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SCIENTIFIC NOTE

TRUCK-MOUNTED NATULAR 2EC (SPINOSAD) ULV RESIDUAL TREATMENT IN A SIMULATED URBAN ENVIRONMENT TO CONTROL *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* IN NORTH FLORIDA

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ABSTRACT. Preemptive treatment of dry habitats with an ultra-low volume (ULV) residual larvicide may be effective in an integrated vector management program to control populations of container-inhabiting *Aedes* mosquitoes, key vectors of Zika, dengue, and chikungunya viruses. We exposed dry, artificial containers placed in exposed and protected locations to Natular 2EC (spinosad) larvicide applied with a truck-mounted ULV sprayer in a simulated urban setting in North Florida, and later introduced water and *Ae. aegypti* or *Ae. albopictus* larvae to conduct bioassays. Up to 50% mortality was observed in bioassays, indicating further analysis of spinosad as a residual treatment application.

KEY WORDS Biorational, container inhabiting, integrated vector management, urban, Zika virus

Aedes aegypti L. and *Ae. albopictus* (Skuse) can transmit a number of important human pathogens such as dengue, chikungunya, and Zika virus (Graham et al. 2011, Kuehn 2014, Duchemin et al. 2017). The risk of pathogens transmitted by these mosquitoes continues to grow due to globalization, increased travel, and habitat incursion by humans (Rose 2001, Imperato 2016). The threat to human populations posed by mosquito-borne pathogens rises every year from the factors listed above, and to mitigate this risk new control strategies are needed. One strategy that can be expanded within integrated vector management (IVM) programs is the targeting of immature stages that develop in artificial containers.

Control of artificial container–developing mosquitoes such as *Ae. aegypti* and *Ae. albopictus* is challenging, given the numerous containers and water sources that may and may not be visible to mosquito control operators. Control is often maintained through the use of large-scale ground or aerial ultra-low volume (ULV) treatments with larvicides that can penetrate large and/or inaccessible areas such as fenced yards and forests (Rose 2001, Pruszynski et al. 2017).

Novel methods, such as the contamination of immature development sites by insecticide-contaminated adult mosquitoes, show some promise (Bibbs et al. 2016). However, typically this type of contamination of immature development sites can only occur if there is 1) water in the container and 2) if there is enough insecticide on the treated adult to contaminate the site. Using Natular 2EC (spinosad) (Clarke, St. Charles, IL), a biorational larvicide derived from bacterial fermentation, and a truck-mounted ULV sprayer, we examined the possibility of pretreating artificial containers in a North Florida environment prior to being inundated with water in order to proactively prevent mosquitoes from developing in them after they have been flooded.

Three truck-mounted ULV spray trials were conducted May 23–24, 2016, at the Military Operations in Urban Terrain (MOUT) training site at the Camp Blanding Joint Training Facility. The MOUT site consists of an array of 1- to 3-story structures constructed of brick and mortar to simulate buildings that would be found in an urban setting. Each structure had window and door openings front and back, and the structures used in Trial 1 included partially opened roofs and several openings (gun ports) built into the wall that allowed for greater airflow. Two sets of 2 adjacent structures were selected for the trials based on prevailing wind such that ULV spray could be applied from the street or back yard, with drift targeted towards the structures. All windows and doors were held open with wood shims to allow wind and air movement to carry ULV droplets through the structures. Aerial images of the study site are shown in Fig. 1.

We established an approximately 60-m spray line along the street (Trial 1; Fig. 1A) and an approximately 50-m spray line along the grass yard (Trials 2 and 3; Fig. 1B, 1C) upwind of each pair of structures. We set up a transect of 9 stations approximately every 3 m perpendicular to the spray line to provide 3 stations in the front (i.e., the upwind side), 3 stations inside, and 3 stations in the back (i.e., the downwind side) of each structure (Figs. 1 and 2). For each trial we placed a pair of empty plastic 473-ml deli cups (DeliPro; TriPak Industrial USA, LLC, White Plains,

