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OPERATIONS NOTE & EQUIPMENT

EVALUATION OF PYRETHROID AND BOTANICAL BARRIER INSECTICIDES AGAINST *AEDES ALBOPICTUS* IN THE LABORATORY AND FIELD

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Subject Editor: Seth Britch

ABSTRACT

Outdoor residual insecticide applications are useful for preventing or reducing mosquito populations at focal areas. Until recently, pyrethroids have been the only option for barrier sprays in mosquito control. In this study, three pyrethroid (Onslaught, Cyzmic CS, DeltaGard) and two botanical (Nature-Cide, Essentria IC^3) outdoor residual insecticides were comparatively tested at low, mid, and high label rates against adult *Aedes albopictus* in both laboratory bioassays and field trials in St. Augustine, FL, from May-August 2017. Bioassays indicated NatureCide and Cyzmic CS were the most toxic across all three dilution ratios followed by DeltaGard, Onslaught, and Essentria IC^3, respectively. In field trials Nature-Cide and Onslaught were the only products that reduced mosquito abundance at the low rate. However, at the mid rate NatureCide and Onslaught caused ~90% percent reduction of adult female *Ae. albopictus* in the field, the highest of all tested products. The performance of DeltaGard (79% reduction in field counts), Essentria IC^3 (64%), and Cyzmic CS (36%) in the field were not similar to the laboratory results. The universally high performance of Nature-Cide indicates that mosquito control operations should expand consideration to botanical based insecticides for field operations.

Key Words: *Aedes albopictus*, mosquito, barrier treatments, pyrethroid, essential oils, passive control

INTRODUCTION

The Asian tiger mosquito *Aedes albopictus* (Skuse) is a highly invasive, peridomestic vector of arboviruses such as dengue and chikungunya (Derraik and Slaney 2015, Wilson and Chen 2015). Its adaptability and vector potential have rendered it a major public health concern while steadily increasing the global burden of vector-borne disease (Bonizzoni et al. 2013). Vector-borne diseases are responsible for more than 17% of all infectious diseases worldwide (World Health Organization 2017a). An estimated 1.38 million suspected cases of chikungunya have been recorded around the world within the last decade (World Health Organization 2017b), and during the 2016 worldwide dengue outbreak the Americas alone reported more than 2.38 million cases (World Health Organization 2017c). Targeting adult mosquito vector populations is still a key process to reduce arbovirus transmission (Manica et al. 2016).

Ground adulticide methods such as applications of a barrier treatment have commonly been used as part of integrated mosquito management (Brown and Xue 2011). Barrier treatments are designed to stop adult mosquitoes entering areas typically used for outdoor human activity while also reducing the need to retreat the area (Fulcher et al. 2008) and treatments have been shown to be effective for focal mosquito control in these areas (Doyle et al. 2009, Brown and Xue 2011, Conover et al. 2015). Many species of adult mosquitoes such as *Ae. aegypti* (L.), utilize foliage structures for a variety of purposes ranging from sheltered resting sites to sources of food (Xue 2008), so barrier treatments leverage resting and feeding behaviors to maximize mosquito-insecticide contact (Fulcher et al. 2008).

Public health mosquito control in the US is restricted to only two classes of mosquito adulticide active ingredient, pyrethroids and organophosphates, which limits the options available for avoiding the evolution of resistance. For example, the majority of outdoor residual insecticides contain synthetic pyrethroid active ingredients such as bifenthrin, deltamethrin, sumithrin, or permethrin. Fortunately, recent work improving the emulsification of essential oils has enhanced development of plant-derived active ingredients, including synergy with existing active ingredients in adulticides and larvicides (Dias and Moraes 2013, Norris et al. 2015, Gross et al. 2017). Botanical "green" alternative insecticides are appealing due to their minimum risk classification, which allows more flexible reapplication procedures and more transparency about all ingredients in a product. To explore and evaluate available EPA exempt barrier insecticidal sprays in comparison with common pyrethroid products, we investigated the relative capabilities of three pyrethroids (type I and type II) and two botanical "green" alternative adulticides for control of adult *Ae. albopictus* through laboratory bioassays and field trials.

MATERIALS AND METHODS

We obtained *Aedes albopictus* for this study from the United States Department of Agriculture, Agricultural Research Service, Center for Medical, Agricultural, and Veterinary Entomology (CMAVE) in Gainesville, FL. Mosquitoes had been maintained in CMAVE insectaries at 26.6 °C, 85± 5% relative humidity (RH), 14 h light:10 h dark photoperiod, and fed on a 10% sucrose solution (Gerberg et al. 1994). Subjects used in bioassays were female, not blood-fed, and 6–8 days old.

We tested five barrier treatment formulations: Nature-Cide All Purpose Concentrate (0.5% clove and 0.5% cottonseed oil; Pacific Shore Holdings, Inc., Canoga Park, CA), Essentria IC-3 (10% rosemary, 5% geraniol, 2% peppermint oil; Envincio LLC, Schaumberg, IL), Onslaught (6.4% esfenvalerate, a type I pyrethroid; McLaughling Gormley King Company, Minneapolis, MN), DeltaGard (2% deltamethrin, a type II pyrethroid; Bayer Environmental Science, Research Triangle Park, NC), and Cyzmic CS (9.7% lambda-cyhalothrin, a type II pyrethroid; Control Solutions, Inc., Pasadena, TX). Each product was tested using label prescribed low, mid, and high application rates across separate trials.

