create a mosquito-free zone of protection. Instead, we observed consistently higher collections of mosquitoes from tents with transfluthrin-impregnated diffusers, and higher rates of mosquito mortality in collections from tents with transfluthrin diffusers, compared to untreated control tents. Based on these findings we do not recommend the use of passive transfluthrin-impregnated diffusers for mosquito protection in two-person US military tents in warm-temperate environments similar to north Florida.

1. Introduction

Mosquitoes transmit pathogens that cause infection and disease to humans and animals and pose a distinct health threat to United States (US) military troops deployed in austere field conditions, where personnel may be sleeping in tents vulnerable to entry of vector and nuisance arthropods such as mosquitoes (Withers and Craig, 2003). Traditional US military mosquito control measures feature the use of personal protection such as application of topical repellents on skin and proper use of repellent-treated uniforms, application of residual pesticides to military tents and other field materiel such as camouflage nets, and pesticide space sprays (AFPMB, 1996). However, additional layers of protection are needed to account for pyrethroid-resistant mosquito populations. One protective layer with potential for development in the US military integrated vector management (IVM) system is that of spatial repellents.

Spatial repellents, unlike residual insecticides, do not require physical contact with target arthropods to be effective (Achee et al., 2012). Spatial repellents create a vapor barrier that a host-seeking arthropod vector such as a mosquito must travel through to contact the host, and rather than causing morbidity or mortality the spatial repellent active ingredient targets mosquito sensory organs to disrupt host orientation and flight (Ogoma et al., 2014; Martin et al., 2020). Spatial repellents can potentially create a zone of protection for one or more humans (Achee et al., 2012; Ogoma et al., 2014; Sukkanon et al., 2021; Verhulst et al., 2021). Because the intended primary mode of action for spatial repellents is to alter the behavior of host-seeking vectors to move away from the protected area around the host, as opposed to causing mortality, their use could be a strategy to both circumvent existing insecticide resistance and not contribute to further evolution of resistance in target mosquito populations (Achee et al., 2012).

Transfluthrin is a fluorinated pyrethroid with low vapor pressure which allows the active ingredient (AI) to disperse at ambient temperatures (Ogoma et al., 2012a; McPhatter et al., 2017; Tambwe et al., 2020) and can be used as a spatial repellent. Previous studies using transfluthrin as a spatial repellent applied to wood furniture, hessian fabric, or other materials placed in exterior environments reduced

---

* Corresponding author.
E-mail address: seth.gibson@usda.gov (S. Gibson).

https://doi.org/10.1016/j.crpvbd.2023.100156
Received 26 May 2023; Received in revised form 2 November 2023; Accepted 20 November 2023

Fig. 1. **A** Visualization of a CDC trap placed in a two-person US military tent with a diffuser. **B** Visualization of paired two-person US military tents to create a collection "site"; one tent containing a transfluthrin diffuser (with either 150-day or 250-day effect) and the other a non-treated control. **C** Visualization of door half open on a two-person US military tent (*Note*: the white post was not part of this experiment but shows an example TFL-250 diffuser).

human-mosquito interactions (Ogoma et al., 2012a; Govella et al., 2015; Mmbando et al., 2018; Mwanga et al., 2019; Masalu et al., 2020; Britch et al., 2020a). However, we have observed variation in the efficacy of transfluthrin and other spatial repellents in the field depending on whether the active ingredient is presented in a partially enclosed space (Britch et al., 2020b, 2021). In this study, we conducted further investigation of transfluthrin in partially enclosed spaces by using passive transfluthrin-impregnated resin diffusers to attempt to reduce the number of mosquitoes entering small two-person US military tents in a warm-temperate field site in north-central Florida. The objectives of this study were (i) to determine the efficacy of transfluthrin diffusers in preventing entry of natural populations of mosquitoes into two-person US military tents and (ii) to assess the duration of efficacy of transfluthrin diffusers with both 150 and 250 purported effect days.

2. Materials and methods

2.1. Field site and US military tents

In August 2019, we erected eight two-person US military tents (COMBAT; Eureka, Binghamton, NY, USA) (Fig. 1), within and outside the edge of a long leaf pine tree (*Pinus palustris*) forest at Camp Blanding Joint Training Center in Starke, Florida (Fig. 2). The tents were paired at four locations in two habitats: two paired sites within the forest canopy (termed *canopy* habitat) and two paired sites in the field outside of the forest (termed *field* habitat). Paired tents at each site were positioned approximately 2–3 m apart with tent doors partially opened to the same cardinal direction and with sites separated by 25 m in each habitat. At the two canopy sites, tents were pitched with doors oriented north-south, while tents at the two field sites had an east-west orientation. Each tent was fitted with a US Centers for Disease Control and Prevention (CDC) trap (Model 512; John W Hock, Gainesville, FL, USA) baited with light and CO_2 (approx. 2 kg of pelletized dry ice) which was delivered from an insulated container (Igloo; John W. Hock, Gainesville, FL, USA). The trap and CO_2 container were suspended from the center of each tent (Fig. 1).

2.2. Experimental design

For each paired site, we established one treatment and one control tent (Fig. 2). The treatment tents received an off-the-shelf commercially produced passive resin diffuser impregnated by the manufacturer with transfluthrin (Dainihon Jochugiku Co., Ltd., Osaka, Japan) with either a 150-day effect (TFL-150) (990 mg transfluthrin technical AI) or a 250-day effect (TFL-250) (1200 mg transfluthrin technical AI) (McMillan et al., 2022). Each diffuser was calibrated by the manufacturer to release transfluthrin at a rate of approximately 5 mg per 24 h and was placed inside a tent corner furthest from the door. Once placed, diffusers remained in the tent for the duration of the experiment.