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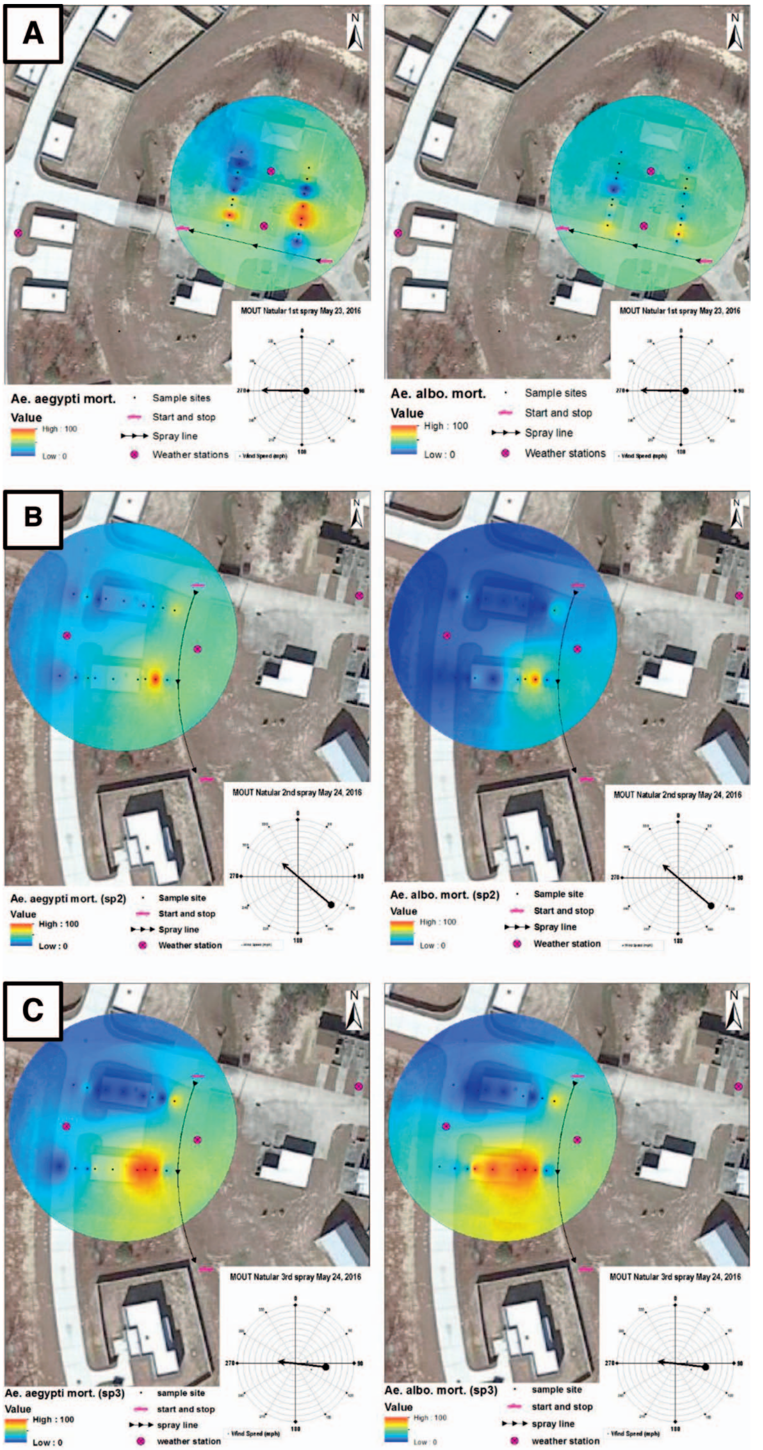

Fig. 1. Interpolated mortality map of Abbott-corrected mortality of immature *Aedes aegypti* and *Ae. albopictus* after exposure to Natular 2EC spray from a truck-mounted Terminator ultra-low volume (ULV) sprayer conducted (A) May 23 (Trial 1), (B) May 24 (Trial 2), and (C) May 24 (Trial 3), 2016, at the Camp Blanding Joint Training facility outside Starke, FL. Inset wind rose shows wind direction and approximate speed for each application. Color ramp represents gradient of mortality.

