ABSTRACT

RICHARDS, STEPHANIE LYNN. Spatial ecology of *Aedes albopictus* in suburban landscapes of a piedmont community in North Carolina. (under the direction of Charles S. Apperson).

The objectives of our study were to investigate the spatial ecology of *Ae. albopictus*, to evaluate some potential management methods, and to characterize its host feeding patterns in a suburban landscape. We monitored *Ae. albopictus* oviposition activity with oviposition traps at fixed stations, conducted container surveys for larvae and pupae, and collected adults from vegetation at residences in eight suburban neighborhoods in Raleigh, NC during the 2002 and 2003 mosquito seasons. Host sources of blood fed mosquitoes were determined by an indirect Enzyme Linked Immunosorbent Assay, using antisera made in New Zealand white rabbits to the blood serum proteins of humans and, domestic and wild animals.

An area-wide management strategy involving a lethal oviposition trap (LOT) placed in the landscape around homes in suburban neighborhoods was evaluated. The trap consisted of a black plastic cup filled with water and containing an oviposition substrate impregnated with an insecticide. Source reduction (SR) of water-filled containers was also evaluated as a management strategy for *Ae. albopictus* in combination with the LOT and as a stand-alone area-wide approach to control. Analysis of variance did not reveal any significant mosquito suppression ($P > 0.05$) resulting from the LOT, SR, or the combination of LOT and SR during the 2002 and 2003 mosquito seasons. We determined that the mosquito production potential of a container was a function of its pupal standing crop, density, and spatial distribution in the landscape. Spatial statistical methods were used to evaluate impacts of SR of man-made, water-holding containers on the spatial structure of the population of *Aedes albopictus* immatures. Spatial analyses considering the presence or absence of pupae revealed that residences with at least one pupa-positive container tended to be dispersed throughout SR areas and clustered throughout control areas, indicating that SR affected the spatial distribution of pupae. We used kriging to show the spatial distribution of oviposition activity within neighborhoods. Areas of high and low egg production existed in most neighborhoods; however, spatial patterns of oviposition changed between seasons.

Oviposition activity peaked in late summer in both the 2002 and 2003 mosquito seasons. *Aedes albopictus* primarily fed on mammals, but took blood meals from 12 different hosts or host classes, including birds, frogs, and turtles. The largest proportion of blood meals was taken from humans, followed by cats and dogs. Host feeding indices were calculated for human and domestic animal hosts based on the proportion of host specific blood-fed mosquitoes per collection in relation to the number of corresponding specific hosts per residence established from a door-to-door host survey. When host abundance was considered solely, host-feeding indices indicated that *Ae. albopictus* was more likely to feed on domestic animals. When feeding indices included host abundance that was time-weighted based on potential exposure to mosquitoes, *Ae. albopictus* fed preferentially upon humans.

SPATIAL ECOLOGY OF *AEDES ALBOPICTUS* IN SUBURBAN LANDSCAPES OF A PIEDMONT COMMUNITY IN NORTH CAROLINA

by

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BIOGRAPHY

Stephanie Lynn Richards was born in Charleston, South Carolina on July 21, 1976. She graduated from East Carolina University with a Bachelor of Science degree in Biology in 1998 and received a Master of Science degree in Environmental Health Sciences, Safety, and Technology in 2001.

During her M.S. program, she became interested in the utility of Geographic Information Systems in Medical Entomology, which lead her to major in Entomology and minor in GIS during the course of her doctoral studies at North Carolina State University.

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SPATIAL ANALYSIS OF *AEDES ALBOPICTUS* (SKUSE) (DIPTERA: CULICIDAE) OVIPOSITION: RELATIONSHIP BETWEEN THE ABUNDANCE OF EGGS, PUPAE, ADULT FEMALES AND LANDSCAPE CHARACTERISTICS IN SUBURBAN NEIGHBORHOODS OF A PIEDMONT COMMUNITY IN NORTH CAROLINA

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ABSTRACT

The spatial distribution of *Aedes albopictus* oviposition activity was investigated in suburban neighborhoods in the City of Raleigh, North Carolina using oviposition traps at fixed sampling stations during the 2002 and 2003 mosquito seasons. Based on spherical variogram models, kriging was carried out to visualize the spatial patterns of oviposition in suburban neighborhoods. Focal areas of high and low egg production were evident in most neighborhoods; however, the spatial patterns of oviposition changed between mosquito seasons. We evaluated our kriging predictions by systematically removing each data point from our dataset and predicting the removed point using the remaining points. The slope of scatterplots of predicted versus observed points varied significantly from one; however, the root mean square (standardized) error values of best fitting variogram models did approximate one and, plots of standardized PRESS residuals showed no distinct pattern for most neighborhoods, indicating that predictions of the spatial distribution of oviposition activity were valid. Oviposition activity of *Ae. albopictus* peaked in late July and late August during the 2002 and 2003 mosquito seasons, respectively. The numerical relationship between the egg abundance in ovitraps and proportions of grass, tree, permeable, and impermeable land cover within prescribed buffers surrounding ovitraps was evaluated since gravid mosquitoes rest and seek oviposition sites in specific areas of the landscape. In general, there was a significant relationship ($P < 0.05$) between all types of land cover and mean numbers of eggs per ovitrap-week, suggesting that gravid females search all areas of the landscape for water-filled containers to lay eggs in. The oviposition biology of *Ae. albopictus* appears to be linked to mosquito production in water-filled containers in backyards. In our study neighborhoods in the 2002 and 2003 mosquito season, we found a significant relationship ($P < 0.05$) between monthly mean total pupal standing crop per residence and mean

numbers of eggs per ovitrap-week. In 2003, we found a significant relationship ($P < 0.05$) between monthly mean total female *Ae. albopictus* per aspiration sample and mean total pupal standing crop per residence. In both mosquito seasons, a significant numerical relationship ($P < 0.05$) between monthly mean total female *Ae. albopictus* per aspiration sample and mean numbers of eggs per ovitrap-week was found. Kriging is an appropriate method for describing the spatial distribution of *Ae. albopictus* oviposition activity and for estimating oviposition activity at unsampled locations.

INTRODUCTION

Aedes albopictus (Skuse) is an exotic container-ovipositing mosquito that was introduced into the United States in Houston, Texas during the mid 1980s (Hawley et al. 1987). This mosquito is native to Asia and was brought into the United States via a shipment of tires. Since its introduction, *Ae. albopictus* has spread across eastern U.S., utilizing man-made and natural containers as oviposition sites (Reiter and Sprenger 1987; Moore and Mitchell 1997).

Oviposition traps (ovitraps) have been used as a standard tool for monitoring the spatial activity and presence of container-inhabiting mosquitoes, such as *Ae. aegypti* (Fay and Perry 1965; Fay and Eliason 1966; Service 1992; Bellini et al. 1996). In the U.S., ovitraps were first used as a monitoring tool in eradication programs for detecting *Ae. aegypti* (Fay and Perry 1965; Fay and Eliason 1966). Ovitraps have subsequently been adapted for *Ae. albopictus* surveillance (Mogi et al. 1990; Hobbes et al. 1991; McHugh 1993; Bellini et al. 1996). Mogi et al. (1990) and Bellini et al. (1996) developed a sequential sampling plan for *Ae. albopictus* surveillance based on presence-absence sampling data from ovitraps.

In recent years, geographic information systems (GIS) have been utilized to study distributions of mosquito populations. Within a GIS, kriging can be used to interpolate mosquito

densities at unsampled locations based on known densities at sampled locations (Isaacs and Srivastava 1989). In Hawaii, Focks et al. (1999) constructed spatially referenced contour maps by kriging ovitrap data for *Ae. albopictus*. By using spatial analysis, areas where oviposition activity was concentrated could be determined so that insecticide treatment could be focused to maximize control of mosquito adults. In estimating the risk of dengue virus transmission in a village in Thailand, Strickman and Kittayapong (2003) constructed maps within a GIS to visualize spatial concentrations of mosquito larvae by kriging larval density values to create contours around georeferenced premises. In a later study in Bangladesh, Ali et al. (2003) used kriging within a GIS to study the association between population clusters of *Ae. albopictus* and dengue cases. Furthermore, an Australian investigation carried out by Ryan et al. (2004) employed weekly kriging maps to explore the dynamics of the spatio-temporal structure of adult mosquito densities to pinpoint control efforts to areas that were 'hot spots' for mosquito production.

In addition to geostatistical analyses, remote sensing data can be utilized within a GIS to identify habitat characteristics related to mosquito abundance (Dale et al. 1998). Aerial photography is a frequently used component of remote sensing (Washino and Wood 1994). When used in conjunction with entomological data, remote sensing can help prioritize areas for mosquito control (Hugh-Jones 1991). Past studies of the distribution of mosquitoes in relation to landscape patterns have traditionally modeled the relationship between remotely sensed larval habitats and mosquito abundance (Dale et al. 1998); however, environmental conditions conducive to production of container-inhabiting mosquitoes are difficult to identify using these techniques as evidenced by a study of *Ae. aegypti* in Australia (Moloney et al. 1998). In contrast, it is feasible to study landscape characteristics related to resting cover for container-

ovipositing adults. Barker et al. (2003) associated egg densities of *Ae. albopictus* and *Ochlerotatus triseriatus* with types of land cover in western Virginia. Significantly higher numbers of *Ae. albopictus* eggs were collected in open grassy areas around homes while oviposition activity of *Oc. triseriatus* was highest in forested habitats adjacent to homes.

Egg densities in ovitraps have been used as a surrogate measure of the abundance of adult mosquitoes (Mogi et al. 1988; Braks et al. 2003). However, the relationship between the numbers of eggs collected in ovitraps and the number of egg-laying females visiting traps is not likely to be linear since females lay some eggs from each clutch in several containers (Rozeboom et al. 1973; Chadee et al. 1990; Colton et al. 2003). Consequently, the number of alternative oviposition sites would affect the number of eggs laid in an ovitrap. Studies on *Ae. aegypti* carried out in the Philippines by Schultz et al. (1993) and in Columbia by Romero-Vivas and Falconar (2005) showed that ovitraps measured the occurrence not the abundance of adults, and that collecting eggs in ovitraps is more sensitive than collecting adults for detection of *Ae. aegypti*, especially at low population levels. Furthermore, in Trinidad, West Indies, Focks and Chadee (1997) advocated using standing crop of *Ae. aegypti* pupae in containers as a measure of adult abundance since pupal survivorship is high.

In our study, we present results of spatial analyses of oviposition activity of *Ae. albopictus* in suburban landscapes. Specifically, we characterized the spatial patterns of *Ae. albopictus* oviposition within suburban habitats and determined whether oviposition activity of *Ae. albopictus* in ovitraps could be used to reliably predict the abundance of eggs at unsampled locations. Also, we determined if oviposition activity was associated with different types of land cover surrounding oviposition traps. The numerical relationship between oviposition intensity and abundance of pupae and adults was also determined.

MATERIALS AND METHODS

Study areas. Raleigh (76.6° W, 35.8° N) is the capital city of North Carolina and is located between the Piedmont and Coastal regions of the state. The City of Raleigh is located in the geographic center of Wake County, which has an area of 2,212 km^2. The eight suburban neighborhoods utilized in our study were primarily comprised of single-family homes. Neighborhoods were composed of 20 – 42 single-family dwellings of comparable age and structure with an average lot size from 0.09 to 0.33 ha. Common flora at study sites included a mixture of deciduous (maple, oak, and elm) and evergreen (pine and cedar) trees with a variety of under story vegetation such as English Ivy and ornamental shrubbery. Neighborhoods were typically comprised of woodland areas interspersed with open, grassy lawns. In general, the types of vegetation found in neighborhoods were similar; however, the proportion of different types of land cover varied between residences and neighborhoods.

