RESIDUAL EFFECTS OF BIFENTHRIN SPRAYED ON PLANT FOLIAGES AGAINST Aedes albopictus and Apis mellifera IN North Central Florida

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ABSTRACT

A field study was conducted to test bifenthrin as a barrier treatment for its residual effects on adult mosquitoes Aedes albopictus and honey bees Apis mellifera in Gainesville, Florida. Plant foliage was treated with an American LongRay misting sprayer machine at the label rate of 0.318 mL/m². Treated plant leaves were then collected at 24 hr, one wk, and two wk post-treatment for laboratory bioassays against adult Aedes albopictus and Apis mellifera. The mortalities of the mosquitoes and honeybees exposed to the bifenthrin-treated plant leaves at 24 hr post-treatment were significantly higher than the mortalities at one wk and two wk post-treatment. There were no significant differences in the mortalities of mosquitoes and honeybees exposed to treated plant leaves at one wk and two wk post-treatment. Also, the results showed that the treated plant leaves away from the spray path resulted in significantly high mortalities of both species at 24 hr post-treatment than the mortalities at 8 m and 11 m at one wk and two wks post-treatment. The commercial product of Talstar P (bifenthrin) sprayed on plant foliage resulted in significantly higher mortalities of mosquitoes and honeybees at 24 hr post-treatment at the 5 m distance. There was no significant residual efficacy of the product one week after post-treatment at any distance.

Key Words: Aedes albopictus, Apis mellifera, bifenthrin, vegetation, barrier treatment

Seven commercial barrier treatment products and compounds against adult mosquitoes Aedes albopictus have been evaluated in the laboratory (Qualls & Xue 2013) and field (Stoops et al. 2019, Bibbs et al. 2020), of which bifenthrin is the most effective insecticide for barrier sprays. Bifenthrin applied to vegetation and has provided effective control of adult mosquitoes in the semi-field and residential areas (Bibbs et al. 2016, Cilek & Hallmon 2008, Qualls et al. 2012, Lloyd et al. 2021). This method has been adopted as a part of operation control by the Anastasia Mosquito Control District (AMCD) in St. Johns County, Florida (Qualls et al. 2013). The residual effects of bifenthrin on plant leaves against adult mosquitoes could last for about 3-4 weeks in the semi-field and field (Doyle et al. 2009, Fulcher et al. 2015). The efficacy of bifenthrin on vegetation is impacted by environmental conditions (Allan et al. 2009). Laboratory bioassays showed that bifenthrin caused significant mortality for honeybees after direct contact (Qualls et al. 2010, Sanchez-Arroyo et al. 2019, 2021). So far, we do not know whether the barrier spraying of bifenthrin on vegetation in the residential areas has any residual impact on the honeybee. Apis mellifera Linnaeus when we aim to control the container-inhabiting mosquitoes, Aedes albopictus Skuse. The present report is about the residual impacts of bifenthrin barrier treatment on vegetation against Aedes albopictus and Apis mellifera around a residential subdivision in north central Florida.

The eggs of Aedes albopictus mosquitoes were obtained from the Anastasia Mosquito Control District and reared and kept in the insectary of Urban Entomology Laboratory, University of Florida (UF), Department of Entomology and Nematology (DEN), Gainesville, Florida. Adult female mosquitoes at 3-5 days old were used in the bioassays. The adult honey bees, Apis mellifera at 5-7 days old were provided by the Bee Laboratory at the UF/DEN.

Whitney Mobile Home Park, a residential subdivision (29°13’56.89”N, 82°22’29.29”W) located in the northern part of Gainesville, Florida was selected for the field experiment. This area was chosen because of its large interior park-like setting, surrounding dense vegetation with similar habitats, and suitable environment for barrier treatments. There are a few pine trees and dense ground vegetation (major species were Melampodium paludosum Melanie and Duranta erecta L. (Golden dewdrop)) around the subdivision.
A misting spray machine (model 3WC-30-4P, American LongRay, San Francisco, CA) was used for the study. The sprayer is powered by a 6.30kw 16-liter diesel engine with 4 adjustable spray nozzles. The flow rate can be continuously varied from 0.83 to 5 L/min. The engine can be turned on and off using a remote control up to 5.5 meters away from the sprayer. The machine was calibrated prior to the treatment. The flow rate was set at 4.7 L/min with a median droplet size diameter (Dv0.5) of 107.5 µm and a mean droplet velocity of 6.9 m/sec. The unit along with the external tank was mounted into a Polaris Ranger (4x4). The sprayer nozzle heads were set at 360° vertically and 330° laterally in order to create a spray pattern 3 m high and 3.1 m wide, respectively, when the Polaris Ranger was stationary. A 168.8 L external tank was added to the unit to ensure the spraying mission would be complete without refilling the tank.

