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# Suppressing *Aedes albopictus*, an Emerging Vector of Dengue and Chikungunya Viruses, by a Novel Combination of a Monomolecular Film and an Insect-Growth Regulator

Mark Nelder, Banugopan Kesavaraju,* Ary Farajollahi, Sean Healy, Isik Unlu, Taryn Crepeau,
Ashok Ragavendran, Dina Fonseca, and Randy Gaugler
*Center for Vector Biology, Department of Entomology, Rutgers University, New Brunswick, New Jersey;
Mercer County Mosquito Control, West Trenton, New Jersey; Monmouth County Mosquito Extermination Commission,
Eatontown, New Jersey; Department of Animal Sciences, Purdue University, West Lafayette, Indiana*

*Abstract.* The Asian tiger mosquito *Aedes albopictus* (Skuse) is rapidly increasing its global range and importance in transmission of chikungunya and dengue viruses. We tested pellet formulations of a monomolecular film (Agnique) and (S)-methoprene (Altosid) under laboratory and field conditions. In the laboratory, Agnique provided 80% control for 20 days, whereas Altosid, in combination with Agnique, provided 80% control for > 60 days. During field trials, the 1:1 pellet ratio of combined products provided > 95% control for at least 32 days and 50% control for at least 50 days. Altosid remained effective after a 107-day laboratory-induced drought, suggesting that the product serves as a means of control during drought conditions and against spring broods in temperate regions. Agnique and Altosid, when used in tandem for cryptic, difficult-to-treat locations, can provide long-term control of *Ae. albopictus* larvae and pupae. The possible additive or synergistic effects of the combined products deserve further investigation.

## INTRODUCTION

The Asian tiger mosquito *Aedes albopictus* (Skuse) is an important vector of dengue virus (DENV, the causative agent of dengue), of which there are globally 100 million infections per year.[1] *Ae. albopictus* is the primary rural and suburban vector in the DENV foci of Asia and the Pacific Islands.[2] More recently, *Ae. albopictus* has become the primary vector of chikungunya virus (CHIKV), an arboviral disease infecting over 1 million people in India and the Indian Ocean islands.[3,4] *Ae. albopictus* has created concern in areas where this mosquito has invaded and caused epidemics of CHIKV, such as Italy and La Réunion.[5,6] In Gabon, *Ae. albopictus* is the primary vector of CHIKV and DENV-2 in urban, suburban, and rural areas, where 8 of 3,211 individuals have been diagnosed with concurrent infections.[7] Since its introduction into the continental United States in 1985, *Ae. albopictus* has spread to 37 states, creating public health concerns.[8] Cache Valley virus, eastern equine encephalitis virus, Jamestown Canyon virus, La Crosse virus (LACV), and West Nile virus (WNV) have all been detected in North American *Ae. albopictus*.[9–12] The coupling of vector and biting nuisance status underscores the importance of control of *Ae. albopictus* for public health worldwide.

Monomolecular films (MMF) such as alcohol-ethoxylated surfactants (Agnique and Arosurf) have been used against immature mosquitoes since the early 1980's.[13] After being added to water, they interfere with the surface tension, preventing larvae and pupae from remaining on the surface and breathing. Insect-growth regulators, such as (S)-methoprene (Altosid), have been used to combat immature mosquitoes since the mid 1970's. (S)-methoprene is a mimic of juvenile hormone III (naturally found in mosquitoes). It targets fourth instars by interfering with diploid cell division and apoptosis in polytene cells, and it creates midguts in pupae that are similar to larval midgets, preventing normal development.[14] MMFs produce rapid mortality (less than 48 hours), whereas insect-growth regulators may take up to 7 days, depending on the age structure of the immature community (most effective against late instars). The concept of combining an MMF with an insect-growth regulator,[15] however, has not been evaluated.

The success of *Ae. albopictus* as an invader is partly because of the pervasiveness and varied nature of its immature habitats, which include artificial (e.g., trash, tarps, plastic toys, and tires) and natural containers (e.g., tree holes and bamboo internodes).[16] More importantly, this mosquito is found primarily in urban areas, where inspection and control by mosquito-abatement personnel are logistically difficult. For effective control of *Ae. albopictus* immatures, operationally feasible, cost-effective, and long-acting measures are needed. Over most parts of its range, there are no interventions specifically directed against *Ae. albopictus* by mosquito control programs. Considering the relatively large number of containers that tend to be hidden or difficult to reach and treat in urban residential centers, we preferred to use a relatively quick acting agent (i.e., where dead larvae and pupae are visualized in 24–48 hours) and a long residual. Currently, products available to treat *Ae. albopictus* and mosquitoes of similar life histories are either quick-acting pesticides with little residual effect or slower-acting pesticides with a residual effect. We tested the dual use of a MMF (Agnique) and an insect-growth regulator (Altosid) against larvae and pupae of *Ae. albopictus* under field and laboratory conditions to evaluate their combined effectiveness. Our study is the first report combining these insecticides for combating *Ae. albopictus*, showing a potentially novel tool for controlling container-inhabiting mosquitoes.

