*Journal of the American Mosquito Control Association*, 34(1):63–66, 2018


# SCIENTIFIC NOTE

## ULTRA-LOW VOLUME APPLICATION OF SPINOSAD (NATULAR 2EC) AS A RESIDUAL IN A HOT-ARID ENVIRONMENT AGAINST *AEDES AEGYPTI*

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ABSTRACT. The invasive *Aedes aegypti* is an important disease vector increasing in frequency in hot-arid regions of the USA such as the Southwest. Within hot-arid surroundings this mosquito may be confined to peridomestic locations that tend to be cooler and humid, such as in lush, irrigated ornamental vegetation surrounding homes. However, to reach these habitat refugia, ultra-low volume (ULV) applications of insecticides targeting this mosquito must retain efficacy after being sprayed from the air or street where hot-arid conditions are prevalent. We investigated the efficacy of a biologically based larvicide, spinosad (Natular 2EC), applied as a ULV in a hot-arid environment targeting *Aedes aegypti*. We found that this pesticide is able to penetrate this environment and has the potential to act as a residual.

**KEY WORDS** *Aedes aegypti*, container inhabiting, desert, peridomestic, pretreatment



*Aedes aegypti* (L.) is an invasive mosquito species to North America that is a major vector of Zika and dengue viruses (Kyle and Harris 2008, Duchemin et al. 2017) and has been increasing in hot-arid ecological regions of the USA such as Arizona and southern California (Metzger et al. 2017). It is difficult to suppress populations of this peridomestic mosquito because immature stages inhabit containers and exploit microhabitats found around a typical home that are nearly impossible to completely eliminate (Kittayapong and Strickman 1993).

In extreme hot-arid environments, percent relative humidity (% RH) may be low with temperatures over 38°C (100°F), and adult *Ae. aegypti* would die out in the open (Anderson et al. 2006). Therefore, the adults may be confined to shaded, relatively cooler, and more humid areas such as in lush, well-irrigated ornamental vegetation around homes. Such homes could be found in the southern California cities of Riverside, San Jacinto, and the Coachella Valley where *Ae. aegypti* have recently made incursions (Metzger et al. 2017). Abundant microhabitat for oviposition is also provided by these landscaped residential yards. These factors encourage *Ae. aegypti* to thrive and pose a public health risk despite the surrounding desert environment. To control *Ae. aegypti*, ultra-low volume (ULV) sprays of insecticides conducted from the air or the ground as part of an integrated vector management (IVM) program must be able to penetrate through the hot-arid environment and remain efficacious when they reach cool-humid microhabitats.

In this kind of an IVM program it would be advantageous to apply larvicides that could act immediately with standing water in containers, or form a residual in similar but dry habitat. Natular 2EC (Clarke, St. Charles, IL) is a biologically based liquid larvicide containing spinosad (20.6% AI) specifically marketed to combat container-breeding mosquitoes such as *Ae. aegypti*. Larvae can absorb a fatal dose of spinosad through the cuticle or through ingestion, thereby potentially controlling early and late instars (Bond et al. 2004, Hertlein et al. 2010). We conducted a study in southern California to investigate the capability of ULV-applied Natular 2EC to disperse through an extreme hot-arid environment, persist as a residual, and control *Ae. aegypti* larvae upon inundation with water.

The research plot, described in Britch et al. (2009), was located in the Coachella Valley near Thermal, CA, and consisted of mixed xeric vegetation of varying density and mostly under 2 m high. We conducted ULV spray trials in the central area of the research plot along an approximately 60-m (200 ft) spray line oriented north–south (Fig. 1). We used a Twister XL3 backpack sprayer (Curtis Dyna-Fog, Westfield, IN) with a flow rate of 45 ml/min from #19 orifice. From preliminary tests we estimated that the Twister XL3 could disperse a visible ULV plume up to approximately 6 m (approximately 20 ft) from the applicator, thus producing a treatment area of 0.09 acres along the 60-m spray line. The flow rate and treatment area were used to calculate walking speed of 20 sec per 10 m of the 60-m spray line to apply the intended maximum label rate of 182.6 ml/ha (2.8 fl oz/acre) of Natular 2EC to the treatment area for each trial in approximately 2 min.