The two canopy habitat treatment tents were located within the tree canopy on the east side of the experiment site with doors half opened to the north, with one tent receiving a TFL-150 diffuser and one tent receiving a TFL-250 diffuser (Fig. 2). The two field habitat treatment tents were located in an open field on the west side of the experiment site with doors half opened to the east, with one receiving a TFL-150 diffuser and one receiving a TFL-250 diffuser (Fig. 2). The control tents received

Fig. 2. Aerial image of the study area at Camp Blanding Joint Training Center, Starke, Florida, showing locations of two pairs of tents in the *canopy* habitat and two pairs of tents in the *field* habitat.

no diffusers and doors were left half open to the north or east, matching their paired treatment tents in both habitats.

To monitor both biotic and abiotic ambient environmental conditions, respectively, we set two CDC traps baited with light and dry ice (termed *ambient* traps) and erected three kestrel weather stations (Model 5500; Nielsen-Kellerman, Boothwyn, PA, USA) to monitor temperature, relative humidity (RH), wind speed, and wind direction at a height of 1.2 m (Fig. 2). The two ambient traps were placed approximately 30 m to the north of the midpoint of the two field habitat sites and 30 m to the west of the midpoint of the two canopy habitat sites (Fig. 2). One weather station was placed between each of the two sites in the canopy and the field habitats (Fig. 2). The third weather station was placed 30 m north of the canopy habitat site at the junction of two access roads north of the study site (Fig. 2).

2.3. Measuring efficacy (Objective 1) and duration (Objective 2) of transfluthrin diffusers preventing entry into tents

Mosquito collections using CDC traps were initiated on August 20, 2019 (week 0) and terminated January 17, 2020 (week 21). Collections were conducted on the following schedule: weekly collections week 0 through week 4; collections every two weeks from week 5 through week 10; and monthly collections from week 11 through week 21. All traps were set in the morning between approximately 10:00 h and 12:00 h to run overnight for 24 h and collection containers retrieved and stored at −20 °C in the USDA Agricultural Research Service Center for Medical, Agricultural, and Veterinary Entomology (USDA-ARS-CMAVE) Laboratory for later counting and identification.

2.4. Mortality observations in collected specimens

After the first two collections (week 0 and 1) we observed high mosquito mortality in trap collections from tents with transfluthrin diffusers. Starting week 3, we estimated the percentage of dead mosquitoes in each trap collection upon retrieval from the experiment site. Mosquitoes were considered dead if they were on the bottom of the trap container and not moving or unable to fly after agitation.

2.5. Statistical analysis

Collection data were analyzed using R (version 4.2.1) (2022-06-23 ucrt) (R Core Team, 2023) and the RStudio GUI (version 2022.07.0548 "Spotted Wakerobin"). To adjust for non-normal distributions of counts and over-dispersion of the data, a general linear model (GLM) with family quasi-Poisson was generated to compare mean collections and assess location bias. Means were separated using general linear hypothesis tests with multiple comparison Tukey's *post-hoc* test using the *multcomp* package. Mean collection numbers were evaluated by Ryan-Einot-Gabriel-Welsch multiple range test using the *agricolae* package to assess differences between the number of mosquitoes collected from control tents and treatment tents. Additionally, a quasi-Poisson GLM followed by a General Linear Hypothesis Test with Tukey's for mean separation were conducted to determine whether the presence of diffusers significantly influenced the mortality of mosquitoes following trapping. Finally, collections were pooled and ecologically analyzed to assess a difference in mosquito community structure associated with treatment using species richness, abundance, rarefaction, diversity, and evenness. Collection data were visualized using the *ggplot2* package in R.

3. Results

3.1. Mosquito collections

Collection and evaluation periods were truncated at Day 149 due to the loss of one of the treatment tents and its diffuser. Furthermore, additional trapping could not be conducted to schedule due to the COVID-19 pandemic limiting activities of federal employees and laboratories. Mosquito collection data are described by location and date in Supplementary Table S1.