## MATERIALS AND METHODS

**Laboratory trials.** *Colony maintenance.* The *Ae. albopictus* used in all laboratory trials was $F_4$ generation, initially collected as larvae during August 2007 from an auto-salvage yard in Trenton, NJ. Maintenance of the colony and rearing of test larvae followed established protocols.[17] Briefly, egg papers were submerged in 2 L of tap water (1 paper per enamel tray) containing 0.15 g of lactalbumin/Brewer's yeast (1:1 ratio by mass), and eggs were allowed to hatch at 27°C under a 16-hour light to 8-hour dark photoperiod. Egg papers with unhatched eggs were removed from trays after 24 hours to ensure uniform hatch (i.e., production of larvae of similar age). Developing

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* Address correspondence to Banugopan Kesavaraju, Salt Lake City Mosquito Abatement District, 2020 North Redwood Road, Salt Lake City, UT 84116. E-mail: banu@slc-mosquito.com

larvae were fed on finely ground rat chow (day 3 = 0.5 g in 50 mL tap water; days 5, 7, and 9 = 0.5 g in 50 mL tap water) until they reached the fourth instar. Only fourth instars were used in all trials.

*Combined efficacy of Agnique and Altosid.* Pellet formulations of the MMF, Agnique, were obtained from Cognis Corp. (Cincinnati, OH), and the (S)-methoprene, Altosid, was from Wellmark International (Schaumburg, IL). Twenty randomly selected pellets were weighed to establish mean pellet mass for each product (Agnique = 0.06 ± 0.006 g; Altosid = 0.13 ± 0.009 g).

All trials were performed in 400-mL Tri-Pour (VWR, West Chester, PA) lidded containers with 250 mL of tap water, 10 fourth instars, and 0.04 g of lactalbumin/Brewer's yeast (1:1 ratio). Insecticide treatments, each with five containers, were as follows: Treatment 1 (T1), 0.06 g Agnique/0.00 g Altosid (Agnique only); T2, 0.03/0.065; T3, 0.03/0.13; T4, 0.03/0.26; T5, 0.06/0.065; T6, 0.06/0.13; T7, 0.06/0.26; T8, 0.12/0.065; T9, 0.12/0.13; T10, 0.12/0.26; and T11, 0.00/0.13 (Altosid only). Controls (C) consisted of 10 cups without insecticides. Insecticides were applied to containers 2 hours before starting the experiment. Survival was measured as the number of adults that emerged from pupae in 10 days. After 10 days, all remaining larvae, pupae, and adults were removed, and a fresh batch of 10 fourth instars was introduced. The procedure was repeated 11 times for a test duration of 120 days. All trials were conducted at 25–27°C with a 12-hours light and 12-hours dark photoperiod. Water was maintained at 250 ± 10 mL by adding water at 10-day intervals. Number of adults emerging in each period was converted to proportions and analyzed in SAS (Cary, NC) using the REPEATED function in PROC MIXED[18] with days (time) as a continuous variable.[19] Proportions were arcsine-square root transformed before statistical analysis to ensure normality. Multiple comparisons were performed by adjusting with the Tukey's least significant difference (LSD) method. We predicted a significant treatment by time interaction that would indicate that the slopes for each treatment were not parallel.

*Efficacy of Agnique and Altosid after drying in the laboratory.* We further tested the hypothesis that efficacy of Altosid would decrease after prolonged drying. Accordingly, at the end of the 120-day trial, the covered containers were allowed to dry. After 107 days, on March 6, 2009, the containers were flooded with 250 mL of tap water, 10 fourth instars were added per container, and 0.04 g of lactalbumin/Brewer's yeast (1:1 ratio) was added. Survival was measured as in previous trials, and experimental conditions remained the same. The proportion (arcsine-square root transformed before statistical analysis) surviving was analyzed by analysis of variance (ANOVA). Multiple comparisons with Tukey's adjustment were performed as follow-up tests. We also compared the proportion surviving (arcsine-square root transformed) at the end of the 120-day trials pre-drying and post-drying with flooding; a *t* test was used for each treatment.

*Stage-specific mortality using Agnique.* We also tested the hypothesis that Agnique efficacy would decrease as larval age and size increased. Ten larvae (first, second, third, and fourth instars) each were placed in each container (10 containers per stage, 5 controls, 5 treatments, and 400 mL of Tri-Pour) with 250 mL of tap water and 0.04 g of lactalbumin/Brewer's yeast (1:1 ratio). Ten control containers with 10 larvae per container were tested for each stage. One-half of an Agnique pellet (average mass = 0.03 g) was applied to each container, and survival was measured after 48 hours. Experimental conditions followed the protocols established in Agnique and Altosid combination trials. Data were analyzed with an ANOVA with number surviving as the dependent variable and treatment (control, Agnique), instar, and their interaction as the independent variables.

