EFFECTS OF PLANT SPECIES, INSECTICIDE, AND EXPOSURE TIME ON THE EFFICACY OF BARRIER TREATMENTS AGAINST Aedes albopictus

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ABSTRACT. The effect of 5 plant species (arborvitae [Thuja occidentalis], boxwood [Buxus sp.], Japanese honeysuckle [Lonicera japonica], rhododendron [Rhododendron sp.], and zebra grass [Miscanthus sinensis]) and 2 rates of lambda-cyhalothrin (3.13 ml and 6.25 ml active ingredient [AI]/liter) on knockdown (1 h) and mortality (24 h) of adult female Aedes albopictus was evaluated over an 8-wk period. A significant difference in knockdown was observed between the 2 rates of lambda-cyhalothrin on the 5 plant species, with the highest proportion of knockdown observed on zebra grass and rhododendron treated at the higher rate. Although mortality was ≥60% and 85% on the 5 plant species at the low and high rates of lambda-cyhalothrin, respectively, a significant difference between the 2 rates was only observed on boxwood and Japanese honeysuckle (P < 0.0001). We also tested the residual toxicity of 3 barrier sprays (lambda-cyhalothrin, bifenthrin, and deltamethrin) and evaluated the efficacy of a short (5-min) exposure to the insecticides on knockdown and mortality of adults over time. Significantly higher knockdown was observed with lambda-cyhalothrin compared with bifenthrin and deltamethrin (P < 0.0001). Mean knockdown was ~98%, 92%, and 20% for lambda-cyhalothrin, bifenthrin, and deltamethrin, respectively, at week 2, and ~98%, 0%, and 44%, respectively, 8 wk after treatments were applied. Adult mortality from the 3 chemical treatments, however, remained above 90% throughout the study. Lastly, the trends in mean proportion of knockdown were similar for mosquitoes exposed for either 5 min or 24 h to the 3 chemicals. An overall decline in mean mortality over time, however, was observed for mosquitoes exposed for 5 min to the chemicals compared with mortality from the 24-h exposure. The results suggest that lambda-cyhalothrin can be an effective barrier spray treatment against Ae. albopictus adults because its efficacy is limited little by plant species, it has long residual toxicity, and it is effective following only 5 min of exposure.

KEY WORDS Aedes albopictus, bifenthrin, deltamethrin, lambda-cyhalothrin, plant species

INTRODUCTION

Aedes albopictus (Skuse) is an invasive vector mosquito in many parts of the world (Bonizzoni et al. 2013) and a competent vector for several viruses (Benedict et al. 2007). An aggressive human biter, Ae. albopictus is often the primary mosquito species eliciting complaints from the public in areas where it occurs (Farajollahi and Price 2013). Because this species readily utilizes artificial containers for breeding, it is well adapted to suburban and urban habitats (Barker et al. 2003). In fact, Ae. albopictus has become the most important and sometimes the only mosquito vector in many urban areas (Bonizzoni et al. 2013). Once Ae. albopictus becomes established in an area it is very difficult to eradicate. Standard area-wide control methods such as the use of spray trucks to administer insecticides (Mount et al. 1996) offer little control of this species since these methods are generally directed at crepuscular species and not diurnal species like Ae. albopictus. Because Ae. albopictus is a major biting pest in suburban yards and has the potential to transmit viruses such as Zika, homeowners in the United States have become increasingly concerned about its presence around their homes and are constantly in search of new methods of control.

A common recommendation for mosquito population control is reduction of larval development sites. However, mosquito oviposition sites may be cryptic. Because many species of adult mosquitoes utilize plant foliage and other vegetation as resting and feeding sites, barrier treatments with residual pesticides can be an effective method for reducing populations (Trout et al. 2007, Amoo et al. 2008, Doyle et al. 2009, Richards et al. 2017). Barrier treatments applied to plant foliage can reduce the use of broad-scale applications of chemicals in the environment and prevent mosquitoes from entering into untreated areas (Cilek and Hallmon 2006, Cilek 2008). Common barrier spray treatments have included lambda-cyhalothrin (Trout et al. 2007, Unlu et al. 2017), bifenthrin (Trout et al. 2007, VanDusen et al 2016, Richards et al 2017), deltamethrin (Cilek and Hallmon 2006, Richards et al. 2017), and permethrin (Cilek and Hallmon 2006, Amoo et al. 2008). Treatments are typically applied every 21–30 days in the United States. Li et al. (2010) found that barrier applications using lambda-cyhalothrin lasted up to 9 wk and provided an 83–98% reduction in Ae. albopictus landing rate counts. In Queensland, Australia, Muzari et al. (2014) conducted weekly sweep-net collections in lambda-cyhalothrin–treated and untreated areas and observed an 87–100% reduction in mosquito numbers for 9 wk postspray.
The effectiveness of barrier spray treatments, however, can be impacted by several factors, including plant species (Doyle et al. 2009), type of sprayer (Farooq et al. 2010), environmental conditions (Allan et al. 2009), and repellency of the chemical (Manda et al. 2013), which is related to the level of exposure of the mosquito to the chemical.