Study design. This study was carried out in four (N-1, N-2, N-3, N-4) and eight (N-1, N-2, N-3, N-4, N-5, N-6, N-7, N-8) neighborhoods during the 2002 and 2003 mosquito seasons, respectively. Egg-laying activity was monitored continuously at each residence using a standard oviposition trap as described below. Each oviposition trap was georeferenced with a handheld global positioning system (GPS) unit (Geo-explorer® III, Trimble, Sunnyvale, CA) fitted with an external antenna. Approximately 40 geographic positions were recorded at one-second intervals for each ovitrap to improve accuracy of measurements. Shapefiles were created from the georeferenced points for spatial analysis in a GIS and for geostatistical analyses described later. The coordinates for points within each shapefile were differentially corrected using positional data downloaded from a GPS base station in Raleigh, NC (http://www.ncgs.state.nc.us/basestation.html#raleigh) (North American Datum 1983).

Pathfinder software (ver. 2.5.1, Trimble, Sunnyvale, CA) was used to differentially correct ovitrap coordinates as described by the manufacturer. The attribute table within each shapefile for each neighborhood was comprised of form fields that contained the alphanumeric code for each residence, along with coordinates for the oviposition trap. Adults were collected from the landscape surrounding a subset of homes within each neighborhood on a weekly or bimonthly basis as described below. Monthly container surveys were completed by a field crew of 4-5 people, searching the home grounds of each premise for containers, which were examined for mosquitoes as described below. Properties were thoroughly canvassed or searched out to a distance of 50 m from the primary dwelling if the property was large. Form fields for the adult and egg abundance variables were added to the attribute tables so that spatial analyses of the distribution of *Ae. albopictus* eggs and adults could be carried out.

Assessment of oviposition activity. Egg-laying activity was monitored using an oviposition trap, which consisted of a black plastic cup (500 ml nominal) half filled with water with an oviposition substrate of red velour paper (1 x 7 cm) clipped inside and three drainage holes drilled 4 cm from the lip. In both mosquito seasons, one oviposition trap was placed continuously on the ground adjacent to or underneath vegetation on the home grounds of each residence. Oviposition strips were replaced twice in each seven-day week in 2002 and every seven days in 2003. When each trap was serviced, water was added and the oviposition strip was replaced. Used oviposition strips were transported back to the laboratory where eggs on each strip were identified to species (Linley 1989 a,b), counted, and the totals added to the appropriate form field for each residence in the attribute table of the shapefile.

Container surveys. The content of each wet container was poured out into a white enameled pan and examined for mosquitoes. If mosquitoes were obscured by dark water or

debris, tap water was added to the pan to improve observation of mosquitoes. This process was repeated until the entire container was emptied. All pupae and a sample of approximately 10 larvae were transferred to Whirl-Pak sample bags (Fisher Scientific, Pittsburgh, PA) and samples were labeled with date of collection, house identification code, and type of container. If the volume of water in the container required what was considered an excessive sampling time, then the container was sampled for a standard time of five minutes.

After collection, samples were placed on wet ice and transported to the lab where mosquitoes were killed in hot water and transferred to labeled vials filled with ethanol. Later, the larvae and pupae in each sample were identified to species using standard keys (Stojanovich 1962; Slaff and Apperson 1989). *Aedes albopictus* pupae were differentiated from pupae of other species by the presence of a fringe of hairs on the edge of the anal paddle (Harrison 2005). Once identified, pupae were counted and the counts, along with the container type, added to a database for subsequent statistical analyses.

Collection and processing of adult mosquitoes. On a weekly basis in 2002 and on an approximate bimonthly basis in 2003, landscape vegetation on the grounds of ten residences in each neighborhood was aspirated for 10 min. each using a large-bore aspirator (Nasci 1981). Mosquitoes were collected from shaded areas containing tall grass, herbaceous plants, or other knee-high vegetation. The aspirator was moved from side-to-side through the vegetation while the collector walked at a pace of approximately 0.5 m per second. Immediately after collection, each sample bag was placed in a cooler on wet ice. Samples were transported to the laboratory for processing within three hours of collection. In both mosquito seasons, the same personnel were used to aspirate vegetation. Residences within each neighborhood were not selected randomly for sampling, because we wanted to maximize the numbers of mosquitoes collected.

Consequently, sampling was restricted to residences that had landscapes comprised of vegetation that we thought would be attractive to mosquitoes that were seeking resting cover.

In the laboratory, each sample bag was placed in a freezer to kill insects collected from foliage. Subsequently, mosquitoes were sorted from debris under 10X magnification against a white background, transferred to labeled vials, and stored in the freezer. Later, mosquitoes in each sample were identified to species (Slaff and Apperson 1989) and sex, and counted. In addition, females were examined to determine their gonotrophic status (unfed, blood fed, gravid).

Land cover characterization. Neighborhood land cover was analyzed since woodland areas and shrubbery provide resting cover for adult mosquitoes and man-made containers used for oviposition are scattered throughout suburban environments. One-meter resolution digital aerial photographs of study sites taken in 1999 were acquired from the Wake County GIS website (http://www.wakegov.com/county/propertyandmapping/gisdigitaldata.htm). We examined the photographs and determined that no substantial land use changes had occurred within our study neighborhoods since the landscape was photographed. Color photographs were available in Mr. Sid format (Lizard Tech, Seattle, WA, 2002). The *Feature Analyst* extension of ArcMap was used for "wall-to-wall" digitizing of land cover in each neighborhood. We hand-digitized examples of specific types of land cover and then defined custom learning settings within *Feature Analyst*. Through "hierarchical learning", incorrectly and correctly classified areas of land cover were highlighted by hand and, *Feature Analyst* software was "trained" to identify unique types. Land cover categories included woodland regions and open areas with grass and shrubbery, along with roads and buildings. Some of the land cover classes were consolidated for further analyses as follows. Wooded locations and open areas with grass and shrubbery were grouped into "permeable" surfaces, while roads and buildings were grouped as

"impermeable" surfaces. Subsequently, land cover was quantified for each neighborhood to determine potential effects on oviposition activity as measured with ovitraps. With the *Geo-processing* extension in ArcMap, buffers corresponding to *Ae. albopictus* flight ranges of 10, 25, 50, and 100 meters were created around the geographic location of each ovitrap. With the *Feature Analyst*, land cover contained within the buffers was "intersected" with the buffer and a new data layer created. Intersection involved "cutting out" a piece of the land cover layer using the household buffer polygon as an "overlay" layer. A new field was added to the attribute tables for the buffer and intersected land cover layers. The area (m^2) within the intersected land cover and buffer layers were calculated using a visual basic script formula within the ArcGIS platform. This procedure allowed the proportions of different types of land cover within each buffer to be calculated and these data were input into a database.

Data analysis. *Aedes albopictus* production variables (Table 1) were analyzed at the neighborhood and household level over each sampling week and monthly container survey for each entire mosquito season. Oviposition activity at each household was standardized to seven-day (weekly) mean numbers of eggs per ovitrap. Box plots were constructed so that mosquito production and landscape characterization data could be examined for normality. The general lack of normality was verified with Kolmogorov-Smirnov tests (PROC UNIVARIATE, SAS Institute 1998). Prior to statistical analyses, assumptions of normality were met by a 2 [arcsin (sqrt x)] transformation of the proportion of specific land cover with buffer zones , and $\log_{10}(x + 1)$ transformations of the mean total pupal standing crop per residence, mean pupal standing crop per container per residence, mean numbers of pupae-positive containers per residence, mean numbers of females per aspiration sample, and mean numbers of eggs per ovitrap-week.

Oviposition phenology. With analysis of variance (ANOVA), using a general linear model (PROC GLM, SAS Institute 2000), we determined if seasonal changes in the weekly mean numbers of eggs per ovitrap in each neighborhood were significant ($P < 0.05$). We also used ANOVA to determine if the mean numbers of eggs per ovitrap-week per month were significantly different between months in the mosquito seasons of 2002 and 2003.

Spatial analysis. We standardized oviposition activity between neighborhoods using the procedures of Ryan et al. (2004). The mean numbers of eggs per ovitrap-week for each ovitrap in each neighborhood were classified by 25^{th}, 50^{th}, 75^{th}, and 100^{th} percentiles before analyses. The same procedure was followed for mean numbers of eggs per ovitrap-week per month to investigate seasonal differences in oviposition activity within each neighborhood. Subsequently, we input these values into ArcMap and used the Geostatistical Analyst extension to perform universal kriging to predict the mean numbers of eggs per ovitrap-week at locations where ovitraps were not located, based on the mean oviposition intensity at sampled locations within neighborhoods. Neighborhood areas classified in the 75^{th} – 100^{th} percentile were considered "hot spot" zones. Our universal kriging method was based on the assumption that the residual error follows an intrinsic stationary isotropic process (Isaacs and Srivastava 1989). We evaluated the validity of different variogram models using the predicted residuals sum of squares (PRESS) statistic and root mean square (standardized) error (RMSSE) and created kriging surfaces based on our best fitting models. To ensure that models used to create kriging surfaces were valid, we performed cross-validation tests. First, we kriged surfaces for egg densities using R geospatial analysis software (GeoR package, R Development Core Team, Austria, 2004) and ArcMap (Geostatistical Analyst extension, ArcMap, California, 2002). Next, we systematically removed each data point from our dataset and predicted the removed point using the remaining

data (Isaacs and Srivastava 1989). Finally, we examined scatter plots of predicted versus observed data and, if the slope was close to one, our model was considered appropriate. We used RMSSE as another validation tool where a value close to one indicated a good model. If the slope of predicted versus actual values was not close to one, but the RMSSE was close to one, we assumed that the predictive variances were large and carried out an additional model validation analysis using the PRESS statistic (GeoR package, R Development Core Team, Austria, 2004). Consequently, plots of standardized PRESS residuals were used to determine the goodness of fit of our models. If the plots did not show any pattern, then our models were valid. We overlaid symbols on the GIS that were proportional to counts of mean numbers of eggs per ovitrap-week, as well as to the percentile classification of egg densities to further validate our predictive kriging maps.

Oviposition activity in relation to abundance of pupae. In each neighborhood, we carried out linear regression analyses (PROC REG, SAS Institute 2000) to determine if there was a numerical relationship between monthly mean numbers of eggs per ovitrap-week, mean total pupal standing crop per residence, mean pupal standing crop per container per residence, and mean numbers of pupae-positive containers per residence. For these analyses, weekly mean numbers of eggs per ovitrap-week were averaged for each month in each neighborhood and regressed against monthly measures of pupal production in each neighborhood for each mosquito season separately.

Oviposition activity in relation to abundance of females. For households where adult mosquito collections were made, we carried out linear regression analyses for each mosquito season to determine if there was a significant association between weekly mean numbers of eggs per ovitrap-week and weekly mean numbers of unfed, gravid, bloodfed, or total numbers of

female *Ae. albopictus* per aspiration sample. We repeated regression analyses after adult abundance data had been time lagged one and two weeks. We also used regression analyses to examine the relationship between the season-long mean number of eggs per ovitrap-week and the season-long mean numbers of unfed, gravid, bloodfed, or total numbers of female *Ae. albopictus* per aspiration sample at each residence. In addition, we used regression analyses to determine if there was a significant association between monthly mean number of eggs per ovitrap-week and mean numbers of female *Ae. albopictus* per aspiration sample (PROC REG, SAS Institute 2000).

