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EVALUATION OF BARRIER SPRAYS FOR CONTROLLING MOSQUITOES IN EASTERN NORTH CAROLINA: CAN LAND COVER AND SPATIAL ANALYSES IMPROVE PREDICTIONS OF EFFICACY?

JUSTIN P. BUNN,[1] JO ANNE G. BALANAY[1] AND STEPHANIE L. RICHARDS[1,2]

[1] *East Carolina University, Department of Health Education and Promotion, Environmental Health Science Program, 3403 Carol Belk Building, 300 Curry Court, Greenville, NC 27858*

ABSTRACT. Mosquitoes can be a nuisance and can transmit pathogens causing numerous diseases. Homeowners may hire private companies that use barrier sprays to alleviate mosquito-related issues, especially in areas where government funding for mosquito control programs is limited. Here, the spatial distribution of mosquitoes was evaluated in a suburban neighborhood during successive treatments with either Bifen Insecticide/Termiticide (active ingredient: bifenthrin) or Suspend Polyzone (active ingredient: deltamethrin) from May 17 to November 8, 2016. A total of 15,083 adult mosquitoes and 18,054 mosquito eggs were collected. Analysis of variance ($P < 0.05$) was used to analyze differences in abundance of key species between weeks, traps, and treatments. Weather trends were analyzed in relation to mosquito abundance using time-lagged weekly average temperatures and total rainfall. Kriging showed hot spots of mosquito abundance. The spatial pattern of abundance was different for oviposition and adults, and this was expected because of different types of traps used here. A land cover analysis was performed within the geographic information system (GIS) file to determine the extent to which land cover type could predict mosquito abundance. We show an uneven distribution of host-seeking mosquito abundance and that, in general, mosquitoes preferred areas that were lightly wooded or composed of small collections of trees or bushes, compared with larger densely wooded areas. Analyses of spatial distribution, land cover, and weather can be used to supplement an integrated mosquito management approach.

KEY WORDS Bifenthrin, deltamethrin, GIS, land cover analyses, mosquito

INTRODUCTION

In North Carolina, La Crosse encephalitis virus, West Nile virus, and Eastern equine encephalitis virus are the most common mosquito-borne viruses (NCDDHS 2016). Female mosquitoes are also nuisances due to their propensity to blood feed on humans and leave itchy welts. Hence, homeowners may hire private pest management professionals to conduct barrier insecticide sprays on vegetation surrounding residences. Barrier sprays may lower mosquito abundance for up to few weeks, depending on environmental conditions (Cilek 2008, Doyle et al. 2009, VanDusen et al. 2016, Richards et al. 2017).

Barrier sprays are applied to foliage and other surfaces where mosquitoes rest and nectar feed, potentially killing adult mosquitoes (Allan et al. 2009, Doyle et al. 2009, Fulcher et al. 2015). The formulation dries on the leaves where residual active ingredient is present on the leaf surface, allowing it to come into contact with mosquitoes when they rest or nectar feed (Allan et al. 2009). A previous study testing the effectiveness of barrier sprays and ultra–low-volume (ULV) application showed that a single barrier spray of TalstarP® (7.9% bifenthrin 1.5 liters/min) at week 0 had a greater reduction (84%) of mosquito populations (8 species) over 6 wk compared with a ULV treatment of 1:5 Aqualuer® (20% permethrin 150 ml/min) that reduced mosquitoes by 52% for up to 5 wk (Qualls et al. 2012). The study also concluded that barrier sprays were cheaper ($80 per application; $0.39/ha) than ULV treatments ($350 per application; $0.92/ha) for approximately the same area treated.

Many environmental factors, such as sunlight, rainfall, density of plant vegetation, and type of plants, can affect the ability of barrier sprays to control mosquitoes. A study by Doyle et al. (2009) examined barrier spray efficacy of TalstarOne® (bifenthrin 7.9%) on 5 different types of foliage (azalea, beauty berry, holly bush, sand cord grass, and southern magnolia) applied using a handheld pump. This study found that 24 h posttreatment, exposure to sand cord grass resulted in 15.6% mortality of 5–7-day-old *Aedes albopictus* (Skuse) exposed to treated leaves compared with >90% mortality among the other plant types. The reduced effectiveness was attributed to the narrowness and arrangement of the sand cord grass blades compared with the leafy/bushy makeup of the other plants studied. Researchers speculated that the narrowness of sand cord grass leaves made it hard to direct the spray onto the leaves using a handheld pump, resulting in an improper coating of the leaves (Doyle et al. 2009).

Bifen Insecticide/Termiticide® (Bifen I/T) (7.9% bifenthrin) is a Type I pyrethroid labeled for use outdoors and indoors (e.g., bed nets) (Barta et al. 2009, Control Solutions Inc. 2016). Bifen I/T is the generic formulation of TalStar® Termiticide/Insecti-

[2] To whom correspondence should be addressed.

cide (7.9% bifenthrin) (DoMyOwn 2018). A study of Bifen I/T barrier sprays administered in eastern North Carolina found an average reduction of 54% of host-seeking mosquito populations in treated properties compared with untreated controls (VanDusen et al. 2016). The same study used backpack mist sprayers to apply Bifen I/T every 3 wk to 5 treatment properties. Leaves from treatment sites in the same study were also collected weekly, and insecticide residue was assessed by gas chromatography. The levels of bifenthrin found on foliage ranged from 0 to 25.6 ng/μl and did not show a correlation with mosquito abundance. Factors such as environmental exposures, small sample size, and inconsistency of plant species among sites could have affected the results (VanDusen et al. 2016).

Suspend® Polyzone is a Type II pyrethroid containing 4.75% deltamethrin that has a microscopic polymer layer that increases the surface distribution of active ingredient (Bayer 2016). Results from a study published in manufacturer brochures for Suspend Polyzone (0.06% concentration) showed that the product resulted in 100% mortality at 30 min for *Ae. aegypti* L. after mosquitoes were exposed to a 56-day residual glazed tile for 3 min (Bayer 2016). Suspend Polyzone (0.03% concentration) resulted in approximately 64% mortality of mosquitoes under the same conditions. Results recorded 24 h after mosquito exposure to 56-day-old residual glazed tiles for 3 min for the other active ingredients tested, Demand CS® (0.03% lambda-cyhalothrin), Cy-Kick® (0.05% cyfluthrin), and TalstarOne (0.03% bifenthrin), showed that all had a mosquito mortality rate of 20% or less (Bayer 2016).

The type of vegetation or land cover can influence the occurrence and abundance of mosquitoes (Chuang et al. 2011, Landau and van Leeuwen 2012). Thus, understanding the environmental characteristics that mosquitoes prefer can help target control measures. A study in Sioux Falls, South Dakota, using 24 CO_2-baited Centers for Disease Control and Prevention (CDC) light traps performed an analysis of 5 land cover types (urban, cultivated crops, grass/hay, forest, and wetland) and multiple buffer radii (200, 400, 600, 800, and 1,000 m) (Chuang et al. 2011). The study found a positive correlation between wetland land cover and *Ae. vexans* Meigen mosquitoes (Chuang et al. 2011). *Culex tarsalis* Coquillett showed a negative correlation with urban land cover and a positive correlation with grass/hay land cover (Chuang et al. 2011).