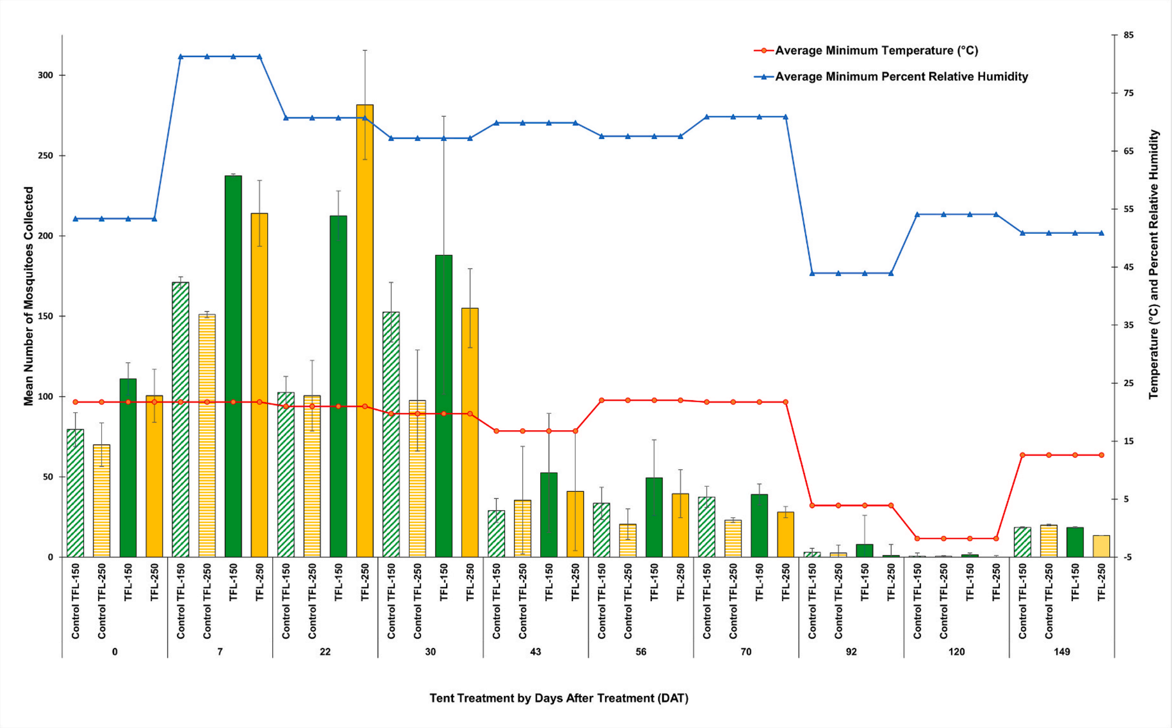


Fig. 3. Average number of mosquitoes collected inside tents containing transfluthrin diffusers with either a 250-day effect (TFL-250) or 150-day effect (TFL-150) or their paired control tents by days after treatment (DAT), August 20, 2019 to January 17, 2020, with standard error (SE) and simultaneously plotted average minimum temperature and relative humidity.

In tents containing transfluthrin-impregnated diffusers we collected 5881 mosquitoes, encompassing 20 species, with 10 (150-day diffuser) and 13 (250-day diffuser) species collected from the canopy habitat, and 14 (150-day diffuser) and 15 (250-day diffuser) species collected from the field habitat (Supplementary Table S2). Five species constituted the majority (94.1%) of the collection totals across both habitats: *Culex nigripalpus* accounted for 61.6% of total mosquitoes collected (67.7% across all canopy collections, 56.2% across all field collections), followed by *Anopheles crucians* (10.6% total; 9.7% canopy, 11.4% field), *Cx. erraticus* (10.2% total; 9.8% canopy, 10.5% field), *Coquillettidia perturbans* (8.5% total; 6.5% canopy, 10.2% field), and *Aedes tormentor* (3.2% total; 1.7% canopy, 4.0% field) (Supplementary Table S3). Additional species were collected, and their prevalence based on treatment and habitat type is described in Supplementary Table S2.

3.2. *Location bias*

Location bias was evaluated by comparing the mean number of mosquitoes collected at each site; ambient traps were treated as individual sites. The average number of mosquitoes collected at each site per trap night was 138.1 ± 18.0 (mean ± standard error, SE) (range: 103.8 ± 30.6 SE to 174.6 ± 51.4 SE). There was no significant difference in the mean number of mosquitoes collected between any of the sites, indicating that there was no site location bias (GLM-Quasi Poisson: $F_{(5,54)} = 0.2451$, $P = 0.94048$).

3.3. *Objective 1: Efficacy of preventing mosquito entry into tents*

We consistently collected more - although not statistically significantly more - mosquitoes in tents containing transfluthrin diffusers (89.6 ± 14.9 SE) than in control tents (57.4 ± 8.8 SE) (Fig. 3). While there was no significant difference in the number of mosquitoes collected between transfluthrin-treated and control tents, ($t_{(78)} = 1.902$, $P = 0.0608$), this trend persisted until cooler weather reduced collection counts. Given the low *P*-value (0.0608), additional trapping, as was planned, may have given better resolution between the treatment and control tent mosquito collections.

There was no significant difference in the mean number of mosquitoes collected in tents with either diffuser treatment (TFL-150: $t_{(38)} = 1.192$, $P = 0.241$; TFL-250: $t_{(38)} = 1.473$, $P = 0.149$) and their paired control tents. However, collections from tents containing diffusers, TFL-

Table 1
Ryan, Einot, and Gabriel and Welsch multiple range test results comparing mean numbers of mosquitoes collected for 21 trap nights distributed over 0–149 days after deployment of transfluthrin spatial repellent diffusers in two-person US military tents deployed in warm temperate Florida canopy and field habitats.