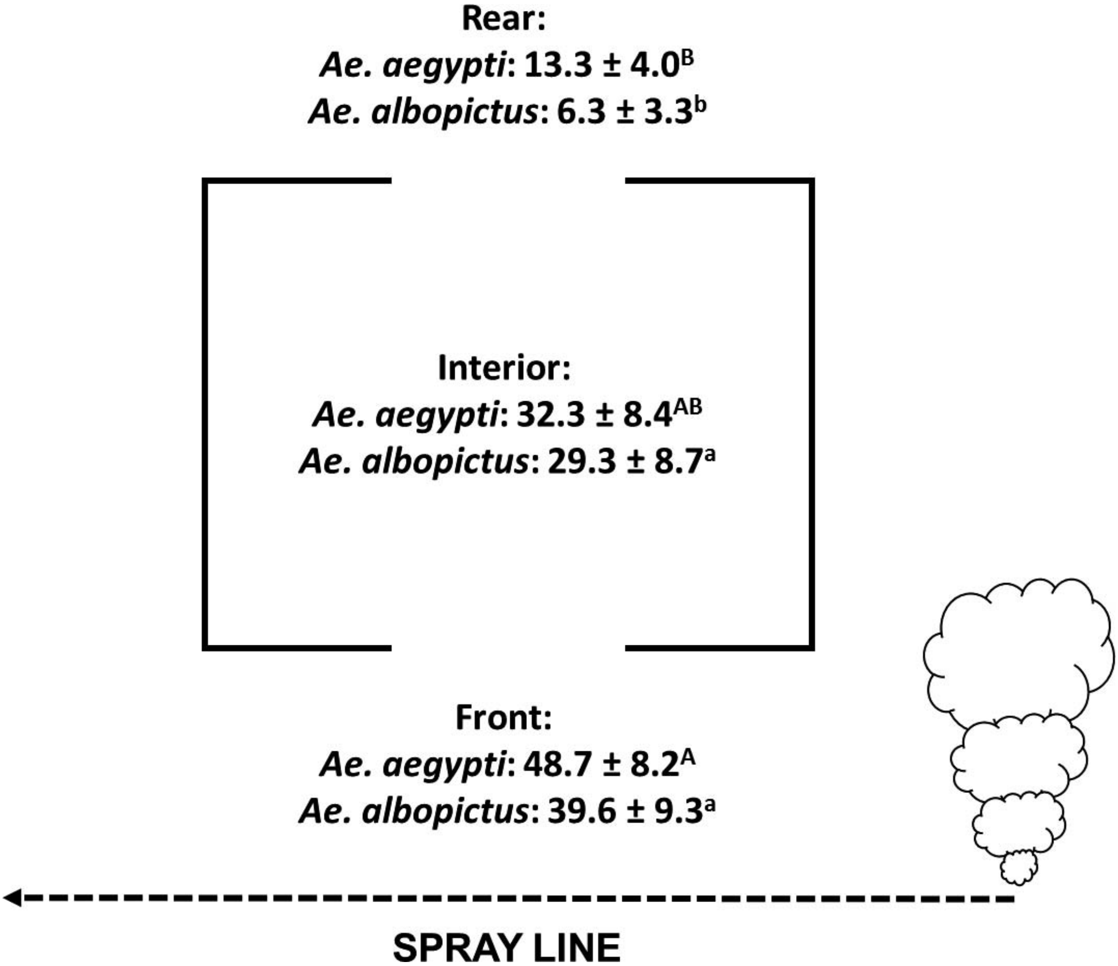

Fig. 2. Cartogram of average Abbott-corrected percent mortality (± SEM) of *Aedes aegypti* and *Ae. albopictus* grouped by location (average of 18 cups per trial per front, interior, and rear locations) across 3 spray trials covering 2 adjacent buildings per spray, $N = 108$ total cups, following truck-mounted ultra-low volume (ULV) application of Natular 2EC. The open square represents a generalized building with the front (spray-side) and rear doors open. Mortalities with shared uppercase (*Ae. aegypti*) or lowercase (*Ae. albopictus*) letters are not significantly different.

NY) at ground level at each station to collect ULV-applied larvicide droplets; one cup for bioassays with *Ae. aegypti* larvae and one for *Ae. albopictus* larvae at each station. Empty cups were utilized for this these spray trials following pilot studies (Aldridge, Linthicum, and Britch, unpublished data) that found negligible difference in mortality among larvicide bioassays conducted with dry, with water-filled, and with larvae and water-filled cups. Natular 2EC was mixed with water and swath widths were calculated to ensure a maximum application rate of 2.8 fl oz (79.5 ml)/acre using a truck-mounted diesel-powered Terminator ULV sprayer (ADAPCO, Sanford, FL). Sixteen untreated control sentinel cups were placed 50 m upwind of the spray area for each trial to provide 8 bioassay control cups for each species.

Following a spray trial, sentinel cups were left in place for 10 min before being covered with a fitted plastic lid, labeled, and collected into large black trash bags for storage in a controlled climate. Within a week of collection from the field, we inundated each sentinel cup with 400 ml of insectary tap water and introduced 50 susceptible *Ae. aegypti* (Orlando strain) or *Ae. albopictus* (Gainesville strain) 2nd–3rd instar larvae. Mosquito larvae were then reared on modified protocols described by Gerberg et al. (1994) and maintained at 28°C, 30% RH, and 12:12 h (L:D) interval. Cups were covered with either fine nylon tulle or the emergence portion of a mosquito breeder cup (Bioquip, Rancho Domingo, CA) for rearing, and we recorded the number of adults that emerged from each cup. Cups were left until a majority of mosquito larvae had emerged as adults before being tallied, and were subsequently checked every day until 14 days past the introduction of larvae.