**Field trials.** *Site descriptions and climate.* Suburban and urban field plots (100 m$^2$) were selected in Mercer County and Monmouth County, New Jersey. Mercer County is located northeast of Philadelphia, PA, and it borders the Delaware River in the city of Trenton. Monmouth County is south of New York City, NY, and it borders the Atlantic Ocean and Raritan Bay. These sites were chosen based on county surveillance reports indicating established *Ae. albopictus* populations. Experiments were conducted July 24, 2008 through October 31, 2008 (in Mercer County, trials ended October 24, 2008) using local populations of *Ae. albopictus*.

For Mercer County, urban sites were located in Trenton. Site 1 was an active automotive repair shop located within an industrial area (40°14′ N, 74°44′ W). Site 2 was an automotive repair shop within a suburban neighborhood (40°14′ N, 74°44′ W). Residential homes in site 2 were comprised of row houses of similar age and size. Tall trees (*Quercus* and *Acer* spp.) and shrubs along the fencing provided shade for resting *Ae. albopictus* for both experimental sites. Further information on selected sites in Mercer County has been described in detail previously.[20] Precipitation and temperature data, during the trials, were obtained from the closest weather station to study sites (Figure 1).

In Monmouth County, site 1 was located in a suburban location in Keyport Marine Basin, a marina northwest of Keyport, NJ (40°34′ N, 74°21′ W). This site was the initial location in New Jersey where *Ae. albopictus* was detected in 1995,[21] and it maintains high populations of *Ae. albopictus*. The canopy was dominated by deciduous trees (*Quercus* spp.), and the underbrush was weeds, grasses including reeds (*Phragmites* spp.), and poison ivy (*Toxicodendron radicans* L.). Site 2 was within Earle Naval Weapons Station located in Leonardo, NJ (location information withheld). American sweetgum (*Liquidambar styraciflua* L.), oaks (*Quercus* spp.), and maples (*Acer* spp.) dominated the canopy at the site with a variety of grasses and small shrubs comprising the underbrush.

*Experimental design.* Our simulated field habitats with natural *Ae. albopictus* populations consisted of 11-L black, plastic buckets. At each field site, 10 control and 10 treatment buckets were paired and randomly placed in shaded areas. On July 24, 2008, buckets received 8 L of tap water with 5 g of crushed oak leaves for nutrients.[22,23] Water levels were maintained throughout field trials by drilling four 0.5-cm holes just above the 8-L water-level mark to allow for overflow, and 5-day aged tap water was added as needed. Buckets were placed in field locations 15 days pre-treatment to allow for sufficient natural *Ae. albopictus* oviposition.

A pre-treatment collection was made August 8, 2008 from each bucket using an aquarium fish net (25 × 55 cm). Captured larvae and pupae were reared in the laboratory for identification. Immediately thereafter, one pellet of Agnique and one pellet of Altosid were applied to each treatment bucket. Post-treatment collections were made at 7-day intervals. Briefly, for all collections, the net was dipped into the bucket perpendicular to the water surface and turned 360° two times to make two

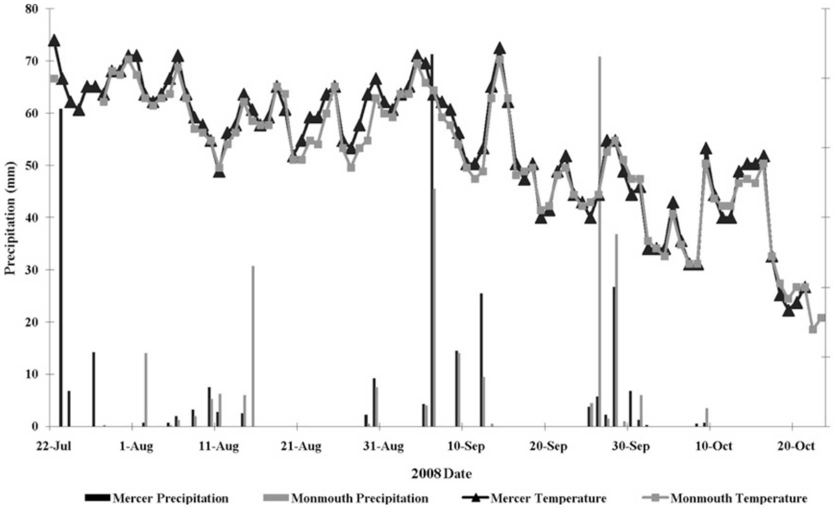

FIGURE 1. Temperature and precipitation for Mercer and Monmouth County sites.

complete circles, and then, the net was removed perpendicular to the water surface. Only pupae were collected, because they received maximal (S)-methoprene exposure, whereas larvae would have received a reduced dose. If more than 15 pupae were collected, a random block was used in the larval tray to select 15 pupae. The pupae collected were taken to the laboratory and allowed to develop in 200 mL of tap water (in Tri-Pour cups with a cover to contain emerging adults) until emergence or mortality. Pupae were held at 22–26°C under a 12-hour dark and 12-hour light photoperiod regimen. Containers were checked daily for 14 days for adult emergence, and pupal survival was scored as in the laboratory trials. The first collection was performed on August 8, 2008, and collections continued weekly for 70 days for Mercer County sites and 77 days for Monmouth County sites.