Pupal production in relation to abundance of females. For households where adult mosquito collections were made, we carried out linear regression analyses for each mosquito season to determine if there was a significant association between monthly mean total pupal standing crop per residence and mean numbers of female *Ae. albopictus* per aspiration sample (PROC REG, SAS Institute 2000).

Landscape factors. To determine if landscape characteristics affected oviposition activity, we regressed mean numbers of eggs per ovitrap-week and mean numbers of eggs per ovitrap-week classified into 25^{th}, 50^{th}, 75^{th}, and 100^{th} percentiles on the proportions of woodland, grass / shrub, permeable, and impermeable land cover within 10, 25, 50, and 100 m buffers around georeferenced ovitraps. We evaluated results from the Type I sum of squares model since we did not have a randomized complete block design without missing values (Steel et al. 1997).

Environmental variables. Weekly rainfall totals and weekly mean maximum and minimum temperatures were downloaded from the State Climate Office, Raleigh, NC for May-

October 2002 and 2003. Weather variables were monitored at the Raleigh-Durham International Airport, which is located 10-15 km from study neighborhoods.

RESULTS

Weather. Mean maximum weekly temperatures were reached during mid- and late August in the 2002 (35^{o}C) and 2003 (33^{o}C) mosquito seasons, respectively. Weekly rainfall amounts peaked in mid-October (12.3 cm) in 2002 and in the beginning of August (9.5cm) in 2003 (Figure 1). In the mosquito season from May-October, rainfall totals were substantially higher in 2003 (92.7 cm) compared to 2002 (65.7 cm).

Seasonal distribution of oviposition activity. A low level of oviposition activity was detected when ovitraps were placed in the field at the beginning of May 2002. In 2003, egg-laying was not detected until late May. The seasonal profile of egg laying activity indicated a later and higher seasonal peak in oviposition activity occurred during the 2003 mosquito season relative to the 2002 season (Figure 2). Although we observed variation in oviposition intensity between mosquito seasons, there was generally a large peak in egg-laying over two to three consecutive weeks in mid summer of both seasons (Figure 2). In 2002, oviposition intensity peaked in late July (mean = 161.8 eggs per ovitrap-week), while in 2003, egg-laying peaked in late August (mean = 182.3 eggs per ovitrap-week). The weekly prevalence of egg-positive ovitraps in both mosquito seasons ranged from zero to 99 percent. In 2002, the peak in ovitrap prevalence preceded the peak in oviposition intensity, while in 2003, peaks in prevalence and intensity were concurrent. In all neighborhoods, there was a significant difference in mean numbers of eggs per ovitrap-week over the period of our study in both the 2002 (df = 21, 66; F = 36.93; $P < 0.0001$) and 2003 mosquito seasons (df = 23, 165; F = 30.52; $P < 0.0001$). In 2002, there was no significant difference in the monthly mean number of eggs per ovitrap-week

between months ($P > 0.05$); however, there was a significant difference in ovitrap intensity between months in the 2003 season (df = 5, 18; $F = 5.07$; $P = 0.005$). Egg-laying was significantly higher ($P < 0.05$) in August compared to other months.

Spatial distribution of oviposition activity. Oviposition intensity was grouped by 25th, 50th, 75th, and 100th percentile classes for each neighborhood during the 2002 and 2003 mosquito seasons. In the respective 2002 and 2003 mosquito seasons, mean numbers of eggs per ovitrap-week ± 1 standard error within percentile classes were 5.9 ± 0.9 and 6.1 ± 0.8 (25th), 36.2 ± 1.3 and 38.4 ± 0.9 (50th), 63.7 ± 1.3 and 63.2 ± 0.9 (75th), and 88.2 ± 1.4 and 88.6 ± 1.0 (100th). The data were fit to spherical semivariogram models, based on the assumption that the spatial process was isotropic and intrinsically stationary (Isaacs and Srivastava 1989). Universal kriging maps illustrated the spatial distribution of season-long oviposition activity and showed locations in each neighborhood where egg laying activity was intense (Figs. 3 and 4). Kriging maps for monthly oviposition activity revealed seasonal variation in oviposition activity between residences with some areas exhibiting repetitive monthly clustering (data not shown). Every neighborhood exhibited some degree of spatial clustering of season-long egg abundance; however, N-1 during the 2002 season and N-5 during the 2003 season exhibited the most uniform spatial distribution of oviposition activity compared to other neighborhoods. Spatial patterns of egg-laying changed between mosquito seasons in N-1 and N-3. Cross-validation analyses produced plots of predicted versus observed points with a slope that did not approximate one for most neighborhoods; however, RMSSE values did approximate one for most neighborhoods, indicating that predictions of the spatial distribution of oviposition activity were valid. Cross-validation indicated RMSSE values were 1.09, 0.96, 1.17, and 0.99 for N-1, N-2, N-3, and N-4, respectively, in 2002. In 2003, RMSSE values were 0.90, 1.01, 1.03, 1.09,

1.07, 0.99, 0.92, and 1.00 for N-1, N-2, N-3, N-4, N-5, N-6, N-7, and N-8, respectively. The slopes of plots of predicted versus observed points were not approximately one and RMSSE values were approximately one, indicating large predictive variances. However, subsequent plots of standardized PRESS residuals showed no distinct pattern and verified that our kriging estimates were valid. The relationship between observed and predicted values of mean numbers of eggs per ovitrap-week was strongest when the semivariogram range was < 200 m, which is within the local dispersal range of container-inhabiting *Aedes* mosquitoes (Reiter et al. 1995; Honorio et al. 2003). When we overlaid proportional symbols for mean numbers of eggs per ovitrap-week and percentile rank of egg densities on our kriging maps, the symbols generally coincided with predictive contour shading (data not shown).

Oviposition activity and abundance of pupae. For both mosquito seasons, we used simple linear regression analyses to establish the numerical relationship between monthly oviposition intensity and measures of pupal production (mean numbers of pupae-positive containers per residence, mean total pupal standing crop per residence, mean pupal standing crop per container per residence) (Figures 5-7). In 2002, egg abundance in ovitraps was generally related to level of mosquito production in backyard containers (Fig. 5-7). Mean numbers of eggs per ovitrap-week were significantly associated with mean numbers of pupae-positive containers ($t = 3.59$; $P = 0.003$; $R^2 = 0.479$) and mean total pupal standing crop per residence ($t = 2.77$; $P = 0.015$; $R^2 = 0.354$); however, there was no significant association between mean numbers of eggs per ovitrap-week and mean pupal standing crop per container per residence ($t = 0.93$; $P = 0.368$; $R^2 = 0.058$). In 2003, we found a significant relationship between oviposition intensity and mean total pupal standing crop per residence ($t = 6.16$; $P < 0.0001$; $R^2 = 0.469$), mean pupal standing

crop per container per residence ($t = 3.62$; $P = 0.001$; $R^2 = 0.234$), and mean numbers of pupae-positive containers per residence ($t = 5.98$; $P < 0.0001$; $R^2 = 0.454$) (Figs. 5-7).

Oviposition activity and abundance of females. Results of linear regression analyses of weekly ovitrap intensity and female abundance data revealed no significant numerical associations ($P > 0.05$) between oviposition intensity and non-time-lagged or time-lagged unfed, gravid, or total number of female *Ae. albopictus* per aspiration sample. We also found no significant relationship ($P > 0.05$) between season-long oviposition intensity and numbers of unfed, gravid, or total females per aspiration sample.

However, averaging ovitrap intensity and female abundance data over each month during the mosquito season improved the significance of regression analyses. A significant relationship ($P < 0.05$) was found between the monthly mean number of eggs per ovitrap-week and mean number of female *Ae. albopictus* per aspiration sample in 2002 ($t = 3.75$; $P = 0.001$; $R^2 = 0.389$) and 2003 ($t = 5.37$; $P < 0.0001$; $R^2 = 0.419$) (Figure 8).

Pupal production and abundance of females. In the 2002 season, linear regression analyses indicated no significant association between monthly mean total pupal standing crop per residence and mean numbers of female *Ae. albopictus* per aspiration sample ($t = 1.87$; $P = 0.201$; $R^2 = 0.201$;). However, in the 2003 season, we found a significant association between monthly mean total pupal standing crop per residence and mean numbers of female *Ae. albopictus* per aspiration sample ($t = 3.46$; $P = 0.001$; $R^2 = 0.222$) (Figure 9).

Oviposition activity in relation to land cover. For both mosquito seasons, linear regression analyses were carried out to determine the association between season-long mean numbers of eggs per ovitrap-week classified in 25th, 50th, 75th, and 100th percentiles, mean numbers of eggs per ovitrap-week not classified into percentile groups, and the proportion of

each type of land cover within prescribed buffer distances of ovitraps. In 2002, we did not find any significant associations ($P > 0.05$) between oviposition intensity and land cover. Again, in 2003, we did not find any significant associations between mean numbers of eggs per-ovitrap-week classified in 25th, 50th, 75th, and 100th percentiles; however, there was a significant relationship between oviposition intensity and woodland (25 m buffer: $t = 2.75$; $P = 0.006$; $R^2 = 0.081$; 50 m buffer: $t = 1.96$; $P = 0.051$; $R^2 = 0.081$), grass / shrub (100 m buffer: $t = 2.49$; $P = 0.013$; $R^2 = 0.053$), permeable (25 m buffer: $t = 2.49$; $P = 0.014$; $R^2 = 0.035$; 50 m buffer: $t = 2.60$; $P = 0.010$; $R^2 = 0.053$), and impermeable (25 m buffer: $t = 2.30$; $P = 0.022$; $R^2 = 0.036$; 50 m buffer: $t = 2.54$; $P = 0.012$; $R^2 = 0.036$) land covers.

DISCUSSION

Seasonality. Oviposition activity of *Ae. albopictus* peaked in either late-July (2002 season) or early-August (2003 season). The peak of oviposition activity in 2002 coincided with seasonal high temperatures, but was not associated with increased amounts of preceding rainfall. Conversely, during 2003, the high point of oviposition activity corresponded to periods when both high temperatures and large amounts of rainfall occurred in previous weeks. The intensity of oviposition activity, temperature, and rainfall varied between mosquito seasons. There was more rainfall and a greater number of weeks with rainfall in 2003 when compared to 2002. Higher egg densities found in 2003 is surprising since more rainfall would produce more water-filled containers that would divert gravid females away from ovitraps, decreasing the numbers of eggs laid in ovitraps. We speculate that higher amounts of rainfall led to production of larger mosquito populations and correspondingly higher egg densities in ovitraps. Sulaiman and Jefferey (1986) found seasonal patterns of *Ae. albopictus* egg laying in ovitraps in Kuala Lampur, Malaysia to be closely associated with rainfall. In our analyses, lagging adult

abundance back to earlier sampling dates did not increase the statistical significance of the relationship between the weekly abundance of females and egg densities in ovitraps. However, averaging the data by month apparently smoothed deviations between abundance of females and egg densities in ovitraps, making the numerical relationship between these variables significant ($P < 0.05$). The significant relationship between the abundance of pupae and adult female *Ae. albopictus* in 2003 supports previous research findings that pupal standing crop can be used to estimate adult abundance (Focks and Chadee 1997). Furthermore, the significant relationship between the abundance of pupae and egg densities in ovitraps in 2003 suggests that ovitrap collections could be an indirect measure of local mosquito production in container habitats.