For laboratory bioassays, we designed a cylindrical chamber using a 55 mL petri dish base covered with an inverted 266 mL (9 oz) polystyrene cup (Fig. 1). We used a hot metal probe to melt a hole through the base of the cup for aspiration and to support a sucrose solution wick, and several smaller holes around all sides of the cup for ventilation. For each of the low, mid, and high label rates, we applied 1 mL of formulation diluted in reverse osmosis (RO) water with a pipette to filter paper (Whatman No. 1; GE Healthcare Bio-Sciences, Pittsburgh, PA) 24 h in advance of bioassays. Controls consisted of RO water with no formulation. To begin the bioassay trials we placed treated filter papers into Petri dish bases and covered with the ventilated cups, with the cup then taped to the base as shown in Fig. 1. We introduced 15 adult female mosquitoes to each cup and fitted cotton balls saturated with 10% sucrose solution in the aspiration hole. We recorded total knockdown at 30 min and mortality at 24 hours. For each repetition we used 3 cups per formulation and five control

Figure 1. Bioassay chamber constructed of a Petri dish base, a pesticide-treated filter paper nested in the dish, and a ventilated polystyrene cup with sucrose solution wick, and containing 15 non-blood-fed, 5-7 d old female *Aedes albopictus* (Skuse).

cups, and conducted 3 repetitions per low, mid, and high label rates.

For field tests, we selected 10 suburban sites (5 treatment paired with 5 control) in St. Augustine, FL, similar to the one shown in Fig. 2 based on the presence of harborage suitable for *Ae. albopictus*, with a minimum of 402 m between each paired treatment and control site. Each site was an average distance of 2.2 km from a central weather station where we recorded weekly rainfall summaries (Fig. 4) to provide context for patterns of mosquito population change across all sites. We conducted 3 weeks of pre-treatment surveillance at each site using BioGents Sentinel (BGS) mosquito traps (BG-2; BioGents AG, Regensburg, Germany) baited with CO_2 for 24 h per week to confirm presence of *Ae. albopictus* at all treatment and control sites. We identified collections from each trap weekly and continued surveillance in this way for the duration of the study.

We used a battery powered backpack sprayer (REC 15 ABZ; Birchmeier Sprühtechnik AG, Stetten, Switzerland) to apply the barrier treatments at the 5 sites, with the machine set to 5 bar flow pressure to achieve a 1,350 mL/min flow rate. We delivered each treatment at an approximately 7-8 km/h walking pace and calibrating each formulation-rate to a 450 mL application. Each site received separate but consecutive treatments for the low, mid, and high rates, in that order, with each rate left in place with surveillance for 4 weeks. We randomly assigned the 5 formulations to the 5 treatment sites, one formulation per site. Following each treatment we flushed the backpack sprayer with 3.785 L of water to prevent cross-contamination among formulations.

Figure. 2. Image of representative suburban field site selected based on the presence of suitable harborage for *Ae. albopictus* such as moderate to dense foliage, many adult resting areas, and various artificial containers for development of immature mosquitoes.

We analyzed laboratory bioassay data using an ANOVA and Tukey's HSD. For the field data, we used Mulla's formula (Mulla et al. 1971) to calculate the percent reduction in the relative abundance of wild mosquitoes as measured by adult surveillance: $^{\%}R = 100 \times [(C_1/T_1) \times (T_2/C_2)] \times 100$; where C_1 = pre-treatment measure of mosquito abundance in the associated control site, C_2 = post-treatment mosquito abundance in the control site, T_1 = pre-treatment mosquito abundance in the treated site, and T_2 = post-treatment mosquito abundance in the treated site. We also analyzed adult surveillance with a generalized linear model to investigate differences among treatments relative to time elapsed during the study.

RESULTS AND DISCUSSION

Results from the laboratory bioassays are summarized in Fig. 3. We found significant performance differences among the 5 formulations for both knockdown (F = 11.67, df = 4, 44, $P < 0.0001$) and mortality (F = 28.39, df = 4, 44, $P < 0.0001$). Nature-Cide and Cyzmic CS caused the highest knockdown across all three dilution rates with 20-50% knockdown at the low rate, 100% knockdown at mid and high rates, and a mean mortality of ≥ 90% at all rates. DeltaGard, Onslaught, and Essentria IC^3 had 0% knockdown and less than 20% mortality at the low rate. DeltaGard performed better at mid and high rates than Onslaught and Essentria IC^3, with the latter two formulations performing poorly overall.

