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SCIENTIFIC NOTE

SPATIAL REPELLENTS PROTECT SMALL PERIMETERS FROM RICELAND MOSQUITOES IN A WARM-HUMID ENVIRONMENT

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ABSTRACT. Recent experiments suggest spatial repellents may significantly reduce biting pressure from host-seeking riceland mosquitoes, such as *Anopheles quadrimaculatus*, in a warm-humid open-field habitat. However, little is known regarding efficacy of these formulations in partially enclosed spaces where US military personnel may be sheltered or concealed in an operational environment. In this study we investigated the capability of 3 spatial repellents—metofluthrin, linalool, and d-*cis*/*trans* allethrin—to reduce mosquito incursion into small open-top enclosures of US military camouflage netting. We found that metofluthrin was more effective in partially enclosed spaces compared with the open field, whereas both linalool and d-*cis*/*trans* allethrin provided superior protection in the open. These findings support strategic selection of spatial repellents depending on the environment immediately surrounding the host.

KEY WORDS Integrated vector management, military operational entomology, passive control, residual pesticide, resistance management

Protection of deployed US military personnel from vector-borne disease is greatly complicated by logistic 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). However, passive control techniques could supplement or replace active control in military field scenarios. For example, Britch et al. (2011) reported reduction of mosquito numbers inside open-top enclosures of US military camouflage netting in a hot-arid environment in southern California when the surface of the fabric wall was treated with a residual insecticide. The significance of this finding is that a passive layer of control may be automatically situated in the field by pretreatment of materials such as camouflage netting, tent exteriors (Frances 2007), or blast wall geotextile (Britch et al. 2018) that are already organic to deploying US military units.

On the other hand, potential shortfalls of this passive technique are that 1) residual pesticides induce mortality and thus the opportunity for evolution of resistance; and 2) host-seeking female mosquitoes must touch the treated surface to be affected by the pesticide and could fly directly to human hosts without contacting the treatment, or contact the treatment and still bite humans before the pesticide dose takes effect (Britch et al. 2020b). Certain pyrethroids with spatial repellent properties have been discovered (Achee et al. 2012) that could be synergized with or replace standard residual pesticide treatments to keep mosquitoes away from protected perimeters (Britch et al. 2020a, 2020b). In theory, an effective spatial repellent would create an unfavorable environment for a host-seeking mosquito, compelling it to leave the area without contacting humans and without the requirement of touching any surfaces, and before accumulating a fatally toxic dose.

Recent field work with a variety of spatial repellents suggested 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. However, although these formulations could potentially protect US military personnel in the field, that experiment was conducted in an open-field environment with free airflow and was not designed to investigate the efficacy of spatial repellents in partially enclosed spaces where military personnel may be sheltered or concealed in an operational environment. In this study we investigated the same 3 commercial emanator devices containing the spatial repellent formulations (metofluthrin, linalool, and d-*cis*/*trans* allethrin) used by Dame et al. (2014), but this time positioned them to potentially reduce incursion of natural populations of riceland mosquitoes into small open-top perimeters of US military camouflage netting that we refer to here and subsequently as "enclosures."

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Fig. 1. Aerial view of study site showing the 12 enclosures arrayed along the ecotone between a forested swamp and irrigated fields of rice, soybean, and corn on the grounds of the University of Arkansas Rice Research and Education Center (RREC) in Stuttgart, AR. White arrows show relationship between aerial and on-ground images for enclosures 1 and 2. Upper right inset shows a view of several of the enclosures looking southeast with the woodland to the left in the photo. Lower left inset shows the interior of one of the enclosures with a Centers for Disease Control and Prevention (CDC) trap suspended from crossed ropes at the center.

We selected the study site previously described in Dame et al. (2014) along the ecotone between a forested swamp and irrigated fields of rice, soybean, and corn on the grounds of the University of Arkansas Rice Research and Education Center (RREC) in Stuttgart, AR (Fig. 1; 34.280°N, 91.241°W). In early July 2010 we constructed 12 5-ft × 5-ft × 6-ft (1.5-m × 1.5-m × 1.8-m) open-top enclosures with 50-ft (15.2-m) spacing as shown in Fig. 1, using US military desert-pattern radar-scattering ultralight-weight camouflage netting system (ULCANS) material following the methods of Britch et al. (2010). We treated selected ULCANS enclosures on both the inner and outer surfaces with λ-cyhalothrin (Demand CS; Syngenta Crop Protection, Inc., Greensboro, NC) at the maximum label rate of 0.02 liter formulation per 1,000 ft^2 (92.9 m^2) in water using a cold-mist backpack sprayer (Model SR420; Stihl, Inc., Norfolk, VA).