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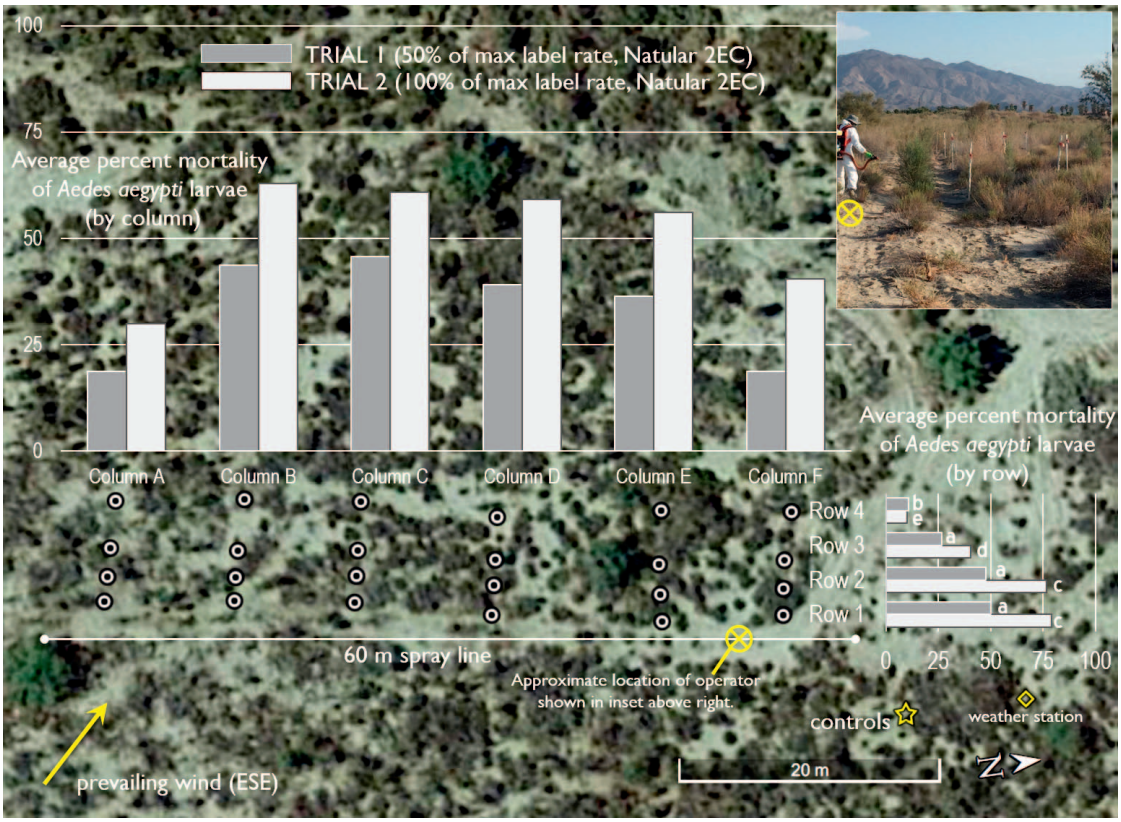


Fig. 1. Aerial view of study area in the Coachella Valley near Thermal, CA, where Natular 2EC (spinosad) in water was applied with a backpack ultra-low volume (ULV) sprayer targeting empty plastic collection cups to investigate efficacy against *Aedes aegypti* larvae after moving through this extreme hot-arid environment and forming a residual. Inset panel shows operator walking south along the spray line parallel to a grid of cup stations, partially visible to his right and marked with upright plastic posts. Yellow crossed-circle symbol shows operator's location on the aerial view in the main panel, where small white circle symbols show locations of cup stations arrayed in columns (A–F) and rows (1–4). Histograms of percent larval mortality for the 2 trials are superimposed on the aerial view to show relative efficacy by column (upper chart; no significant difference across columns for either trial) and by row (right-hand chart; significant differences by row are indicated by letters, a–b for Trial 1 and c–e for Trial 2).