Numbers of adults emerging for each weekly collection were converted to their proportions and analyzed using the REPEATED statement in PROC MIXED.[18] The independent variables in the model were site, treatment, time, and their appropriate interactions. The interaction between site and treatment was not significant for both counties and subsequently, was removed from the models. Proportions were arcsine-square root transformed before statistical analysis. Least square estimates were obtained and compared for each period using Tukey's adjustment. We predicted a significant interaction between treatment and time, indicating that the slopes were not parallel.

## RESULTS

**Laboratory trials.** *Combined efficacy of Agnique and Altosid.* Time, treatment, and their interaction were significant, indicating that the slopes for each treatment differed (Tables 1 and 2). Survival was highest in the control and lowest in the Altosid groups (T2–T11), and the Agnique alone (T1) group was in between the other groups (Table 2). Multiple comparisons revealed that survival in Agnique (T1) and control (C) differed until day 60 but was not different from the 70th day to the 110th day, indicating that the insecticidal effects of Agnique disappeared after 70 days. Survival in the Altosid group (T2–T11) was different from the Agnique alone (T1) and control (C) groups from the 10th day to the 110th day, indicating that the effects of Altosid did not dissipate. There was no significant difference in survival among the Altosid groups (T2–T11) from the 10th day to the 50th day. Nevertheless, the treatment that received the lowest Agnique and Altosid (T2) amount was different from the treatments that received the highest Altosid amounts (T4 and T10) from the 60th day to the 110th day. In several treatments, the combined Agnique and Altosid treatments were more effective against fourth instar *Ae. albopictus* than Agnique or Altosid only.

*Efficacy of Agnique and Altosid after drying in the laboratory.* The treatment was significant, indicating that larval survival was not the same for the treatment containers that were allowed to dry (treatment, $F_{11,48} \leq 0.0001$). Multiple comparisons revealed that survival was not different between control, Agnique alone, and the T2 treatment, which had the lowest Altosid levels among the combinations (Figure 2). All other treatments, which had higher levels of Altosid (T3–T11), were not significantly different from each other (Figure 2). There

TABLE 1

Repeated measures analyses of laboratory and field experiments using combinations of Agnique and Altosid against immature *Ae. albopictus*

| Variable | DF | F | *P* |
|---|---|---|---|
| Laboratory experiments | | | |
| Combined efficacy (Agnique and Altosid) | | | |
| Time | 1 | 99.74 | < 0.0001 |
| Treatment | 11 | 37.17 | < 0.0001 |
| Time × treatment | 11 | 4.15 | < 0.0001 |
| Field experiments | | | |
| Mercer County | | | |
| Site | 1 | 8.52 | 0.0062 |
| Time | 1 | 23.30 | < 0.0001 |
| Treatment | 1 | 81.92 | < 0.0001 |
| Time × treatment | 1 | 18.49 | 0.0001 |
| Monmouth County | | | |
| Site | 1 | 2.27 | 0.1382 |
| Time | 1 | 0.27 | 0.6054 |
| Treatment | 1 | 34.03 | < 0.0001 |
| Time × treatment | 1 | 8.55 | 0.0044 |

DF = degrees of freedom.

Table 2

Results from multiple comparison analyses of the laboratory trial on the combined efficacy of Agnique and Altosid against *Ae. albopictus*