Analysis of field collections indicated significantly different performance among the 5 formulations ($\chi^2 = 10148$, df = 15, $P < 0.0001$). Weekly changes in relative abundance of adult *Ae. albopictus* at field sites are shown in Fig. 4. Unfortunately, we were not able to conduct field trials at the high label rate because of limitations of time. Collections of adult female *Ae. albopictus* from Nature-Cide and Onslaught treat-

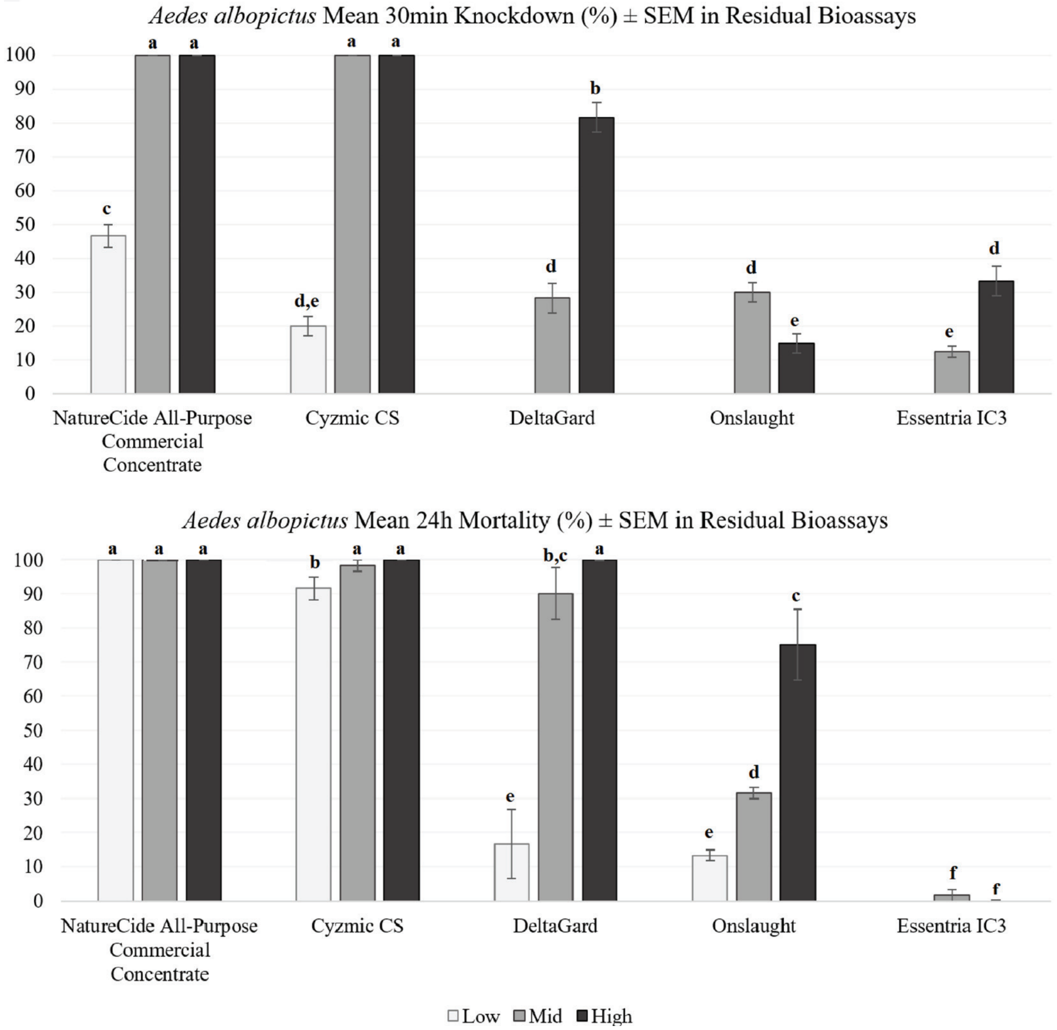

Figure 3. Average percent 24 h mortality with standard errors of the mean (ANOVA/Tukey HSD at 95% confidence, $P < 0.001$) of *Aedes albopictus* (Skuse) for each of five residual spray formulations, NatureCide All-Purpose Commercial Concentrate (clove oil, cottonseed oil; 25-100 mL/L), Cyzmic CS (lambda-cyhalothrin; 1.5-3.0 mL/L), DeltaGard (deltamethrin; 2-12 mL/L), Onslaught (fenvalerate; 4-8 mL/L), and Essentria IC^3 (Rosemary oil, peppermint oil; 23-47 mL/L) applied at low, mid, and high label rates. Control bioassays produced 0% mortality.

ment sites showed a net reduction of 80% by Week 8 (i.e., 4 weeks post treatment with the low rate). On the other hand, after 4 weeks with the low rate the site treated with Cyzmic had no meaningful change in relative abundance, while sites treated with DeltaGard and Essentria IC^3 had a net increase in *Ae. albopictus* between 10% and 20%. With mid-rate applications, however, sites treated with Nature-Cide and Onslaught had 90% net reductions in mosquito collections 4 weeks post treatment, compared to DeltaGard (79% net reduction), Essentria IC^3 (64%), and Cyzmic (36%). In the GLM for the week-by-week comparison the treatment used ($\chi^2 = 6554.87$, df = 5, $P < 0.0001$) explained most of the variation, followed by the duration of weeks across the study ($\chi^2 = 3593.13$, df = 10, $P < 0.0001$).