For each of 2 trials we deployed empty 473-ml (16 fl oz) plastic larval sentinel cups (DeliPro; TriPak Industrial USA, LLC, White Plains, NY) at ground level in a grid to the west of the transect to collect ULV larvicide droplets for later bioassays. In order to test the efficacy of the spray at increasing distances from the spray operator, we placed a series of 4 cups along a "column" perpendicular to the spray line at 3 distances falling within the visible ULV plume, 1.8 m (6 ft), 3.7 m (12 ft), and 5.5 m (18 ft), and 1 distance beyond the plume at 9.1 m (30 ft) as shown in Fig. 1. We observed varying density of vegetation in the spray area so we set up a total of 6 replicate columns approximately 10 m (35 ft) apart, labeled A–F (Fig. 1). We designated 4 columns (A, C, E, and F) running through relatively sparse vegetation and 2 columns (B and D) through relatively more dense vegetation to test the hypothesis that vegetation challenge would reduce spray efficacy. This arrangement of 6 replicate columns naturally formed a series of 4 rows (labeled 1–4; Fig. 1) each with 6 replicate cups at each distance from the spray line, for a total of 24 cups per trial. Column A was situated approximately 5 m (15 ft) from the start of the spray line, and the end of the spray line was marked approximately 5 m beyond Column F to increase the likelihood that all cups would be reached by the ULV spray despite the expected natural variation in wind direction during each spray trial. An untreated control station with 6 sentinel cups per trial was located approximately 10 m upwind and to the northeast, away from the spray line, and a Kestrel 4500NV Weather Tracker (Nielsen-Kellerman, Boothwyn, PA) was positioned about 10 m north of the control area (Fig. 1).

The spray operator walked along the spray line, holding the spray nozzle level with the ground at waist height approximately 1.8 m from the 1st row of sentinel cups and perpendicular to the spray line. The wind was predominantly from the east and east-

southeast during both trials so the ULV cloud was generally directed into the grid of 24 collection cups. After each spray trial, all sentinel cups were quickly labeled by trial number and position, collected and capped, and stored in a cool dry environment in large plastic bags. Cups were shipped within 1 wk to the USDA-ARS Center for Medical, Agricultural, and Veterinary Entomology in Gainesville, FL, to initiate bioassays.

The larval bioassay consisted of introducing 400 ml of insectary tap water, 50 2nd- to 3rd-stage *Ae. aegypti* larvae (susceptible Orlando strain, 1952), and 2.5 ml of insectary diet suspension to each cup from the field, and covering each with an emergence funnel (1425 Mosquito Breeder; Bioquip, Rancho Dominguez, CA). Larvae were then left to rear in an incubator under insectary conditions of 12:12 h (light:dark) cycle at 28°C and 30–70% RH. Efficacy of the larvicide was determined by calculating percent mortality in each cup as $100 \times [(50 - A)/50]$, where A is the number of emerged adults from each cup. All mortality data were Abbott-corrected (Abbott 1925) before data analysis with ANOVA and *t*-tests, using the observed control mortalities of 4.0% and 4.3% in Trials 1 and 2, respectively.

For Trial 1 with Natular 2EC, conducted late afternoon on August 2, 2016, the operator walked the spray line twice (total spray time of approximately 4–5 min), but the level in the formulation tank at the end of the trial still indicated that the operator would have needed to walk the spray line several more times to apply the planned maximum label rate. Based on the volume of formulation used, we estimated that approximately 50% of the maximum label rate was applied to the treatment area in Trial 1. Weather conditions were 2.3 m/sec (5.1 mph) winds, 38.5°C (101.3°F), and 44.4% RH. Under these parameters, the average mortality was 33.8% across the 24 cups. For Trial 2, which took place early afternoon, August 3, 2016, the operator walked the spray line 15 times (total spray time of approximately 35 min) to achieve the planned maximum label rate, confirmed by monitoring levels in the formulation tank, which resulted in 51.6% average mortality. Average weather conditions during Trial 2 were 2.0 m/sec (4.5 mph) winds, 39.1°C (102.4°F), and 36.1% RH.

Mortality data analyzed with *t*-tests from both Trial 1 and Trial 2 indicated that Rows 3 and 4, farthest from the sprayer, had significantly lower mortality as indicated by letter symbols on the right-hand histograms in Fig. 1. In Trial 1, cups located in Row 1 and Row 2, closest to the spray path and $\leq$4 m from the sprayer, had similar average mortalities of 50.0% and 47.6%, respectively. Average mortality in Row 3 at 5.5 m from the sprayer was lower (26.7%) but not significantly different from Rows 1 or 2. However, average mortality dropped significantly to 10.8% ($P = 0.012$ and 0.002 compared with Rows 1 and 2, respectively) at Row 4, which was 9.1 m from the sprayer (Fig. 1). A similar pattern was observed in Trial 2: average mortalities in Rows 1 and 2 were not significantly different at 79.1% and 76.7%, respectively, then dropped significantly to 40.4% ($P = 0.003$ and 0.029 compared with Rows 1 and 2, respectively) across Row 3, and to 10.2% ($P \leq 0.003$ compared with Rows 1–3) in Row 4. These observations somewhat match our initial estimation based on the visible ULV plume that the effective range of the Twister XL3 in this environment was no more than 6 m (approximately 20 ft).