We investigated the efficacy of 3 spatial repellent formulations in commercial emanator devices suspended inside selected enclosures: Off Clip-On (31.2% metofluthrin; S.C. Johnson, Racine, WI), Mosquito Cognito (linalool, formulation 5155847, Conceal Metagel; BioSensory, Inc., Putnam, CT), and Therma Cell (21.97% d-*cis*/*trans* allethrin;

Schwabel Corp., Bedford, MA). We divided the 12 enclosures into 4 groups: 1) $N = 3$ residual pesticide–treated ULCANS (no spatial repellent), 2) $N = 3$ residual pesticide–treated ULCANS (spatial repellent present), 3) $N = 3$ untreated ULCANS (spatial repellent present), and 4) $N = 3$ untreated ULCANS (no spatial repellent). Each spatial repellent formulation alone and in combination with a residual pesticide was tested 4 days in each of 3 sets in a 3-block Latin square design, providing 12 replicates during the 12 continuous-day test period July 7–18, 2010.

To simulate the presence of a human and sample local populations of multiple species of riceland mosquitoes entering enclosures, we suspended Centers for Disease Control and Prevention (CDC) traps (Model 512; John Hock, Gainesville, FL) with no light and baited with an octenol strip (BioSensory, Inc.) and CO_2 (2 lb [0.9 kg] dry ice) 4.5 ft (1.4 m) aboveground and centered in the interior of each enclosure (Fig. 1: lower inset). For enclosures containing spatial repellent emanators, we suspended the emanator alongside the octenol bait. Traps were activated at approximately 1945–2000 h each evening and discontinued after sunrise. All collected mosquitoes were identified to species at RREC, and collections from inside enclosures with and without residual insecticide treatment or spatial repellents were compared using *t*-tests in Wizard 1.9.43 (Evan Miller, http://www.wizardmac.com; Chicago, IL).

The habitat surrounding the study area included irrigated riceland, dry cropland, woodland swamps, reservoirs, and human habitation with both temporary and permanent mosquito breeding sites and innumerable resting sites. The identified species collected by the unlighted and CO_2/octenol–enhanced CDC traps included *Aedes vexans* (Meigen), *Ae. fulvus pallens* Ross, *An. crucians* Wiedemann, *An. quadrimaculatus*, *Coquillettidia perturbans* (Walker), *Culex erraticus* (Dyar and Knab), *Cx. nigripalpus* Theobald, *Cx. quinquefasciatus* Say, *Cx. salinarius* Coquillett, *Cx. territans* Walker, *Psorophora ciliata* (Fabricius), *Ps. columbiae* (Dyar and Knab), *Ps. cyanescens* (Coquillett), *Ps. howardii* Coquillett, and *Uranotaenia sapphirina* (Osten Sacken). Of the total 513,324 specimens collected, 284,704 (55% of the total collection) were *An. quadrimaculatus*, 17,369 (3%) were *Cx. erraticus*, and 209,161 (41%) were *Ps. columbiae*; leaving 2,090 (approximately 0.5%) specimens of the remaining 12 species that were not included in analysis.

Statistical analysis of mosquito collections from residual pesticide–treated enclosures without spatial repellents showed no difference from untreated control enclosures without spatial repellents for any of the 3 focal species—*An. quadrimaculatus* (t (46) = 0.868; C = 2.013; $P = 0.390$), *Cx. erraticus* (t (46) = 0.171; C = 2.013; $P = 0.865$), and *Ps. columbiae* (t (46) = 0.664; C = 2.013; $P = 0.510$)—in paired *t*-tests. Similar analyses between treated and untreated enclosures with each spatial repellent present also showed no statistical differences. Subsequent laboratory analysis of cuttings taken the day of the residual pesticide treatment of the ULCANS enclosures using the bioassay process described in Aldridge et al. (2013) showed a mean effect of only 30.2% corrected 24-h mortality (Abbott 1925) on susceptible colony-reared *Cx. quinquefasciatus*. Findings from previous studies with cuttings from similarly treated material in a warm-temperate environment showed a mean 100% corrected mortality in similar bioassays (Britch, Linthicum, and Aldridge, unpublished data), indicating that the treatment conducted at RREC had been compromised. A rainstorm had passed through the study site immediately following the residual pesticide spray, most likely washing most of the treatment off of the ULCANS before it had dried and bonded to the fabric.