Comparison between treatment means	Difference	*P*-value	LCL	UCL
Canopy TFL-250 - Canopy Control TFL-250	20.5	0.992	−87.500	128.500
Canopy TFL-250 - Canopy TFL-150	4.8	1	−112.076	121.676
Canopy TFL-250 - Canopy Control TFL-150	21.5	1	−100.228	143.228
Canopy TFL-250 - Canopy Ambient Control	−56.1	0.879	−181.114	68.914
Canopy TFL-250 - Field Control TFL-150	13.8	1	−113.669	141.269
Canopy TFL-250 - Field TFL-150	−27.6	0.999	−157.014	101.814
Canopy TFL-250 - Field Control TFL-250	36.1	0.996	−94.914	167.114
Canopy TFL-250 - Field TFL-250	−14	1	−145.014	117.014
Canopy TFL-250 - Field Ambient Control	−23.4	1	−156.932	110.132
Canopy Control TFL-250 - Canopy TFL-150	−15.7	0.998	−123.700	92.300
Canopy Control TFL-250 - Canopy Control TFL-150	1	1	−115.876	117.876
Canopy Control TFL-250 - Canopy Ambient Control	−76.6	0.516	−198.328	45.128
Canopy Control TFL-250 - Field Control TFL-150	−6.7	1	−131.714	118.314
Canopy Control TFL-250 - Field TFL-150	−48.1	0.958	−175.569	79.369
Canopy Control TFL-250 - Field Control TFL-250	15.6	1	−113.814	145.014
Canopy Control TFL-250 - Field TFL-250	−34.5	0.997	−165.514	96.514
Canopy Control TFL-250 - Field Ambient Control	−43.9	0.978	−174.914	87.114
Canopy TFL-150 - Canopy Control TFL-150	16.7	0.997	−91.300	124.700
Canopy TFL-150 - Canopy Ambient Control	−60.9	0.703	−177.776	55.976
Canopy TFL-150 - Field Control TFL-150	9	1	−112.728	130.728
Canopy TFL-150 - Field TFL-150	−32.4	0.996	−157.414	92.614
Canopy TFL-150 - Field Control TFL-250	31.3	0.998	−96.169	158.769
Canopy TFL-150 - Field TFL-250	−18.8	1	−148.214	110.614
Canopy TFL-150 - Field Ambient Control	−28.2	0.999	−159.214	102.814
Canopy Control TFL-150 - Canopy Ambient Control	−77.6	0.276	−185.600	30.400
Canopy Control TFL-150 - Field Control TFL-150	−7.7	1	−124.576	109.176
Canopy Control TFL-150 - Field TFL-150	−49.1	0.918	−170.828	72.628
Canopy Control TFL-150 - Field Control TFL-250	14.6	1	−110.414	139.614
Canopy Control TFL-150 - Field TFL-250	−35.5	0.994	−162.969	91.969
Canopy Control TFL-150 - Field Ambient Control	−44.9	0.977	−174.314	84.514
Canopy Ambient Control - Field Control TFL-150	69.9	0.386	−38.100	177.900
Canopy Ambient Control - Field TFL-150	28.5	0.992	−88.376	145.376
Canopy Ambient Control - Field Control TFL-250	92.2	0.274	−29.528	213.928
Canopy Ambient Control - Field TFL-250	42.1	0.976	−82.914	167.114
Canopy Ambient Control - Field Ambient Control	32.7	0.997	−94.769	160.169
Field Control TFL-150 - Field TFL-150	−41.4	0.852	−149.400	66.600
Field Control TFL-150 - Field Control TFL-250	22.3	0.998	−94.576	139.176
Field Control TFL-150 - Field TFL-250	−27.8	0.997	−149.528	93.928
Field Control TFL-150 - Field Ambient Control	−37.2	0.989	−162.214	87.814
Field TFL-150 - Field Control TFL-250	63.7	0.488	−44.300	171.700
Field TFL-150 - Field TFL-250	13.6	1	−103.276	130.476
Field TFL-150 - Field Ambient Control	4.2	1	−117.528	125.928
Field Control TFL-250 - Field TFL-250	−50.1	0.723	−158.100	57.900
Field Control TFL-250 - Field Ambient Control	−59.5	0.726	−176.376	57.376
Field TFL-250 - Field Ambient Control	−9.4	1	−117.400	98.600

Abbreviations: LCL, lower control limit = one confidence interval lower than the difference; UCL, upper control limit = one confidence interval higher than the difference.

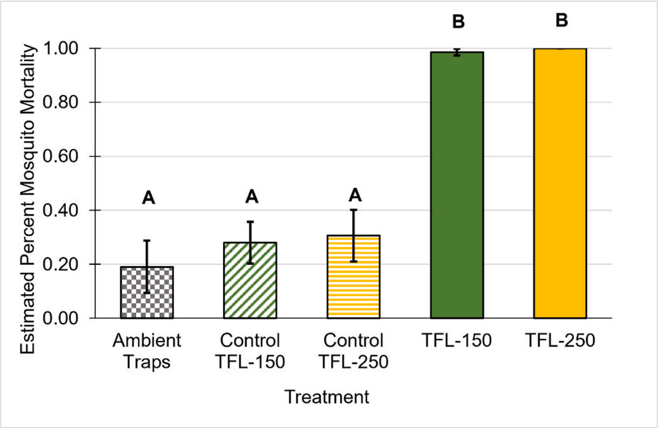


Fig. 4. Average estimated percent mortality, with standard error (SE), of mosquitoes collected in the ambient environment or inside tents containing transfluthrin diffusers with either a 250-day effect (TFL-250) or 150-day effect (TFL-150) or their paired control tents from September 11, 2019 to January 17, 2020. *Note*: Histogram bars with the same letter ("A" or "B") are not significantly different; bars with different letters are significantly different, at $P < 0.001$.

150 (91.8 ± 20.8 SE) or TFL-250 (87.4 ± 21.9 SE), contained *c.*30 more mosquitoes per trap/night compared to collections from their paired control tent, 62.8 ± 13.5 SE (TFL-150 control) and 52.1 ± 11.6 SE (TFL-250 control) (Table 1).

We assessed the interaction between habitat (field or canopy) and tent treatment. Mosquito collection numbers from treatment tents placed in either the field (101.2 ± 23.0 SE) or the canopy (78.0 ± 19.2 SE) habitats were higher than the control tents in either the field (55.5 ± 11.5 SE) or the canopy (59.4 ± 13.6 SE) habitats, but not significantly more ($t_{(76)} = 0.698$, $P = 0.487$). We also did not find any significant difference in mosquito collections between the field tents (78.3 ± 13.2 SE) or the canopy tents (68.7 ± 11.7 SE) ($t_{(76)} = -0.187$, $P = 0.852$).

