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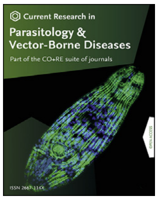

Assessing transfluthrin mortality against *Aedes aegypti* and *Culex quinquefasciatus* inside and outside US military tents in a northern Florida environment

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ABSTRACT

Mortality caused by passive resin transfluthrin diffusers (~5 mg AI per 24 h release rate) suspended in small 2-person tents was measured for colony-reared sentinel pyrethroid susceptible *Aedes aegypti* and *Culex quinquefasciatus* female mosquitoes, as well as a pyrethroid-resistant strain of *Aedes aegypti*, in a USA military field camp scenario. Mortality effects were investigated for impact by factors such as sentinel cage location (inside tent, tent doorway and outside tent), exposure time (15, 30, 45 and 60 min), and environmental temperature (°C), all of which were examined over an 8-week period. Analyses determined there was a significant interaction between mosquito strain and transfluthrin susceptibility, with the two susceptible strains experiencing significantly greater mean mortality than the resistant *Ae. aegypti* strain. Significant differences were likewise observed between the mosquito strains over the 8-week study period, where study week and temperature were both positively correlated with an increase in observed mean mosquito mortality. Mosquito proximity to the transfluthrin diffusers was also influenced by week and showed that sentinel cage placement in the environment demonstrates different mortality measurements, depending on the environmental conditions. The length of exposure to transfluthrin, however, was determined to not significantly impact transfluthrin efficacy on the examined mosquito strains, although increased exposure did result in increased susceptible strain mortality. These results suggest that transfluthrin is highly effective in causing mortality against susceptible *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes under field conditions but is minimally effective against pyrethroid-resistant *Ae. aegypti* mosquitoes. Transfluthrin-infused devices are influenced by environmental factors that can combine to impact mosquito mortality in the field.

1. Introduction

Recent studies have shown that spatial repellents can be incorporated into integrated vector management (IVM) systems to protect personnel from arthropod disease vectors during USA military field operations (Britch et al., 2020a, b; 2021). These studies demonstrated that, similar to conventional residual sprays applied as barrier treatments, spatial repellents can protect defined areas from a variety of nuisance and disease vector biting arthropods in the field. Unlike conventional residual pesticide applications, however, spatial repellents do not require physical contact with target arthropods to be effective and may not contribute to the evolution of insecticide resistance if mortality is limited (Achee et al., 2012). Spatial repellents such as transfluthrin can also be incorporated into small controlled-release devices that can be rapidly deployed and are easily portable to new locations. Although such devices have recently been investigated in the field and show promise for potential application in US military field scenarios (Dame et al., 2014; Britch et al., 2021), the limitations of spatial repellent controlled release devices with respect to their operational temperature range and diffusion/effect radius in a military scenario are not yet known.

Mosquitoes, such as *Aedes aegypti* (L.), *Culex quinquefasciatus* (Say), and *Anopheles albimanus* (Wiedemann) can transmit an array of pathogens to livestock and humans (Goddard, 2016; Herricks et al., 2017). Furthermore, they can cause painful bites that disrupt US military field activities (Mehr et al., 1997). To control vectors such as these, the Department of Defense (DoD) designed a management programme to

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protect USA military personnel which involved overlapping control strategies to manage the vector – and therefore the pathogens they transmit. Traditionally, this is an IVM programme, and these programmes can include strategies such as limiting personnel activities during times of high vector activity (e.g. crepuscular periods). Contrary to conventional IVM programmes in civilian scenarios or in military installation scenarios in the continental USA, a military IVM programme for deployed USA military personnel against vector-borne disease may be complicated by logistical and operational constraints that limit or prevent source reduction or active control measures, such as adulticide applications or treatment of larval habitats (Burkett et al., 2013).

Passive control techniques could supplement or replace active controls in military field scenarios. For example, residual pesticide treatment of US military uniforms and materials have been leveraged against mosquitoes in military IVM because these materials, such as camouflage netting or tents, are found ubiquitously among US military personnel in the field (Britch et al., 2010). However, widespread resistance of mosquitoes to available pesticide active ingredients, such as organophosphates and synthetic pyrethroids (Weill et al., 2003; Liu, 2015), can limit the efficacy of these products in field settings. Potential shortfalls of contact residual pesticides are that (i) residual pesticides induce mortality and thus the opportunity for the evolution of resistance, and (ii) host-seeking female mosquitoes must touch the treated surface to be affected by the pesticide, and therefore could fly directly to human hosts without contacting the treatment, or contact the treatment and still bite humans before the acquired pesticide dose takes effect (Britch et al., 2020b).

Recent advancements have uncovered spatial repellent properties of some volatile pyrethroids such as transfluthrin (TFL; USEPA, 2018) that may interfere with the host-seeking behavior of hematophagous arthropods (Achee et al., 2012; Ogoma et al., 2012) and reduce biting pressure in protected areas. Recent work has demonstrated that mosquito biting pressure can be reduced with transfluthrin on US military materials, such as camouflage netting in field scenarios (Britch et al., 2018). In theory, an effective spatial repellent (i) would create an unfavorable environment for a host-seeking mosquito, (ii) would compel the mosquito to leave the area without contacting human hosts, (iii) would not require touching any surfaces in the environment, and (iv) would achieve all described conditions before accumulating a fatally toxic dose.