Seasonal peaks of *Ae. albopictus* oviposition in mid to late summer in NC are consistent with late summer peaks of egg-laying observed for populations in VA (Barker et al. 2003) and Illinois (Swanson et al. 2000). Joy et al. (2003, 2004) conducted studies on *Ae. albopictus* immatures in WV and found that mosquito prevalence in containers was highest in August and September.

Land cover analysis. There are few studies relating land cover with mosquito oviposition activity. In general, we found a significant association between *Ae. albopictus* oviposition intensity and the "open grass / shrub", "woodland", "permeable", and "impermeable" types of land cover. These observations suggest that *Ae. albopictus* will lay eggs in any habitat in suburban landscapes. Barker et al. (2003) in VA reported that a greater number of *Ae. albopictus* eggs were collected in ovitraps placed in open compared to forested areas of western VA. Likewise, results of ovitrap surveillance reported by Swanson et al. (2000) suggest that *Ae. albopictus* would be most abundant in artificial containers in open, residential areas, as opposed to wooded areas. However, in these investigations, land cover surrounding ovitraps was

qualitatively described. In our study, the association between mean numbers of eggs per ovitrap-week and 'impermeable' land cover may be related to the availability of blood meal hosts at residences, resulting in an abundance of gravid females in proximity to ovitraps adjacent to residences. The statistically significant relationship between abundance and *Ae. albopictus* eggs and "open grass / shrub", "woodland", and "permeable" land covers may be an artifact of the prevalence of each macro-habitat in the landscape surrounding most ovitraps. In our investigation, we did not measure micro-habitat variables associated with ovitraps. In this regard, Beier et al. (1982) studied the relationship between eight micro-habitat variables and the spatial distribution of eggs in ovitraps and female *Ochlerotatus triseriatus* collected from vegetation in a woodlot in Indiana, using a computer mapping program. The distribution of adults and eggs were restricted to separate, small portions of the woodlot. None of the eight micro-habitat variables were significantly correlated with the abundance of eggs in ovitraps. These results suggest that gravid females may rest in specific areas of the landscape, but search the entire landscape for water-filled containers in which to lay eggs.

The numerical relationship between the abundance of *Ae. albopictus* eggs in ovitraps and resting females collected from vegetation could not be established from our weekly or bimonthly samples. These findings support the conclusions of Focks (2003) that ovitraps indicate the presence or absence of mosquitoes and provide a relative rather than absolute measure of mosquito abundance. The propensity of females to lay a varying number of eggs as well as oviposit in more than one container (Rozeboom et al. 1973; Chadee et al. 1990; Romero-Vivas and Falconar 2005) would make it difficult to precisely estimate the number of females that had visited each ovitrap. However, in both mosquito seasons, we found a significant association between the monthly mean numbers of adults in aspiration samples and mean number of eggs in

ovitraps, indicating that averaging the numbers of adults and eggs in weekly/bimonthly collections smoothed irregularities in the datasets for these two variables.

Our kriging maps revealed local oviposition “hot spots” within most neighborhoods, except N-1 and N-5 during the 2002 and 2003 seasons, respectively. A further examination of oviposition data indicated a neighborhood-wide, rather than local pattern of egg clustering in these neighborhoods. Interpolation error on kriging maps was most prevalent on the outskirts of study areas due to "edge effects" associated with the periphery of neighborhoods. It is likely that variation in spatial patterns of oviposition activity in neighborhoods between mosquito seasons was due to differences in the clustering pattern of discarded containers in backyards combined with higher amounts of rainfall in 2003.

Past research studies have advised caution when interpreting interpolation maps and some have suggested use of proportional symbols to represent insect count data, rather than contour maps (Nansen et al. 2003; Ryan et al. 2004). When we overlaid proportional symbols on neighborhood maps for mean numbers of eggs per ovitrap-week, our kriging maps generally coincided with egg densities, further validating our contour maps. These maps provided a convenient method for viewing the spatial distribution of *Ae. albopictus* oviposition activity and could be used by mosquito control programs as a tool for pinpointing areas of high mosquito production. Our research results demonstrate that spatial modeling within a GIS can be utilized to predict the spatial distribution of *Ae. albopictus* oviposition activity, using egg abundance data collected in permanent ovitrap stations.

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Table 1. Mosquito production and landscape characterization variables analyzed at the neighborhood and household level over each week and over each entire mosquito season.

Variable	Description
Mean ovitrap intensity	Sum of the numbers of eggs collected in all ovitraps / total numbers of all ovitraps with eggs
Mean number of eggs per ovitrap-week	Mean ovitrap intensity / total number of weeks that ovitraps were sampled
Ovitrap prevalence	Number of ovitraps positive for eggs / total number of ovitraps per neighborhood.
Mean total pupal standing crop per residence	Sum of the numbers of pupae collected in all containers or in each specific container type per residence.
Mean numbers of mosquitoes per aspiration sample	Sum of the numbers of unfed, gravid, blood fed, or female mosquitoes collected / total number of aspiration samples taken
Proportion of land cover within buffers	Total area (m^2) of a specific type of land cover within a specified buffer region / total area of the specified buffer region

FIGURE CAPTIONS

Fig. 1. Maximum and minimum air temperatures and rainfall in the City of Raleigh, NC during the 2002 and 2003 mosquito seasons.

Fig. 2. Mean numbers of *Aedes albopictus* eggs per ovitrap-week and proportion of traps positive for eggs at residences in study neighborhoods in the City of Raleigh, NC during the 2002 and 2003 mosquito seasons.

Fig. 3. Universal kriging contour maps of the distribution of *Ae. albopictus* eggs per ovitrap-week in N-1, N-2, N-3, and N-4 during the 2002 mosquito season. Dots signify ovitrap locations, shading corresponds to egg densities in the 25^{th}, 50^{th}, 75^{th}, and 100^{th} percentiles, and property boundaries are delineated.

Fig. 4. Universal kriging contour maps of the distribution of *Ae. albopictus* eggs per ovitrap-week in N-1, N-2, N-3, N-4, N-5, N-6, N-7, and N-8 during the 2003 mosquito season. Dots signify ovitrap locations, shading corresponds to egg densities in the 25^{th}, 50^{th}, 75^{th}, and 100^{th} percentiles, and property boundaries are delineated.

Fig. 5. Regression of monthly mean pupal standing crop per container and mean number of eggs per ovitrap-week for the 2002 and 2003 mosquito seasons.

Fig. 6. Regression of monthly mean total pupal standing crop per residence and mean number of eggs per ovitrap-week for the 2002 and 2003 mosquito seasons.

Fig. 7. Regression of monthly mean numbers of pupae-positive containers per residence and mean number of eggs per ovitrap-week for the 2002 and 2003 mosquito seasons.

Fig. 8. Regression of monthly mean numbers of total female *Ae. albopictus* per aspiration sample and mean number of eggs per ovitrap-week for the 2002 and 2003 mosquito seasons.

Fig. 9. Regression of monthly mean numbers of total female *Ae. albopictus* per aspiration sample and mean total pupal standing crop per residence for the 2002 and 2003 mosquito seasons.

Figure 1

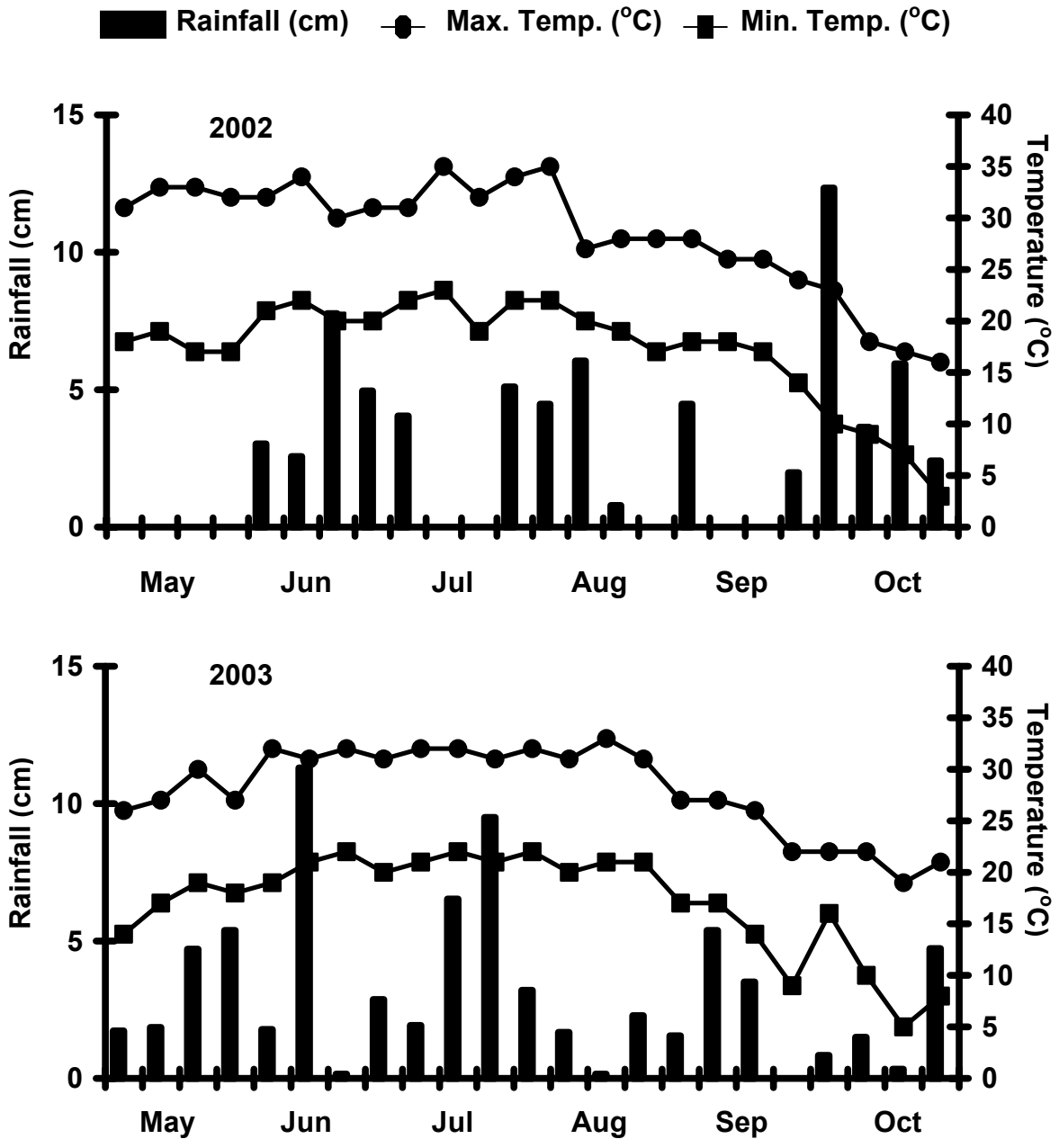

Rainfall (cm)
Max. Temp. (°C)
Min. Temp. (°C)
2002
2003
Rainfall (cm)
Temperature (°C)
0
5
10
15
20
25
30
35
40
May
Jun
Jul
Aug
Sep
Oct