However, although we observed a lack of significance in collection numbers among various groupings as described above, we did observe a general trend between the field and canopy habitats and temperature (Fig. 3). On days when the maximum temperature in the field and canopy habitats was similar (< 2 °C difference), the average number of mosquitoes collected in the canopy habitat (all tents) was higher than for the field habitat. Conversely, when the maximum temperature difference between the field and canopy habitats was greater than 2 °C, the average number of mosquitoes collected in the field habitat was higher than the canopy habitat. The only deviation from this trend was the last night of trapping, 149 days after treatment (DAT), in which the temperature difference was 0.5 °C, but the field habitat tents averaged 21.6 mosquitoes, which was higher than collections in the canopy habitat tents which averaged 19.0 mosquitoes.

3.4. *Objective 2: Duration and mortality observation*

We observed high mean mortality in tents with either diffuser, TFL-250 (100%) and TFL-150 (98.7%), and no significant difference in mean mortality rates between diffuser treatments from 22 DAT through 149 DAT ($z = 0.073$, $P = 1.00$) (Fig. 4). The estimated mean percent mortality in the treatment tents was significantly higher (TFL-150 - ambient trap, $z = 4.509$, $P < 0.001$; TFL-150 - Control TFL-150, $z = 4.612$, $P < 0.001$; TFL-150 - Control TFL-250, $z = 4.431$, $P < 0.001$; TFL-250 - ambient trap, $z = 4.530$, $P < 0.001$; TFL-250 - Control TFL-150, $z = 4.632$, $P < 0.001$; TFL-250 - Control TFL-250, $z = 4.452$, $P < 0.001$) than that observed in the control tents, control TFL-250 (30.7%) and control TFL-150 (28.0%), or the ambient traps (19.1%) as described in Fig. 4. We determined transfluthrin diffuser duration of effect based on the estimated percent mortality observed, since we consistently collected more mosquitoes in treatment tents than in control tents. A reduction in percent mortality was only observed at 149 DAT from the TFL-150 diffuser placed in the field habitat tent. All other tents with a diffuser reported 100% mortality throughout. The decrease in mortality for the TFL-150 diffuser in the field habitat would appear to indicate a drop in efficacy of the diffuser at the expected 150-day limit of its efficacy period. We hypothesize that the canopy habitat may have had a protective effect on weathering the 150-day diffuser placed there, because mortality in collected specimens was still 100% at Day 149 in that tent.

Ecological analysis of the collection data was conducted to assess if there were differences observed between collections regarding species abundance and richness. These assessments yielded the following results as described in Table 2. Species richness values were highest from canopy habitat collections, more specifically from the control (i.e. non-treated) tents (2.02 and 2.58). However, overall species richness was more consistently high among the field habitat collections. Abundance was highest from the ambient trap collections. Furthermore, the abundance values were consistently higher in treatment tent collections than in control tent collections regardless of the habitat. Rarefaction was calculated using 10 individuals and described in Table 2. Overall, the greatest number of individual species that would be collected from a 10-unit subsample are from the ambient traps at 4.83 and 4.95. Furthermore, the collections from the field habitat had higher rarefaction richness values than the canopy habitat, and in both the field and canopy habitats the treatment tents had lower rarefaction richness values compared to their respective control tents. Diversity using a Shannon-Wiener Index and Simpson's index was calculated for all collection sites and described in Table 2. Similar to rarefaction, the greatest index values were from the ambient traps at 1.82, 0.80 and 1.88, 0.80. Furthermore, the collections from the field habitat had higher Shannon-Wiener Index and Simpson's index values than the canopy habitat, and in both the field and canopy habitats the treatment tents had lower Shannon-Wiener Index and Simpson's index values compared to their respective control tents. Finally, evenness was calculated using a Pielou's evenness index and true diversity using Shannon index. Similar to

Table 2
Ecological data analysis to assess differences between species richness, abundance, rarefaction, diversity, and evenness related to treatment type and locale.

Locale	Treatment	Richness		Abundance	Rarefaction	Diversity		Evenness	
		Menhinick	Margalef			Shannon-Wiener Index	Simpson's Index	Pielou's evenness	True diversity - Shannon
Canopy	TFL-250	0.52	2.08	830	3.15	1.09	0.46	0.17	2.97
Canopy	Control	0.77	2.58	490	3.85	1.45	0.65	0.23	4.27
Canopy	TFL-150	0.39	1.50	780	3.21	1.09	0.51	0.21	2.99
Canopy	Control	0.56	2.02	618	3.70	1.34	0.60	0.23	3.82
Canopy	Ambient trap	0.43	2.17	1577	4.83	1.82	0.80	0.28	6.20
Field	Ambient trap	0.48	2.34	1431	4.95	1.88	0.80	0.28	6.53
Field	Control	0.56	2.13	711	4.40	1.66	0.72	0.27	5.28
Field	TFL-150	0.51	2.29	1099	3.93	1.44	0.63	0.22	4.24
Field	Control	0.63	2.10	489	4.55	1.71	0.74	0.28	5.54
Field	TFL-250	0.51	2.18	986	3.84	1.40	0.61	0.22	4.06

rarefaction and diversity, the greatest evenness values were from the ambient traps at 0.28, 6.20 and 0.28, 6.53. Moreover, the collections from the field habitat had higher evenness values than the canopy habitat and in both the field and canopy habitats the treatment tents had lower evenness values compared to their respective control tents.