Recent field work with spatial repellents suggests that they could be effective at reducing biting pressure from host-seeking riceland mosquitoes in a warm-humid environment. Dame et al. (2014) reported significant reductions of mosquitoes such as *Anopheles quadrimaculatus* (Say) in traps augmented with spatial repellents such as metofluthrin or linalool in Arkansas, USA. More recent and compelling results are from a randomized cluster trial in Iquitos, Peru, where Morrison et al. (2021) demonstrated a significant reduction in *Aedes*-borne viral infections transmitted from *Ae. aegypti* populations by utilizing spatial repellents in homes.

This study aims to investigate the operational diffusion/effect radius of a transfluthrin spatial repellent device and how insecticide-susceptible and insecticide-resistant vectors may respond to its deployment in a realistic US military field scenario consisting of protecting personnel in a small 2-person tent. Although transfluthrin is a spatial repellent, it is also a highly toxic agent against mosquitoes at close or prolonged contact with vapors. In this work, we exploited this latter property to evaluate the effective range and temperature sensitivity of this active ingredient by measuring mortality of sentinel colony-reared mosquitoes in a US military field scenario.

2. Materials and methods

2.1. Study location and treatment

Eight LiteFighter® 2 (LiteFighter Systems, LLC., Roswell, GA, USA) 2-person tents were arranged at a field research plot along the western boundary of Camp Blanding Joint Training Center (CBJTC), Starke, Florida, USA, centered on 29.866178°N, 82.040696°W (Fig. 1). Tents were clustered into 4 pairs, each consisting of 1 treatment tent and 1 control tent separated by 5 m, with at least 15 m from the center of one pair to the next. During bioassays, each tent was opened on only one side and each pair of tents opened in the same direction, with 2 pairs facing north and 2 pairs facing east (Fig. 1). For each tent and each sampling period, one sentinel cage containing colony-reared adult mosquitoes was hung in each of the four inside corners, 4 cages were suspended in the doorway, and 4 attached at 0.5 m from the ground on a tread-in post (Field Guardian Fencing Systems, Farm Supply, Barnesville, GA, USA) positioned 0.5 m outside of the doorway (Fig. 2) for a total of 12 cages for each bioassay for each species. A single transfluthrin passive resin diffuser (Dainihon Jochugiku Co., Ltd., Osaka, Japan) with either a 150 day effect (990 mg transfluthrin technical AI, 5 mg per 24 h release rate) or a 250-day effect (1200 mg transfluthrin technical AI, 5 mg per 24 h release rate), was suspended from the center of the ceiling of each of the treatment tents using 550-paracord (generic; manufacturer unknown) to provide equidistant exposure for all inside sentinel cages inside treatment tents and consistent exposure distance for all doorway and outside sentinel cages. Diffusers were set into treatment tents on 14 January 2021 (week 0) and remained in place throughout the study until 11 March 2021 (week 8).

Sentinel cage bioassays were performed on the same day each week during the early afternoon and temperature data were collected from the outside and inside each of tent (see *Section 2.4*.) throughout the study period. For each tent during each bioassay, the rainfly was secured open on one side of the tent and the inner mesh door was opened to a consistent marker (approximately 25% open; Fig. 2) to allow sentinel cages to be hung in the doorway of the tent. Apart from during the weekly bioassay sessions, all tent rainflys and inner doors were kept closed throughout the study period. Bioassays with sentinel cages were conducted for a total of 9 weekly sessions between 14 January 2021 and 11 March 2021.

2.2. Mosquitoes

Sentinel mosquitoes were sourced from three colony strains maintained at the USDA Agricultural Research Service Center for Medical, Agricultural, and Veterinary Entomology (USD-ARS-CMAVE) insectaries between 26–28 °C, 70–80% relative humidity (RH), and a 14:10 h (L:D) photoperiod per Gerberg et al. (1994): a pyrethroid-susceptible Orlando 1952 *Ae. aegypti* strain (Schreck, 1977), a pyrethroid-resistant Puerto Rico 2012 *Ae. aegypti* strain (Estep et al., 2017), and a pyrethroid-susceptible Gainesville 1996 *Cx. quinquefasciatus* strain (Allan et al., 2005).

2.3. Sentinel cages

Sentinel cages were constructed and prepared in accordance with the methods outlined by Britch et al. (2019) for each mosquito strain. Each sentinel cage was loaded with ten 7–10-day-old non-blood-fed adult female mosquitoes one day prior to transportation to the field and provided access to cotton balls soaked with a 10% sucrose solution. Each strain was kept in separate insulated picnic coolers lined with a wet terrycloth to prevent desiccation.

At each field location, sentinel cages were arranged as described in *Section 2.1* (Fig. 2). and monitored for mortality at 15, 30, 45 and 60 min of exposure. Control sentinel cages were similarly monitored in identical but untreated tents for comparison against treatment-exposed sentinel cages.

Mosquitoes were monitored for approximately 10 min after the final sampling for each test day, but no mosquitoes recovered from their exposure to transfluthrin throughout the study.