Treatment mortality data were adjusted with control mortality using Abbott's correction (Abbott 1925) and were statistically analyzed with Wizard 1.9.8 (Evan Miller, http://www.wizardmac.com; Chicago, IL). Corrected mortality data were visualized with inverse distance weighting interpolation on aerial imagery of the study site using ArcGIS v10.1 (Environmental Systems Research Institute, Redlands, CA) (Fig. 1). Wind speed and direction were digitally recorded each minute using Kestrel weather stations (Nielsen-Kellerman, Minneapolis, MN) situated on 48-in. tripods at the control and treatment locations (Fig. 1). For each trial, weather station data were averaged across 10 min prior to treatment until 15 min after the treatment.

Applications of larvicide with truck-mounted ULV spray equipment reduced *Ae. albopictus* as well as *Ae. aegypti* populations, similar to the findings reported by Williams et al. (2014), after their application of *Bacillus thuringiensis israelensis* deBarjac. Average mortality of both *Ae. aegypti* and *Ae. albopictus* significantly varied by distance from the spray line as illustrated in Fig. 2. For *Ae. aegypti*, larval mortality significantly varied ($P < 0.001$) from 48.7 ± 8.2% to 13.3 ± 4.0% when comparing the front of the structure with the back of the structure, respectively, a span of approximately 90 ft (approximately 27 m) from the spray line (Fig. 2). However, the mortality inside the building did not differ significantly from mortality in the front or the back of the structures. For *Ae. albopictus*, larval mortality significantly varied ($P = 0.003$) from 39.6 ± 9.3% to 6.3 ± 3.3% when comparing the front and back of the building, respectively (Fig. 2). Mortality for this species also varied significantly ($P = 0.044$) between the inside (26.3 ± 8.7%) and the back (6.31 ± 3.3%) of the building, but not between the inside and the front of the building.

Mortality and penetration of Natular 2EC into structures was similar when compared between *Ae. aegypti* and *Ae. albopictus* larvae (Fig. 2). Significantly higher mortality was achieved in containers placed at the front of rather than behind structures for both *Ae. aegypti* ($P < 0.001$) and *Ae. albopictus* ($P = 0.003$). The average mortality across all 3 sprays was compared for each species by ANOVA and there was a significant difference for *Ae. aegypti* ($F_{2,51} = 5.815$; $P = 0.005$) and *Ae. albopictus* ($F_{2,51} = 4.595$; $P = 0.015$) mortality from cups placed at the front, the interior, and behind structures. Slightly higher (but not significant) mortality was observed for both species in cups placed at the back of the structure used for the 1st trial (Fig. 1A) compared with the structure used for the 2nd and 3rd trials (Fig. 1B, 1C; data not shown). We speculate that the vegetation present near the 1st structure may have trapped and redirected some of the ULV plume around the structures and towards the back even though the angle of attack for spray penetration through the structures was not ideal for the 1st trial (Fig. 1A). In contrast, the wind direction and speed in the 2nd and 3rd trials were more perpendicular to the spray line and perhaps the ULV plume had less opportunity to move around the structures (Fig. 1B, 1C). Additionally, for the 3rd spray (Fig. 1C), the door closest to the spray line of the North building had shut itself halfway through the spray treatment. We speculate that lower mortality from the cups may have resulted from the reduction of wind that penetrated through the building, carrying with it the larvicide.

Our conclusions are that Natular 2EC could be used to treat immature mosquito habitats using a truck-mounted sprayer immediately prior to the onset of a rain event, as part of an IVM program. Penetration into and through structures is possible, but average control even directly in front of the sprayer remains below 50% and generally declines through and behind buildings for both *Ae. aegypti* and *Ae. albopictus*. Optimal control occurred in the front of structures (i.e., within 10 m of the spray line) for both *Ae. aegypti* and *Ae. albopictus*, with no significant difference in average mortality between the front and interior of structures. Further study on the longevity of Natular 2EC to effectively pretreat larval development sites prior to rain events should be conducted. Although Pérez et al. (2007) showed that technical spinosad in solution rapidly lost toxicity when placed outdoors in sunlight, they also found that toxicity was maintained when placed outdoors in shaded areas common to *Ae. aegypti* larval habitat for up to 30 days. In future studies we plan to investigate the capability of spinosad to maintain toxicity in a variety of environments prior to inundation. Even if this larvicide applied as a residual is less effective than if applied directly to water-filled habitat, the possibility of enhancing an IVM program with preemptive control should not be discounted. Pretreatment of dry larval habitats could provide a bridge between high-risk mosquito development periods and the operational limits of an IVM program.

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