4. Discussion

Based on results from the total numbers of collected mosquitoes among treatment and control tents, the commercially produced transfluthrin-impregnated resin diffusers placed in two-person US military tents did not prevent mosquitoes from entering the tents. We emphasize that these results are not conclusive for all applications of the active ingredient transfluthrin and represent only one transfluthrin deployment and use scenario. Our findings might be explained by the effect described by Ogoma et al. (2012b, 2014) as excito-repellency, where transfluthrin-exposed mosquitoes are compelled to perform high levels of flight and other movement activity. It is expected that when mosquitoes encounter a spatial repellent plume they move away from the area (Achee et al., 2012); however, the combination of the diffuser location and the half-opened tent door might not allow for a sufficient zone of protection (Sukkanon et al., 2021; Yan et al., 2023). McMillan et al. (2022) observing differences in temperature-related mortality in caged sentinel mosquitoes placed across an interior-to-exterior gradient in similar two-person US military tent entrances speculated that convection currents might be occurring at the tent entrance due to temperature differences inside and outside the tent. If these convection currents exist, they may prevent a stable protection zone from forming, allowing mosquitoes attracted by a CO_2 trail to fly through this intermittent barrier.

McMillan et al. (2022) further speculated that transfluthrin accumulated on surfaces inside the tent from point sources of transfluthrin such as diffusers or treated cloth strips placed in or hanging from the inside of the tent. In this scenario, once mosquitoes enter a tent that currently contained or had in the past contained a transfluthrin source and rested on any surface, they would contact transfluthrin and possibly receive a lethal dose. Yan et al. (2023) showed that, in non-contact trials with *An. minimus* using filter paper treated with 0.065%, 0.2%, and 1.5% transfluthrin, mosquitoes had a strong escape response of 62.50%, 49.85%, and 63.49%, respectively. However, in scenarios with contact at the same transfluthrin rates, the escape response was depressed to 35.29%, 17.3%, and 0.0%, respectively (Yan et al., 2023). In the current investigation, the combination of an unstable spatial repellent plume and mosquito contact with surfaces potentially contaminated with transfluthrin once inside the tent might explain the higher numbers of mosquitoes collected in treated tents compared to the control.

The placement of the transfluthrin diffuser in tents in the present investigation could also be an important consideration (Ogoma et al., 2014; McPhatter et al., 2017; Martin et al., 2020; Rajagopal et al., 2023). Our placement of the transfluthrin diffuser in a back corner, away from the tent opening may be a contributing factor in the number of mosquitoes entering and later collected in the treated tents. McPhatter et al. (2017) placed two types of transfluthrin-impregnated strips at the entrance of three-person tents and was able to prevent 66–88% of *Ae. aegypti* entering. Ogoma et al. (2014) suggested that creating a transfluthrin "bubble" with multiple devices rather than using one single source would be more effective. Rajagopal et al. (2023) reduced entry of *Ae. aegypti*, *Ae. taeniorhynchus*, *An. quadrimaculatus*, and *Cx. quinquefasciatus* by 85–95% by placing transfluthrin-activated controlled release passive devices at the entrance as well as the center and rear of large 3 × 4 m multi-person US military tents.

In addition to higher numbers of mosquitoes in treated tents, we observed significantly higher average percentages of mortality in collected mosquitoes in treated tents than those from the control tents (99.14% and 28.33%, respectively). Martin et al. (2020) found that airborne transfluthrin induced toxic effects and achieved a maximum knockdown/mortality of ~80% or higher at 2 h when cages of mosquitoes were placed 2 and 4 m from a transfluthrin source. Masalu et al. (2020) and Rajagopal et al. (2023) observed nearly 100% mortality at 24 h with caged mosquitoes placed near but not in contact with any of the transfluthrin sources. Because of the high mortality in treatment tents, we suspect that all interior surfaces of the tent, including the trap, battery, collection bag, and CO_2 reservoir, were contaminated with transfluthrin in addition to the airborne transfluthrin. Once inside the tent, mosquitoes could encounter a stable transfluthrin plume at least at floor level and contact transfluthrin, thus experiencing excito-repellency leading to a reduced escape response and more opportunities for contacting a treated surface, allowing more mosquitoes to be collected in traps in transfluthrin-treated tents. We speculate that mosquitoes, once inside the tent with a diffuser, were overstimulated and/or disoriented due to excito-repellency and not able to be repelled as expected. The transfluthrin-induced flight activity may have increased the likelihood of becoming captured by the trap, and continued exposure to transfluthrin while confined in the collection container resulted in high rates of mortality.

Additionally, although our investigation was not designed to evaluate biting rates, we speculate that mosquitoes collected in tents with a transfluthrin diffuser even if not repelled from the area would be less likely to bite occupants within as well as nearby the tent (Lucas et al., 2007; Ogoma et al., 2014; McPhatter et al., 2017). Masalu et al. (2020) found that painting transfluthrin on the underside of chairs placed outdoors could reduce mosquito biting by 70–85%. When using transfluthrin-treated hessian fabric placed around outdoor cooking kitchens, outdoor biting by mosquitoes was reduced by up to 81% in the immediate area and reduced biting occurred in nearby enclosures by up to 43% (Masalu et al., 2020). However, further investigation is needed to determine whether mosquitoes exposed to transfluthrin in partially enclosed spaces have a diminished biting potential. In the context of the current investigation, further experiments could illuminate whether the mosquitoes accumulating transfluthrin doses in treated tents would have still attempted to bite human occupants before being killed by the active ingredient.

Similar to observations reported in Britch et al. (2020b) that not all mosquito species in an area may be equally present in collections from traps protected with spatial repellents, we observed a difference in the number of species collected between the field (14–15 species, depending on diffuser type) and canopy (10–13 species, depending on diffuser type) habitats (Supplementary Table S2). We were not able to distinguish a difference in collection abundance for single-specimen species that may have been singularly impacted by spatial repellent-treated tents.