The current study evaluated 2 products, Bifen I/T (7.9% bifenthrin) and Suspend Polyzone (4.75% deltamethrin), applied by backpack mist blowers in a suburban eastern North Carolina neighborhood. The objectives of this study were to 1) compare mosquito abundance between areas treated with Bifen I/T and Suspend Polyzone barrier sprays, 2) determine spatiotemporal hot spots of mosquito abundance using a geographic information system (GIS), 3) explore the extent to which land cover may impact

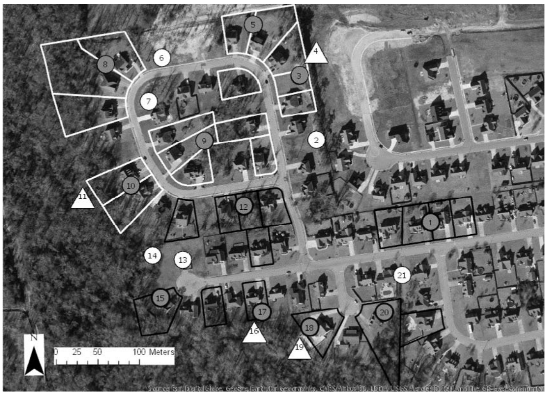

Fig. 1. Aerial view of study area. White outlines represent lots treated with deltamethrin, while black outlines indicate lots sprayed with bifenthrin. Shaded circles represent treated area CDC and oviposition traps. White circles indicate both control CDC and oviposition traps. White triangles represent control oviposition traps only.

mosquito abundance and spatial distribution, and 4) assess the potential correlation between environmental variables (rainfall, temperature) and mosquito abundance.

MATERIALS AND METHODS

Recruitment of participants

Properties within a neighborhood in Pitt County (eastern North Carolina) were recruited based on known mosquito abundance (Richards et al. 2017). Door-to-door and email inquiries were used to recruit participants. If homeowners were home, investigators provided a flyer and verbal information about the study. If homeowners were not home, an informational flyer was left with contact information for the investigator. We asked residents at every neighborhood home to participate in the study; however, not all residents agreed to participate, hence we assigned treatments based on availability of participant lots. Participants were not informed which type of barrier spray treatment was applied on their property. A total of 31 homes (including some vacant lots) were used for the current study. Homes and vacant lots were grouped (in most cases) by acreage into clusters (range of cluster acreage from 1,133 to 8,903 m^2) (Fig. 1; Table 1). Control traps were located at least 50 m from treatment lots.

Barrier spray application

The Mosquito Authority of Eastern North Carolina, a franchisee of The Mosquito Authority (Hickory, North Carolina; https://www.mosquito-authority.com/) collaborated on this study. Certified public health pest control operators applied Bifen Insecticide/Termiticide (active ingredient [AI] =

Table 1. Locations of mosquito traps in Magnolia Ridge neighborhood in Winterville, North Carolina (Pitt County).

Trap	Longitude	Latitude
1	35.519205	−77.41822
2	35.520132	−77.419701
3	35.520872	−77.419903
4	35.521086	−77.419904
5	35.521381	−77.42049
6	35.520978	−77.421781
7	35.520598	−77.421944
8	35.520895	−77.422456
9	35.520065	−77.421171
10	35.519554	−77.422282
11	35.519416	−77.422551
12	35.519385	−77.420712
13	35.518776	−77.421507
14	35.518763	−77.421843
15	35.518396	−77.421779
16	35.518039	−77.420565
17	35.518153	−77.42041
18	35.518044	−77.419802
19	35.517826	−77.420011
20	35.518164	−77.418819
21	35.518594	−77.41864

bifenthrin) and Suspend Polyzone (AI = deltamethrin) to treat foliage on participating properties. Bifen I/T (approximately \$10–17 per 0.5 liter [depending on supplier]) is less expensive than Suspend Polyzone (approximately \$50–53 per 0.5 liter [depending on supplier]); hence, we wanted to test the efficacy of Suspend Polyzone at lower concentration and frequency, for practical reasons. The foliage of properties was treated every 21 days with Bifen I/T (30 ml/3.8 liter [high label rate, 0.06% bifenthrin] so approximately \$6–10 for 30 ml and 8 treatments for this study) and every 28 days with Suspend Polyzone (22 ml/3.8 liter [mid label rate; 0.03% deltamethrin] so approximately \$22–23 for 22 ml and 6 treatments for this study) using a backpack mist blower. Suspend Polyzone is labeled for 90-day efficacy, but was sprayed every 28 days to test the ability of the product to withstand environmental conditions. Label instructions were followed, and operators applied the finished solution (8–19 liters per 305 m^2) in circular patterns to vegetation until runoff. All foliage on treatment properties (but not grass or structures) was treated, i.e., vegetation around perimeter of properties, as well as around structures. Foliage was not considered when properties were selected for treatments (only the participants' agreement to be included in the study) and this was analyzed after the study was completed. The first treatments took place on May 27, 2016.

Host-seeking mosquito collection

Mosquitoes were sampled weekly from May 16 to November 8, 2016 (26 wk) using 17 (6 control traps, 6 traps in bifenthrin zone, 5 traps within deltamethrin zone) CDC light traps with bulbs removed (BioQuip, Rancho Dominquez, CA) (Fig. 1). Traps were baited with dry ice (1.4 kg) as a CO_2 source in a 1-liter cooler and placed in shaded areas of the property/cluster close to its center (within the barrier). Traps were hung using a 2-m shepherd style metal plant hanger. Traps were set in the field weekly between 3:00 p.m. and 5:00 p.m. and retrieved the following morning between 8:30 a.m. and 9:30 a.m. Nets were placed in a freezer to kill mosquitoes, and specimens were subsequently transferred to petri dishes coded by trap site and date. Mosquitoes were identified to species and counted using a Leica S6E dissecting microscope (Wetzlar, Germany) and a dichotomous key (Harrison et al. 2016). Data were organized in a spreadsheet by trap number, week, treatment, and mosquito species.

Mosquito oviposition

Oviposition of container inhabiting mosquitoes (*Ae. albopictus, Ae. triseriatus* Say, and *Ae. japonicus* Theobald) was monitored weekly at the same 17 trap locations and 4 additional locations outside the spray area (control traps: 2 each were placed outside of the barrier near bifenthrin and deltamethrin spray zones). The reason for these 4 additional oviposition traps was to further analyze the pattern of oviposition both inside and immediately outside of the barrier zone. Oviposition traps consisted of black plastic cups (500 ml) half-filled with water with a drain hole drilled 7 cm from the lip. Each oviposition trap contained an oviposition strip (ovistrip) of seed germination paper (8 × 22 cm) encircling the circumference of the cup. Oviposition traps were zip-tied to the bottom of the same plant hangers used to hang CDC light traps. Oviposition strips were set weekly at the same time as the CDC light traps and collected the following week when a new strip was placed in the cup. This process lasted for the entirety of the study. Tap water was dumped from the cup and refilled each week. Ovistrips were transported back to the laboratory in individually labeled Ziploc® bags. Eggs were counted and data were added to data sheets by week, trap number, and treatment. For weeks 0–6, eggs were counted as a total collected per trap. For weeks 7–24, eggs were identified to species for *Ae. albopictus* (shiny black) and *Ae. triseriatus* (dull/matte black). Collection was performed in this manner because of the presence of 2 *Ae. japonicus* specimens identified from egg strips that were reared in the lab during week 3 of the study. Since it may be difficult to distinguish *Ae. triseriatus* and *Ae. japonicus* eggs (Bova et al. 2016), throughout the study, a subset of egg strips was hatched each week and reared to adult for identification. There were no further collections of *Ae. japonicus* noted for the remainder of the study.