The goal of this study was to assess the effectiveness of barrier spray treatments applied under natural environmental conditions using laboratory bioassays. The specific objectives were to study the effect of plant species on the residual toxicity of lambda-cyhalothrin; to compare the residual toxicity of 3 commonly used barrier spray insecticides: lambda-cyhalothrin (Demand® CS), deltamethrin (Suspend® Polyzone®), and bifenthrin (Talstar® Professional); and to evaluate the effects of exposure time of the insecticide treatments against *Ae. albopictus*.

### MATERIALS AND METHODS

#### Effect of plant species

Five plant species common to residential landscapes in southwestern Virginia were selected for this study: *Miscanthus sinensis* Andersson (zebra grass), *Buxus* sp. (boxwood), *Rhododendron* sp. Linnaeus (rhododendron), *Thuja occidentalis* Linnaeus (arborvitae), and *Lonicera japonica* Thunberg (Japanese honeysuckle). Plants of each species in 11.4-liter pots and of comparable size and age were purchased from the Christiansburg Garden Center (Christiansburg, VA). Potted plants of each species were assigned to 1 of 3 spray treatment groups consisting of Demand CS (7.9% lambda-cyhalothrin; Syngenta Crop Protection, Greensboro, NC) at 3.13 ml active ingredient (AI) liter or 6.25 ml AI/liter, and water as a control. The treatments were applied to plants using a backpack mist blower (SR200, Stihl Corp.) to runoff ensuring that both the upper and lower surfaces of leaves were treated. Plants were grouped by treatment, planted evenly spaced in a 1-m² plot, and lower surfaces of leaves were treated. After treatment, plants were grouped by treatment, planted evenly in a 2-m² plot under natural environmental conditions, and watered at the base as needed. All plants were located at the Prices Fork Research Center, Blacksburg, VA.

Leaves were sampled from plants in each treatment group between September 7 and November 2, 2015, with the first samples collected the day following treatment (i.e., week 0). Additional leaf samples were collected at weeks 1, 2, 4, 6, 8, 10, and 12. At each sampling, 5 leaves were selected from the plants in each treatment group, avoiding new growth. Leaves were selected and processed as described above (see “Effect of plant species”). The leaf samples were used in bioassays to test the residual toxicity of barrier spray treatments on knockdown at 1 h and mortality at 24 h of *Ae. albopictus* (see “Leaf bioassays”).

#### Comparative residual toxicity of barrier sprays

*A. rhododendron* sp. plants in 11.4-liter pots were purchased from Fast Growing Tree Nursery (www.fast-growing-trees.com; Fort Mill, SC. [accessed October 6, 2017]). Groups of 2 plants were selected and assigned to 1 of 4 treatment groups consisting of 3 chemical pesticides and a water control. The spray treatments were lambda-cyhalothrin (Demand CS; AI 7.9%; Syngenta Crop Protection) at 6.25 ml/liter of water; bifenthrin (Talstar Professional; AI 7.9%; FMC Professional Solutions, Philadelphia, PA) at 7.81 ml/liter of water, and deltamethrin (Suspend Polyzone; AI 4.75%; Bayer Crop Science, Research Triangle Park, NC) at 11.72 ml/liter of water. The 3 chemicals and the water treatments were applied using a backpack mist blower (SR200, Stihl Corp.) to runoff ensuring that both the upper and lower surfaces of leaves were treated. Plants were grouped by treatment, planted evenly spaced in a 1-m² plot, and watered as needed. All plants were located at the Prices Fork Research Center, Blacksburg, VA. Leaves were sampled from treatment plants between July 27 and October 19, 2016, with the first samples collected the day following treatment (i.e., week 0). Additional leaf samples were collected at weeks 1, 2, 4, 6, 8, 10, and 12. At each sampling, 5 leaves were selected from the plants in each treatment group, avoiding new growth. Leaves were selected and processed as described above (see “Effect of plant species”). The leaf samples were used in bioassays to test the residual toxicity of barrier spray treatments on knockdown at 1 h and mortality at 24 h on *Ae. albopictus* (see “Leaf bioassays”).