It was surprising to find that Nature-Cide, formulated with clove and cottonseed oil as a multi-purpose insecticide, outperformed all other products in both laboratory (Fig. 3) and field (Fig. 4) trials. In contrast, the other tested botanical product, Essentria IC^3, had zero to low effects

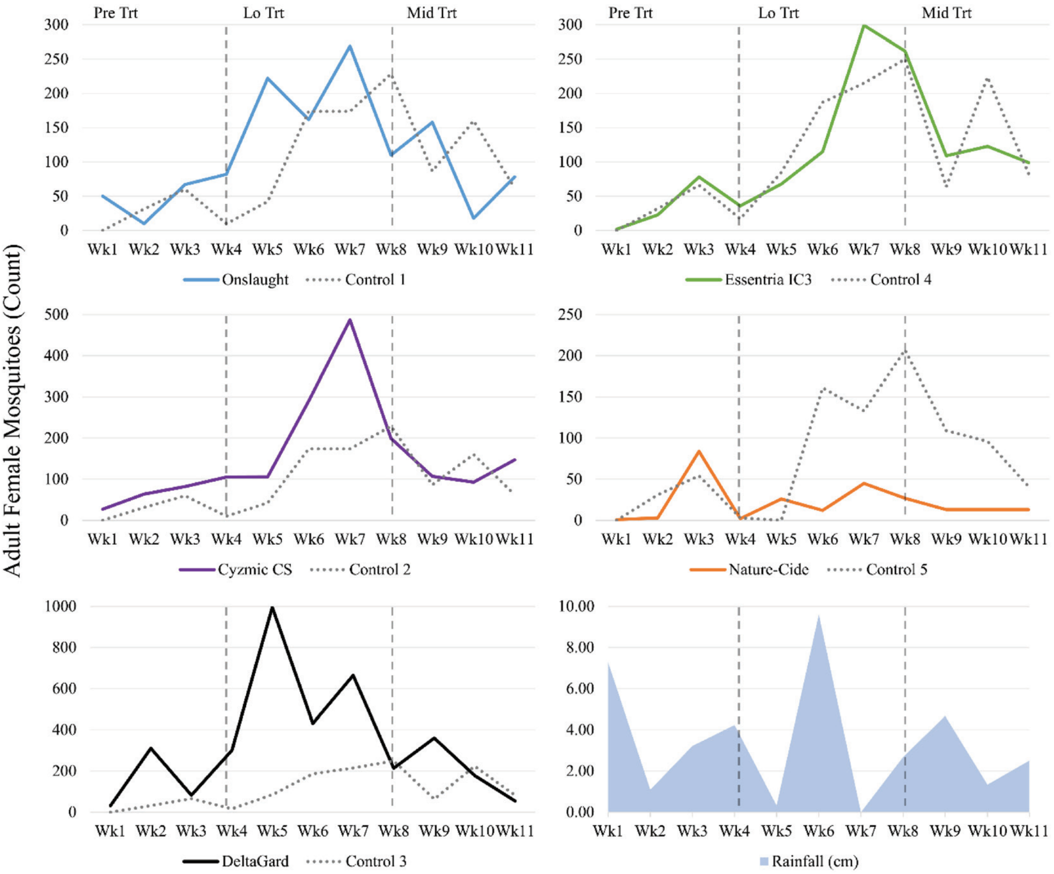

Figure 4. Field collections of adult *Ae. albopictus* from 5 sites treated with residual insecticides paired with 5 untreated control sites, with rainfall data (cm) from a centrally located weather station to provide context for patterns of mosquito population change. Each graph includes results from collections for 3 weeks prior to application of the residual treatment that confirmed presence of *Ae. albopictus* at all treatment and control sites. We initiated experimental treatments at Week 4 with the low rate which, with the exception of Nature-Cide, did not substantially reduce *Ae. albopictus* abundance. We applied mid rate treatments at Week 8 resulting in *Ae. albopictus* reduction at all treatment sites: Nature-Cide and Onslaught (~90% reduction), DeltaGard (~79%), Essentria IC3 (64%), and Cyzmic CS (~36%). We were not able to conduct field trials at the high label rate because of time limitations. Reduction was quantified using Mulla's formula (see text for details).

in laboratory bioassays yet low to moderate efficacy for reducing field populations of *Ae. albopictus* which could imply effects besides toxicity in a field environment. The rosemary, geraniol, and peppermint in Essentria IC^3 could be stronger as repellents than insecticides, but we did not collect outside the treatment sites to determine if mosquito populations in adjacent areas may have increased. In comparison, the very high efficacy of Cyzmic CS, DeltaGard, and Onslaught in laboratory bioassays was not mirrored in field collections. Cyzmic CS and DeltaGard, both containing type II pyrethroids, completely failed to reduce mosquitoes when applied at the low label rate and at the mid rate performed below Onslaught, the only type I pyrethroid formulation we tested.

Pyrethroids are the most commonly used insecticides for adult mosquito control because of low environmental impact, high insecticidal potency, and good mammalian safety profiles (Amoo et al. 2008). However, the Federal, Insecticide, Fungicide, and Rodenticide Act (FIFRA) restricts the frequency that pyrethroids may be applied to the environment for adult mosquito control, spurring demand for research emphasizing green chemistry. The

Environmental Protection Agency (EPA) allows minimum risk pesticides to be exempt from FIFRA (40 C.F.R. §152.25 2015). Therefore, exempt pesticides containing for example the botanical ingredients described above can be applied more frequently than FIFRA labeled products. This intrinsically appeals to mosquito control programs when treatments need frequent reapplication, for example during significant mosquito outbreaks or when mitigating arbovirus transmission. Furthermore, exempt pesticides could provide different chemical classes for mosquito control programs, potentially reducing the risks of both resistance and environmental impact.

