Journal of the American Mosquito Control Association, 00(00):000–000, 2025

SCIENTIFIC NOTE

NOVEL SYNERGIZED ARYL AMIDE SPATIAL REPELLENT SIGNIFICANTLY REDUCES COLLECTIONS OF *AEDES ALBOPICTUS* IN A WOODED NORTH FLORIDA SUBURBAN RESIDENTIAL YARD

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ABSTRACT. *Aedes albopictus* is a significant vector of dengue and chikungunya to humans. Increasing evidence of resistance of this species coupled with their use of cryptic habitat pose significant obstacles to effective control. We investigated efficacy against natural populations of *Ae. albopictus* of a novel aryl amide spatial repellent (Compound 53) and a novel synergist (trans-chrysanthemic acid; TCA) that in previous work had shown high capability to repel *Ae. aegypti* in a laboratory environment. We observed a significant reduction in collections of *Ae. albopictus* in a wooded north Florida residential yard when synergized Compound 53/TCA was present. We conclude that this novel synergized aryl amide demonstrates potential and should be investigated further.

KEY WORDS *Aedes* (*Stegomyia*) mosquito, integrated vector management, passive control, resistance management, spatial repellent

Aedes albopictus (Skuse) is a high-risk species capable of transmitting dengue and chikungunya to humans (Vanlandingham et al. 2016). Increasing evidence of resistance (Parker et al. 2020) coupled with their use of cryptic habitat near human dwellings (Nava and Debboun 2020) pose significant obstacles to effective control of this species. However, spatial repellents may supplement traditional integrated vector management (IVM) by creating a repellent effect over distance that can protect defined areas from mosquito incursion (Bibbs and Kaufman 2017, Norris and Coats 2017).

Previous studies demonstrate that select aryl amides are repellent and toxic against *Ae. aegypti* L. in laboratory glass tube bioassays (Richoux et al. 2020, Bloomquist et al. 2021). Of these, 2,2,3,3,3-pentafluoro-N-(4-fluorophenyl) propenamide (Compound 53) was among the most potent, with a median effective concentration (EC_{50}) of 4.5 μg/cm^2. Although an EC_{50} value of 4.5 μg/cm^2 was among the lowest reported for the aryl amide class developed and researched previously, it was still not as low as that of transfluthrin (EC_{50} of 0.5 μg/cm^2) and metofluthrin (EC_{50} of 0.3 μg/cm^2) (Yang et al. 2019). Transfluthrin and metofluthrin are two highly successful spatial repellent and toxic pyrethroids already utilized in a number of products and demonstrated to be potent arthropod repellents in the laboratory (Yang et al. 2020) and field (Britch et al. 2020a, 2020b, 2021; Aldridge et al. 2024). However, given its favorable performance in the laboratory, our eventual goal was to determine the spatial repellent efficacy of Compound 53 in real-world field conditions.

To maximize the repellent effect in the field, we aimed to evaluate Compound 53 when synergized with another agent. Although this would not inform the field efficacy of either Compound 53 or the synergist alone, we sought to investigate Compound 53 with a hypothesized maximum effect to take advantage of the timing of natural populations in the study site. In theory, pairing this compound with a repellent synergist or other repellent agent may significantly increase repellent efficacy of the mixture than that of the aryl amide (Compound 53) alone. Previous work demonstrates that select pyrethroid and pyrethrum acids are potent synergists of an array of repellent compounds (Yang et al. 2020). One of these—trans-chrysanthemic acid (TCA)—was shown to synergize pyrethroids when co-applied directly to the mosquito nervous system (Bloomquist et al. 2022) and was also shown to possess some repellency by itself against other arthropods (e.g., *Amblyomma americanum* [L.]; Le Mauff et al. 2024) demonstrating its broad potential as an additive in future field-deployed repellent formulations. In this study, we deployed Compound 53 and TCA together in a north Florida study site harboring natural populations of *Ae. albopictus* to determine the potential utility of pairing this novel aryl amide spatial repellent and novel synergist in the field.