Figure 2

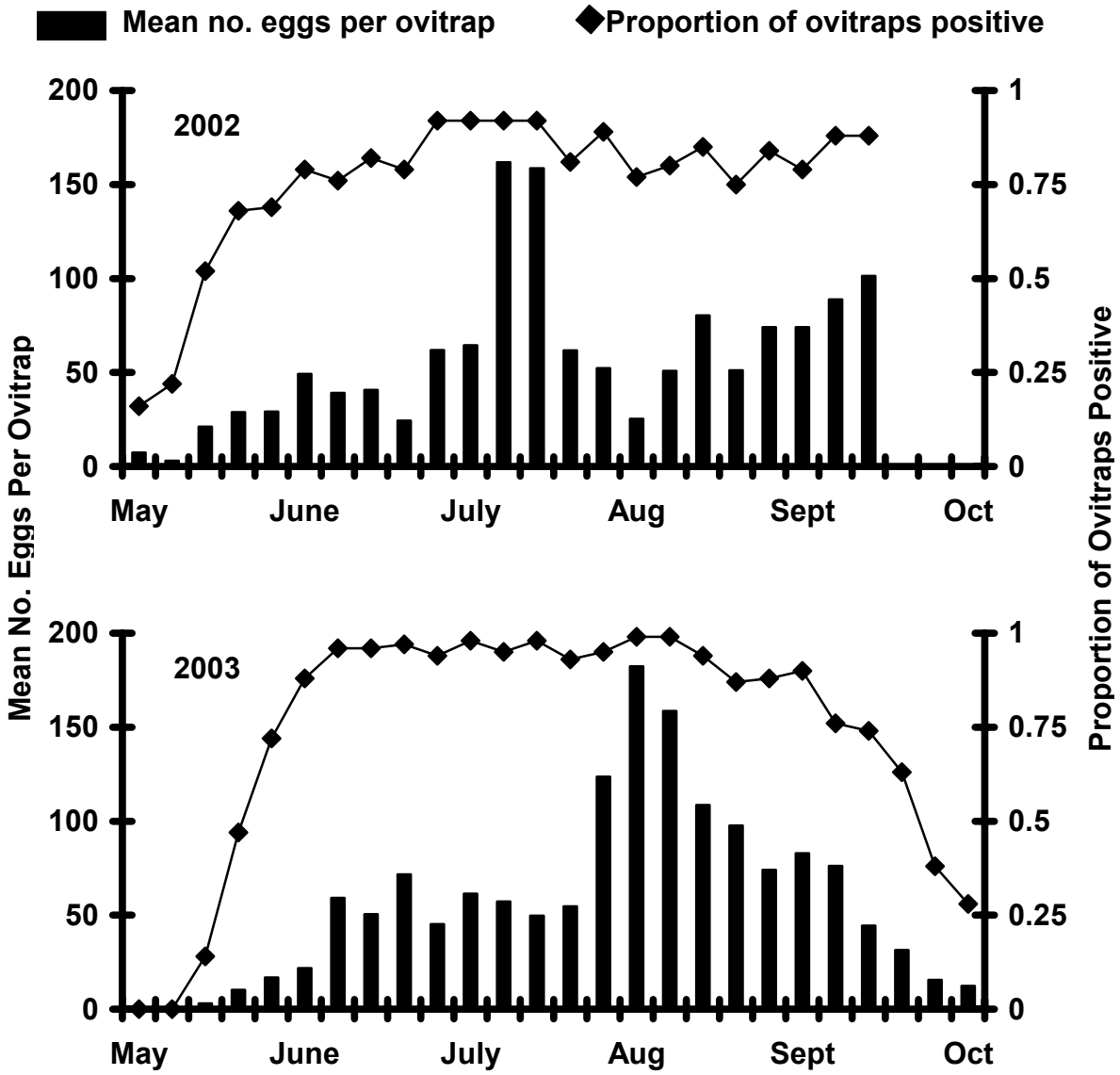

Mean no. eggs per ovitrap
Proportion of ovitraps positive
2002
2003
Mean No. Eggs Per Ovitrap
Proportion of Ovitraps Positive
200
150
100
50
0
1
0.75
0.5
0.25
0
May
June
July
Aug
Sept
Oct

Figure 3

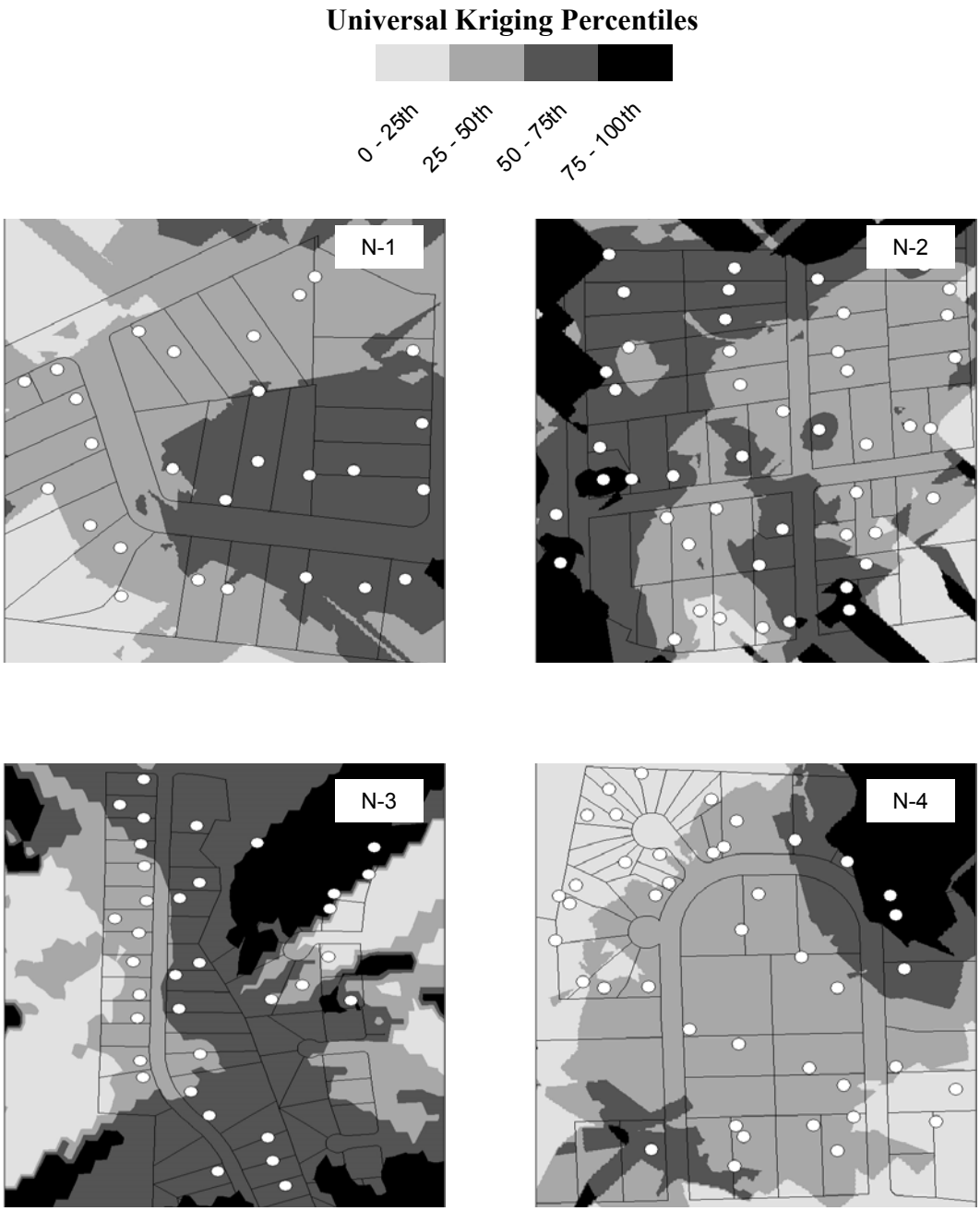

Universal Kriging Percentiles
0 - 25th
25 - 50th
50 - 75th
75 - 100th
N-1
N-2
N-3
N-4

Figure 4

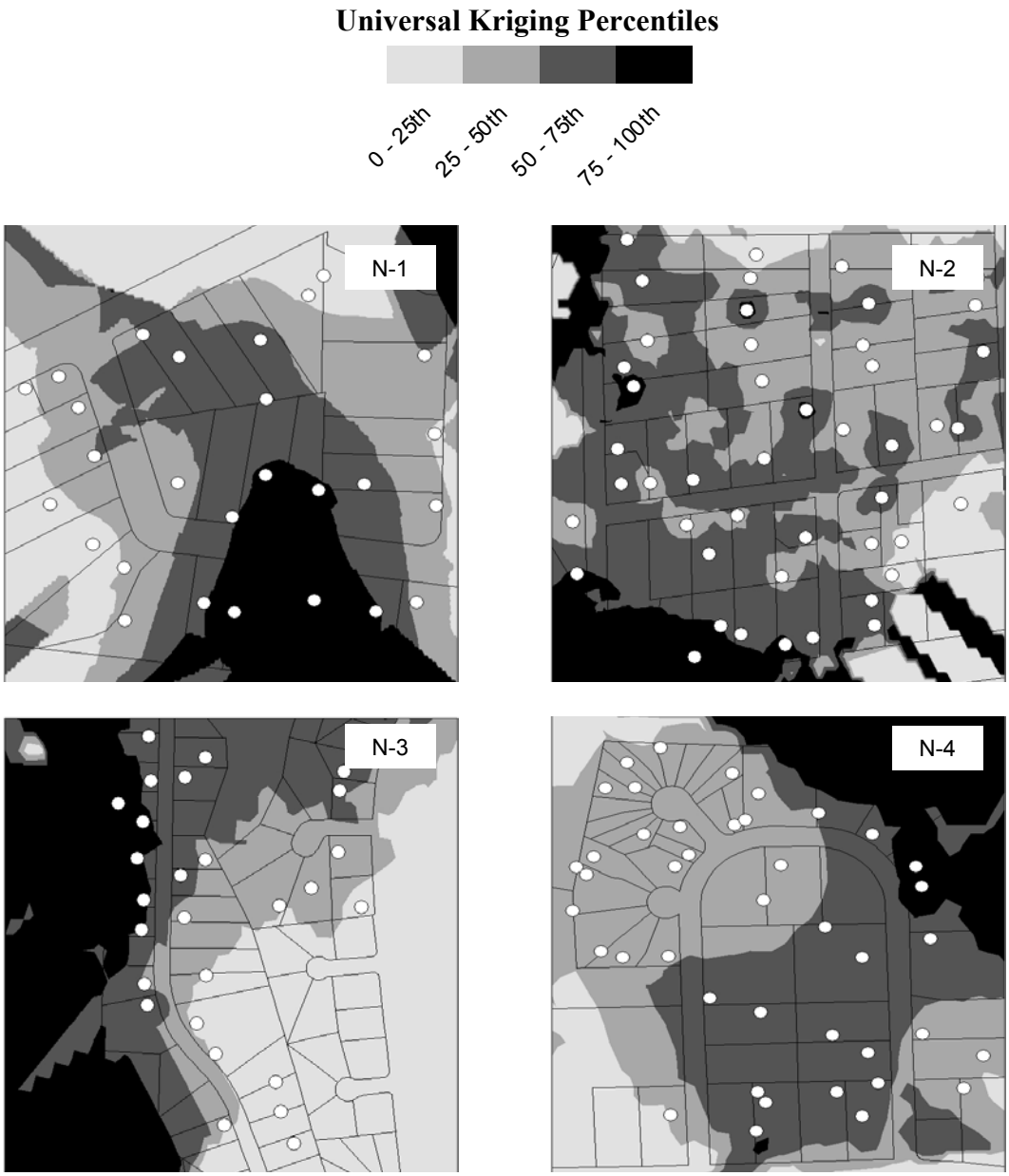

Universal Kriging Percentiles
0 - 25th
25 - 50th
50 - 75th
75 - 100th
N-1
N-2
N-3
N-4

Figure 4, cont.