In the literature there are recent and accumulating examples of botanical oils used for mosquito control, with various ingredients functioning as repellents (Gross and Coats 2015), enhancers of other active ingredients (Gross et al. 2017), or acting as a synergist for toxicity (Tong and Bloomquist 2013, Gross et al. 2017). Plant-derived active ingredients for pesticides have generated enough interest to prompt the screening of 361 essential oils from 269 plant species as larvicides against *Ae. aegypti* (L.) (Dias & Moraes 2013). Phytochemicals have also become important in adulticide development due to the success of microemulsion formulations (Montefuscoli et al. 2013, Gross et al. 2017). Commercially available plant essential oils have been screened as adulticides against *Ae. aegypti* and *Anopheles gambiae* Say with favorable results (Norris et al. 2015). Despite these impressive developments centered on plant-derived compounds for public health vector control, key botanically based products suitable for mosquito control programs such as ultra-low volume (ULV) cold aerosol space sprays are not yet developed for operational use. The positive results using Nature-Cide as an outdoor residual treatment in this study demonstrate that botanically based formulations are ready to be investigated further and possibly incorporated operationally into mosquito control programs.

ACKNOWLEDGEMENTS

We thank Jason Conrad and Univar for their assistance with selecting and sourcing the formulations chosen for this study. This is a research report only; specific mention of commercial products does not imply endorsement by the Anastasia Mosquito Control District.

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SEMI-FIELD ULV EVALUATION OF AN ALL-PURPOSE BOTANICAL INSECTICIDE CONTAINING CEDARWOOD AND CINNAMON OILS AGAINST ADULT *AEDES AEGYPTI*

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Subject editor: Eva Buckner

ABSTRACT

Public health mosquito control operates with only two classes of mosquito adulticides: pyrethroids and organophosphates. Recent work improving the emulsification of essential oils has increased the potential for development of plant-derived active ingredients. There is a growing body of literature on essential oils for various roles in mosquito management. NatureCide Pest Management (NCPM), a product available in private and commercial home pest control, uses a mixture of 25.3% cedarwood oil and 12.7% cinnamon oil as a Federal Insecticide, Fungicide, Rodenticide Act (FIFRA) exempt insecticide for both indoor and outdoor use. Recent investigations by the Anastasia Mosquito Control District of St. Johns County have found other FIFRA exempt products to be effective as a residual spray on vegetation. In continuing the exploration of botanical insecticides, NCPM was used in ULV tests against *Aedes aegypti* (L.) within its 35-122 ml per L of water label rate. Applications at 35 ml/L resulted in 60-70% knockdown after 1 hr and mortality after 24 hr. Increasing the rate to 70 ml/L resulted in 100% knockdown and mortality across all replications. Crystalline precipitation of the microemulsion was observed in mix tanks after standing for at least 2 wk, but it was not apparent that the efficacy of the product was reduced as a consequence. Cedarwood oil and cinnamon oil are a beneficial combination for ULV adulticiding against mosquitoes and could have a beneficial role for integrated mosquito management.

Key Words: *Aedes aegypti*, mosquito, botanical, insecticide, essential oils

INTRODUCTION

Botanical ingredients are attractive alternatives in formulated repellents (Gross and Coats 2015), toxicants (Gross et al. 2017), and synergists (Tong and Bloomquist 2013; Gross et al. 2017). The sustained demand for plant-derived active ingredients in pesticides has prompted the screening of over 350 plant essential oils as larvicides against *Aedes aegypti* (L.) (Dias & Moraes 2013). Phytochemicals have become increasingly viable for product development since successful formulation in microemulsions (Gross et al. 2017), and microemulsion formulations were demonstrated in pilot work as effective against *Culex pipiens* (Montefuscoli et al. 2013). In consequence, essential oils also are being screened as adulticides against *Ae. aegypti* and *An. gambiae* (Norris et al. 2015). Despite this effort, few products exist for mosquito management that use plant-derived active-ingredients, particularly for ultra-low volume (ULV) cold aerosol space sprays.

Amidst the emphasis on green chemistry underlies the principle cause of the demand for this research: EPA allows minimum risk pesticides to be exempt from FIFRA (40 C.F.R. §152.25 2015). This exemption is ideal for green products because environmental impact is minimal, and the product may be used more frequently than a FIFRA labeled product. This fundamentally appeals to desires for reapplication treatments when managing a significant mosquito outbreak or when mitigating arbovirus transmission. Furthermore, mosquito control is currently limited to two chemical classes for adulticides, which are the FIFRA regulated pyrethroids and organophosphates. However, exempt pesticides would provide different active ingredients for minimizing both resistance and environmental impacts.