We conducted the investigation in June–July 2023 at a study site located in a wooded residential yard approximately 10 km west of Gainesville, Florida. Preliminary surveillance in this yard indicated presence of a variety of mosquito species including *Ae. albopictus*. Here we set up 2 BG-Sentinel 2 traps (BGS2; Biogents AG, Regensburg, Germany) targeting *Ae. albopictus* approximately 50 m apart, the maximum separation

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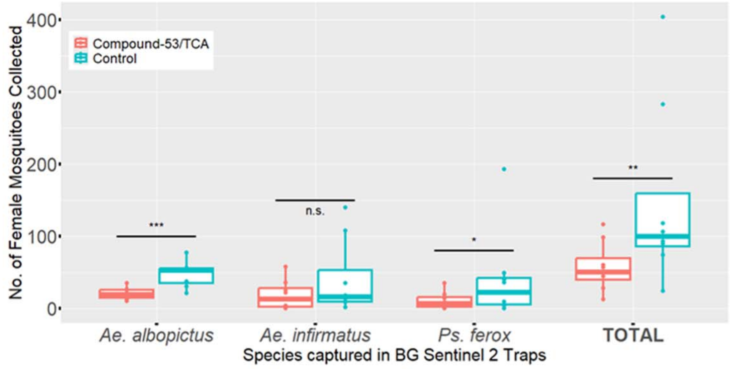

GLM - Response Var.	Variable	Param. Est.	S.E.	Z	p
Ae. albopictus - collected	Treatment	-0.8517	0.1862	-4.575	<0.001***
Ae. infirmatus - collected	Treatment	-0.8139	0.6621	-1.229	0.219
Ps. ferox - collected	Treatment	-1.3774	0.6509	-2.116	0.0343*
Total count - collected	Treatment	-0.9543	0.3398	-2.809	0.00497**

Significance levels: * = 0.05 - 0.01; ** = 0.009 - 0.001; *** = < 0.001

Fig. 1. Plots of mosquito collections in a wooded north Florida residential yard from a modified BG-Sentinel 2 trap baited with CO_2 (dry ice) and a BG-Lure (human skin odor) and protected by a 1:1:1 solution of aryl amide 2,2,3,3,3-pentafluoro-N-(4-fluorophenyl)propenamide (Compound 53) synergized with trans-chrysanthemic acid (TCA) in acetone. Significance is indicated from comparison to collections from an untreated control trap separated by 50 m. Table indicates GLM variables, associated estimates, and *P* values compared by species. Inset: BG-Sentinel 2 trap in the study site modified with 10 cotton sachet assemblies, each containing 2 ml of the synergized spatial repellent, hanging from rivet mandrils around the lid. This arrangement produces a standardized spatial repellent perimeter intended to reduce incursion of host-seeking female mosquitoes into the trap.

distance practical in the property boundaries of the site. We baited traps with a BG-Lure (human skin odor) placed inside each trap for optimal diffusion paired with CO_2 sublimated from ~2 kg dry ice in a 4 liter perforated plastic insulated reservoir suspended ~0.3 m above the trap on a 1.2 m plastic tread-in post (Fig. 1).

We mixed a 1 g:1 ml:1 ml solution of Compound 53, TCA, and acetone, respectively, to deploy in cotton ball sachets on the treatment BGS2 trap. We prepared cotton ball sachets as described in Aldridge et al. (2024), each made from an approximately 10 cm × 10 cm square of fine nylon mesh tulle wrapped around a cotton ball saturated with 2 ml of the Compound 53/TCA acetone solution, secured with a 32 mm steel binder clip. Both BGS2 traps were modified by fitting 10 0.64-mm aluminum rivets (with mandril retained) in predrilled holes spaced evenly around the outer edge of the 40 cm dia. lid (Fig. 1). The treatment setup consisted of suspending 10 spatial repellent treated sachets by their binder clip handles from the 10 rivet mandrils to create a perimeter of synergized spatial repellent around the upper edge of the trap. The untreated control BGS2 trap was fitted with 10 dry, untreated cotton ball sachets. The purpose of acetone was to aid in uniform solvation of both compound 53 and TCA, allowing a homogenous mixture to penetrate the cotton ball, more or less evenly. Preliminary trials indicated that acetone volatilizes away within several minutes and is not present prior to placement on the trap in the field. Untreated control cotton balls were deployed dry based on this fact.