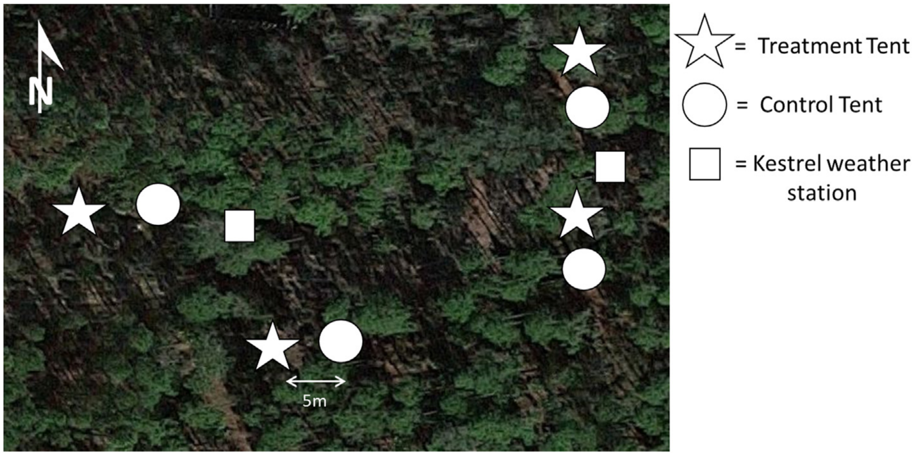

Fig. 1. Overview image of study area and tent layout for field investigation of mortality effects of transfluthrin vapor targeting colony-reared sentinel mosquitoes placed in a variety of locations in and near US military 2-person tents. This warm-temperate study site was located at Camp Blanding Joint Training Center (CBJTC), Starke, FL, USA, centered on 29.866178°N, 82.040696°W. Tents on eastern side of the study site opened to face north and tents on the western side opened to face east. Square symbols show the two outside locations for weather recorders; an additional weather recorder was also placed on the center of the floor inside each of the 8 tents.

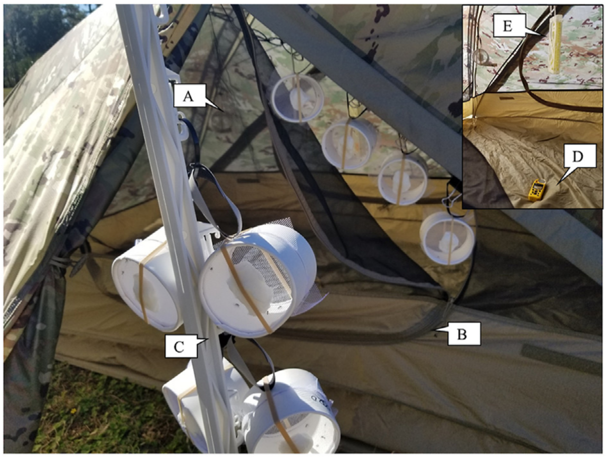

Fig. 2. Example of inside tent (**A**), tent doorway (**B**), and outside tent (**C**) arrangements of adult *Aedes aegypti* and *Culex quinquefasciatus* sentinel cages. Inset image shows the interior of a treatment tent containing a Kestrel® 5500 Weather Meter on the floor for temperature measurements (**D**) and the transfluthrin diffuser hanging from the center of the ceiling (**E**). Control tents have identical arrangements but with no transfluthrin diffuser present.

2.4. Environmental data

Air temperature (°C) inside and outside tents was recorded over the entire study period using ten Kestrel® 5500 Weather Meter (Kestrel Instruments, Nielsen-Kellerman Company, Boothwyn, PA, USA): 1 placed on the floor in the center of each tent and 2 mounted on tripods in the environment outside tents as shown in Fig. 1. Temperature data were averaged by week and location and analyzed for relationships with other treatment variables.

2.5. Statistical analysis

Data on the vapor toxicity of transfluthrin from the diffusers against the three sentinel mosquito species consisted of mean mosquito mortality after Abbott's correction. Sentinel mortality frequencies were analyzed using a linear mixed model for repeated measures ANOVA (Littell et al., 2000; Feazel-Orr et al., 2016; McMillan et al., 2021). The model examined sampling week (0, 1, 2, 3, 4, 5, 6, 7 and 8), sentinel cage location (inside tent, tent doorway or outside tent), treatment (transfluthrin diffuser or control), mosquito strain (*Ae. aegypti* (Orlando), *Ae. aegypti* (Puerto Rico) or *Cx. quinquefasciatus*), exposure time (15, 30, 45 and 60 min), and their 2- and 3-way interactions as the fixed effect factors. In the presence of a significant 3-way interaction, we analyzed one of the 2-way interactions at each level of the third factor and repeated this process for each of the other two factors, as suggested by Ott & Longnecker (2001). Before each analysis, the response variable measurement, proportion mortality, was tested for normality and transformed using a Box-Cox transformation (Osborne, 2010). Shapiro-Wilk W-test and/or the skewness and kurtosis values were used to judge the goodness-of-fit of the transformed data when compared to the normal distribution (Thode, 2002; Zar, 2010). Multivariate analyses were conducted to determine correlations between inside and outside temperature and other variables, with Pearson's and Spearman's ρ examinations performed for any significant results. For interactive effects, it was not possible to conduct *post-hoc* comparisons using temperature data, and thus "week" was included to serve as the nominal variable for this correlated pair. *Post-hoc* multiple comparison tests were carried out with the Tukey's HSD. All statistical analyses were performed on the means of 4 replicates using JMP Pro v15 (SAS Institute, 2019) at a significance level of $\alpha = 0.05$.