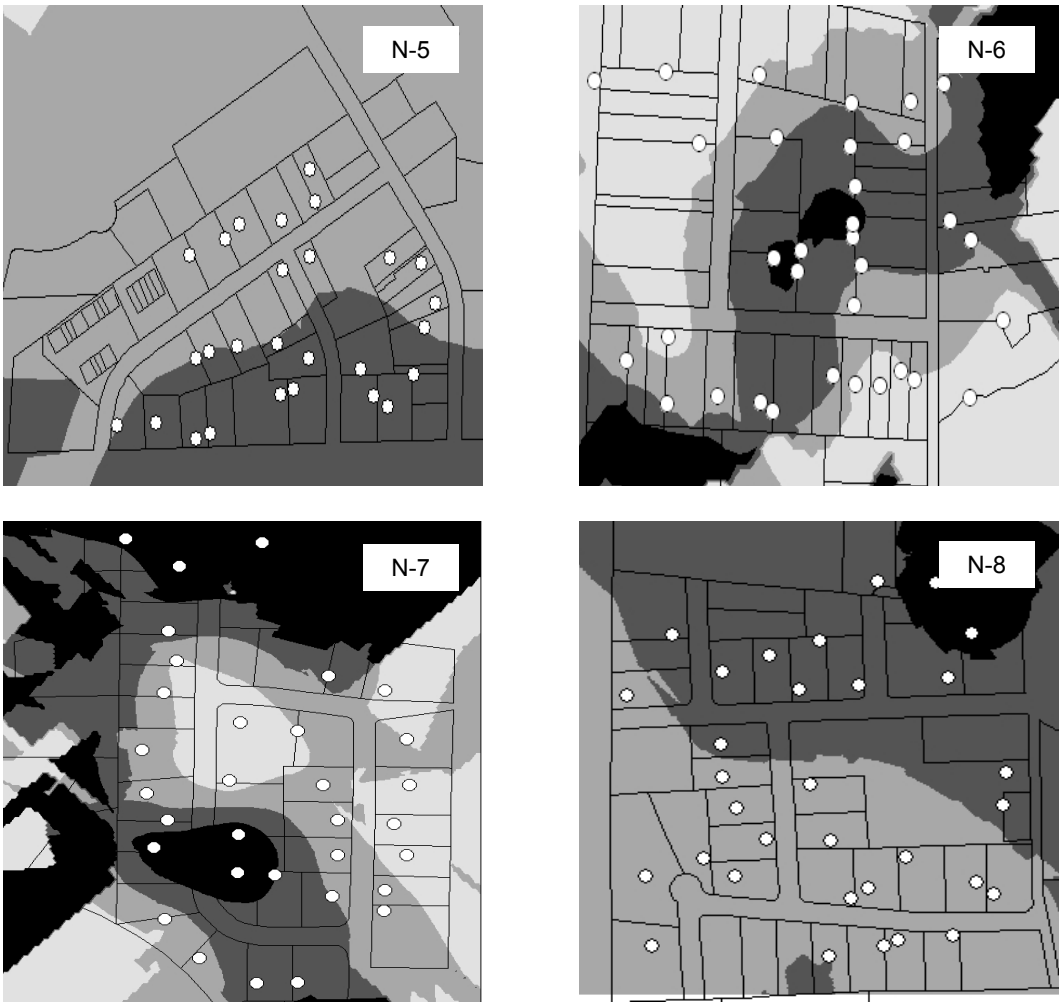
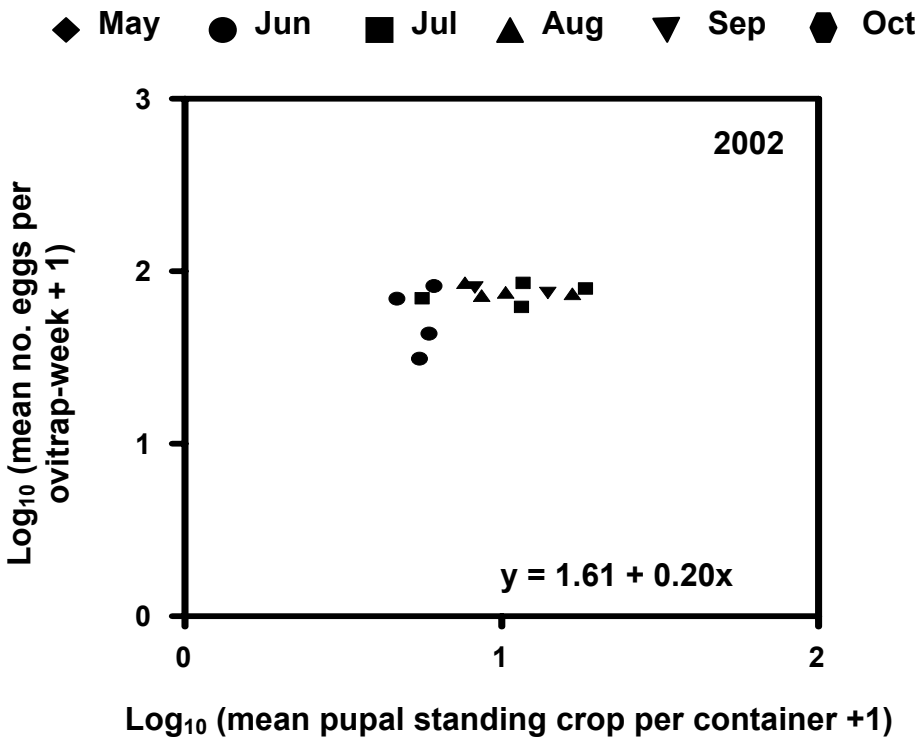


Figure 5


May
Jun
Jul
Aug
Sep
Oct
2002
y = 1.61 + 0.20x
Log10 (mean no. eggs per ovitrap-week + 1)
Log10 (mean pupal standing crop per container +1)
0
1
2
3

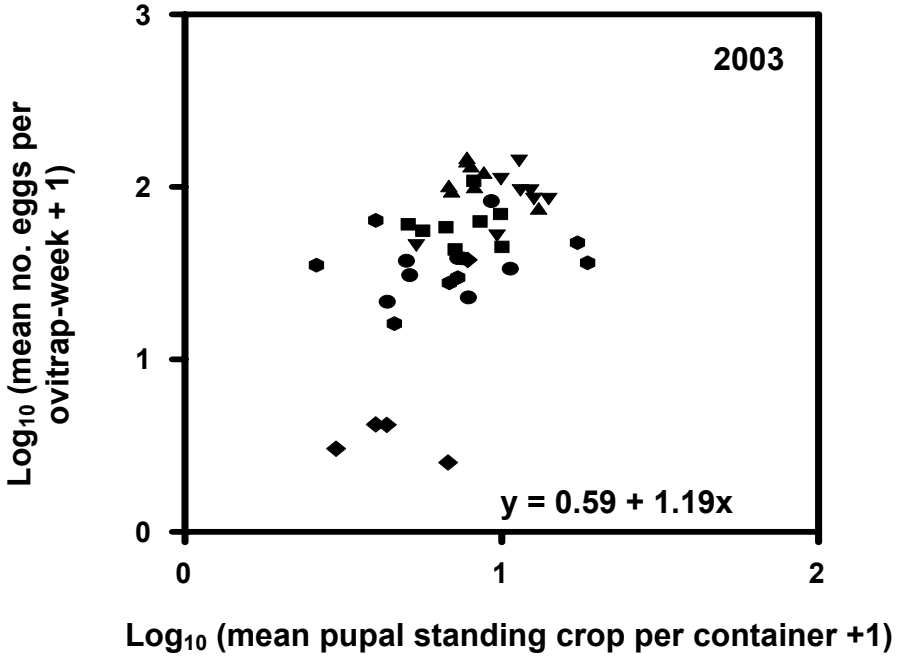

2003
y = 0.59 + 1.19x
Log10 (mean no. eggs per ovitrap-week + 1)
Log10 (mean pupal standing crop per container +1)
0
1
2
3

Figure 6

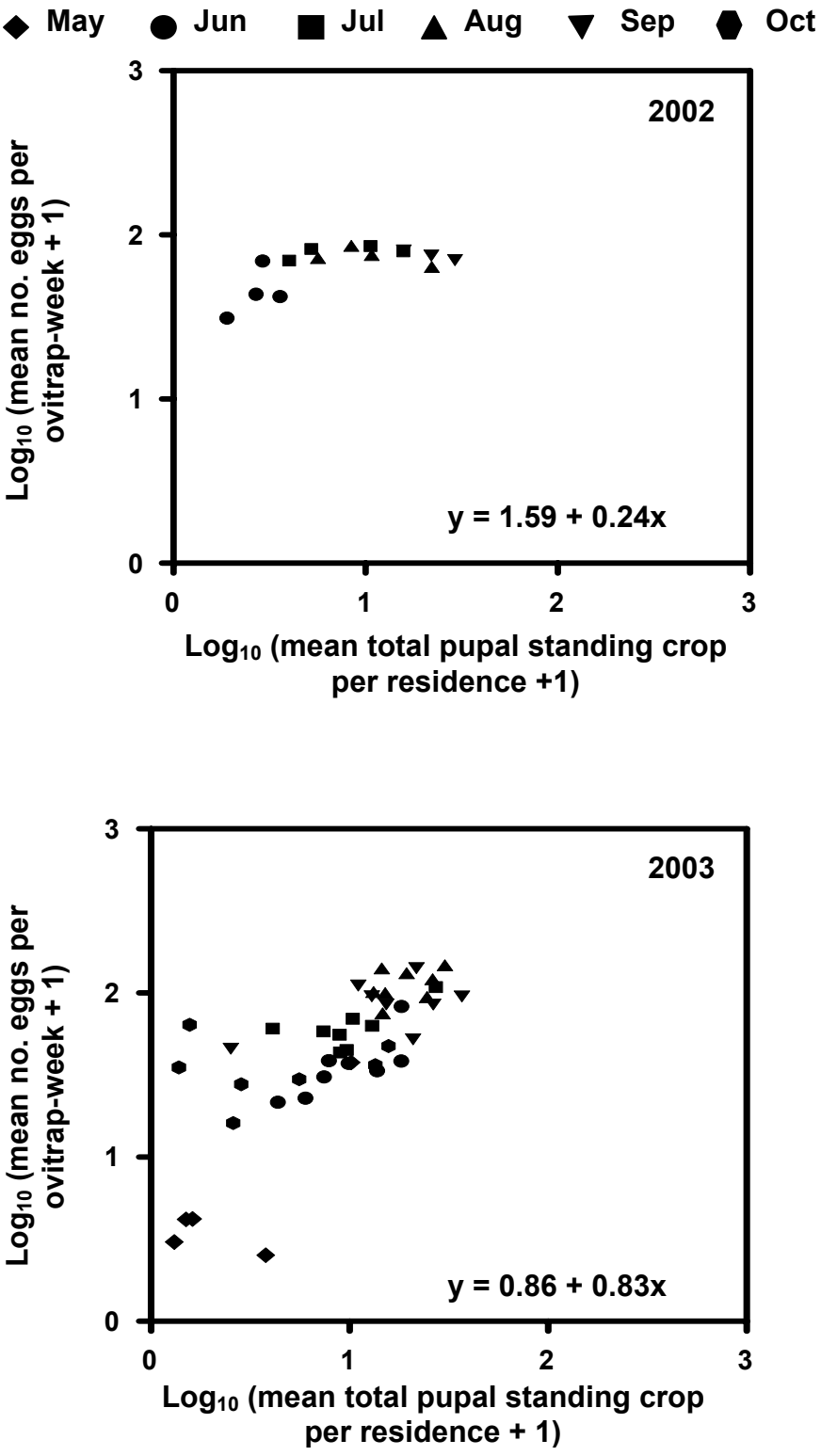
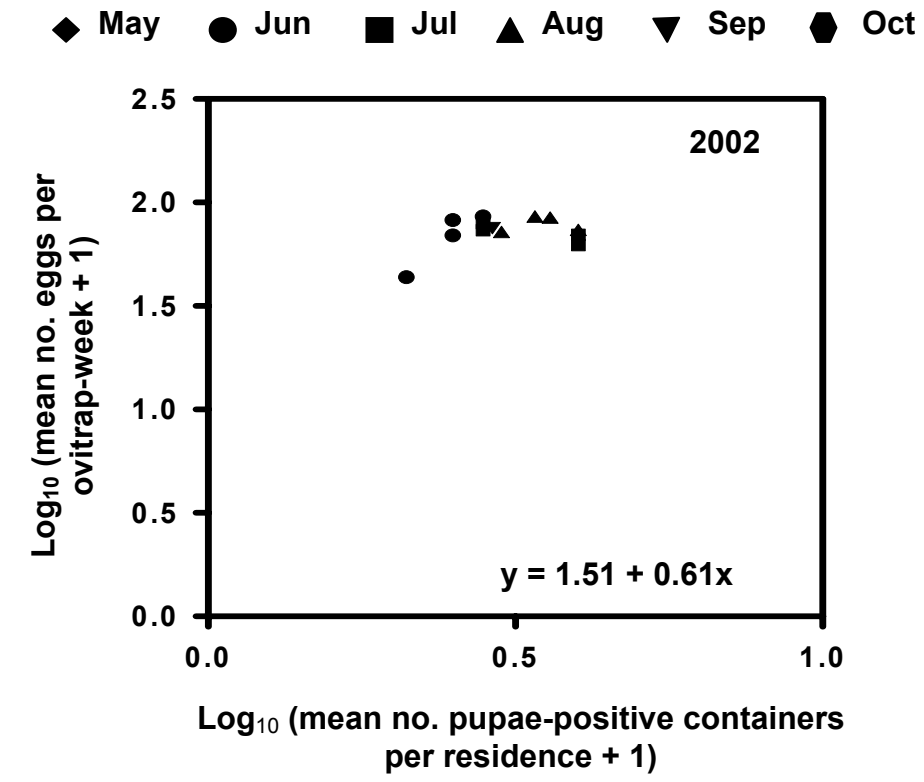