We conducted mosquito collections with the two BGS2 traps for an 18–24 h period once a week for 2 months (8 collections). Each collection was conducted with a new set of 10 spatial repellent sachets. Following each collection period, we retrieved the BGS2 trap collection bags from the field, froze them at −20°C, and identified and tallied mosquito collections to species. We reversed the positions of the control and treatment BGS2 traps before each collection to reduce location bias. We measured efficacy of the Compound 53/TCA synergized spatial repellent by comparing percent reduction of mean *Ae. albopictus* collection numbers from the treatment versus the control BGS2 trap. We considered a reduction in collections at the treated BGS2 trap of at least 61% as indicative of spatial repellent efficacy per expectations of field efficacy established in an in-depth review of spatial repellent investigations by Miller et al. (2022). To determine whether presence of the spatial repellent caused a statistically significant reduction in collections compared to controls, the treatment variable was fitted to a generalized linear model (GLM) configured with a negative binomial error structure and a log link function in R (MASS package). Selection of variables inserted was determined by lowest AIC score and plots generated through R and RStudio (ggplot2 package).

The total number of mosquitoes collected over the 8 sample periods was 1,651. Of these, approximately 88% (N = 1,449) were *Ae. albopictus* (N = 545), *Ae. infirmatus* Dyar and Knab (N = 482), and *Psorophora ferox* Von Humbolt (N = 422). Approximately

12% (N = 202) of the total collection consisted of 14 other mosquito species (*Ae. fulvus pallens* Ross, *Ae. tormentor* [Dyar and Knab], *Ae. triseriatus* (Say), *Ae. vexans* (Meigen), *Anopheles crucians* Wiedemann, *Culex coronator* Dyar and Knab, *Cx. erraticus* [Dyar and Knab], *Cx. nigripalpus* Theobald, *Cx. quinquefasciatus* Say, *Cx. salinarius* Coquillett, *Coquillettidia perturbans* [Walker], *Mansonia dyari* [Belkin, Heinemann, and Page], *Ma. titillans* [Walker)], *Wyeomyia mitchellii* [Theobald], and 28 unidentifiable specimens of the *Aedes*, *Psorophora*, and *Toxorhynchites* genera), and were not included in the analysis. Plots displaying median collection numbers and statistical significance of the treatment variables for *Ae. albopictus* (57.3% reduction; $P < 0.001$), *Ae. infirmatus* (55.7% reduction; not significant), *Ps. ferox* (74.8% reduction; $P = 0.03$), and total across all species (61.5% reduction; $P < 0.005$) are shown in Fig. 1. Meteorology for the 8 collection days based on data from Gainesville Regional Airport (18.5 km to the east) ranged from 16.1 to 35.0° C, 37 to 100 RH, and wind speed 0–34 kph.