3. Results

There were significant interactions between the following variables: week × sentinel cage location × treatment × mosquito strain ($F_{(32,\ 2759)} = 3.7394$, $P < 0.0001$); week × sentinel cage location × treatment × temperature ($F_{(16,\ 2759)} = 2.8392$, $P = 0.0001$); week × sentinel cage location × mosquito strain × temperature ($F_{(32,\ 2759)} = 2.1252$, $P = 0.0003$); week × treatment × mosquito strain × temperature ($F_{(16,\ 2759)} = 2.7205$, $P = 0.0003$); and week × sentinel cage location × treatment × mosquito strain × temperature ($F_{(32,\ 2759)} = 2.6430$, $P < 0.0001$).

3.1. Effect of exposure time

There was no significant effect due to exposure time observed in this study ($P > 0.05$). All sentinel cages in treated tents experienced significant mosquito mortality after their initial deployment ($t = 0$ min), but there were no significant differences between sentinel cage mortality measurements for any time points afterward when controlling for other study variables ($P > 0.05$).

Sentinel cage mosquitoes did experience increased mortality with increased exposure time, but this relative increase was not influenced by the study week, was consistent across mosquito strains, was unaffected by the sentinel cage location, and was present for both treatment and control sentinel cage groups ($P > 0.05$).

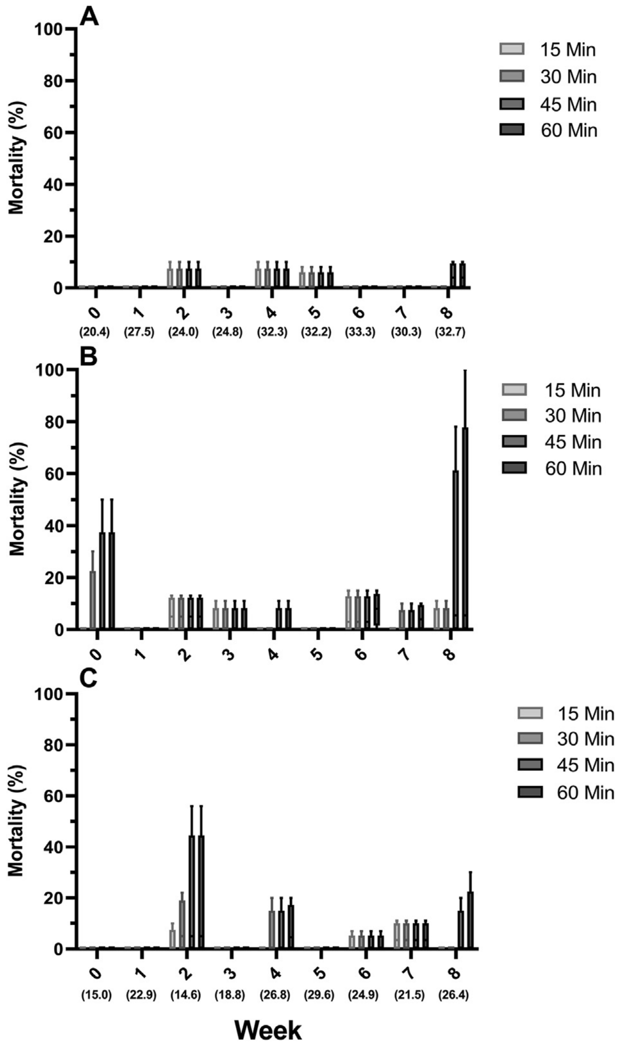

Fig. 3. Box-and-whisker plots for percent mortality of transfluthrin-exposed *Ae. aegypti* mosquitoes (pyrethroid-resistant Puerto Rico strain) over the 8-week (January 14, 2021 to March 11, 2021) study period at Camp Blanding Joint Training Center, FL, USA. Each week is expanded by exposure time and graphs (means ± SE) are separated by sentinel cage location: **A** inside tent; **B** tent doorway; **C** 0.5 m outside tent. Weekly average temperature (°C) during experiments recorded inside ($n = 8$) and outside ($n = 2$) tents are shown below the x-axis of graphs **A** and **C**, respectively. Temperature was not recorded in tent doorways.