#### Effect of exposure time

Leaf samples for this study were collected in a similar manner as described earlier (see “Comparative residual toxicity of barrier sprays”). Mosquitoes in this experiment were exposed to the treated leaves in vials for 5 min, after which the mosquitoes were removed, placed into 220-ml plastic containers (Corning, Inc., Corning, NY), and observed for knockdown and mortality at 1 and 24 h, respectively (see “Leaf bioassays”).

#### Leaf bioassays

Two- to 3-day-old adult female *Ae. albopictus* were obtained from the Virginia Tech Medical Entomology Lab colony. This colony was established from mosquitoes collected in Blacksburg, VA, and has been maintained in the lab for approximately 3 years. The mosquitoes were reared in an insectary at 24°C and 80% relative humidity, with a photoperiod
of 16 h light and 8 h dark using the procedures described by Munstermann and Wasmuth (1985).

To test the effect of plant species and residual toxicity of barrier spray treatments, 10 mosquitoes were added to a 7-dram borosilicate glass shell vial (Thomas Scientific, Swedesboro, NJ) containing a single leaf from a plant in each treatment group. Leaves were positioned so that both sides were accessible to the mosquitoes. Mesh netting was placed over the open end of each vial and secured with a rubber band; a wet paper towel was placed over the vial to prevent desiccation of the mosquitoes. Knockdown and mortality were evaluated after 1 h and 24 h, respectively. Knockdown was assessed at 1 h because it was impossible to ascertain the difference between knocked-down and dead mosquitoes. Mosquitoes were considered either dead or apparently healthy when assessed at 24 h.

To study the effect of exposure time on the toxicity of barrier spray treatments, the mosquitoes were exposed to treated leaves in the vials for 5 min, after which they were transferred to sterile 220-ml plastic containers (Corning, Inc.) and covered with mesh netting. Knockdown and mortality were evaluated after 1 h and 24 h, respectively.

The vials and plastic containers with mosquitoes were stored in the insectary for observation. Mosquitoes were considered knocked down if they were unable to fly within the vial or container, and were considered dead if they were unable to stand after slight agitation.

Collection of environmental data

Data on rainfall (mm), temperature (°C), relative humidity, and light intensity (PAR μmol/m²s) were collected at a central location at the edge of plots containing the potted plants using an m50G Wireless Cellular Data Logger (Decagon Devices, Pullman, WA).

Statistical analysis

The effects of plant species on knockdown and mortality of adult female *Ae. albopictus* were determined using a linear mixed model for repeated measures ANOVA with an autoregressive covariance structure (Littell et al. 2000, Traver et al. 2018). In the model, plant species (arborvitae, boxwood, Japanese honeysuckle, rhododendron, and zebra grass), spray treatment (lambda-cyhalothrin at 3.13 and 6.25 ml AI/liter, and water) on mosquito knockdown after a 1-h exposure over an 8-wk sampling period. The analysis showed that there was a significant interaction of plant species × spray treatment (F<sub>8,26,5</sub> = 2.9337, P = 0.0174; Fig. 1A) and spray treatment × sampling week (F<sub>12,157.6</sub> = 45.7643, P < 0.0001, Fig. 2A) with respect to mosquito knockdown after 1 h. The highest proportion of knockdown (>80%) was observed on zebra grass and rhododendron at the higher rate of lambda-cyhalothrin. A significant decline in 1-h knockdown was observed for the lower rate of lambda-cyhalothrin after week 0. However, knockdown remained relatively high up to week 4 at the higher rate, after which it declined significantly (Fig. 2A).

A significant interaction of plant species × spray treatment (F<sub>6,28,9</sub> = 8.9627, P < 0.0001; Fig. 1B) and spray treatment × sampling week (F<sub>12,153.0</sub> = 12.4952, P < 0.0001; Fig. 2B) was also observed for 24-h mortality. Post hoc analysis showed that the significant interaction of plant species × spray treatment resulted mainly from differences between the 2 rates of lambda-cyhalothrin on boxwood and Japanese honeysuckle (Fig. 1B) with higher mortality observed at the higher rate. A significant decline in 24-h mortality after week 2 occurred at both rates of lambda-cyhalothrin although mortality at the higher rate remained above 80% throughout the study (Fig. 2B).