In this investigation we demonstrated that the novel aryl amide Compound 53 synergized with the pyrethrum acid TCA can significantly reduce the incursion of a natural population of host-seeking *Ae. albopictus* in a north Florida wooded suburban habitat. Although this investigation was designed to focus on *Ae. albopictus*, two other aggressive, high-threat vector and day-biting species, *Ae. infirmatus* (Walter Reed Biosystematics Unit 2024b) and *Ps. ferox* (Walter Reed Biosystematics Unit 2024a), were collected in sufficient numbers to calculate percent reductions in treated areas. Percent reduction of *Ps. ferox* (74.8%) was the only one of the 3 species that met or surpassed the Miller et al. (2022) proposed spatial repellent efficacy benchmark of 61% reduction. Reductions of *Ae. albopictus* (57.3%) and *Ae. infirmatus* (55.7%) were substantial but did not meet the 61% benchmark. Nevertheless, traps with synergized Compound 53/TCA spatial repellent collected significantly fewer mosquitoes, including *Ae. albopictus* and *Ps. ferox*, than unprotected traps. Thus, despite not reaching the 61% benchmark, this novel spatial repellent system could significantly reduce contact between humans and mosquitoes and therefore significantly reduce risk of transmission of pathogens.

Insecticide resistance to pyrethroids in Florida *Ae. albopictus* appears to be less than that for Florida *Ae. aegypti* (Estep et al. 2018, Parker et al. 2020), yet this novel spatial repellent and novel synergist could be a valuable tool to supplement traditional pyrethroid control measures. Integrated vector management of mosquitoes such as *Ae. aegypti* (Bibbs and Kaufman 2017) or *Culex pipiens* L. (Fotakis et al. 2017) with reduced response to pyrethroid-based toxicants and/or spatial repellents could immediately benefit from the Compound 53/TCA novel synergized spatial repellent. Spatial repellents should pose less of a risk for evolution of resistance compared to IVM strategies designed to kill (Norris and Coats 2017). To evaluate risk of evolution of resistance from the novel Compound 53/TCA synergized spatial repellent system, future studies should investigate for example host seeking and fecundity of *Ae. albopictus* and other mosquito species post-exposure. Future work with this system should also determine efficacy in field environments around the world, and formulations and delivery methods that could enhance its capability against additional arthropod vector species.

This research was supported by United States Department of Agriculture—Agricultural Research Service (USDA-ARS) and US Department of Defense (DoD) Deployed War-Fighter Protection Program (DWFP). Mention of trade names or commercial products is to provide specific information and does not imply recommendation or endorsement by USDA, DoD, or DWFP. Findings and conclusions are those of the authors and do not necessarily represent the views of USDA, DWFP, or DoD. The USDA is an equal opportunity provider and employer.

REFERENCES CITED

Aldridge RL, Pagac AA, Norris EJ, Kline DL, Geden CJ, Linthicum KJ. 2024. Point protection with transfluthrin against *Musca domestica* L. in a semi-field enclosure. *Insects* 15:277.

Bibbs CS, Kaufman PE. 2017. Volatile pyrethroids as a potential mosquito abatement tool: A review of pyrethroid-containing spatial repellents. *J Integr Pest Manag* 8:21.

Bloomquist JR, Coquerel QR, Richoux JR, Tsikolia M, Yang L. 2021. Aryl and pyridyl amide pesticides and compositions thereof. *World Patent WO-2021041927-A1*.

Bloomquist J, Jiang S, Norris E, Richoux G, Yang L, Linthicum KJ. 2022. Novel pyrethroid derivatives as effective mosquito repellents and repellent synergists. *Advances in Arthropod Repellents*. Elsevier, 19–32. https://doi.org/10.1016/B978-0-323-85411-5.00004-2

Britch SC, Dame DA, Meisch MV, Kline DL, Walker TW, Allan SA, Urban J, Aldridge RL, Linthicum KJ. 2021. Spatial repellents protect small perimeters from riceland mosquitoes in a warm-humid environment. *J Am Mosq Control Assoc* 37:41–45.

Britch SC, Kline DL, Linthicum KJ, Urban J, Dickstein E, Aldridge RL, Golden FV. 2020a. Transfluthrin spatial repellent on US military camouflage netting reduces Tabanids in a warm-temperate environment. *J Am Mosq Control Assoc* 36:212–215.