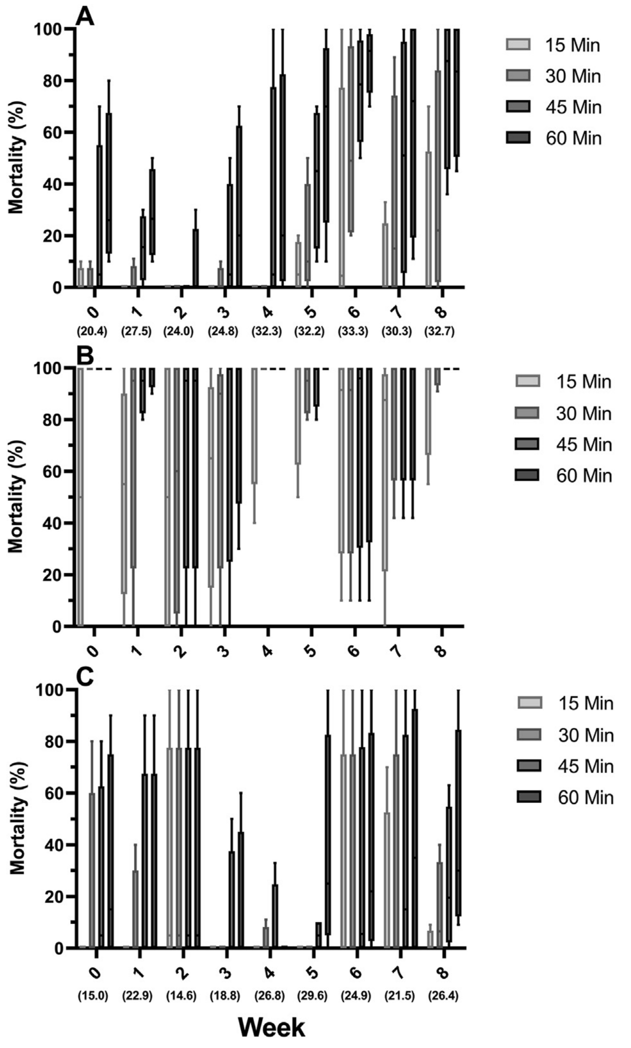

Fig. 4. Box-and-whisker plots for percent mortality (%) of transfluthrin-exposed *Aedes aegypti* mosquitoes (pyrethroid-susceptible Orlando strain) over the 8-week (January 14, 2021 to March 11, 2021) study period at Camp Blanding Joint Training Center, FL, USA. Each week is expanded by exposure time and graphs (means ± SE) are separated by sentinel cage location: **A** inside tent; **B** tent doorway; **C** 0.5 m outside tent. Weekly average temperature (°C) during experiments recorded inside ($n = 8$) and outside ($n = 2$) tents are shown below the x-axis of graphs **A** and **C**, respectively. Temperature was not recorded in tent doorways.

3.2. Effect of sentinel cage location

Sentinel cage location had a significant effect on mean sentinel cage mortality when combined with other study variables ($F_{(2,\ 2759)} = 18.7920$, $P < 0.0001$).

Sentinel cages 0.5 m outside of the treatment tents experienced the least mortality, followed by those cages inside the treatment tents, and the sentinel cages inside the treatment tent doorways experienced the greatest mortality ($P < 0.0001$; Figs. 3–5). Control sentinel cages were not significantly influenced by their proximity to the control tents ($P > 0.05$).

Sentinel cage mortality was influenced by the study week ($F_{(16,\ 2759)} = 3.2251$, $P < 0.0001$), which was correlated with increasing temperatures (see *Section 3.4*.). Sentinel cage mortality increased inside treatment tents over the study but remained constant 0.5 m outside of the treatment tents and in the treatment tent doorways (Figs. 3–5).

The impact of sentinel cage location was also dependent on the mosquito strain, with the two susceptible strains (*Ae. aegypti* (Orlando) and *Cx. quinquefasciatus*) showing significant differences in mortality based on location, and the resistant *Ae. aegypti* strain exhibiting no differences in mortality due to sentinel cage location ($F_{(4,\ 2759)} = 5.8366$, $P = 0.0001$; Figs. 3–5).

3.3. Effect of transfluthrin treatment

Transfluthrin treatment had a significant effect on mean sentinel cage mortality when combined with other study variables ($F_{(1,\ 2759)} = 128.3504$, $P < 0.0001$). Sentinel cages paired with treatment tents exhibited significant mortality when compared to control sentinel cages. Sentinel cage mortality was influenced by the location of the sentinel cages to the experimental tents, as described in *Section 3.2*.

Transfluthrin treatment was influenced by the study week ($F_{(8,\ 2759)} = 3.8906$, $P = 0.0001$), which was correlated with increasing temperatures (see *Section 3.4*.). Sentinel cage mortality significantly increased as the study progressed for treatment tents (Figs. 3–5), but there was no significant increase in sentinel cage mortality for control tents during the same period ($P > 0.05$).