One example of an exempt product, NatureCide Pest Management (NCPM), uses 25.3% cedarwood oil and 12.7% cinnamon oil as active ingredients. Cedarwood oil has been explored as a repellent against mosquitoes, ticks, and ants (Khanna and Chakraborty 2018; Eller et al. 2014), but has consistently shown high proclivity for killing arthropods, especially public health pests (Khanna and Chakraborty 2018; Eller et al. 2014; Singh et al. 1984). Cinnamon oil is an octopaminergic insecticide (Kostyukovsky et al. 2002) that expressed the greatest toxicity of eight adulticidal essential oils screened against adult *Culex quinquefasciatus* (Say) and *Musca domestica* (L.) (Benelli et al. 2018). It is also a synergist that increases the bioefficacy of other essential oils when presented together (Reegan et al. 2014).

The cedar and cinnamon oil mixture of NCPM is labeled for use against a variety of indoor and outdoor pests, including ants, fleas, filth flies, and other arthropods. Both of the aforementioned NatureCide products are not labeled for use as a space spray, instead being prescribed at rates for outdoor residual sprays. There is limited exploratory work with this and similar commercial products. However, utilization as a cold aerosol for ULV would provide more options to mosquito control. Therefore, we tested NCPM, which was recommended by the manufacturer for mosquito management, at the low end of its label rate to help determine the ULV potential of this alternative tool.

MATERIALS AND METHODS

The mosquito strain selected for testing was the 1952 Orlando strain *Aedes aegypti* sourced from the United States Department of Agriculture, Agricultural Research Service, Center for Medical, Agricultural, and Veterinary Entomology and reared in the insectaries of the Anastasia Mosquito Control District of St. Johns County. Mosquitoes were maintained at 26 ± 1.0°C, 65-80% relative humidity, and a photoperiod of 14:10 hr (L:D). The adult mosquitoes were provided 10% sugar solution as needed. Once mosquitoes were 5-7 d old, non-blood-fed females were selected for testing. To conduct assays, twenty females were transferred into cylindrical screened cages (4 x 10 cm) with the use of a HEPA-filtered mouth aspirator. Caged mosquitoes were acclimated to outdoor conditions for a minimum of 20 min prior to the start of any applications.

Treatments were carried out using NatureCide Pest Management (25.3% Cedarwood oil, 12.7% cinnamon oil, Pacific Shore Holdings, Inc., Canoga Park, CA). The label prescribed recommendation was to mix the product at a range of 35-122 ml per liter of water. For these tests, dilutions were arbitrarily selected at 35 ml/L and 70 ml/L. The formulation was applied by a truck-mounted single nozzle ULV cold aerosol sprayer (Guardian 95 ES, ADAPCO, LLC, Sanford, FL). The machine was calibrated to dispense droplets with an average size of 18 microns, spanning VMD of 10-30 microns (10 µ ≤ Dv 0.5 ≤ 30 µ), at 296 ml/min (10 oz/min). For each treatment, a row of polyvinyl chloride pipe stands, 1.2 m in height, held the mosquito cages mounted at 0.8-1 m above ground level. Stands were placed in three equidistant rows approximately 30 m, 60 m, and 90 m downwind from the truck drive path. Tests were conducted in the morning (0700 h-1100 h), with wind direction, wind speed, temperature, and relative humidity recorded on site. Spray trucks were driven at an average of 16 kilometers per hour in a straight line perpendicular to the length of the hanging field cage line. The treatment started 30 m prior to the first pipe stand and the treatment was shut off at 30 m past the last stand to ensure coverage during variable wind conditions. After the treatment, 15 min was allowed for drift to ensure passage of the spray plume downrange past the test plot before cages were gathered and returned to the laboratory for processing. Both dilutions were evaluated across three replications each. Once returned to the laboratory, mosquitoes were provided with 10% sucrose solution (in water) overnight using saturated cotton balls. Knockdown was recorded at 1 h and mortality was recorded 24 h post-treatment. Sets of 3 control cages per replicate were handled in an identical manner except being placed 30m upwind of the truck during application.

Data was corrected for control mortality below 10% by using Abbott's formula (Abbot 1925). Variation between field tested dilutions were analyzed in JMP 13.1.0 (SAS Institute, Inc., Cary, NC) using analysis of variance (ANOVA) and Tukey HSD test.

RESULTS AND DISCUSSION

Weather conditions averaged 27.5°C air temperature, 77.2% RH, and persistent south-southwest wind direction at 3.7 km/hr. Day conditions were clear and sunny with no persistent cloud cover or precipitation. Field assay data are summarized with mean and standard error of the mean (SEM) knockdown and mortality rates provided for 35 ml/L and 70 ml/L of NCPM in Fig. 1. There were no significant differences between the position in the 3 × 3 test array nor at discrete distances (30m, 60m, 90m) within knockdown ($F_{2,26}$ = 1.278, p = 0.3072) or mortality ($F_{2,26}$ = 2.4967, P = 0.1159) for 35ml/L. Treatments made at 70ml/L resulted in 100% knockdown and mortality at all distances and all replications (p < no variance). The lowest rate, 35 ml/L, averaged 60-70% knockdown