Comparative residual toxicity of barrier spray insecticide bioassays

The results showed that there was a significant interaction of spray treatment × sampling week with respect to 1-h knockdown (F<sub>21,92.8</sub> = 14.9768, P <
Knockdown was >90% in the bifenthrin and lambda-cyhalothrin treatments up to weeks 2 and 8, respectively (Fig. 3A). The proportion of mosquito knockdown in the deltamethrin treatment varied between 10% and 70% for the first 10 wk of the study. Knockdown declined for all chemical treatments after week 10 but remained above 60% for the lambda-cyhalothrin treatment.

Fig. 1. (A) Mean proportion of knockdown and (B) mean proportion of mortality of *Ae. albopictus* to lambda-cyhalothrin (Demand® CS)-treated leaves by plant species. Knockdown evaluated at 1 h and mortality evaluated at 24 h. Means within each graph followed by the same letter are not significantly different. Tukey’s HSD test ($P > 0.05$). Mean is based on 3 replications.
No significant differences were observed among the lambda-cyhalothrin, bifenthrin, and deltamethrin treatments with respect to mean 24-h mortality ($P > 0.05$), with mortality in these treatments consistently $\geq 90\%$. Mortality in the water control treatment was significantly lower ($\bar{x} = 8.25\%$) throughout the study compared with the 3 chemical treatments ($F_{21,88.9} = 3.7155, P < 0.0001$; Fig. 3B).

Fig. 2. (A) Mean proportion of knockdown and (B) mean proportion of mortality of *Ae. albopictus* after exposure to 2 treatments of lambda-cyhalothrin (Demand® CS) for all plants over time. Knockdown evaluated at 1 h and mortality evaluated at 24 h. Means within each graph followed by the same letter are not significantly different. Tukey’s HSD test ($P > 0.05$). Mean is based on 3 replications.
Short exposure time bioassays

Mean proportion of 1-h knockdown after mosquitoes were exposed to the treated leaves for only 5 min (short exposure) varied significantly over time among the spray treatments ($F_{21,46,3} = 11.0540, P < 0.0001$; Fig. 4A). Proportion of knockdown was $>75\%$ in the lambda-cyhalothrin treatment for the first 8 wk of the study; knockdown in the bifenthrin treatment was $>80\%$ up to week 2, after which a significant decline was observed. Knockdown in the deltamethrin treatment varied but was $<40\%$ throughout the duration of the study.

The trends in 24-h mortality after a short exposure to treated leaves were similar to those observed for the 1-h knockdown bioassay, with mean 24-h mortality after 5-min exposure varying significantly over time among the treatments ($F_{21,48,0} = 6.8808, P < 0.0001$; Fig. 4B). Mortality in bifenthrin treatment declined after week 2, but remained above 60% in the lambda-cyhalothrin treatment up to week 8. Relatively low mortality

Fig. 3. (A) Mean proportion of knockdown and (B) mean proportion of mortality of Ae. albopictus to treated leaves over time. Knockdown evaluated at 1 h and mortality evaluated at 24 h. Means within each graph followed by the same letter are not significantly different. Groups marked with an asterisk (*) are statistically similar. Tukey’s HSD test ($P > 0.05$). Mean is based on 5 replications.
(<40%) was observed throughout the study in the
deltamethrin treatment although mortality spiked
to ~60% at week 8.

**Environmental data**

Mean temperature, mean percentage of relative
humidity, and total precipitation during the study
period in 2015 were 13.5°C, 83%, and 311 mm,
respectively (Table 1). Likewise, mean temperature,
mean percentage of relative humidity, and total
precipitation in 2016 were 20.3°C, 79%, and 43.3
mm, respectively (Table 1).

**DISCUSSION**

Barrier spraying is the application of insecticide
treatments to vegetation to reduce or prevent adult
mosquitoes from entering the area of treated foliage,
and has become a more appealing control option for
managing pest mosquito populations (Anderson et al.
1991, Cilek and Hallmon 2006, Trout et al. 2007). To
The higher rate remained above 80% throughout the week 2 across all plant species although mortality at honeysuckle. A significant decline in mortality of lambda-cyhalothrin on boxwood and on Japanese elces were observed between the low and high rates was recorded on all plant species, although differ-
ences were observed among the treatments. Examining the results of the study, for lambda-cyhalothrin, deltamethrin, and bifenthrin, there was little difference in mortality among the treatments. Examining the results of the