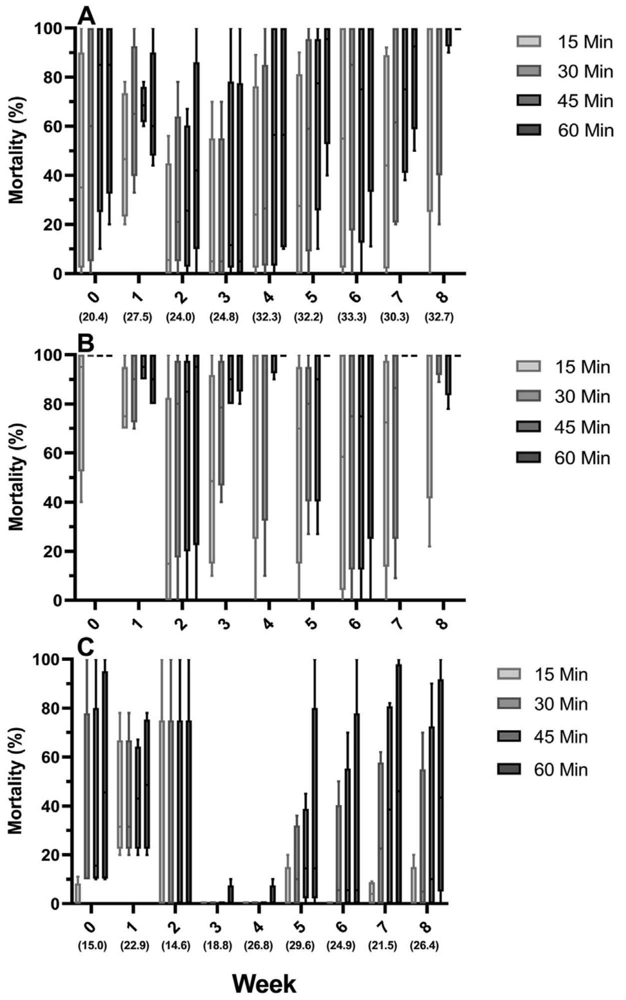

Fig. 5. Box-and-whisker plots for percent mortality of transfluthrin-exposed *Culex quinquefasciatus* pyrethroid susceptible mosquitoes over the 8-week (January 14, 2021 to March 11, 2021) study period at Camp Blanding Joint Training Center, FL, USA. Each week is expanded by exposure time and graphs (means ± SE) are separated by sentinel cage location: **A** inside tent; **B** tent doorway; **C** 0.5 m outside tent. Weekly average temperature (°C) during experiments recorded inside ($n = 8$) and outside ($n = 2$) tents are shown below the x-axis of graphs **A** and **C**, respectively. Temperature was not recorded in tent doorways.

The impact of the transfluthrin treatments was also characterized by the mosquito strain, with the two susceptible strains (*Ae. aegypti* (Orlando) and *Cx. quinquefasciatus*) showing significant differences in mortality based on exposure to the treatment, and the resistant *Ae. aegypti* strain exhibiting no differences in mortality due to treatment exposure ($F_{(2,\ 2759)} = 20.7147$, $P < 0.0001$; Figs. 3–5).

3.4. *Effect of study week*

Study week had a significant effect on mean sentinel cage mortality when combined with other study variables ($F_{(8,\ 2759)} = 10.8834$, $P < 0.0001$). Sentinel cages experienced increased mortality as the study progressed, but these increases were not uniform for each location. Sentinel cage mortality was influenced by the location of the sentinel cages to the experimental tents, as described in *Section 3.2*.

Sentinel cages associated with treatment tents experienced significant increases in mortality over time when compared to control sentinel cages, as described in *Section 3.3*.

Sentinel cage mortality increased as the study progressed and as temperatures in the environment increased (Figs. 3–5). Overall, there was a positive correlation between the variables of week and environmental temperature (Spearman's $\rho = 0.5752$, $n = 2760$, $P < 0.0001$) and a positive correlation between environmental temperature and mean mortality in treated tents (Spearman's $\rho = 0.1708$, $n = 2760$ $P < 0.0001$).

Study week impact was also characterized by the mosquito strain, with the two susceptible strains (*Ae. aegypti* and *Cx. quinquefasciatus*) showing significant differences in mortality based on the week of the study and the resistant *Ae. aegypti* strain exhibiting no differences in mortality due to changes over time ($F_{(16,\ 2759)} = 16.1550$, $P < 0.0001$; Figs. 3–5).

3.5. *Effect of mosquito strain*

Mosquito strain had a significant effect on mean sentinel cage mortality when combined with other study variables and those interactions are described in the sections above.

3.6. *Effect of environmental temperature*

The mean mortality observed each week in treated tents was influenced by environmental temperature differently depending on the location of the sentinel cages: sentinel cages inside the tents or outside the tents both showed increases in mean mortality as temperatures increased, but sentinel cages in the doorway of the tent did not demonstrate a consistent pattern related to environmental temperature (Fig. 6). Mean mosquito mortality was similarly affected by environmental temperature for the interaction of week × treatment, where treatment-exposed mosquitoes experienced significantly greater mortality as temperatures rose, compared to the constant low mortality observed in the control populations under the same environmental conditions (Fig. 6). The interaction of mosquito strain × week was also influenced by environmental temperatures: *Cx. quinquefasciatus* and the susceptible strain of *Ae. aegypti* both experienced increases in mean mortality as temperatures increased, but the observed mortality in the resistant strain of *Ae. aegypti* remained consistent throughout the study.

4. Discussion

Recent studies have shown that spatial repellents could protect US military personnel vulnerable to transmission of pathogens from local vector populations (Dame et al., 2014; Britch et al., 2021), but more information was needed on the radius of efficacy of these active ingredients and possible effects of temperature. This study examined how environmental temperature, sentinel cage locations, exposure time, age of the device in the field, and mosquito strain all influence susceptibility to a transfluthrin-emitting device when deployed over time.

Previous research investigating field efficacy of transfluthrin treatments has shown that it significantly reduces exposure to *Anopheles* (Ogoma et al., 2012; Masalu et al., 2017; Masalu et al., 2020), *Aedes* (Estrada et al., 2019), and *Culex* mosquitoes (Britch et al., 2020b). The results of Lee (2007) and Jeyalakshmi et al. (2014) are supported by the findings of the present study: transfluthrin-exposed *Ae. aegypti* mosquitoes of a susceptible strain experienced significant mortality over the course of the 8-week study, and susceptible strain of *Cx. quinquefasciatus* mosquitoes exposed to the transfluthrin treatments experienced equal or greater mortality than the susceptible *Ae. aegypti* strain, depending on the exposure time (Figs. 4 and 5). This study also initially included observations for *Anopheles albimanus* mosquitoes, but the observed mortality in controls of this species in the field was unacceptably high, and thus the data were excluded (data not shown).

The location of the sentinel cages was shown to have a significant impact on the mortality of the susceptible mosquito strains examined, independent of other experimental variables. Previous research into the impact of distance on the dispersion and efficacy of transfluthrin as either a toxicant or a repellent is currently limited, but has been conducted for *Anopheles* mosquitoes in Vietnam (Martin et al., 2020). Martin et al.