5. Conclusions

Based on the collection data presented herein, we conclude that the commercially produced passive transfluthrin diffusers we deployed in two-person tents in a warm temperate environment do not provide a spatial repellent effect sufficient to protect tents from entry by mosquitoes. These findings corroborate those of Dame et al. (2014), Britch et al. (2021), and McMillan et al. (2022) in that a single spatial repellent formulation and/or delivery method will likely not be sufficient to protect personnel from all mosquito species present in an area and will likely not be appropriate for all defined spaces that require protection. However, we emphasize that these results are not conclusive for all applications of transfluthrin and represent only one transfluthrin deployment and use scenario. Spatial repellents have shown potential in preventing human-vector interactions as observed by Ogoma et al. (2012a), Britch et al. (2020a, b), and McMillan et al. (2022) and could help mitigate current shortfalls in IVM. However, our findings in the present investigation indicate that over-confidence in spatial repellents could bring about unintended harmful effects such as accumulating mosquitoes in a space intended for protection. Whether these accumulated mosquitoes would have a reduced biting behavior following exposure to transfluthrin should be investigated in future studies. Future refinements of spatial repellent formulation, delivery method, and placement could provide operational protection from mosquito and other arthropod vectors and contribute to solving the significant challenges in insecticide resistance and disease transmission that impact the efficacy of current IVM systems (Revay et al., 2013; Ogoma et al., 2017).

Funding

This research was supported by the USDA-ARS and the U.S. Department of Defense (DoD) Deployed War-Fighter Protection Program (DWFP).

Ethical approval

Not applicable.

Disclaimer

Mention of trade names or commercial products in this publication is solely to provide specific information and does not imply recommendation or endorsement by USDA, DoD, the Florida Army National Guard, or the DWFP. The USDA is an equal opportunity provider and employer. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the USDA.

CRediT authorship contribution statement

Barbara E. Bayer: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Robert L. Aldridge:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Bianca J. Moreno:** Investigation, Methodology, Writing – review & editing. **Frances V. Golden:** Investigation, Methodology, Writing – review & editing. **Seth Gibson:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. **Jeffrey L. Wahl:** Conceptualization, Project administration, Resources. **Kenneth J. Linthicum:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing, All authors read and approved the final manuscript.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data supporting the conclusions of this article are included within the article and its supplementary files. Data, analysis, and interpretation from this investigation will be posted to the Mobile Pesticide App operational entomology decision support system database (https://ars.usda.gov/saa/cmave/PesticideApp).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crpvbd.2023.100156.

References

Achee, N.L., Bangs, M.J., Farlow, R., Killeen, G.F., Lindsay, S., Logan, J.G., et al., 2012. Spatial repellents: From discovery and development to evidence-based validation. Malar. J. 11, 164. https://doi.org/10.1186/1475-2875-11-164.

AFPMB, 1996. Armed Forces Pest Management Board, Technical Guide No. 36. Personal protective techniques against Insects and other arthropods of military significance. Armed Forces Pest Management Board, Washington, DC.

Britch, S.C., Dame, D.A., Meisch, M.V., Kline, D.L., Walker, T.W., Allan, S.A., et al., 2021. Spatial repellents protect small perimeters from riceland mosquitoes in a warm-humid environment. J. Am. Mosq. Control Assoc. 37, 41–45. https://doi.org/10.2987/20-6974.1.

Britch, S.C., Kline, D.L., Linthicum, K.J., Urban, J., Dickstein, E., Aldridge, R.L., Golden, F.V., 2020a. Transfluthrin spatial repellent on US military camouflage netting reduces tabanids in a warm-temperate environment. J. Am. Mosq. Control Assoc. 36, 212–215. https://doi.org/10.2987/20-6933.1.

Britch, S.C., Linthicum, K.J., Kline, D.L., Aldridge, R.L., Golden, F.V., Wittie, J., et al., 2020b. Transfluthrin spatial repellent on US military materials reduces *Culex tarsalis* incursion in a desert environment. J. Am. Mosq. Control Assoc. 36, 37–42. https://doi.org/10.2987/19-6894.1.

Dame, D.A., Meisch, M.V., Lewis, C.N., Kline, D.L., Clark, G.G., 2014. Field evaluation of four spatial repellent devices against Arkansas rice-land mosquitoes. J. Am. Mosq. Control Assoc. 30, 31–36. https://doi.org/10.2987/13-6379.1.

Govella, N.J., Ogoma, S.B., Paliga, J., Chaki, P.P., Killeen, G., 2015. Impregnating hessian strips with the volatile pyrethroid transfluthrin prevents outdoor exposure to vectors of malaria and lymphatic filariasis in urban Dar es Salaam, Tanzania. Parasites Vectors 8, 322. https://doi.org/10.1186/s13071-015-0937-8.

Lucas, J.R., Shono, Y., Iwasaki, T., Ishiwatari, T., Spero, N., Benzon, G., 2007. U.S. laboratory and field trials of metofluthrin (SumiOne) emanators for reducing mosquito biting outdoors. J. Am. Mosq. Control Assoc. 23, 47–54. https://doi.org/10.2987/8756-971x(2007)23[47:ulafto]2.0.co;2.

Martin, N.J., Nam, V.S., Lover, A.A., Phonbg, T.V., Tu, T.C., Mendenhall, I.H., 2020. The impact of transfluthrin the spatial repellency of primary malaria mosquito vectors in Vietnam: *Anopheles dirus* and *Anopheles minimus*. Malar. J. 19, 9. https://doi.org/10.1186/s12936-019-3092-4.