Given the lack of efficacy of the residual pesticide treatment on the ULCANS enclosures, we pooled results from residual pesticide–treated and untreated ULCANS enclosures in analyses. With this adjustment we were only able to evaluate the effects of each spatial repellent in untreated ULCANS enclosures compared with untreated ULCANS enclosures lacking spatial repellents. Results from collective analysis of all treatments and controls are shown in Fig. 2.

The histograms in Fig. 2 suggest that the 3 spatial repellent formulations investigated could provide some protection within partially enclosed spaces with limited airflow in the field against host-seeking *An. quadrimaculatus*, *Cx. erraticus*, and *Ps. columbiae* mosquitoes. However, even though the percent reduction in most collections was indicative of some level of efficacy there were only 2 cases with statistically significant reductions in collections owing to the presence of a spatial repellent, i.e., metofluthrin reducing incursion of *Cx. erraticus* and *Ps. columbiae* (Fig. 2A).

As was observed in the earlier study (Dame et al. 2014), we found that not all formulations were effective against all 3 of these mosquito species. Thus, the best choice of a spatial repellent formulation may depend on the mosquito community composition in the environment where personnel in the field need to be protected. Species in the genus *Anopheles* have been found to be the most difficult to repel in both laboratory and field topical repellent trials (Schreck 1985, Frances et al. 1993). In the majority of field scenarios, multiple spatial repellent active ingredients may need to be used simultaneously to adequately protect personnel from the spectrum of host-seeking mosquito species present. Also, even the most effective formulations against any one of the species in this study, e.g., 85.4% reduction of *Cx. erraticus* by metofluthrin (Fig. 2A), did not provide sufficient protection to obviate the need for personal protective measures such as the application of repellent on exposed skin. Despite these general similarities between Dame et al. (2014)