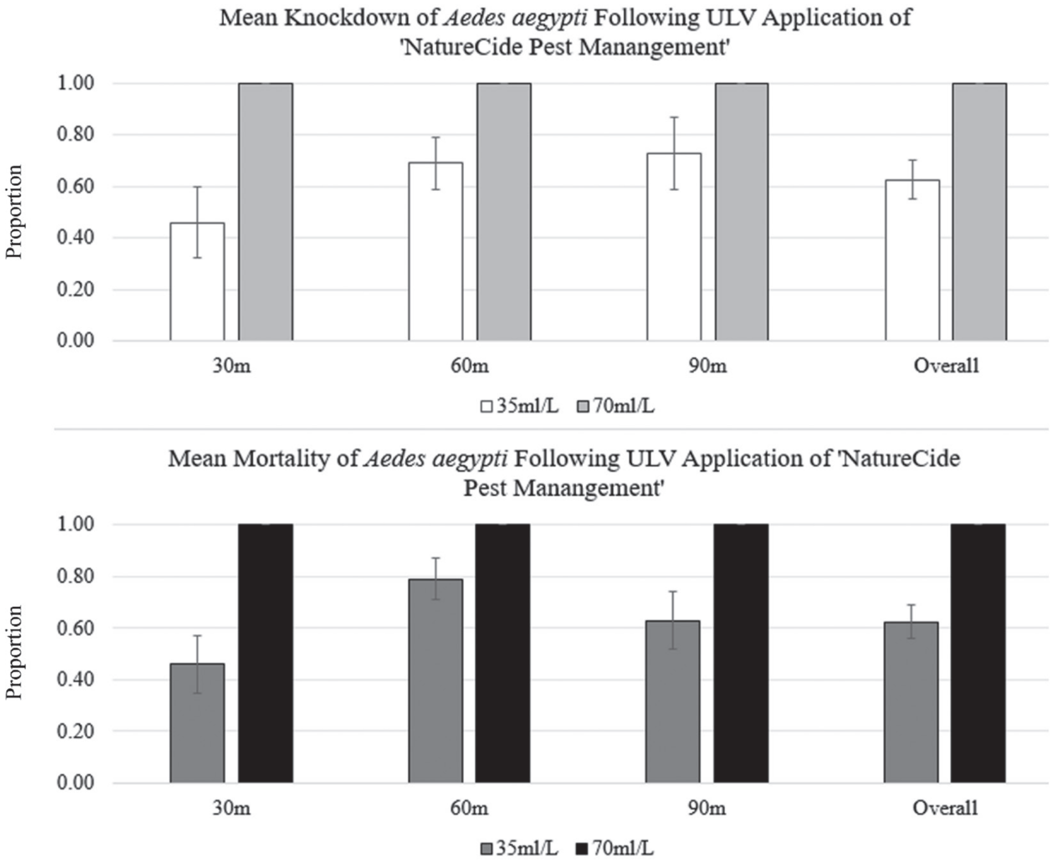

Figure 1. Significant mean (+ SEM) 1 hr knockdown and 24 hr mortality of *Aedes aegypti* (L.) were observed following ULV treatment with 35 ml/L and 70 ml/L of NatureCide Pest Management (25.3% cedarwood oil, 12.7% cinnamon oil) in a 3 × 3 grid with 30 m equidistant separations between mosquito cages (F = 5.34, df = 5, 54, p < 0.0005). There were no significant differences between the position in the 3 × 3 test array nor at discrete distances (30m, 60m, 90m) within knockdown ($F_{2,26}$ = 1.278, p = 0.3072) or mortality ($F_{2,26}$ = 2.4967, P = 0.1159) for 35ml/L. Treatments made at 70ml/L resulted in 100% knockdown and mortality at all distances and all replications (p < no variance). Treatments with 35 ml/L and 70 ml/L fell inside the low end of the label allowed rates of 35-122ml per liter of water.

and mortality among the exposed *Ae. aegypti* (Fig. 1). In contrast, all values for 70 ml/L were 100% for knockdown and mortality regardless of distance or position (Fig. 1). Knockdown and mortality were significantly greater at 70 ml/L than 35 ml/L, which was significantly greater than observed in the controls (F = 5.34, df = 5, 54, $p < 0.0005$). Control mortality was 0% in all trials.

Unexpectedly, crystalline precipitates were found on the surface of the liquid (Fig. 2) in the mix tank after the solution aged on the truck ULV assembly for 2 wk. Replicates using precipitated mixture were omitted from the data analysis, however there did not appear to be an obvious toxicity change when using precipitated mixtures. Freshly diluted product was used for each replicate and mix tanks were held for 6 wk after use. The crystalline precipitation occurred in all mixes regardless of which dilution. Agitation did not appear to resolve the precipitation of aged mixtures. Precipitation did not occur when mixtures were kept in cooler, laboratory conditions.