| Treatment | | Proportion surviving days post-treatment | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 | 110 |
| Control | Mean | 0.976 | 0.974 | 0.612 | 0.875 | 0.639 | 0.910 | 0.939 | 0.711 | 0.907 | 0.720 | 0.950 |
| | SE | 0.005 | 0.003 | 0.013 | 0.016 | 0.004 | 0.005 | 0.005 | 0.007 | 0.008 | 0.016 | 0.006 |
| | | A | A | A | A | A | A | A | A | A | A | A |
| T1: Ag only (0.06 g) | Mean | 0.079 | 0.174 | 0.584 | 0.887 | 0.872 | 0.861 | 0.768 | 0.732 | 0.948 | 0.856 | 0.925 |
| | SE | 0.006 | 0.017 | 0.005 | 0.008 | 0.025 | 0.014 | 0.019 | 0.022 | 0.021 | 0.012 | 0.013 |
| | | B | B | B | B | B | A | A | A | A | A | A |
| T2: Ag (0.03 g) + Al (0.065 g) | Mean | 0 | 0.004 | 0.037 | 0.040 | 0.095 | 0.079 | 0.188 | 0.084 | 0.368 | 0.225 | 0.268 |
| | SE | 0 | 0.004 | 0.006 | 0.018 | 0.057 | 0.045 | 0.025 | 0.018 | 0.030 | 0.082 | 0.032 |
| | | C | C | C | C | C | B | B | B | B | B | B |
| T3: Ag (0.03 g) + Al (0.13 g) | Mean | 0 | 0 | 0 | 0.032 | 0.004 | 0.009 | 0.137 | 0.013 | 0.234 | 0.147 | 0.048 |
| | SE | 0 | 0 | 0 | 0.014 | 0.004 | 0.009 | 0.032 | 0.013 | 0.003 | 0.010 | 0.009 |
| | | C | C | C | C | C | BC | BC | BC | BC | BC | BC |
| T4: Ag (0.03 g) + Al (0.26 g) | Mean | 0 | 0 | 0.009 | 0.009 | 0.048 | 0 | 0.016 | 0.032 | 0.073 | 0.034 | 0.037 |
| | SE | 0 | 0 | 0.009 | 0.009 | 0.009 | 0 | 0.006 | 0.014 | 0.028 | 0.013 | 0.006 |
| | | C | C | C | C | C | C | C | C | C | C | C |
| T5: Ag (0.06 g) + Al (0.065 g) | Mean | 0 | 0 | 0 | 0.004 | 0.016 | 0.004 | 0.105 | 0.052 | 0.144 | 0.053 | 0.073 |
| | SE | 0 | 0 | 0 | 0.004 | 0.006 | 0.004 | 0.012 | 0.021 | 0.025 | 0.020 | 0.014 |
| | | C | C | C | C | C | BC | BC | BC | BC | BC | BC |
| T6: Ag (0.06 g) + Al (0.13 g) | Mean | 0 | 0 | 0 | 0.004 | 0.004 | 0.037 | 0.073 | 0.009 | 0.192 | 0.061 | 0.128 |
| | SE | 0 | 0 | 0 | 0.004 | 0.004 | 0.006 | 0.014 | 0.009 | 0.003 | 0.011 | 0.025 |
| | | C | C | C | C | C | BC | BC | BC | BC | BC | BC |
| T7: Ag (0.06 g) + Al (0.26 g) | Mean | 0 | 0 | 0.009 | 0 | 0.016 | 0.004 | 0.095 | 0.079 | 0.196 | 0.009 | 0.037 |
| | SE | 0 | 0 | 0.009 | 0 | 0.006 | 0.004 | 0.007 | 0.006 | 0.002 | 0.009 | 0.006 |
| | | C | C | C | C | C | BC | C | C | C | BC | BC |
| T8: Ag (0.12 g) + Al (0.065 g) | Mean | 0 | 0 | 0.004 | 0.004 | 0.037 | 0 | 0.009 | 0.024 | 0.232 | 0.270 | 0.048 |
| | SE | 0 | 0 | 0.004 | 0.004 | 0.006 | 0 | 0.009 | 0.010 | 0.004 | 0.006 | 0.009 |
| | | C | C | C | C | C | BC | BC | BC | BC | BC | BC |
| T9: Ag (0.12 g) + Al (0.13 g) | Mean | 0 | 0 | 0 | 0.009 | 0.024 | 0.016 | 0.004 | 0.184 | 0.100 | 0.004 | 0.102 |
| | SE | 0 | 0 | 0 | 0.009 | 0.010 | 0.006 | 0.004 | 0.027 | 0.019 | 0.004 | 0.018 |
| | | C | C | C | C | C | BC | C | C | C | BC | BC |
| T10: Ag (0.12 g) + Al (0.26 g) | Mean | 0 | 0 | 0 | 0 | 0 | 0.004 | 0 | 0.004 | 0.137 | 0.034 | 0.016 |
| | SE | 0 | 0 | 0 | 0 | 0 | 0.004 | 0 | 0.004 | 0.001 | 0.013 | 0.006 |
| | | C | C | C | C | C | BC | C | C | C | C | C |
| T11: Al only (0.13 g) | Mean | 0 | 0 | 0.004 | 0.040 | 0.004 | 0.004 | 0 | 0.024 | 0.258 | 0.048 | 0.037 |
| | SE | 0 | 0 | 0.004 | 0.018 | 0.004 | 0.004 | 0 | 0.010 | 0.001 | 0.009 | 0.006 |
| | | C | C | C | C | C | BC | C | C | C | C | BC |

Columns with similar letters under each period (days) do not differ significantly. SE = standard error; Ag = Agnique; Al = Altosid.

were no differences between the 120-day pre-drying survival and the post-drying with flooding survival (Table 3). Our results indicate that drying of habitats, under laboratory conditions, does not neutralize the effects of Altosid against fourth instar *Ae. albopictus*.

*Stage-specific mortality using Agnique alone.* The interaction between treatment and instar stage was significant, indicating that survival was dependent on both treatment and instar (treatment × instar, $F_{3,72} \leq 0.0001$). Multiple comparisons revealed that survival was highest for first to third instars and lowest for fourth instars in the Agnique (Figure 3); therefore, Agnique has limited impact on *Ae. albopictus* first to third instars.