A commercial product Talstar P (A.I. 7.9% of bifenthrin, FMC Corporation, Philadelphia, PA) was diluted to 7.8 mL of formulation per liter of well water and applied at the label rate of 0.318 mL/m². During the application, the Polaris Ranger carrying the spray machine with the nozzle was at approximately 0.5 m from the nearest vegetation and the driving speed was 8 Km/h. The application rate resulted in 0.025 g A.I./m² of the vegetation surface at the 5 m distance away from the machine. The control plot was untreated and about 500 m away from the treated site. Runoff of insecticides was not observed during the treatment.

Residual effects of bifenthrin treatment on the vegetation were measured using the leaf bioassay method (Xue 2008). Leaf clippings from bushes of treated and untreated sites were used for the bioassays against *Ae. albopictus* and *A. mellifera*. Nine leaves (six *M. paludosum* and three *D. erecta*) were excised from three different rows (3 leaves from each row) 3 m apart from each other at 5 m, 8 m, and 11 m from the line of travel of the swath of the sprayer. Each sampled leaf had a similar surface area, thickness, and waxiness. Upon return to the laboratory, each leaf was contained in a 60 mL cup. For each bioassay, ten adult female *Ae. albopictus* mosquitoes (3–5 days old) and ten adult *A. mellifera* honeybees at 5-7 days old were knocked down using carbon dioxide (CO₂) anesthetization and placed in the respective leaf-cup through forceps manipulation. Cups with cotton balls, saturated with 10% sucrose solution for mosquitoes and 50% sucrose for honeybees, were stored in a temperature-controlled room (24°C). Mortality as indicated by complete non-response to the stimulus, was recorded at 24 hr of continuous exposure. Each trial was composed of 3 cups for the treatment and 3 cups for the control. Similar bioassays were carried out weekly after the treatment. When the mortality in the treatment group was less than 50% the weekly experiment was stopped. The trial was repeated three separate times.

The treatment mortalities were corrected for any control mortalities using the Abbott formula (Abbott 1925) and the data were analyzed by computer software. One-way and Two-way ANOVA were used appropriately to compare the mortalities among the two species for 3 different post-treatment time periods, and three different distances.

The experiment was stopped at three wk post-treatment due to the detection of low mortality (less than 50%) for both species of insects in the treatment group. The data analysis (Table 1) showed that the treatment resulted in significant differences in the mortalities of mosquitoes, *Ae. albopictus* (*F* = 5.520, df =10, *P* < 0.05) and the honeybees *A. mellifera* (*F* =6.613, df = 10, *P* < 0.05) after exposed to the treated plant leaves. The mortality at 24 hr was 45.6 ± 20.9 for mosquitoes and 60.3 ± 12.7 for honey bees and the differences were significant compared to corresponding controls. The mortalities of both species at 24 hr were significantly higher than the mortalities in the control.

Table 1. Mean mortalities (% ±SD) of *Aedes albopictus* and *Apis mellifera* exposed to foliage sprayed by bifenthrin at 24 hr, one wk, and two wk post-treatment

<table>
<thead>
<tr>
<th>Post-treatment period</th>
<th><em>Aedes albopictus</em></th>
<th><em>Apis mellifera</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>24 hr</td>
<td>0.7±0.06</td>
<td>45.6 ±20.9a</td>
</tr>
<tr>
<td>one wk</td>
<td>0.0</td>
<td>17.8 ±31.1c</td>
</tr>
<tr>
<td>two wk</td>
<td>0.3±0.03</td>
<td>13.3 ±16.6c</td>
</tr>
</tbody>
</table>

Different letters in the column indicate significant differences.
the one wk and two wk post-treatment, but the mortalities did not show any significant differences between the one wk and two wk post-treatment.