We intended to test farther into the label range for NCPM, however it was surprising to see it was not necessary to go higher than 70 ml/L, and perhaps not even necessary to go much higher than 35 ml/L. We did not test larvicide potential in this study, but it is also possible that exempt products made from botanical ingredients may be equally useful for larvicides as they are for adulticides (Norris et al. 2015). Furthermore, there may be additional benefits of NCPM in broader integrated management questions. Several examples of botanical oils for mosquito control are functional as repellents (Gross and Coats 2015) or synergists (Tong and Bloomquist 2013; Gross et al. 2017). Intensive screening of 361 essential oils from 269 plant spe-

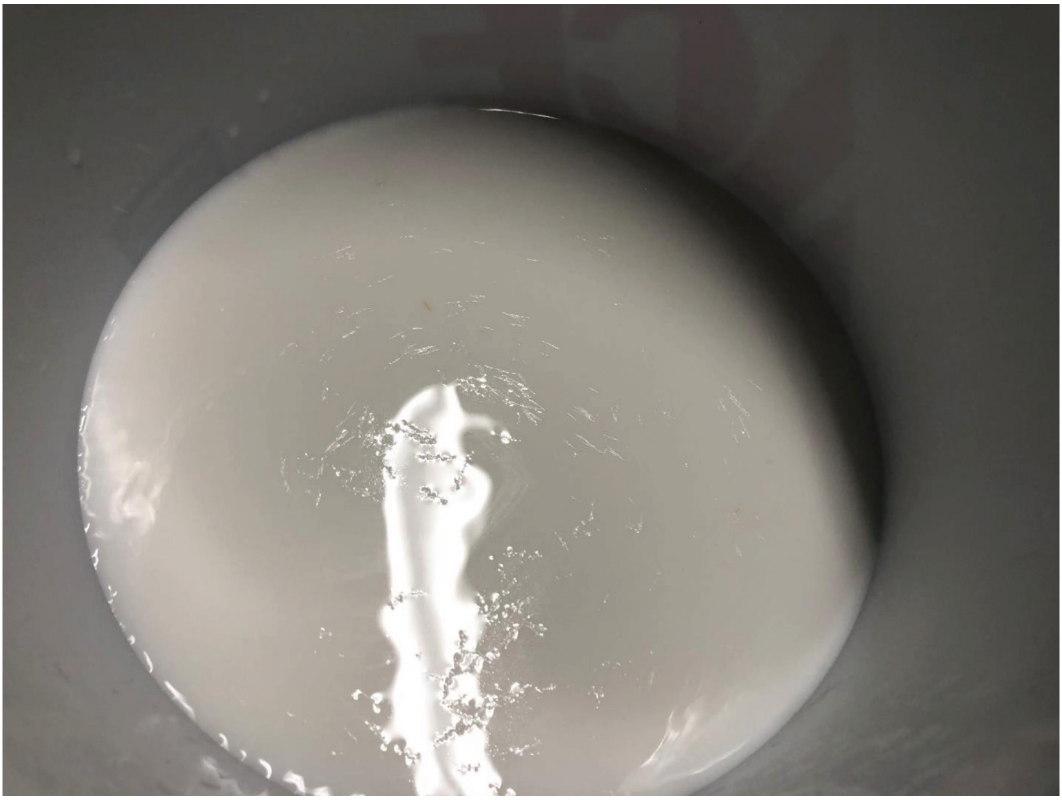

Figure 2. Crystalline precipitation in the mix tank for NatureCide Pest Management (25.3% cedarwood oil, 12.7% cinnamon oil) after 2 wk of storage after a replicate of truck mounted ultra-low volume cold aerosol treatment. Mixtures were left on the truck between the conclusion of treatment and the time of this image.

cies revealed dozens of potential larvicides against *Ae. aegypti* (Dias & Moraes 2013). Despite the aforementioned evidence, mosquito control has been slow to acquire botanical products for residual treatments or ultra-low volume (ULV) cold aerosol space sprays. By translating NatureCide Pest Management or similar products into public health operations, mosquito control can gain wider access to "green" alternative adulticides that do not have reapplication restrictions.

NatureCide Pest Management an EPA exempt product currently labeled for indoor and outdoor residual spot treatments against an assortment of urban and peridomestic insect pests. Meanwhile, the active ingredients, essential oils, appeal to eco-friendly proponents of botanical insecticides while still presenting a potentially effective mosquito adulticide. There may be broader utility in using these products if it also expands the circumstances or land area in which intervention can be made to reduce mosquitoes. The success of microemulsion formulations appears to be one reason that products may become more available from the discovered bioactive essential oils (Montefuscoli et al. 2013, Gross et al. 2017). Other FIFRA exempt products also have shown high comparative efficacy. Evaluation of an exempt sister product, NatureCide All-Purpose Commercial Concentrate containing clove and cottonseed oil, showed that when used as a vegetative barrier spray it outperformed Essentria IC^3 (rosemary oil, peppermint oil), Onslaught (fenvalerate), DeltaGard (deltamethrin), and performed equivalently with Cyzmic (lambda-cyhalothrin) (Smoleroff et al. 2019).

However, the stability of the microemulsions is not well understood in an operational context. The precipitation we observed in the mix tanks may imply that precautions need to be made with NCPM or similar essential oil emulsions if incorporating them into the machinery used in mosquito control operations. As an additional consideration, understanding non-target effects may in turn facilitate expansion of the label and trust in the blend of active ingredients in NCPM and similar products. Given the exemption status and consequent potential to reapply this insecticide frequently, it is critical to understand the non-target impacts of application on key pollinators or to water ecology. Regardless of the gaps in knowledge, we believe our positive results using NatureCide Pest Management as a ULV treatment highlights that some botanicals are ready to be incorporated into mosquito control programs.

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