Statistical analysis

Statistical analysis of host-seeking mosquito abundance and oviposition was carried out using SAS (SAS Institute, Cary, NC). Comparisons with *P*

< 0.05 were considered significant. Kolmogorov–Smirnov tests were used to determine whether the numbers of mosquitoes collected in different treatments and weeks were normally distributed. To improve normality, data were log transformed [log (x + 1)]. Analysis of variance (ANOVA) was used to determine the extent to which abundance of total adult mosquitoes, key species (*Anopheles crucians* complex, *An. punctipennis* Say, *Cx. pipiens/quinquefasciatus*, *Psorophora columbiae* Dyar and Knab), total mosquito eggs, *Ae. albopictus* eggs, and *Ae. triseriatus* eggs differed between traps, treatment/control areas, and between weeks. Key species were the most abundant (>1,000 specimens) throughout the study. Because we found environmental factors may contribute to differences observed in the northern (deltamethrin) compared with southern (bifenthrin) part of the neighborhood, 2 additional ANOVAs were carried out as follows: 1) total adult mosquitoes compared between control lots that were in the southern part of the neighborhood, deltamethrin, and bifenthrin lots, and 2) total adult mosquitoes compared between control lots that were in the northern part of the neighborhood, deltamethrin, and bifenthrin lots.

Weather

Daily average temperatures and total precipitation data were retrieved from Weather Underground (Langston Farms: KNCWINTE12; Weather Underground 2017). The KNCWINTE12 station is approximately 4.5 km from the study site. Analyses were carried out using SPSS 23 (IBM SPSS Statistics, Chicago, IL), and comparisons with $P < 0.05$ were considered significant. A multiple linear regression analysis was carried out to determine the association between environmental variables (rainfall and temperature) time-lagged 0, 1, 2, 3, and 4 wk and mosquito (adults and eggs) abundance.

Spatiotemporal analysis of hot spots (kriging)

ArcGIS 10.4 (Esri, Redlands, CA) was used to determine areas with high mosquito abundance for the entire study site. The spatial analysis tool "kriging" was used to predict mosquito abundance at locations that were not sampled based on the ability of the tool to provide linear unbiased prediction (Ryan et al. 2004). Ordinal linear kriging was used because this type of analysis is appropriate for a small scale (e.g., neighborhood level). This tool weights the values provided (i.e., trap totals of adult mosquitoes and eggs collected) with the distance in between these values to create predicated intermediate values. The predicted areas of higher mosquito abundance indicated hot spots of mosquito abundance.

Land cover analysis

Land cover analyses were performed using ArcGIS 10.4 and SPSS 23 (IBM SPSS Statistics, Chicago, IL). ArcMap was used to place points at the location of the traps and saved as a point layer file. An aerial photo of the site was downloaded from the U.S. Geological Survey and added to the ArcMap file (US Geological Survey 2016). A red-green-blue (RGB) composite was performed on the aerial image to classify types of land covers. Five classes were created based on land cover types. A train iterative self-organizing (ISO) cluster was then used to name the classes based on the types of land cover each specific color represented: 1) grass, 2) trees/bushes, 3) roads, 4) dense vegetation, and 5) homes, and these classes were also verified on the aerial photo and ground truthed. The train ISO tool uses an RGB image to divide the image into different classes based on the color of each pixel in the image. Pixels that are the same class and adjacent to each other were grouped together into sections of each category. The classification layer was then transferred from raster data to vector data by using the raster to polygon feature within ArcMap. The "buffer" tool was used to apply 18- and 36-m buffer zones to the points simulating the approximate range of mosquito detection of CO_2 (this range will vary by species and was an estimate based on previous studies) (Gillies and Wilkes 1969, 1970). The "intersect" tool was used to create 2 new layers containing all of the created polygons within the 18- and 36-m radius of the traps. The area of each of these polygons was calculated, and the area of all the polygons in the same class within the same trap buffer was divided by the total buffer area of the trap. This was done in order to have information for each land cover type for each mosquito trap. Percentages for the 18- and 36-m buffers were then analyzed in SPSS and compared with the following mosquito abundance variables: total adult mosquitoes, mosquito eggs, and adult female *Ae. albopictus*, *An. crucians* complex, *An. punctipennis*, *Cx. pipiens/quinquefasciatus*, and *Ps. columbiae* trapped in each trap by week. The relationship between land cover and mosquito abundance was analyzed using linear regression.

RESULTS

Host-seeking mosquitoes

In total, 15,083 adult female mosquitoes (20 species) were collected from May 16 to November 8, 2016 (Table 2). During the 2 wk prior to the first treatments, there were no significant differences in total mosquito adults between control, bifenthrin, or deltamethrin traps (df = 2; $F = 0.33$; $P = 0.721$). The total number of mosquitoes collected was significantly higher in traps located in the control and deltamethrin-treated areas, compared with bifenthrin-treated areas (df = 2; $F = 3.90$; $P = 0.021$) (Fig. 2). The total number of mosquitoes collected during the study was significantly highest in traps collected during the week of June 14, 2016 (df = 23; $F = 17.37$; $P < 0.0001$). No other weeks experienced significantly high mosquito counts.

Table 2. Number of adult mosquitoes collected from CDC light traps by mosquito species and treatment.

Mosquito species	Bifenthrin	Deltamethrin	Control
Aedes albopictus	9	30	38
Ae. atlanticus	106	241	447
Ae. fulvuspallens	0	2	5
Ae. infirmatus	85	233	390
Ae. triseriatus	3	17	121
Ae. tormentor	110	152	721
Ae. vexans	248	502	470
Anopheles crucians complex	693	359	753
An. punctipennis	365	408	655
An. quadrimaculatus	165	150	227
Coquilletidia perturbans	3	1	7
Culiseta inornata	0	1	0
Culex erraticus	48	28	28
Cx. pipiens/ quinquefasciatus	1,115	1,081	1,772
Cx. salinarius	113	233	275
Orthopodomyia signifera	1	1	0
Psorophora ciliata	8	11	25
Ps. columbiae	411	702	1,244
Ps. ferox	8	51	210
Ps. howardii	0	1	0
Total	3,491	4,204	7,388

An analysis of adult mosquito trap counts from the northern part of the neighborhood (deltamethrin treatment zone compared with control traps in the northern part of the neighborhood) showed no significant differences between control and treatment traps (df = 1; $F = 0.37$; $P = 0.546$). However, an analysis of adult mosquito trap counts from the southern part of the neighborhood (bifenthrin treatment zone compared with control traps in the southern part of the neighborhood) showed significantly higher trap counts in control compared with treatment traps (df = 1; $F = 8.61$; $P = 0.004$).