**Field trials.** In Mercer County, time and the interaction between time and treatment were significant, indicating that the slopes of control and the Agnique and Altosid combination were different. Multiple comparisons revealed that *Ae. albopictus* survival was different between a combination of Agnique and Altosid and the control from 7 to 70 days post-treatment (Figure 4). In Monmouth County, time was not significant, but the interaction between time and treatment was significant, indicating that the slopes were different. Multiple comparisons revealed that the survival was different from control and Agnique and Altosid combination from 7 to 70 days post-treatment. There was no difference between control and Agnique and Altosid combination on the 77th day, indicating that activity diminished after 70 days (Figure 4).

The pre-treatment collection in Mercer County yielded five species: *Culex pipiens pipiens* L. (50% of collections), *Ae. albopictus* (45%), *Culex restuans* Theobald (2%), *Aedes japonicus japonicus* Theobald (2%), and *Aedes triseriatus* Say (1%). The pre-treatment collection in Monmouth County yielded four species: *Ae. albopictus* (70%), *Cx. p. pipiens* (25%), *Cx. restuans* (4%), and *Toxorhynchites rutilus septentrionalis* Dyar and Knab (1%).

## DISCUSSION

Agnique and Altosid used simultaneously provided effective and long-lasting control against *Ae. albopictus*. The addition of Agnique to Altosid provided a minimal improvement in efficacy, but more work is needed to elucidate potential additive or synergistic effects of Agnique. Tandem use suppressed *Ae. albopictus* for at least 120 days in the laboratory and 32 days in the field. Combinations of the MMF Arosurf with other insecticides, including *Bacillus thuringiensis* var. *israelensis*, diesel oil, diesel-isopropyl mix, temephos, or *Bacillus sphaericus*, were tested in the laboratory in the 1980s against *Anopheles albimanus* Wiedemann and *Culex quinquefasciatus* (Say) with favorable results.[24–28] These combinations provided

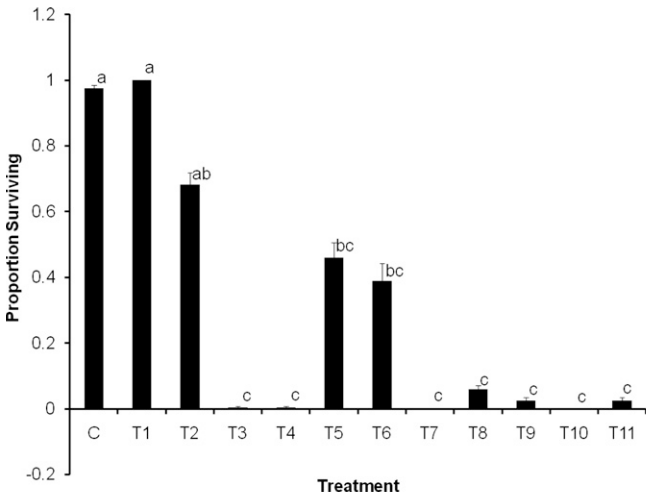

FIGURE 2. Survival (back transformed means ± SE) of *Ae. albopictus* using 11 different combinations of Agnique and Altosid under laboratory drought conditions (107 days). Treatments with different letters are significantly different ($P < 0.05$).

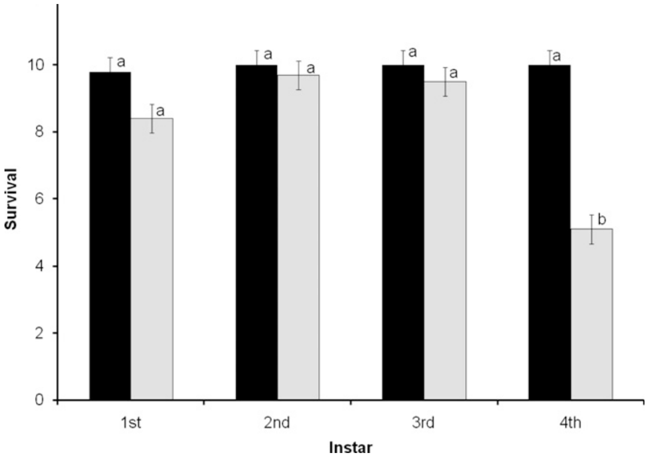

FIGURE 3. Instar-specific survival (mean ± SE) of *Ae. albopictus* larvae after 48 hours in the laboratory (treated with Agnique [gray] and untreated control [black]). Ten larvae were tested in 10 containers for each instar ($N = 100$). Means within a treatment or control followed by different letters are significantly different at $P < 0.05$.

greater control than either product alone, indicating additive or synergistic effects. Arosurf in combination with *Bacillus thuringiensis* var. *israelensis* (several formulations) and nonanoic and oleic acids have been field tested against *An. albimanus*, *Cx. quinquefasciatus*, *Culex tarsalis* Coquillett, and *Culex peus* Speiser with efficacy that matched laboratory studies.[25,29] The new combination of existing products targeting *Ae. albopictus* that we tested provides new options for controlling this container mosquito.