May
Jun
Jul
Aug
Sep
Oct
2002
y = 1.59 + 0.24x
Log10 (mean no. eggs per ovitrap-week + 1)
Log10 (mean total pupal standing crop per residence +1)
2003
y = 0.86 + 0.83x
Log10 (mean no. eggs per ovitrap-week + 1)
Log10 (mean total pupal standing crop per residence + 1)

Figure 7


May
Jun
Jul
Aug
Sep
Oct
2002
y = 1.51 + 0.61x
Log10 (mean no. eggs per ovitrap-week + 1)
Log10 (mean no. pupae-positive containers per residence + 1)
0.0
0.5
1.0
1.5
2.0
2.5

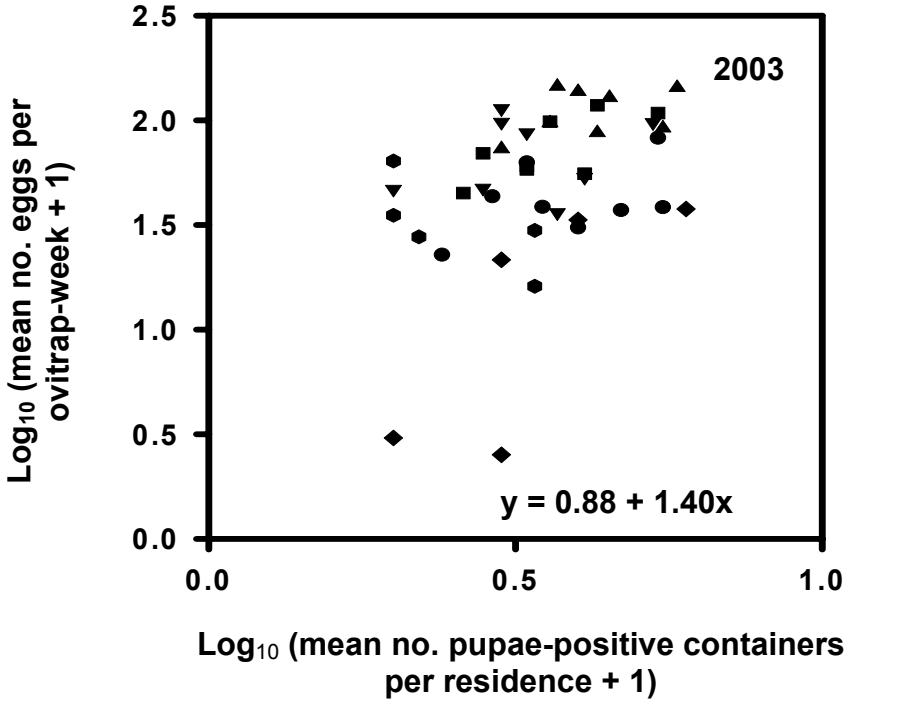

2003
y = 0.88 + 1.40x
Log10 (mean no. eggs per ovitrap-week + 1)
Log10 (mean no. pupae-positive containers per residence + 1)
0.0
0.5
1.0
1.5
2.0
2.5

Figure 8

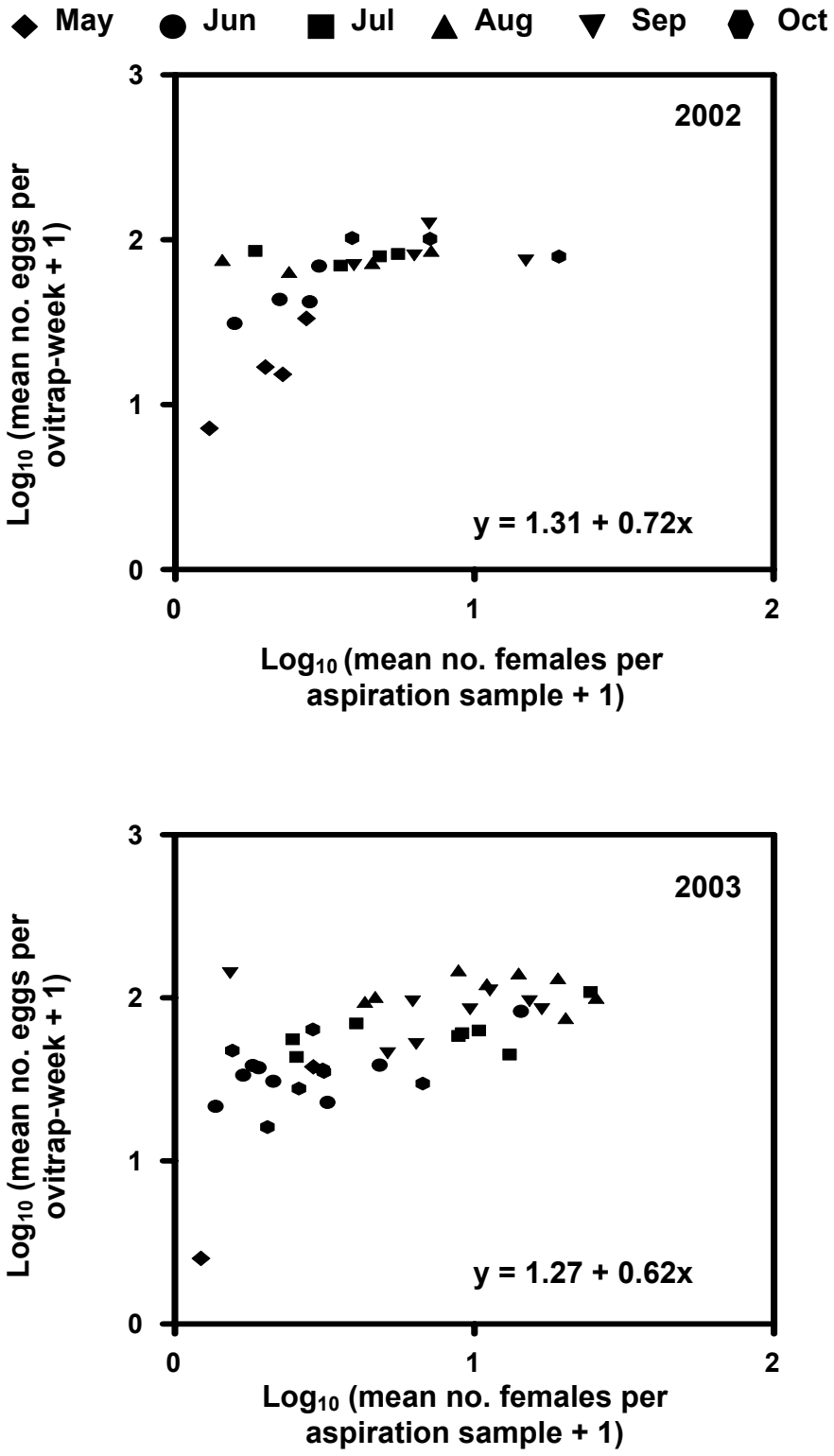
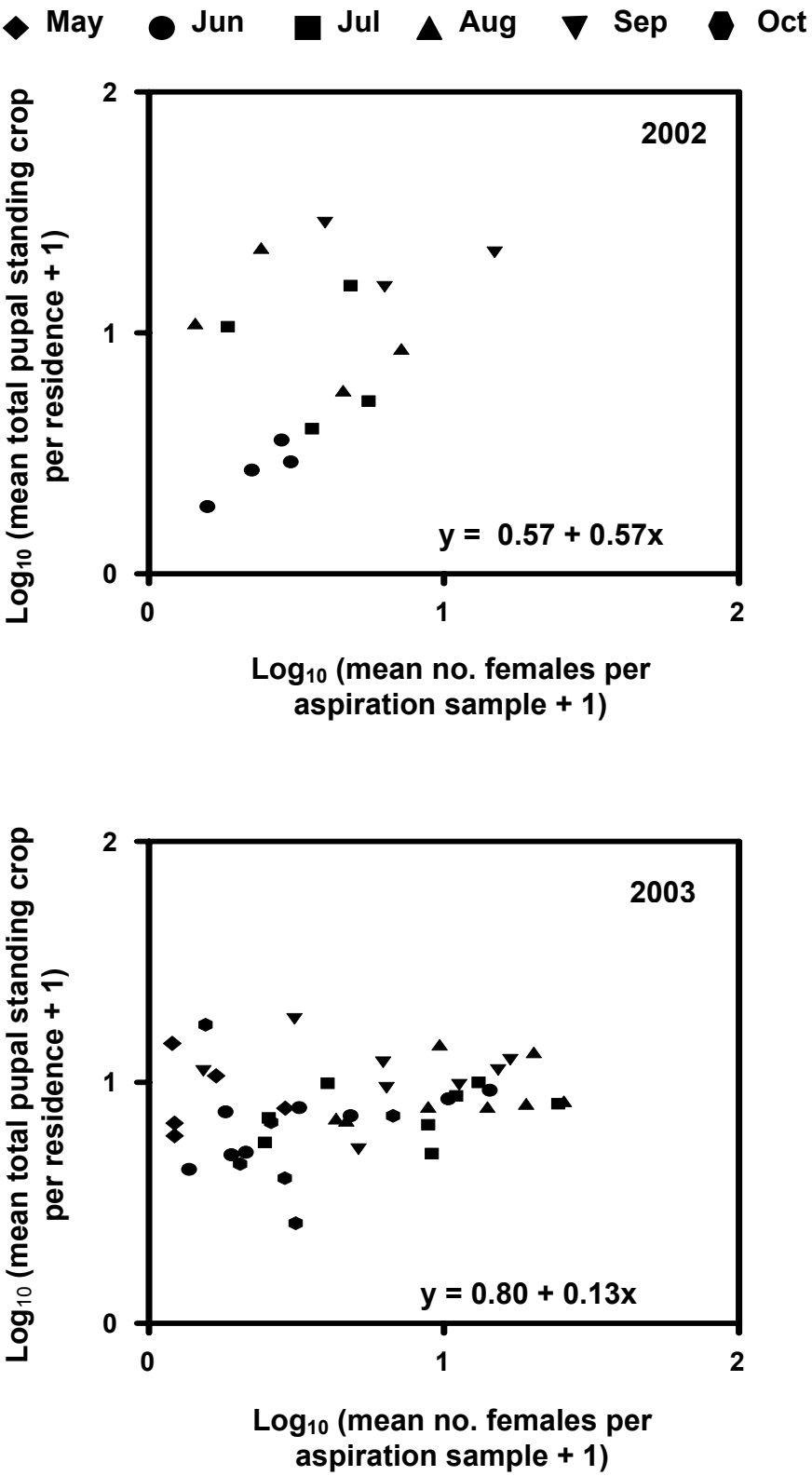