When we analyzed total mosquitoes from only control lots in the northern part of the neighborhood compared with all deltamethrin and bifenthrin lots, we found no significant differences between control, deltamethrin, and bifenthrin lots (df = 2; $F = 1.05$; $P = 0.353$). Conversely, when we analyzed total mosquitoes from only control lots in the southern part of the neighborhood compared with all deltamethrin and bifenthrin lots, we show that mosquitoes in control lots were significantly higher than mosquitoes collected from deltamethrin and bifenthrin lots (df = 2; $F = 6.87$; $P = 0.001$).

Traps in control areas showed significantly more *Ps. columbiae* than traps in bifenthrin or deltamethrin areas (df = 2; $F = 3.74$; $P = 0.026$), and this species was significantly most abundant in traps collected during the week of June 8, 2016 (df = 21; $F = 11.72$; $P < 0.0001$). No significant differences were observed in abundance of other key species between treatments (Fig. 3). During the week of June 14, 2016, there was a statistically significant increase in the number of *An. crucians* complex (df = 24; $F = 8.43$; $P < 0.0001$) and *An. punctipennis* (df = 23; $F = 10.33$; $P < 0.0001$) adults collected, but no other significant weekly spikes were observed. Significantly more *Cx. pipiens/quinquefasciatus* were collected during the week of May 24, 2016 (df = 22; $F = 17.43$; $P < 0.001$), but no other weeks. Weekly means of mosquitoes collected per trap night in control, bifenthrin, and deltamethrin lots are shown in Fig. 4.

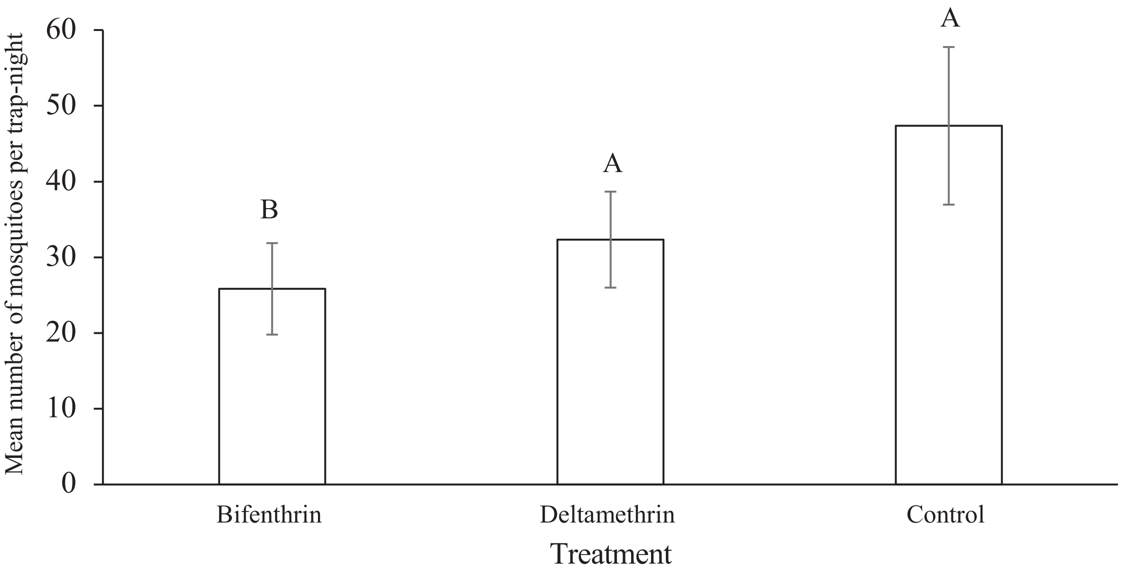

Fig. 2. Mean numbers of mosquitoes (all species) per trap night. Shown with standard error bars. Different letters indicate a significant difference ($P < 0.05$).

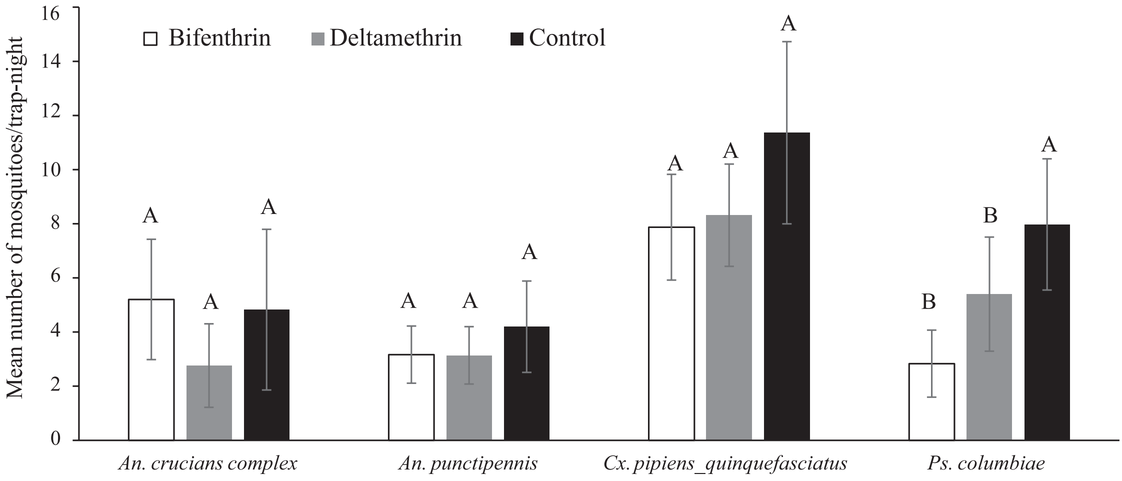

Fig. 3. Mean number of mosquitoes per trap night by key species and treatment. Shown with error bars. Different letters indicate significant differences ($P < 0.05$) between treatments for each species.

Mosquito oviposition

In total, 18,054 mosquito eggs were collected during the study, consisting of 3 different species: *Ae. albopictus*, *Ae. triseriatus*, and *Ae. japonicus*. Ovitraps in the control area collected significantly more eggs compared with bifenthrin and deltamethrin lots (df = 2; $F = 20.57$; $P < 0.001$) (Fig. 5). For mosquito eggs collected during weeks 7–25 of the study, there were significantly more *Ae. albopictus* (df = 2; $F = 4.51$; $P = 0.013$) and *Ae. triseriatus* (df = 2; $F = 0.002$; $P = 0.002$) in the traps placed in deltamethrin and control areas compared with those placed in the bifenthrin area (Fig. 6). *Aedes albopictus* egg abundance was significantly highest during collections the week of July 25, 2016 (df = 17; $F = 2.23$; $P = 0.006$). *Aedes triseriatus* egg abundance was significantly highest during collections from both the weeks of July 25, 2016, and September 22, 2016 (df = 17; $F = 2.21$; $P = 0.006$).