(S)-methoprene remained active under artificial drought conditions in the laboratory, suppressing *Ae. albopictus*. Although field validation is needed, this compound may remain effective even when containers dry and reflood. For example, fall and winter (S)-methoprene treatments would affect the subsequent spring generation through residual action. Immature habitats of *Ae. albopictus*, especially artificial containers, routinely dry in the heat of summer. (S)-methoprene will degrade when exposed to ultraviolet light, but the charcoal matrix of Altosid might provide UV protection and prolong its larvicidal properties. Altosid may be useful under drought and flooding conditions with implications for combating spring emergence and populations in container habitats.

TABLE 3

Back transformed mean ± SE and *t* test comparisons for 120 days pre-drying and post-drying with flooding survival in the drying experiment

| Treatment | Pre-drying (120 days) survival (mean ± SE) | Post-drying with flooding survival (mean ± SE) | *P* values (*t* test) |
|---|---|---|---|
| Control | 0.976 ± 0.010 | 0.957 ± 0.017 | 0.7728 |
| T1: Ag only (0.06 g) | 1.000 ± 0 | 0.925 ± 0.013 | 0.0705 |
| T2: Ag (0.03 g) + Al (0.065 g) | 0.681 ± 0.038 | 0.268 ± 0.032 | 0.0612 |
| T3: Ag (0.03 g) + Al (0.13 g) | 0.004 ± 0.004 | 0.048 ± 0.009 | 0.0947 |
| T4: Ag (0.03 g) + Al (0.26 g) | 0.004 ± 0.047 | 0.037 ± 0.006 | 0.1778 |
| T5: Ag (0.06 g) + Al (0.065 g) | 0.460 ± 0.054 | 0.073 ± 0.014 | 0.1637 |
| T6: Ag (0.06 g) + Al (0.13 g) | 0.388 ± 0 | 0.128 ± 0.025 | 0.2132 |
| T7: Ag (0.06 g) + Al (0.26 g) | 0 ± 0 | 0.037 ± 0.006 | 0.0705 |
| T8: Ag (0.12 g) + Al (0.065 g) | 0.059 ± 0.012 | 0.048 ± 0.009 | 0.8349 |
| T9: Ag (0.12 g) + Al (0.13 g) | 0.024 ± 0.010 | 0.102 ± 0.018 | 0.1571 |
| T10: Ag (0.12 g) + Al (0.26 g) | 0 ± 0 | 0.037 ± 0.006 | 0.0705 |
| T11: Al only (0.13 g) | 0.024 ± 0.010 | 0.037 ± 0.006 | 0.6629 |

Ag = Agnique; Al = Altosid.

Agnique alone suppressed fourth instar *Ae. albopictus* with minimal efficacy for first to third instars. Arosurf has also been shown as ineffective against first and second instar *Aedes aegypti* L,[30] *Aedes taeniorhynchus* (Wiedemann), *An. albimanus*, *Anopheles quadrimaculatus* (Say), and *Anopheles stephensi* (Liston).[28,31–33] Early instar culicine mosquitoes can use transcuticular oxygen for respiration, and the proportion of time spent at the surface for respiration increases in successive instars.[34] Our study provides insight into larval susceptibility of *Ae. albopictus* to the MMF Agnique, providing valuable operational information to vector control programs. Control measures against immature *Ae. albopictus*, using Agnique as the sole means, must consider the population dynamics of the immature community and timing of applications.

The tandem use of Agnique and Altosid provided a 95% reduction of *Ae. albopictus* in the field for at least 32 days. According to product labels, Agnique provides up to 22 days of control and Altosid provides 30 days of control, well below the efficacy that we showed under urban and suburban conditions. Artificial containers, as used in our field trials, reduce product exposure to ultraviolet radiation and wind, thereby increasing residual activity of pesticides. Application of one Altosid pellet per tire provided 116 days of *Ae. albopictus* control in Florida.[35] Two grams of Altosid pellets provided 150 days of control against *Ae. albopictus* in small bowls in Louisiana.[36] Longer control was achieved in these studies compared with ours; however, these studies used higher doses based on smaller container volumes.