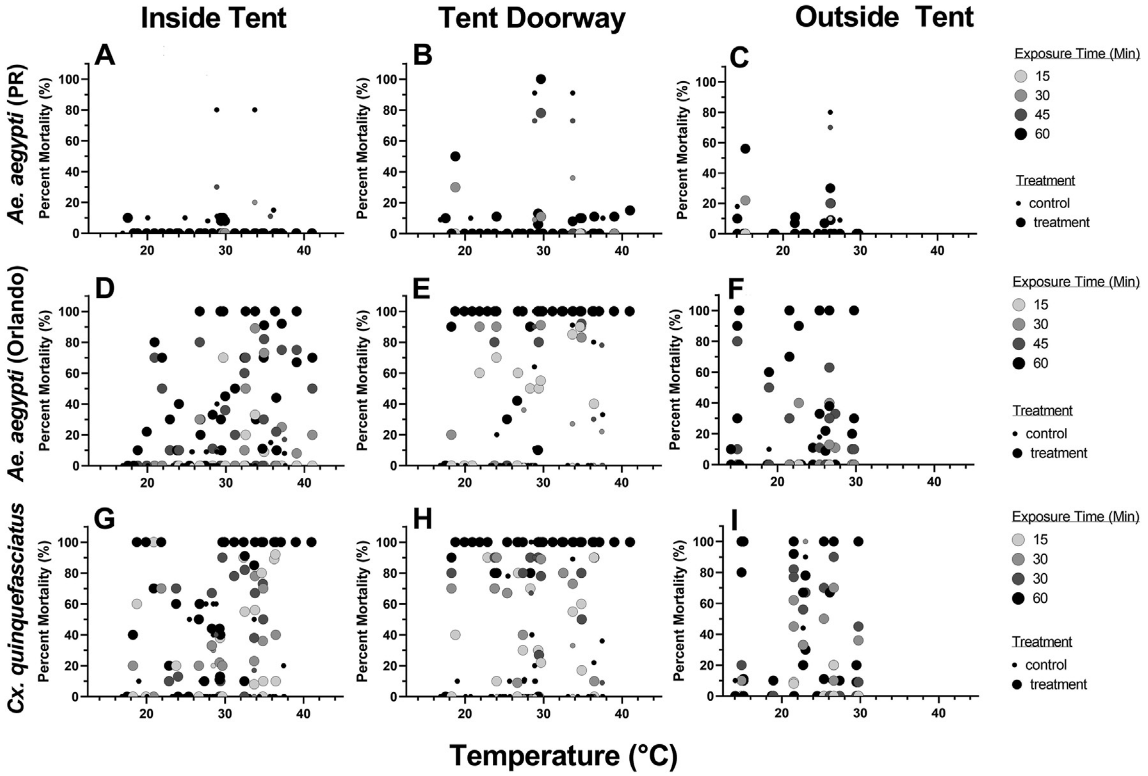

Fig. 6. Relationship between percent mortality (mean ± SE) and temperature (°C) for sentinel mosquitoes, sorted by mosquito strain, sentinel cage location, exposure time, and treatment group. Inside temperature was used for comparisons to inside tent and tent doorway mortality; outside temperature was compared to outside tent mortality. Columns indicate sentinel cage location: left (**A**, **D**, **G**), inside tent; middle (**B**, **E**, **H**), tent doorway; right (**C**, **F**, **I**), 0.5 m outside tent. Rows indicate mosquito species/strain: top (**A**, **B**, **C**), *Ae. aegypti* (resistant); middle (**D**, **E**, **F**), *Ae. aegypti* (susceptible); bottom (**G**, **H**, **I**) *Cx. quinquefasciatus* (susceptible).

(2020) determined that mortality decreased for two examined species of *Anopheles* mosquitoes as distance from the volatilized transfluthrin increased. Differences in the magnitude of the mortality responses between the two species indicate the importance of analyzing different species for their susceptibility to transfluthrin under the same conditions (Martin et al., 2020).

The high mortality observed in week 1 for *Cx. quinquefasciatus* exposed to transfluthrin is likely an artifact related to the sudden change in environmental temperature during that week, as seen by the relatively high average mortality observed in control tents for the same week (Supplementary Table S1). It should also be noted that the temperatures recorded at the beginning of the study period were cooler than those recorded at the end of the study (Figs. 3–5), and that temperature was positively correlated with study week (*Section 3.6*). The interaction between temperature × week was shown in this study to impact mean mosquito mortality: mortality increased as both the number of weeks increased, and ambient temperatures rose in the environment. We hypothesized that this increase in mortality was due to seasonally increasing temperatures over the nine study weeks, not due to some effect of time on increasing the performance of the diffusers.

Similar to findings from Martin et al. (2020) and Pettebone (2014), temperature was determined to have a significant impact on sentinel cage mortality in this study. Pettebone (2014) observed that there was a positive relationship between the volatilization of transfluthrin from hessian fabric and the temperature of transfluthrin. In the present study, both the pyrethroid-susceptible *Ae. aegypti* and *Cx. quinquefasciatus* strains experienced greater mean mortality as the temperatures inside the tents increased.