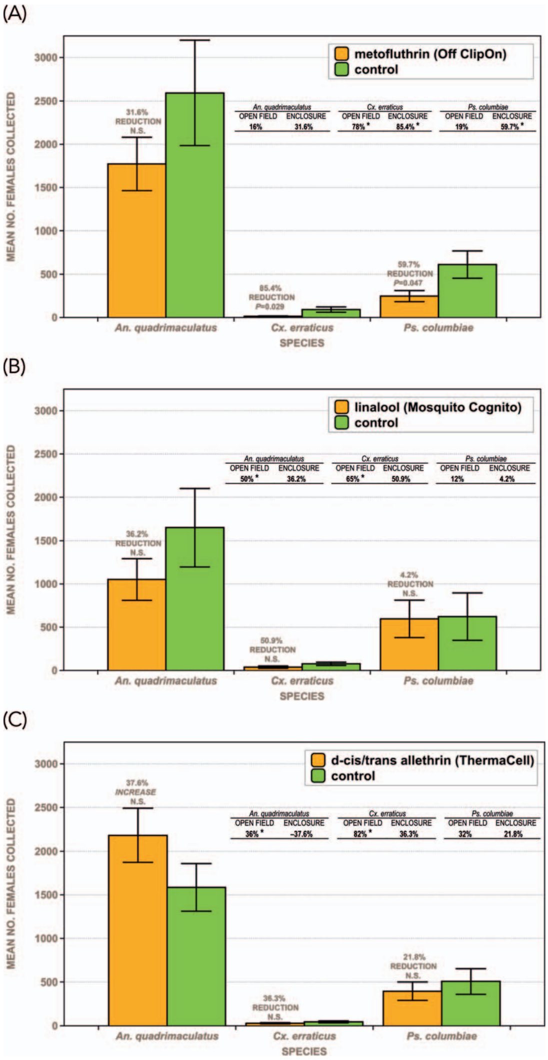

Fig. 2. Histograms showing mean numbers with error bars (standard errors of the mean) of *Anopheles quadrimaculatus*, *Culex erraticus*, and *Psorophora columbiae* females collected in camouflage netting enclosures with and without (A) metofluthrin, (B) linalool, and (C) d-*cis*/*trans* allethrin spatial repellent formulations present. Percent reductions calculated from collection data are printed above each pair of histogram bars as well as a significance value estimated from paired *t*-tests of the differences in collections for each species when the spatial repellent was present or absent. The table printed directly below the legend in each chart compares the percent reductions for that spatial repellent for each species observed in the earlier Dame et al. (2014) study (OPEN FIELD) with the percent reductions in the current study (ENCLOSURE), with statistically significant reductions from either study marked with an asterisk.

and the current study, we observed differences in the efficacy of the 3 tested formulations against the 3 focal species that were not expected given the earlier findings.

For example, the presence of metofluthrin in the open-field scenario investigated in Dame et al. (2014) resulted in lower percent reductions in trap collections for all 3 focal species compared with the enclosure scenario in the current study, which generated the only 2 statistically significant reductions in collections, i.e., $P = 0.029$ (t (22) $= 2.332$, C $= 2.074$) for *Cx. erraticus* and $P = 0.047$ (t (22) $= 2.099$, C $= 2.074$) for *Ps. columbiae* in paired *t*-tests (Fig. 2A: embedded table). These results suggest that the Off Clip-On device could be better suited to protecting personnel positioned in static and partially enclosed shelters. However, given that *An. quadrimaculatus* comprised 55% of collections and that metofluthrin had the lowest relative reductions for this species in enclosures compared with the other 2 focal species, this spatial repellent would need to be augmented with an integrated approach that could include standard residual treatment of the ULCANS (which unfortunately we were not able to measure) and use of personal protective measures such as permethrin-treated clothing and application of repellent to exposed skin.

Conversely, linalool showed higher percent reductions for all 3 species—2 of them statistically significant—in the open-field scenario (Dame et al. 2014) compared with percent reductions from linalool situated within the enclosures in the current study (Fig. 2B: embedded table). These results point to the Mosquito Cognito being potentially suitable for protecting personnel positioned in the open. Echoing the pattern observed with linalool, percent reductions in collections with d-*cis*/*trans* allethrin indicated greater efficacy against all 3 species, 2 with statistical significance, in the open-field environment (Dame et al. 2014) compared with the enclosures (Fig. 2C: embedded table). Most surprising was the observation that the presence of d-*cis*/*trans* allethrin resulted in a nearly 40% increase in *An. quadrimaculatus* collections compared with the control in the enclosure scenario.

One hypothesis for this unexpected apparent reversal in efficacy for d-*cis*/*trans* allethrin is the possible disorienting effect of the spatial repellent on *An. quadrimaculatus* in the protected space of the enclosure. It could be that this species only experiences repellency to this formulation at a close range—i.e., a range equal to or smaller than the distance from the enclosure wall to the central location of the emanator and the CDC trap—yet is physiologically affected, for instance resulting in disorientation, by the active ingredient accumulated and confined in the comparatively lower airflow within the enclosure. After becoming disoriented, individuals of this species could have a higher likelihood of moving close enough to the trap to be drawn in or have a reduced capacity for an escape reflex in the vacuum. In the open-air scenario, we hypothesize that the d-*cis*/*trans* allethrin is strong enough to repel at close range of the trap but does not accumulate sufficiently to disorient *An. quadrimaculatus* mosquitoes.

We have observed similar phenomena of apparent disorientation in trials with transfluthrin in hot-humid, Mediterranean, and warm-temperate environments where collections of mosquitoes and sand flies in partially enclosed as well as open areas with the active ingredient may be similar to or higher than the control (Britch, Linthicum, and Kline, unpublished data). However, in these trials with transfluthrin we observed that nearly all the specimens in the control collections were alive whereas most of those in the treated collections were dead. These unexpected findings with both d-*cis*/*trans* allethrin and transfluthrin in partially enclosed spaces suggest that further study is warranted to calibrate concentrations and release rates of some spatial repellents to specific target species to prevent entry into a zone of active ingredient accumulation where they could become disoriented yet still probe or bite people. Potential changes to biting and bloodfeeding behavior after sublethal exposure to accumulated spatial repellents also need to be investigated.

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