The combination of a MMF (Agnique) and an insect-growth regulator (Altosid) provides effective and long-lasting reduction

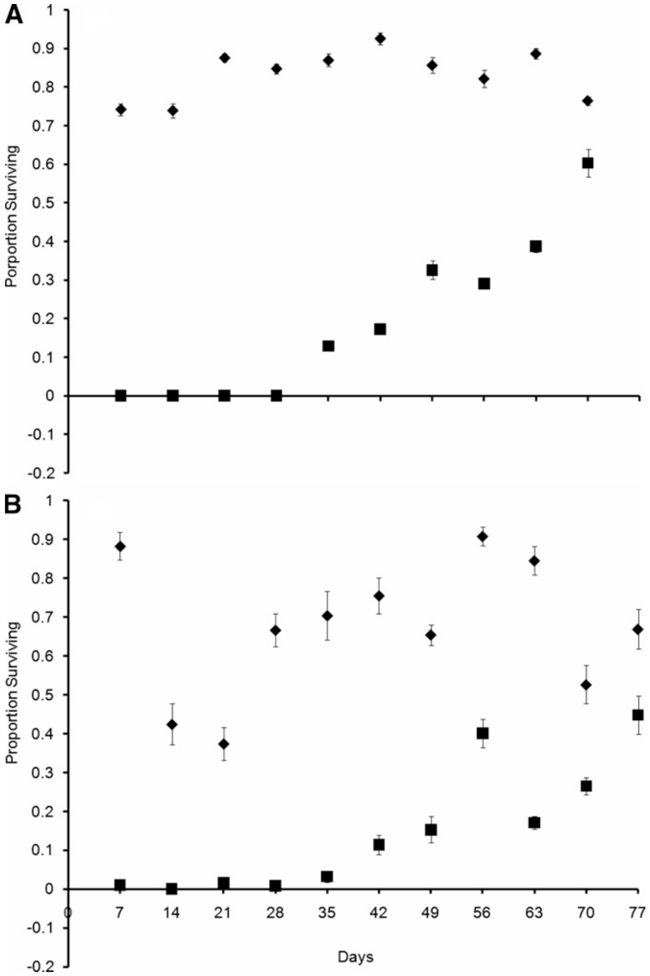

FIGURE 4. (**A**) Survival (back transformed means ± SE) of *Ae. albopictus* when treated with a 1:1 pellet ratio (Agnique:Altosid) at Mercer County sites in New Jersey. Lines with squares indicate treatment, and diamonds are the controls. Multiple-comparison *P* values for control versus treatment: day 7 post-treatment ≤ 0.0001, day 14 ≤ 0.0001, day 21 ≤ 0.0001, day 28 ≤ 0.0001, day 35 ≤ 0.0001, day 42 ≤ 0.0001, day 49 ≤ 0.0001, day 56 ≤ 0.0001, day 63 = 0.0002, and day 70 = 0.0097. (**B**) Survival (back transformed means ± SE) of *Ae. albopictus* larvae treated with 1:1 pellet ratio (Agnique:Altosid) at Monmouth County sites in New Jersey. Lines with squares indicate treatment, and diamonds are the controls. Multiple-comparison *P* values for control versus treatment: day 7 post-treatment ≤ 0.0001, day 14 ≤ 0.0001, day 21 ≤ 0.0001, day 28 ≤ 0.0001, day 35 ≤ 0.0001, day 42 ≤ 0.0001, day 49 ≤ 0.0001, day 56 ≤ 0.0001, day 63 = 0.0002, day 70 = 0.0058, and day 77 = 0.0520.

of *Ae. albopictus* immatures, thereby decreasing the costly labor inherent to frequent applications of short residual products. Considering costs and limited increases in efficacy, we do not recommend combining Agnique with Altosid under all conditions; rather, this provides an impetus for further investigation of possible additive or synergistic effects of the tandem products against *Ae. albopictus*. The autochthonous transmission of CHIKV by *Ae. albopictus* in Europe shows that countries like the United States, which have an established population, are at risk from potential epidemics as expressed by Leroy and others[7] and Farajollahi and Nelder;[37] this highlights the need for effective control measures against vector populations. The combination of products that we tested in these studies should be considered as an important tool in managing and preventing establishment of *Ae. albopictus* during incipient invasions.[36,38] The sustained activity of Altosid after laboratory-induced drought conditions will further lower costs by influencing the spring brood and remaining effective under summer drying conditions. Our study shows the efficiency and efficacy of a combination of two commercially available products against *Ae. albopictus*, providing a valuable tool for integrated management practices for this and other container mosquito species.

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Authors' addresses: Mark Nelder, Ministry of Health and Long-Term Care, Public Health Division, Public Health Protection and Prevention Branch, Enteric, Zoonotic, and Vector-Borne Disease Unit, Toronto, Ontario, Canada. Banugopan Kesavaraju, Salt Lake City Mosquito Abatement District, Salt Lake City, UT, E-mail: banu@slc-mosquito.com. Ary Farajollahi and Isik Unlu, Mercer County Mosquito Control, West Trenton, NJ. Sean Healy and Taryn Crepeau, Monmouth County Mosquito Extermination Commission, Eatontown, NJ. Ashok Ragavendran, Department of Animal Sciences, Purdue University, West Lafayette, IN. Dina Fonseca and Randy Gaugler, Center for Vector Biology, Rutgers University, New Brunswick, NJ.

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