In the tent doorways, however, the mean sentinel cage mortality for the susceptible *Ae. aegypti* and *Cx. quinquefasciatus* strains were both high (> 80%) for all observed environmental temperatures. There was no significant 3-way relationship between temperature, exposure time, and experiment week for the sentinel cages located in the doorways of treatment tents ($P > 0.05$). Therefore, although the two susceptible strains experienced greater mortality when located inside or outside of the treatment tents as the environmental temperatures rose, the tent doorways reliably showed the greatest mortality (Figs. 4B and 5B). It is possible that the temperature differences between the insides of the tents and the environment (Figs. 3–5) created convection causing air to move from the inside of the tents into the environment when the doorways and rain fly remained open, thus possibly increasing the exposure of the doorway sentinels. Since doorway mortality was not as strongly influenced by these convection currents in control tents, we deduce that the relationship between environmental temperature and transfluthrin treatment. Further understanding of how airborne transfluthrin behaves and how that, in turn, affects a mosquito's susceptibility is an important factor in the successful deployment of transfluthrin treatments into uncontrolled environments. Additionally, in this experiment we observed a positive correlation between study week and environmental temperature with regard to sentinel mortality. Therefore, it is not possible to untangle whether transfluthrin was releasing from the diffusers at a higher rate with increased temperature, or whether increasing temperatures had an increasing effect on release rate because the diffusers aged in the environment.

Another possibility is that diffusers appeared to become more potent because of accumulation of released transfluthrin in the tent material over time. In previous preliminary trials at this site with earlier generation non-permethrin treated 2-person US military tents and similar transfluthrin diffusers left in place for several months, we observed that removing diffusers also eliminated sentinel mosquito mortality (data not shown). This does not exclude the possibility, however, that the efficacy of the diffusers left in place is enhanced with transfluthrin accumulation in surrounding materials. Additional investigation is necessary to understand the impact of potential transfluthrin accumulation, aging of the diffusers, or the behavior of the diffusers and/or transfluthrin at higher temperatures.

Existing literature regarding the relationship between transfluthrin susceptibility and temperature for various mosquito species supports the findings of this study. It has been shown that increases in temperature correlate with a reduction in relative biting exposure from some species of *Anopheles*, *Culex* and *Mansonia* (Ogoma et al., 2017), that reductions in temperature reduce the protection provided by transfluthrin-treated materials (Masalu et al., 2017), and that the vaporization of transfluthrin is hindered by lower environmental temperatures (Mmbando et al., 2018). Warmer environmental temperatures increase the vaporization of transfluthrin, which also might increase the impact of minor convection currents throughout the tent. This could explain the high mortality observed in the tent doorways, with lower mortality observed inside or outside of the tents. Mmbando et al. (2018) point out that areas where mosquito-borne illnesses are more prevalent are often higher temperatures climates, and our study illustrates how a long-term transfluthrin-emitting device might fare in an environment between 20 °C and 40 °C. The ability to remain effective across a variety of environmental conditions is necessary for a device implemented to protect the health of US military personnel in diverse scenarios. Although our study outlines the efficacy of a transfluthrin-emitting device against *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes, future research involving other mosquito species and different environmental conditions is encouraged. In addition to sentinel cage mortality with colony-reared specimens, field experiments utilizing CO_2-baited traps might provide additional insight into the efficacy of these devices in the field against natural populations.

The results of this study do provide support for the efficacy of transfluthrin in a small enclosed space against pyrethroid-susceptible *Ae. aegypti* and *Cx. quinquefasciatus*, both vectors responsible for transmitting numerous pathogens to humans, livestock, and wildlife. However, we found that transfluthrin efficacy is influenced by the location of the mosquito relative to the source of the treatment, the target species and its resistance status, and the temperature at which the exposure occurred. Even at the highest efficacies observed in this study, pyrethroid-susceptible mosquito vectors could only be considered reduced and not substantially eliminated. The spatial repellent component of IVM would need to be layered with additional components to further reduce potential biting pressure and risk of pathogen transmission. In particular, our results suggest that even in a very small defined area such as that of a 2-person tent, efficacy is not uniform across the radius of that defined area and is extremely focal, which challenges the concept of a spatial repellent/toxicant. These findings corroborate those of Dame et al. (2014) and Britch et al. (2021) in that a single spatial repellent formulation and/or delivery method will likely not be sufficient for all mosquito vector species present in an area and will likely not be appropriate for all defined spaces that need to be protected. The transfluthrin utilized in this study was impregnated into a passive resin diffuser and calibrated for uniform volatilization, yet efficacy increased with temperature which could mean that more active ingredient was being released at higher temperatures, thus potentially reducing the labeled duration of efficacy of the diffuser.

In this study, mortality was used as a surrogate for repellency, but the study was not designed to determine how mortality and spatial repellency are related, especially in preventing the bite of potential vector mosquitoes. Future research should compare how transfluthrin-impregnated devices compare to other methods for mosquito control and how changes in temperature and possibly other environmental weather conditions affect their efficacy. When utilized in conjunction with other IVM practices, transfluthrin could serve as an additional treatment option to enhance protection from disease vector mosquitoes like *Ae. aegypti* and *Cx. quinquefasciatus* as long as clear expectations are established by determining the variation in pyrethroid resistance in the target populations, and determining whether repellency (as opposed to mortality) is dependent on resistance. The potential inclusion of transfluthrin in such a programme must take environmental conditions, the form of the area to be protected, available modes of delivery, target insect resistance status, and operational objectives into account.

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Ethical approval

Not applicable.

CRediT author statement

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Data availability

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).

Disclaimer

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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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crpvbd.2021.100067.

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