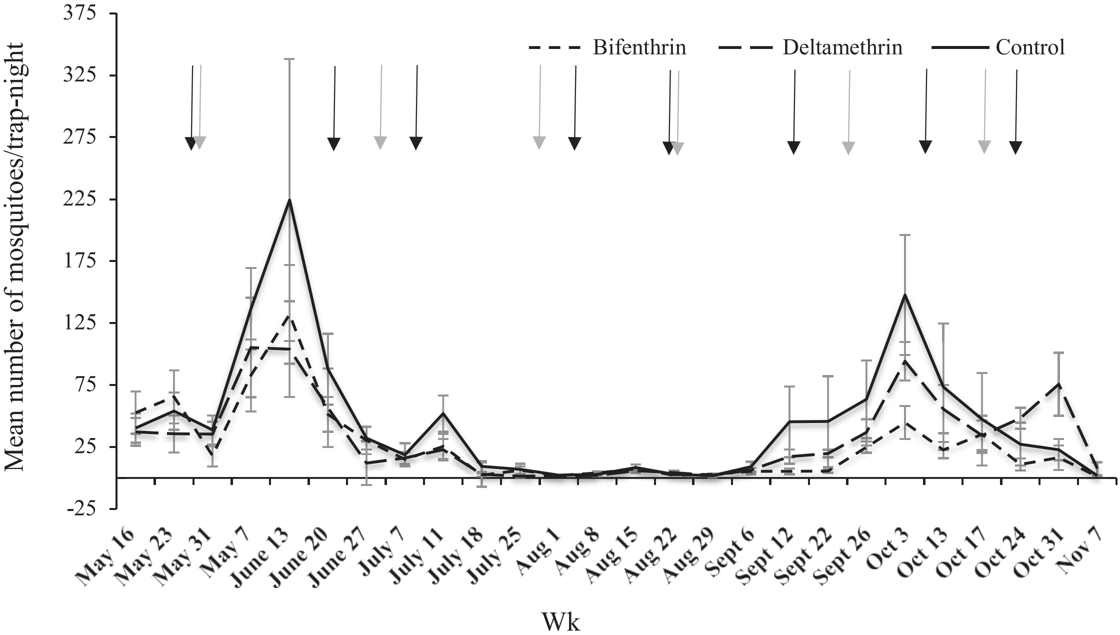

Fig. 4. Mean numbers of mosquitoes per trap night by week and treatment. Shown with error bars. Treatment dates are indicated by black (bifenthrin; 8 treatments; every 21 days) or gray (deltamethrin; 6 treatments; every 28 days) arrows. Initial treatments for both groups were carried out on May 27, 2016.

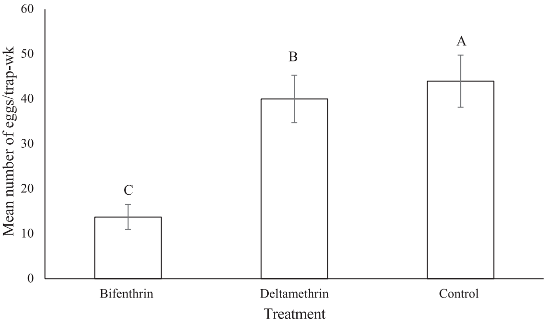

Fig. 5. Mean numbers of mosquito eggs (all species) per trap week by treatment. Shown with standard error bars. Different letters indicate significant differences ($P < 0.05$).

Weather

The relationship between temperature and total adult mosquito abundance was significant ($P < 0.05$) for the week of collection and lag periods 1, 2, 3, and 4 wk prior to collection. In all cases, cooler temperatures (21.2°C–25.8°C) were indicators of higher total adult mosquito collection. Temperature during the week of collection and total mosquito abundance were correlated ($r = -0.435$, $P = 0.027$). Lagged temperatures were correlated (negatively or positively, depending on week) with total adult mosquito abundance (1-wk lag, $r = -0.522$, $P = 0.006$; 2-wk lag, $r = -0.486$, $P = 0.012$; 3-wk lag, $r = -0.573$, $P = 0.002$; 4-wk lag, $r = 0.486$, $P = 0.012$). Rainfall was not a significant indicator of total adult mosquito abundance. The relationship between temperature and total mosquito egg abundance was significant at a lag period 3 wk prior to collection ($r = 0.450$, $P = 0.028$).

Aedes vexans, *An. crucians* complex, *An. punctipennis*, and *Cx. pipiens/quinquefasciatus* showed some level of correlation with either rainfall or temperature. However, abundance of neither *Ae. albopictus* nor *Ps. columbiae* showed a relationship with either rainfall or temperature. The abundance of *Ae. vexans* was significantly negatively correlated with temperature at lag periods 1, 2, 3, and 4 wk prior to collection (1-wk lag, $r = -0.534$, $P = 0.005$; 2-wk lag, $r = 0.551$, $P = 0.004$; 3-wk lag, $r = -0.501$, $P = 0.009$; 4-wk lag, $r = -0.465$, $P = 0.017$). Rainfall was not a significant indicator of *Ae. vexans* abundance. The relationship between abundance of *An. crucians* complex and rainfall was found to be significant for the 2-wk lag period tested ($r = 0.481$, $P = 0.013$). *Anopheles punctipennis* abundance was related to 1-wk ($r = 0.416$, $P = 0.034$) and 2-wk ($r = 0.500$, $P = 0.009$) lag periods. For both species of *Anopheles,* temperature was not an indicator of abundance. The relationship between *Cx. pipiens/quinquefasciatus* mosquitoes and temperature was significant for lag periods 1 wk ($r = 0.586$, $P = 0.002$), 2 wk ($r = -0.479$, $P = 0.013$), 3 wk ($r = -0.482$, $P = 0.013$), and 4 wk ($r = -0.455$, $P = 0.02$) prior to collection. Cooler temperatures for all these periods resulted in greater *Cx. pipiens/quinquefasciatus* at the time of collection. Rainfall was not a significant indicator of *Cx. pipiens/quinquefasciatus* abundance.

Spatiotemporal analysis of hot spots (kriging)

Kriging of total adults captured in each trap for the length of the study shows hotspots were evident in some parts of the neighborhood (Fig. 7). Based on the kriging estimates, the greatest abundance of all host-seeking mosquito species captured was observed in

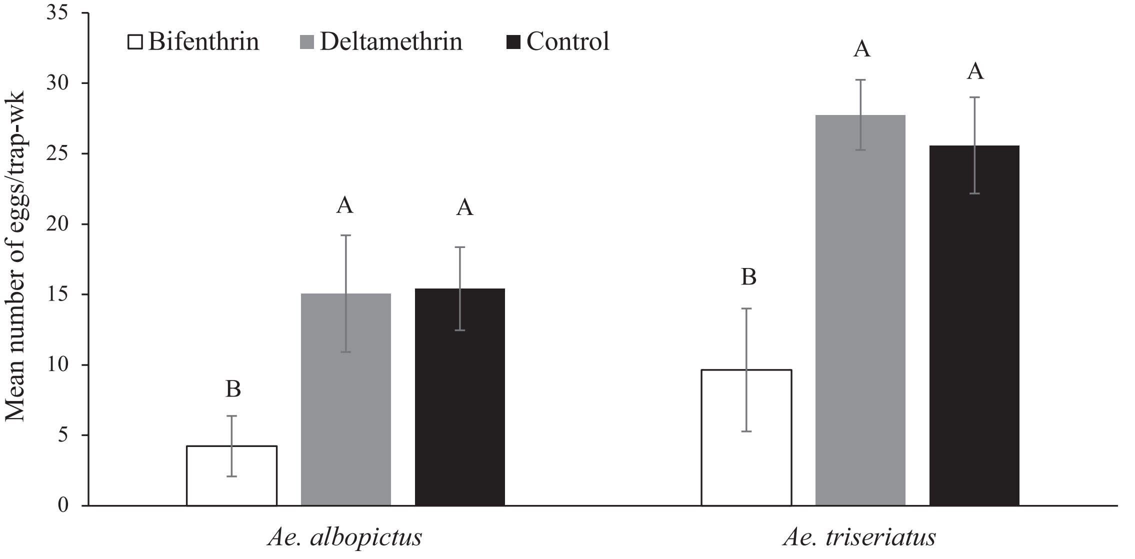

Fig. 6. Mean numbers of mosquito eggs per trap week by species and treatment. Shown with error bars. Different letters indicate significant differences ($P < 0.05$) between treatments for each species.