One way ANOVA showed that there were significant differences in the mortalities of mosquitoes and honeybees in the 5 m distance at 24 hr post-treatment, compared to the mortalities in the 8 m and 11 m (Table 2), but the mortalities at the one wk and two wk post-treatment were not significantly different between the 8 m and 11 m distances. Two-way ANOVA showed that there was no statistically significant interaction between the effect of species (honeybees and mosquitoes) and the distances on mortalities (F2,12 = 0.289, P = 0.754).

Plants are a major part of the mosquito ecosystem (Xue 2008a) and the application of insecticides to perimeter vegetation for the purpose of controlling adult mosquitoes in backyards and other recreational areas has been confirmed to be effective control methods (Stoops et al. 2019; Richards et al. 2017). Bifenthrin has been approved to suppress mosquito populations in treatment areas and previous leaf bioassays have revealed that bifenthrin-treated leaves exhibited > 70% knockdown/mortality against laboratory-reared female Ae. albopictus and Culex quinquefasciatus for 4 weeks (Cilek 2008). Bifenthrin (0.08%) and lambda-cyhalothrin (0.1%) as barrier treatments at their maximum label concentrations has significantly reduced Aedes spp. population up to six wks post-treatment (Cilek 2008, Trout et al. 2007).

Trapping results (CDC light trap) in a study using bifenthrin as a barrier treatment against Ae. aegypti, conducted in St. Augustine, FL, showed a 77% mean reduction in adult mosquito population up to four wks post-treatment and the laboratory leaf bioassays revealed an average mortality of 80% at 2.7 m and 51% at 5.5 m for five wks post-treatment (Fulcher et al 2015). Leaves collected from the treated areas caused significantly higher mortality at distances closer to the sprayer, though the distance and coverage of bifenthrin application was effective up to 5 m (Fulcher et al. 2015). Our study showed similar results for mosquitoes and honeybees exposed to the bifenthrin treated leaves.

<table>
<thead>
<tr>
<th>Distance</th>
<th>Aedes albopictus</th>
<th>Apis mellifera</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>24 hr post-treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.7±0.03</td>
<td>41.0±25.7a</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>22.3±12.9b</td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>13.3±13.5b</td>
</tr>
<tr>
<td>One wk post-treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>66.7±20.3a</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>32.3±31.1b</td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>27.7±27.3b</td>
</tr>
<tr>
<td>Two wk post-treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>66.7±28.9a</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>37.0±36.1b</td>
</tr>
<tr>
<td>11</td>
<td>0.3±0.03</td>
<td>29.0±33.0b</td>
</tr>
</tbody>
</table>

Different letters in the column (within the sampling time) indicate significant differences.
The efficacy of bifenthrin as a barrier treatment on vegetation varied with the species of plants and insect species, application rates of the insecticide, and many other environmental conditions (Allan et al 2009, Britch et al. 2009). The impact of bifenthrin on honeybees, A. mellifera, was shown to be affected by both dose and exposure time (Qualls et al. 2010; Sanchez-Arroyo et al. 2019, 2021). Application dose of 35 µg/mL resulted in 100% bee mortality in the laboratory bioassays (Sanchez-Arroyo et al. 2019). In a study in which bifenthrin was applied at different concentrations to common landscape vegetation of M. paludosum and D. erecta L. (Golden dewdrop), honey bee mortality was significantly higher at high application rates, compared to the mortality at low application rates after 24 hr exposure to the treated vegetation (Qualls et al. 2010). It was not a surprise that the bifenthrin-barrier treatment on plant leaves against Ae. albopictus mosquitoes resulted in a high mortality of non-target honey bees during this experiment.

In summary, bifenthrin as a barrier treatment on vegetation provide effective control of the container-inhabiting Aedes mosquitoes, but also showed a high mortality impact on non-target honeybees at a short distance and a short time direct exposure. Fortunately, the foraging activity of honeybees in the natural environment is not around residential areas, unlike that of container-inhabiting Aedes mosquitoes. This may provide less opportunity for honeybees to be exposed to bifenthrin-treated vegetation when we plan to control the container-inhabiting Aedes mosquitoes to protect the residents from mosquito pressure. Also, this indicates that we have to take caution and limit the impact on nontarget honeybees when we select the barrier-spraying method to control target mosquitoes at residential areas.

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