| Week no. | Rainfall (mm) | Humidity (RH) | Mean temperature (°C) |
|----------|---------------|---------------|----------------------|
| 2015     |               |               |                      |
| 1        | 2.4           | 0.84          | 14.0                 |
| 2        | 19.0          | 0.77          | 16.8                 |
| 3        | 51.2          | 0.91          | 15.7                 |
| 4        | 168.8         | 0.98          | 14.7                 |
| 5        | 10.2          | 0.89          | 14.2                 |
| 6        | 2.4           | 0.67          | 10.3                 |
| 7        | 0.4           | 0.75          | 12.1                 |
| 8        | 56.4          | 0.85          | 10.4                 |
| 2016     |               |               |                      |
| 1        | 49.8          | 80.1          | 24.6                 |
| 2        | 62.5          | 0.90          | 21.8                 |
| 3        | 3.6           | 0.79          | 25.2                 |
| 4        | 8.1           | 0.81          | 21.8                 |
| 5        | 0.3           | 0.77          | 23.7                 |
| 6        | 11.4          | 0.76          | 20.5                 |
| 7        | 0.0           | 0.70          | 22.4                 |
| 8        | 22.1          | 0.79          | 21.3                 |
| 9        | 45.5          | 0.79          | 20.6                 |
| 10       | 100.1         | 0.82          | 17.2                 |
| 11       | 49.8          | 0.78          | 13.7                 |
| 12       | 0.0           | 0.78          | 14.8                 |
short-term exposure bioassays, however, showed that there were significant differences among the 3 pyrethroids. Deltamethrin was less than half as effective as lambda-cyhalothrin, and the efficacy of bifenthrin dropped dramatically after 2 wk. This would indicate that lambda-cyhalothrin has roughly twice the duration of bifenthrin when used as a barrier spray, and that deltamethrin, due to its low efficacy in the short-term exposure trials, may not provide significant levels of control in the field. Additional evaluations of this formulation (Suspend Polyzone), however, may be warranted. Richards et al. (2017) observed that deltamethrin and bifenthrin (Bifen I/T) significantly reduced the abundance of Psorophora columbiae Dyar and Knab adults; however, no other key species, including Ae. albopictus, experienced significant decreases in abundance between treatments. The authors did note this could be attributed, in part, to the low sample size during the mosquito season.

Mosquitoes exhibit distinct daily rhythms in flight activity, feeding, reproduction, and development (Rund et al. 2013), which makes it extremely unlikely that a mosquito will rest on a treated substrate for a full 24 h. The circadian rhythms of Ae. albopictus have been shown to be modulated by reproductive state and feeding status (Lima-Camara et al. 2014), and only mated, blood-fed females showed significant reduction in locomotion. As a result, the mortality assessment in the 5-min exposure trials, although rarely done in bioassays, may be more biologically meaningful than at the longer exposures. The most common evaluation of insecticide efficacy uses the full 24-h exposure timeframe, which may not accurately represent real-world conditions. Current World Health Organization (WHO) guidelines, while initially designed for evaluating insecticides used in bed netting against malaria vectors, does provide some precedent for short exposure periods. The initial screen and assessment of insecticide efficacy is conducted using a WHO cone test in which mosquitoes are exposed to treated material for just 3 min and mortality recorded a day later (WHO 2006).

This study provides additional support for the microencapsulated formulation of lambda-cyhalothrin for use as a barrier spray against Ae. albopictus (Trout et al. 2007). Furthermore, it shows that plant species does not appear to play a major role in the efficacy of the treatment. However, care should be taken to treat all of the target foliage evenly to ensure optimal coverage. Lambda-cyhalothrin remained resistant to environmental degradation and outperformed the other treatments evaluated. The formulation can be differentiated due to the microencapsulation technology that protects the active ingredient from movement into or inactivation by the surface (Wege et al. 1999). Because of its increased residual activity, the frequency of reapplications may be reduced compared to other insecticides, resulting in benefits for the operator and environment.

Future studies could expand upon the list of pyrethroids tested, as well as compare different formulations of active ingredients. A cost analysis could also be performed for these applications to determine the costs associated with treatments. Additionally, differences observed between 5-min and 24-h exposures were significant. These differences have potentially biologically relevant implications, indicating the importance of further investigation. Combined with mosquito surveillance data and an integrated pest management plan, barrier spray applications can be utilized as an effective option to suppress mosquito species such as Ae. albopictus. However, while plant species had minimal impact on efficacy, the performance of an active ingredient and formulation can vary greatly.

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