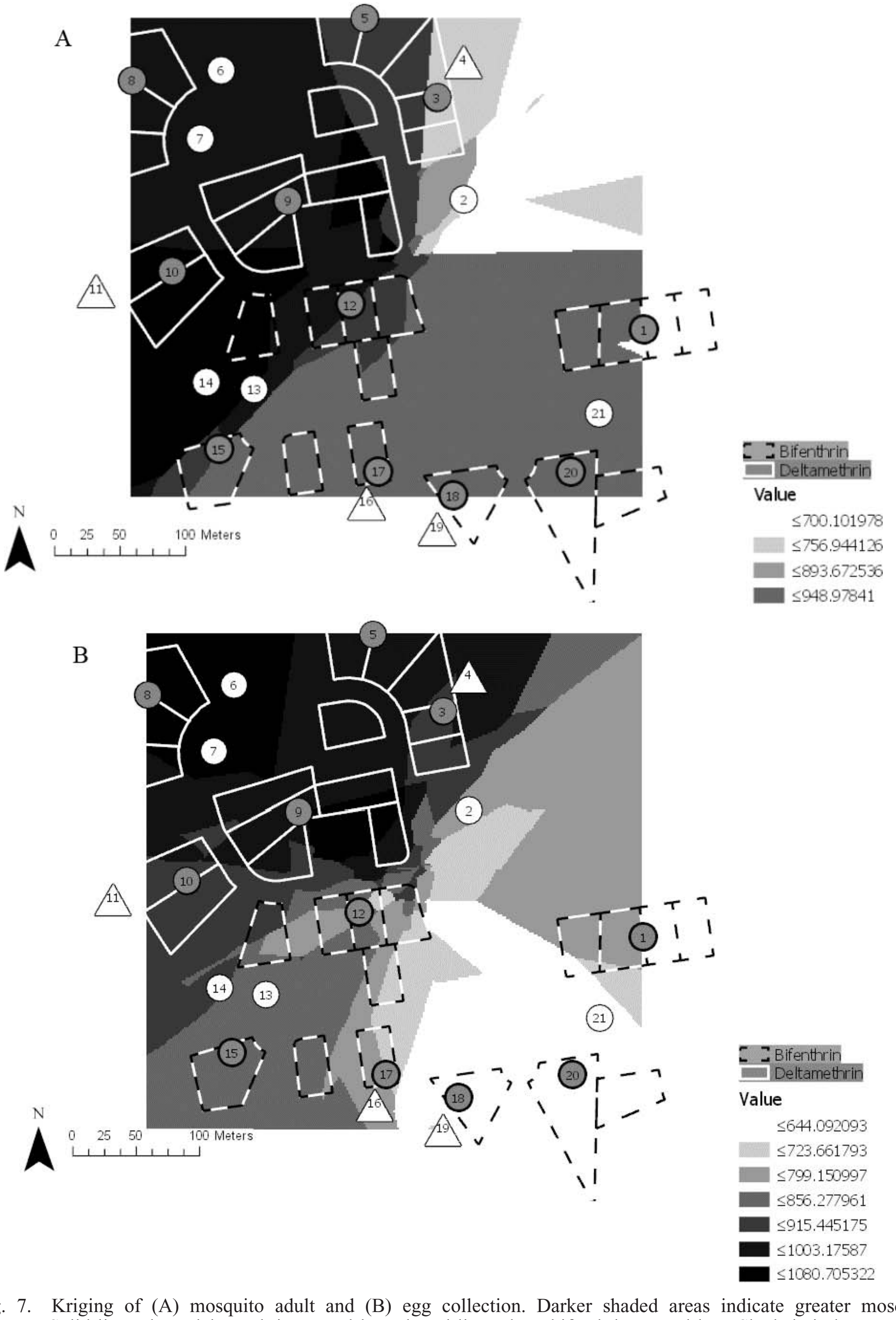

Fig. 7. Kriging of (A) mosquito adult and (B) egg collection. Darker shaded areas indicate greater mosquito abundance. Solid lines show deltamethrin-treated lots, dotted lines show bifenthrin-treated lots. Shaded circles represent treated area CDC and oviposition traps. White circles indicate control CDC and oviposition traps. White triangles represent oviposition at control sites.

Fig. 8. Example of land cover analysis of 18- and 36-m buffer zones. Locations of traps are indicated by the circle with the assigned trap numbers. Circles surrounding the trap locations represent the 18- and 36-m buffer zones. Individual shapes within these circles indicated the combined adjacent pixels that share the same land cover class.

the southwest area of the study located around traps 14 (control property) and 15 (bifenthrin-treated property), and in a small section in the center of the neighborhood. The northwest part of the study area located around traps 6, 7, and 8 was predicted to have high host-seeking mosquito abundance but to a lesser extent than the previously mentioned area. Kriging predictions of oviposition estimated hot spots in the northwest corner of the study area around traps 6 (control), 7 (control), and 8 (deltamethrin-treated property) and a smaller section around trap 9 (deltamethrin-treated property) (Fig. 7).

Land cover analysis

Linear regressions showed an association between each mosquito species and at least 1 land classification (within 18- and 36-m buffers) for all species except *Ae. albopictus* and *Cx. pipiens/quinquefasciatus* (Fig. 8). The total adult mosquito abundance was significantly positively correlated to trees/bushes (18 m, $\beta = 0.103$, $P = 0.03$; 36 m, $\beta = 0.163$, $P = 0.001$) and negatively correlated with homes in both the 18- and 36-m buffer zones (18 m, $\beta = -0.94$, $P = 0.048$; 36 m, $\beta = -0.096$, $P = 0.045$). A negative correlation was observed with total adult mosquito abundance and roads within the 36-m buffer zone ($\beta = -0.103$, $P = 0.031$). *Anopheles crucians* complex abundance was significantly positively correlated to trees/bushes within the 36-m buffer zone and was significantly negative correlated with homes in the 36-m buffer zone (trees/bushes, $\beta = 0.131$, $P = 0.046$; homes, $\beta = -0.164$, $P = 0.012$). *Psorophora columbiae* abundance was significantly positively correlated with trees/bushes at the 18-m buffer zone ($\beta = 0.131$, $P = 0.044$). A significant negative correlation was observed with *Ps. columbiae* and homes in the 18- and 36-m buffer zone (18 m, $\beta = -0.221$, $P = 0.0001$; 36 m, $\beta = -0.132$, $P = 0.043$). The abundance of mosquito eggs was significantly positively correlated to trees/bushes (18 m, $\beta = 0.183$, $P = 0.00$; 36 m, $\beta = 0.161$, $P = 0.001$) and dense vegetation (18 m, $\beta = 0.117$, $P = 0.014$; 36 m, $\beta = 0.128$, $P = 0.007$) within the 18- and 36-m buffer zones. A significant negative correlation was found with mosquito eggs and for grass within 18- and 36-m buffer zones (18 m, $\beta = -0.233$, $P = 0.00$; 36 m, $\beta = -0.215$, $P = 0.00$).