Britch SC, Linthicum KJ, Kline DL, Aldridge RL, Golden FV, Wittie J, Henke J, Hung K, Gutierrez A, Snelling M, Lora C. 2020b. Transfluthrin spatial repellent on US military materials reduces *Culex tarsalis* incursion in a desert environment. *J Am Mosq Control Assoc* 36:37–42.

Estep AS, Sanscrainte ND, Waits CM, Bernard SJ, Lloyd AM, Lucas KJ, Buckner EA, Vaidyanathan R, Morreale R, Conti LA, Becnel JJ. 2018. Quantification of permethrin resistance and *kdr* alleles in Florida strains of *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse). *PLoS Negl Trop Dis* 12:e0006544.

Fotakis EA, Chaskopoulou A, Grigoraki L, Tsiamantas A, Kounadi S, Georgiou L, Vontas J. 2017. Analysis of population structure and insecticide resistance in mosquitoes of the genus *Culex*, *Anopheles* and *Aedes* from different environments of Greece with a history of mosquito-borne disease transmission. *Acta Trop* 174:29–37.

Le Mauff A, Norris EJ, Li AY, Swale DR. 2024. Repellent activity of essential oils to the Lone Star tick, *Amblyomma americanum*. *Parasit Vectors* 17:202.

Miller SL, Swai K, Gowelo S, Opiyo M, Allen IE, Vajda E, Mbando A, Okumu F, Barasa SO, Maia M, Lobo N, Moore SJ, Chen I. 2022. VPrAM (Volatile Pyrethroids Against Mosquitoes): a systematic review and meta-analysis of the association between the use of volatile pyrethroid-based spatial repellents and mosquito bite prevention. Annual Meeting of the American Society of Tropical Medicine and Hygiene, Seattle, WA, November.

Nava MR, Debboun M. 2020. Mosquito species of Texas. *Mosquitoes*, *Communities, and Public Health in Texas*. Elsevier, 9–167. https://doi.org/10.1016/B978-0-12-814545-6.00002-X

Norris EJ, Coats JR. 2017. Current and future repellent technologies: The potential of spatial repellents and their place in mosquito-borne disease control. *Int J Environ Res Public Health* 14:124.

Parker C, Ramirez D, Thomas C, Connelly CR. 2020. Baseline susceptibility status of Florida populations of *Aedes aegypti* (Diptera: Culicidae) and *Aedes albopictus*. *J Med Entomol* 57:1550–1559.

Richoux GM, Yang L, Norris EJ, Tsikolia M, Jiang S, Linthicum KJ, Bloomquist JR. 2020. Structure-activity relationship analysis of potential new vapor-active insect repellents. *J Agric Food Chem* 68:13960–13969.

Vanlandingham DL, Higgs S, Huang Y-JS. 2016. *Aedes albopictus* (Diptera: Culicidae) and mosquito-borne viruses in the United States. *J Med Entomol* 53:1024–1028.

Walter Reed Biosystematics Unit. 2024a. *Psorophora ferox* (von Humboldt, 1819). Available at: https://wrbu.si.edu/vectorspecies/mosquitoes/ferox [Accessed September 30, 2024.]

Walter Reed Biosystematics Unit. 2024b. *Aedes infirmatus* Dyar & Knab, 1906. Available at: https://wrbu.si.edu/index.php/vectorspecies/mosquitoes/infirmatus [Accessed September 30, 2024.]

Yang L, Norris EJ, Jiang S, Bernier UR, Linthicum KJ, Bloomquist JR. 2020. Reduced effectiveness of repellents in a pyrethroid-resistant strain of *Aedes aegypti* (Diptera: Culicidae) and its correlation with olfactory sensitivity. *Pest Manag Sci* 76:118–124.

Yang L, Richoux GM, Norris EJ, Cuba I, Jiang S, Coquerel Q, Demares F, Linthicum KJ, Bloomquist JR. 2020. Pyrethroid-derived acids and alcohols: Bioactivity and synergistic effects on mosquito repellency and toxicity. *J Agric Food Chem* 68:3061–3070.