Contrary to our expectations of vegetation inhibiting efficacy of the ULV-applied residual, we found that average mortality across Columns B and D with the highest vegetation challenge (40.1% in Trial 1; 59.5% in Trial 2) was not significantly different compared with the average mortality across Columns A, C, E, and F with the lowest vegetation challenge (30.6% in Trial 1; 47.6% in Trial 2) (Fig. 1). Although these differences were not significant in this study, the percent mortalities were numerically higher across cups situated in more dense vegetation, which does not support our initial hypothesis that vegetation should impede efficacy of the Natular 2EC ULV application. We observed similar unexpected differences in efficacy of Natular 2EC applied through dense compared with sparse vegetation that were statistically significant in a related study conducted in a hot-humid environment (Britch et al. 2018, this issue). We hypothesize that the presence of thicker vegetation could create pockets of air protected from wind that trap ULV droplets and give them more time to settle, and may also provide shade that could reduce ultraviolet (UV) degradation of the formulation. In contrast, areas with more sparse vegetation may be subject to increased UV exposure, and wind currents may disperse droplets before they have time to settle in the target area.

If we visually compare the spatial distribution of mortality between Trials 1 and 2 from the perspectives of both the column histograms and the row histograms (Fig. 1), we observed that the overall spatial patterns of mortality are alike between the 2 trials if we ignore the greater amplitude of each histogram from Trial 2. Despite the 2 different (unintended) application rates of the 2 trials, there are no significant differences between any Trial 1–Trial 2 pairing of data for any row or column. Nevertheless, we suspect that the apparent difference in amplitude between Trial 1 and Trial 2 for any row or column are not random and due to the higher application rate in Trial 2.

The efficacy of Natular 2EC applied as a residual in this study in a hot-arid environment is not significantly different from the efficacies of Natular 2EC applied as a residual in Britch et al. (2018, this issue) in a hot-humid environment in Thailand (52.0% average mortality) and Aldridge et al. (2018, this issue) in a warm-temperate environment in Florida (48.7% average mortality), if we limit our analysis to comparable data across the 3 studies. In

the Thailand study, conducted with the same model of backpack ULV sprayer, there was only a single row of cups, equivalent to Row 1 in the present study. In the Florida study, conducted with a small truck-mounted ULV sprayer, there were multiple positions of cups in front of and through buildings, so we should only consider data from cups in front of the buildings and nearest the ULV. An ANOVA across these 3 studies (restricted to comparable data as described) shows no significant difference in mortality regardless of whether we consider Trial 1 (50% maximum label rate; 50.0% average mortality Row 1) or Trial 2 (100% maximum label rate; 79.1% average mortality Row 1) data from the current study, or whether we only compare California and Thailand studies that used the same model of sprayer. We suggest that this is a positive outcome of the present study despite the difficulties with the backpack ULV sprayer, pointing to Natular 2EC being capable of efficacy even under extreme climates and suboptimal ULV application. We propose that the observed consistency across studies shows that Natular 2EC is capable of acting as a residual in a hot-arid environment as well as hot-humid and warm-temperate environments, which may enhance its overall efficacy in IVM because in a single application the material could reach flooded habitat as well as dry habitat that could be later inundated.

Future studies should be conducted with a backpack ULV capable of label-rate application with a single pass to reduce possible confounding effects of environmental variables. Future studies should also investigate similar ULV applications of Natular 2EC targeting both dry cups and cups with water and larvae placed in warm-humid microhabitats in residential yards situated in a hot-dry environment. These applications could be conducted from the street with a truck-mounted ULV sprayer to simulate real-world operational conditions. Sentinel cups could be left in place in the study area during bioassays to provide more information on the capability of the residual to persist in field conditions, and on the immediate efficacy of Natular 2EC reaching water-filled cups with larvae after moving through the hot-arid surroundings. Additionally, residential yards with variable vegetation coverage potentially allow some areas to be treated more effectively than others, which could also help quantify vegetation impact and develop application techniques to compensate.

We thank E. V. Moore for expert technical support in the lab and field, and T. Carney, B. Smith, and C. Swain for expert production of immature mosquitoes at the USDA-ARS-CMAVE insectary. This research was supported by the US Department of Agriculture (USDA)–Agricultural Research Service and the US Department of Defense (DOD) Deployed War-Fighter Protection Program (DWFP). Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by USDA, DOD, AFRIMS, or the DWFP. The USDA is an equal opportunity provider and employer.

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