DISCUSSION

In the current study, Bifen IT (30 ml/3.8 liter [high label rate, 0.06% bifenthrin]) sprayed every 21 days significantly reduced the abundance of total adult mosquitoes and *Ps. columbiae* populations compared with untreated control lots. Suspend Polyzone (22 ml/3.8 liter [mid label rate; 0.03% deltamethrin]) sprayed every 28 days significantly reduced *Ps. columbiae* mosquito populations compared with untreated lots. When the total number of adult mosquitoes was analyzed, bifenthrin significantly reduced mosquito abundance. We hypothesize that environmental differences between the northern part (deltamethrin test area) and the southern part (bifenthrin test area) of the neighborhood may have influenced trap counts so that there was more mosquito pressure in the northern region. Our analysis of total mosquito abundance comparing counts in treatment areas with only control lots in the southern part of the neighborhood showed that mosquitoes in control lots were significantly higher than mosquitoes collected from both deltamethrin and bifenthrin lots. This is revealing and illustrates an important point that trap placement, even in a single neighborhood, can influence results. If this neighborhood is used in a future study to evaluate more than 1 treatment method, treatments should be scattered in both the northern and southern parts of the neighborhood, to provide a more even distribution of different treatment types. Hence, this should be considered with interpreting results and also when considering initial study design in other experiments. It should also be noted that the small scale in this neighborhood-sized study could impact mosquito abundance detected in our traps due to factors including but not limited to: variation in outdoor lighting on different properties, pets in back yards attracting host-seeking mosquitoes, artificial shade devices, and outdoor activity of homeowners on properties.

The current study used CO_2-baited light traps, which attract adult host-seeking females from long distances (usually from $\geq$18 m) (Gillies and Wilkes 1969, 1970). Future studies may consider using traps with attractants as well as leaf bioassays for a more local analysis (not attracting mosquitoes from long distances) of efficacy justification. The number of mosquito eggs was significantly higher in oviposition sites located within the control compared with treatment sites. These results are similar to those of Richards et al. (2017) that tested the same 2 products with the same neighborhood at the same label rates (but application rate of every 21 days for both products). Both studies found that both the bifenthrin and deltamethrin products reduced *Ps. columbiae* better than no treatment (control lots) and resulted in equivalent reductions for other key species. The current study showed that reducing the spray frequency of deltamethrin (every 28 days rather than every 21 days) decreased its performance for some species, but not others. This should be considered for future efficacy studies. In the current study, a significant increase in total mosquito abundance was observed during the week of June 13, 2016 compared with all other weeks. During the same week, significantly high abundance was observed for *An. crucians* complex and *An. punctipennis*. The week of May 23, 2016, showed significantly higher *Cx. pipiens/quinquefasciatus* abundance, while during the week of June 7, 2016, significantly higher *Ps. columbiae* populations were observed.

Another barrier treatment study evaluating the efficacy of bifenthrin and lambda-cyhalothrin showed a reduction in *Ae. albopictus*, but not *Culex* spp. of mosquitoes (Trout et al. 2007). The same study hypothesized that targeting upper tree canopies with barrier treatments may have improved efficacy of *Culex* spp. control. In Florida, barrier treatments around a golf course neighborhood also showed a reduction in mosquito populations (e.g., *An. crucians, Ae. atlanticus*) and were also more economical than truck-mounted ULV treatments (Qualls et al. 2012).

Another eastern North Carolina study (Richards et al. 2017) in the same neighborhood studied here compared the effects of both Suspend Polyzone (deltamethrin 4.75%) (Type II pyrethroid) and Bifen I/T (bifenthrin 7.9%) (Type I pyrethroid) as barrier sprays. In the same study, each formulation was applied to every 21 days. CO_2-baited CDC light traps and oviposition traps were set weekly (Richards et al. 2017). The study showed that, in the Magnolia Ridge neighborhood, the insecticide treatments resulted in a significant decrease in mosquito abundance, compared with the control lots (Richards et al. 2017). The study also found that *An. punctipennis* were significantly more abundant in the Bifen I/T areas of Magnolia Ridge than the Suspend Polyzone or control areas (Richards et al. 2017). The same study showed that Magnolia Ridge had significantly higher egg numbers in control traps compared with the treatments (Richards et al. 2017). Cooler temperatures during the time of collection and precipitation events 2 wk prior to collection resulted in significantly greater mosquito abundance (Richards et al. 2017).

Results in the current study are in line with those of the aforementioned study (Richards et al. 2017) in the same neighborhood that found a significantly higher total mosquito abundance in the week of June 15, 2015 compared with all other weeks (Richards et al. 2017). It is believed that lower temperatures in the 4 wk leading up to this period of the year (weekly average, 2015, 20°C–28°C; 2016, 21°C–26°C) played a role in the high abundance of mosquito populations during this period. During periods of much higher temperatures (mid-July through late-August), we observed mosquito activity to be low in all sites, which may indicate that adult mosquito populations were reduced, likely due to mortality and other unknown factors. This indicates that, in this eastern North Carolina area, temperature may be used to predict mosquito abundance, and this could help direct treatment efforts. Knowledge of local patterns of seasonal abundance for different mosquito species in different regions can be used in conjunction with control efforts to maximize targeted control, thereby potentially limiting unnecessary insecticide applications. For example, in the Southeast (Mississippi), *An. punctipennis* typically has peaks in late spring and early summer, while *Ps. columbiae* occurs April–November and peaks in late summer (Goddard et al. 2010). Patterns of abundance should be tracked for each species within mosquito surveillance programs and compared with temperature/rainfall data each year to better time insecticide treatments. As mentioned previously, significant weather events (e.g., hurricanes or tropical storms) may impact abundance for some populations, such as flood water species of *Ae. atlanticus*, depending on time of year.

Oviposition was significantly highest during the week of July 25, 2016. As expected, higher levels of rainfall 3 wk prior to egg collection may have contributed to egg abundance of the container species collected here. With heavy rainfalls, artificial containers may fill with water, providing substrate for mosquito growth (i.e., approximately 7 days required to reach adulthood from the egg stage, depending on environmental conditions) and may wash off pesticide from foliage (Sivanathan 2006). The weather analysis performed in this study showed that relatively cooler temperatures were related to higher mosquito abundance, while rainfall had no significant effect on total adult mosquito abundance. Similar findings were indicated by Richards et al. (2017) in the same neighborhood for the previous year. Conversely, a study on *Cx. quinquefasciatus* from Louisiana showed that mosquito populations increased with increasing temperature (Moise et al. 2018). A study in Georgia showed that weather variables were not predictive of *Cx. quinquefasciatus* (Buckner et al. 2011).