Masalu, J.P., Finda, M., Killeen, G.F., Ngowo, H.S., Pinda, P.G., Okumu, F.O., 2020. Creating mosquito-free outdoor spaces using transfluthrin-treated chairs and ribbons. Malar. J. 19, 109. https://doi.org/10.1186/s12936-020-03180-1.

McMillan, B.E., Britch, S.C., Golden, F.V., Aldridge, R.L., Moreno, B.J., Bayer, B.E., Linthicum, K.J., 2022. Assessing transfluthrin mortality against *Aedes aegypti* and *Culex quinquefasciatus* inside and outside US military tents in a northern Florida environment. Curr. Res. Parasitol. Vector Borne Dis. 2, 100067 https://doi.org/10.1016/j.crpvbd.2021.100067.

McPhatter, L.P., Mischler, P.D., Webb, M.Z., 2017. Laboratory and semi-field evaluations of two (transfluthrin) spatial repellent devices against *Aedes aegypti* (L.) (Diptera: Culicidae). U.S. Army Med. Dep. J. 1–17, 13–22. https://www.ncbi.nlm.nih.gov/pubmed/28511270.

Mmbando, A.S., Ngowo, H., Limwagu, A., Kilalangongono, M., Kifungo, K., Okumu, F.O., 2018. Eave ribbons treated with the spatial repellent, transfluthrin, can effectively protect against indoor-biting and outdoor-biting malaria mosquitoes. Malar. J. 17, 368. https://doi.org/10.1186/s12936-018-2520-1.

Mwanga, E.P., Mmbando, A.S., Mrosso, P.C., Stica, C., Mapua, S.A., Finda, M.F., Okumu, F.O., 2019. Eave ribbons treated with transfluthrin can protect both users and non-users against malaria vectors. Malar. J. 18, 314. https://doi.org/10.1186/s12936-019-2958-9.

Ogoma, S.B., Mmando, A.S., Swai, J.K., Horstmann, S., Malone, D., Killeen, G.F., 2017. A low technology emanator treated with the volatile pyrethroid transfluthrin confers long term protection against outdoor biting vectors of lymphatic filariasis, arboviruses and malaria. PLoS Negl. Trop. Dis. 11, e0005455 https://doi.org/10.1371/journal.pntd.0005455.

Ogoma, S.B., Moore, S.J., Maia, M.F., 2012b. A systematic review of mosquito coils and passive emanators: defining recommendations for spatial repellency testing methodologies. Parasites Vectors 5, 287, 10.1186%2F1756-3305-5-287.

Ogoma, S.B., Ngonyani, H., Simfukwe, E.T., Mseka, A., Moore, J., Killeen, G.F., 2012a. Spatial repellency of transfluthrin-treated hessian strips against laboratory-reared *Anopheles arabiensis* mosquitoes in a semi-field tunnel cage. Parasites Vectors 5, 54. https://doi.org/10.1186/1756-3305-5-54.

Ogoma, S.B., Ngonyani, H., Simfukwe, E.T., Mseka, A., Moore, J., Maia, M.F., et al., 2014. The mode of action of spatial repellents and their impact on vectorial capacity of *Anopheles gambiae sensu stricto*. PLoS One 9, e110433, 10.1371%2Fjournal.pone.0110433.

R Core Team, 2023. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. R-project.org/.

Rajagopal, N.R., Bowman, A.R., Aldana, F.J., Batich, C.D., Hogsette, J.A., Kline, D.L., 2023. Semi-field evaluation of a novel controlled release device using transfluthrin as spatial repellent to prevent entry of mosquitoes into military tents. Curr. Res. Parasitol. Vector Borne Dis. 3, 100113 https://doi.org/10.1016/j.crpvbd.2023.100113.

Revay, E.E., Junnila, A., Xue, R.D., Kline, D.L., Bernier, U.R., Kravchenko, V.D., et al., 2013. Evaluation of commercial products for personal protection against mosquitoes. Acta Trop. 125, 226–230. https://doi.org/10.1016/j.actatropica.2012.10.009.

Sukkanon, C., Tisgratog, R., Muenworn, V., Bangs, M.J., Hii, J., Chareonviriyaphap, T., 2021. Field evaluation of a spatial repellent emanation vest for personal protection against outdoor biting mosquitoes. J. Med. Entomol. 58, 756–766. https://doi.org/10.1093/jme/tjaa213.

Tambwe, M.M., Moore, S.J., Chilumba, H., Swai, J.K., Moore, J.D., Stica, C., Saddler, A., 2020. Semi-field evaluation of freestanding transfluthrin passive emanators and the BG sentinel trap as a "push-pull control strategy" against *Aedes aegypti* mosquitoes. Parasites Vectors 13, 392, 10.1186%2Fs13071-020-04263-3.

Verhulst, N.O., Cavegn, J.C., Mathis, A., 2021. Spatial repellency and vapour toxicity of transfluthrin against the biting midges *Culicoides nubeculosus* and *C. sonorensis* (Ceratopogonidae). Curr. Res. Insect Sci. 1, 100002 https://doi.org/10.1016/j.cris.2020.100002.

Withers, B.G., Craig, S.C., 2003. The historical impact of preventive medicine in war. Military Preventive Medicine: Mobilization and Deployment Vol. 1, 21–57.

Yan, C., Hii, J., Ratchadawan, N., Ahebwa, A., Saeung, M., Chareonviriyaphap, T., 2023. The effect of transfluthrin-treated jute and cotton emanator vests on human landing and fecundity of *Anopheles minimus* in Thailand. Acta Trop. 242, 106904 https://doi.org/10.1016/j.actatropica.2023.106904.