Along with mosquito trapping, mosquito control personnel may use a localized weather monitoring system to monitor temperatures in an area to determine, in part (along with mosquito surveillance), whether treatment is necessary. The relationship between weather variables, mosquito surveillance of typical patterns of mosquito abundance for different species, and treatments should be considered by mosquito control personnel and may provide a more targeted approach to control. While not indicated in the current study, Richards et al. (2017) found a positive correlation between rainfall and mosquito abundance within the same neighborhood, suggesting heavy rainfall may have contributed to the barrier spray product washing off the vegetation to some degree. Heavy rainfall may have washed some barrier spray residue from leaves in the current study, but not at significant levels. The current study did not quantify the amount of residual AI on leaves. However, this should be considered in future studies. The study area experienced 2 major hurricanes during this study. Hurricane Hermine impacted on September 1, 2016, with a total rainfall of 116.3 cm over a 3-day period. Hurricane Matthew impacted the study area on October 8, 2016, with a total rainfall of 198.7 cm over a 3-day period. These hurricanes may have washed barrier spray products off of the vegetation, as well as greatly reducing much of the adult mosquito population during the storm period. In addition, increased rainfall enhanced abundance of flood water mosquito species that may not have otherwise increased in years with typical rainfall amounts.

Increased mosquito control measures such as surveillance-based targeted adulticides/larvicides and reduction of oviposition sites are needed in order to manage mosquito abundance. Mosquito abundance during July and August are likely suppressed due to higher temperatures, hence less frequent spraying may be required during these periods in some regions. More work should be done to evaluate additional environments and neighborhoods where mosquito occurrence/seasonality and abundance may vary. Furthermore, the CDC traps used in the current study are not ideal for trapping host-seeking *Ae. albopictus* adults; therefore, BG Sentinel traps may be considered for future studies on this species.

In the previous study (Richards et al. 2017), the quantities of mosquitoes collected were much lower (bifenthrin, 6.0 mosquitoes/trap night; deltamethrin, 4.6 mosquitoes/trap night; control, 8.0 mosquitoes/trap night). In the current study, these numbers experienced a dramatic increase (bifenthrin, 25.8 mosquitoes/trap night; deltamethrin, 32.3 mosquitoes/trap night; control, 44.6 mosquitoes/trap night). This increase between successive years in the same neighborhood could be due to seasonal differences in temperature, rainfall, and/or other factors. It is possible that residents may have altered their source reduction efforts, and this could have led to greater mosquito abundance (Dumont and Thuilliez, 2016); however, homeowners were not surveyed in the current study.

The results of the land cover analysis of the study area showed that, in general, adult mosquitoes (all species) prefer areas that are lightly wooded or composed of small collections of trees or bushes, compared with larger densely wooded areas. As expected, host-seeking adult mosquitoes were less likely to be collected from areas with high amounts of built structures/homes and roadways. This is likely because resting areas and favorable habitats are more prevalent among trees and bushes. Mosquito resting habits indicated by Reiskind et al. (2017) found that *Cx. pipiens/quinquefasciatus* and *Ae. infirmatus* Dyar and Knab prefer to rest in shrubs, while *Cx. salinarius* and *An. quadrimaculatus* Say prefer shrub and grassland equally. Together these 4 species represent 38.98% (*Cx. pipiens/quinquefasciatus*, 26.4%; *Ae. infirmatus*, 4.7%; *Cx. salinarius*, 4.1%; *An. quadrimaculatus*, 3.8%) of the total mosquitoes captured in the aforementioned study. Here, the dense vegetation land cover class consisting of pine trees may have impacted abundance due to oils in pine trees that may have repelled mosquitoes (Ansari et al. 2005). A land cover analysis performed in Tucson, Arizona, using 11 classes and 5 radii (10, 20, 30, 40, and 50 m) compared land cover to abundance of *Ae. aegypti* and *Cx. quinquefasciatus* (Landau and van Leeuwen 2012). The 30-m radius was determined as the best scale for the study because it had the strongest relationship to land cover variables. Others have shown that CO_2 alone can attract mosquitoes at 18–36 m; however, animal hosts can increase this range ($\geq$36 m), depending on mosquito species (e.g., *Anopheles* spp. may be able to detect baits from longer distances than *Culex* spp.) (Gillies and Wilkes 1969, 1970). The 18–36-m buffer zones were used in our study since these are the shortest distances that have been shown attractive to mosquitoes when CO_2 bait (dry ice used here) is used; however, future studies could consider testing the relative attractiveness of different CO_2 flow rates using tanks with flow regulators. The Arizona study found that *Ae. aegypti* was positively associated with structures and medium height trees, while bare earth had a negative association. A positive association was also found with *Cx. quinquefasciatus* and pavement, as well as medium height trees, while shrubs had a negative association (Landau and van Leeuwen 2012). A study in Georgia showed an association with *Cx. quinquefasciatus* and anthropogenic areas without a high degree of natural cover (Buckner et al. 2011). In our study, *Cx. pipiens/quinquefasciatus* was not associated with any land cover types within the 18- or 36-m buffer. We showed that, within both our 18- and 36-m buffers, total host-seeking mosquito counts were associated with trees and homes. High mosquito egg abundance was positively associated with trees/bushes and dense vegetation, but negatively associated with grassy areas. This is likely due to gravid mosquitoes preferring to rest and oviposit in shaded areas with nectar-feeding potential, similar to host-seeking adult mosquitoes collected in the CDC traps. Another possible reason could be that oviposition sites in grassy areas are exposed to more sunlight than those set in the shade. This could lead to a higher degree of evaporation each week, hence reducing potential oviposition. Additional work should be done to evaluate the association of different mosquito species with a variety of different types of land cover. It should be considered that the different types of traps used here attract different types of mosquitoes, i.e., oviposition traps selectively collect eggs of container ovipositing mosquitoes such as *Ae. albopictus* and *Ae. triseriatus*, and these species are generally not attracted to CDC light traps. Future studies could consider using a combination of CDC light traps and BG Sentinel traps (attract adult host-seeking container species, such as *Ae. albopictus*).

The results of the kriging show that it is possible to map areas of higher mosquito abundance with similar results to the analysis of each barrier spray product. Both the spatial and statistical analyses found greater mosquito abundance within the deltamethrin area. The high presence of the trees and bushes land class in the deltamethrin-treated area may have contributed to higher abundance of mosquitoes in traps, hence providing a challenging environment for the deltamethrin, especially since it was applied at a lower frequency than bifenthrin. This makes it difficult to draw definitive conclusions about the comparative efficacy of deltamethrin and bifenthrin. To test this, a future study in this neighborhood could randomly scatter different treatments to lots within the northern and southern part of the neighborhood, rather than separating the treatments. It is also possible that applying barrier sprays in some parts of the neighborhood affected the location of mosquito hotspots within the neighborhood, effectively "pushing" mosquitoes out of some areas and into other areas. Since bifenthrin and deltamethrin were not applied at the same frequency here, the treatment dates for each AI did not necessarily fall on the same days. A future study evaluating hot spots of mosquito abundance in the neighborhood (without barrier spray applications) could be done to gain a better understanding of the natural baseline of abundance.

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