May
Jun
Jul
Aug
Sep
Oct
2002
y = 1.31 + 0.72x
Log10 (mean no. eggs per ovitrap-week + 1)
Log10 (mean no. females per aspiration sample + 1)
2003
y = 1.27 + 0.62x
Log10 (mean no. eggs per ovitrap-week + 1)
Log10 (mean no. females per aspiration sample + 1)

Figure 9


May
Jun
Jul
Aug
Sep
Oct
2002
y = 0.57 + 0.57x
Log10 (mean total pupal standing crop per residence + 1)
Log10 (mean no. females per aspiration sample + 1)
2003
y = 0.80 + 0.13x
Log10 (mean total pupal standing crop per residence + 1)
Log10 (mean no. females per aspiration sample + 1)
0
1
2

EVALUATION OF A LETHAL OVIPOSITION TRAP AND SOURCE REDUCTION ON OCCURRENCE AND ABUNDANCE OF IMMATURE *AEDES ALBOPICTUS* (SKUSE) (DIPTERA: CULICIDAE) IN SUBURBAN LANDSCAPES OF A PIEDMONT COMMUNITY IN NORTH CAROLINA

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ABSTRACT

Management of the container-inhabiting mosquito *Aedes albopictus* (Skuse) is problematic as presently there is a lack of effective strategies. In the 2002 mosquito season, we evaluated a lethal oviposition trap (LO) as a novel strategy of control for *Ae. albopictus* in two suburban neighborhoods in the City of Raleigh, North Carolina. The trap, a black plastic cup filled with water and containing an oviposition strip impregnated with deltamethrin insecticide, was designed to attract and kill gravid *Aedes Stegomyi* mosquitoes. Five traps were distributed around each home in two neighborhoods. Mosquito production in LO treated sites was not significantly different ($P > 0.05$) than in two control (No-TRT) neighborhoods. In 2003, environmental sanitation in the form of source reduction (SR) of man-made containers was carried out in two additional neighborhoods in an effort to increase the number of gravid females visiting LOs by removing alternative oviposition sites. So that impacts of SR treatments on the efficacy of the LO could be understood, SR treatments were carried out in two additional neighborhoods (LO+SR). Treatments were initiated in May-June and, at monthly intervals thereafter, all containers around each residence were examined for mosquitoes. All pupae and a sample of larvae were collected from containers. The control efficacy of LO, SR, and LO+SR treatments were evaluated by comparing mosquito production variables (mean pupal standing crop per container per residence, mean total pupal standing crop per residence, and mean numbers of mosquito-positive containers per residence) between treatment areas. In SR and LO+SR neighborhoods, the contents of containers were 'dumped' or treated with the insect growth regulator methoprene after sampling. In No-TRT and LO neighborhoods, container contents were replaced after sampling. Analysis of variance did not reveal any significant mosquito suppression resulting

from control measures during the 2002 and 2003 mosquito seasons ($P > 0.05$) and, mean pupal standing crop per container per residence and mean total pupal standing crop per residence were highest in LO treatment areas compared to other areas during both mosquito seasons. Containers positive for mosquitoes were identified as one of twelve different types. Mean total pupal standing crop per residence and mean numbers of pupae-positive containers per residence ranked across container types were not concordant (Kendall's Tau-*b*; $P > 0.05$), indicating that the mosquito production potential of container types were different. We determined that the mosquito production potential of a container was a function of its mean pupal standing crop, density, and spatial distribution in the landscape. Results of spatial regression analyses indicated that, in general, mosquito production was highest at 'key premises' in each neighborhood. Based on the clustered distribution of mosquito production within neighborhoods, focusing control efforts on 'key premises' would optimize the efficiency of mosquito control efforts.

INTRODUCTION

Aedes albopictus (Skuse), the "Asian tiger mosquito", was introduced into the United States via a shipment of tires from Asia in the mid 1980s (Hawley et al. 1987). Interstate transportation of tires facilitated the movement of *Ae. albopictus* (Reiter and Sprenger 1987), and the availability of artificial containers as oviposition sites supported the establishment and spread of this mosquito in peri-domestic habitats (Moore and Mitchell 1997). *Aedes albopictus* is presently distributed through much of the southern, eastern, and lower Midwestern U.S. (Moore and Mitchell 1997). Despite the passing of 20 years since *Ae. albopictus* was discovered in Houston, Texas (Sprenger and Wuithiranyagool 1986), knowledge of the field biology of this species that relates to its management is based largely

on studies conducted on Asian populations or with another container-inhabiting mosquito, *Ae. aegypti*. Unquestionably, the rapid spread of this exotic species after its introduction into the U.S. reflects a lack of an effective area-wide management strategy for container-inhabiting mosquitoes, in general. Because adults emerge daily from containers, conventional applications of ultra low volume adulticides achieve only temporary suppression of container-inhabiting mosquitoes, such as *Ae. aegypti* (Focks et al. 1987; Perich et al. 1990; Castle et al. 1999). Use of larvicides is also problematic in that treating containers has generated insecticide resistant populations (Rawlins et al. 1998; Rawlins and Wan 1995; Wirth and Georghiou 1999). Elimination of water-filled containers through environmental sanitation substantially reduced both *Ae. albopictus* and *Ae. aegypti* populations in Singapore (Kai-Lok et al. 1972). However, in general, source reduction (SR) is difficult to implement over large areas because of the manpower required (Kai-Lok et al. 1972; Tun-Lin et al. 1995a). Also, this strategy for *Stegomyia* mosquitoes has not generally been sustainable in communities (Lloyd et al. 1992; Reiter and Gubler 1998) as evidenced by the resurgence of dengue fever in areas of Asia and Latin America (Gubler 1998).

Nevertheless, mosquitoes require water to complete their life cycle, and environmental sanitation of water-holding containers provides a basis for an area-wide management strategy for container-inhabiting mosquito populations (Nathan and Knudsen 1991). It has been established previously in premise surveys for *Ae. aegypti* that prevalence of mosquito-positive containers varies between types of containers (Moore et al. 1978; Focks et al. 1981; Barker-Hudson et al. 1988; Nathan and Knudsen 1991; Focks and Chadee 1997), as well as between residences (Chadee 2004). Accordingly, several methods for reducing the manpower needed for controlling *Ae. aegypti* have been proposed, including environmental

sanitation or treatment efforts directed towards container types that are important sources of adult production and targeting specific residences regarded to be mosquito production 'hot spots' (Tun-Lin et al. 1995b; Chadee 2004). The importance of specific types of containers as production sites has largely been addressed by determining prevalence rates of larva- or pupa-positive containers (Moore et al. 1978; Barker-Hudson et al. 1988; Chadee 2004). A '*larval density index*' (*LDI*), based on the average number of larvae per premise, was used by Chan et al. (1971) to compare the distribution and density of *Ae. aegypti* and *Ae. albopictus* in peri-domestic areas of Singapore. Strickman and Kittayapong (2002) in Thailand calculated an *LDI* for *Aedes* mosquitoes based on visual estimates of larval abundance in containers. In a subsequent investigation, Strickman and Kittayapong (2003) advocated use of a *LDI* for routine surveillance and counts of pupae during dengue epidemics to determine the efficacy of vector control. Focks and Chadee (1997), in dengue endemic areas of Trinidad, West Indies, used mean standing crop of *Ae. aegypti* pupae to estimate container productivity. Because pupal survivorship is high, pupal densities could be used to index adult emergence. Prevalence of larvae-positive containers was regarded to provide a less accurate estimate of mosquito populations because equal weight is given to containers, regardless of the numbers of larvae held. After categorizing containers, Focks and Chadee (1997) ranked them based on their mean pupal standing crop and spatial abundance to provide an overall estimate of the importance of each type of container. In a succeeding study, Focks et al. (2000) discussed how a targeted approach to environmental sanitation based on container productivity could be used to disrupt dengue virus transmission.

Regardless of past failures in sustainable control of container-inhabiting mosquitoes, previous research has laid the groundwork for future improvements in mosquito management

by providing effective means, such as pupal surveys, for measuring populations. Oviposition behavior exhibited by *Ae. albopictus* (Rozeboom et al. 1973) can be exploited for purposes of its control by an ovitrap that is designed to attract this mosquito away from other competing containers. Recently, Zeichner and Perich (1999) developed a novel modification of the traditional ovitrap by fitting it with an oviposition substrate that was treated with a fast-acting insecticide. Under laboratory conditions, this modified ovitrap, called a 'lethal ovitrap' (LO), resulted in high mortality of gravid *Ae. aegypti*. A subsequent investigation in two Brazilian cities carried out by Perich et al. (2003) demonstrated that the LO effectively suppressed *Ae. aegypti* under urban field conditions. Conversely, in a study in Thailand, the LO achieved only moderate control of *Ae. aegypti* in three villages where there were numerous competing containers (Sithiprasasna et al. 2003). While the LO has shown promise in controlling *Ae. aegypti*, there are no published studies of effects of the LO on *Ae. albopictus* populations.

Accordingly, the principal goal of our investigation was to evaluate the impact of the LO on abundance of *Ae. albopictus* immatures in containers in suburban landscapes. We also evaluated effects on mosquito production of removing containers through environmental sanitation. We predicted that the LO would not be effective in significantly reducing mosquito production in man-made containers when alternative oviposition sites were available. Consequently, we also evaluated impacts of the LO on production of larvae and pupae in peri-domestic containers when alternative oviposition sites were reduced through environmental sanitation.

MATERIALS AND METHODS

Study sites. Our study was carried out in eight suburban neighborhoods in the City of Raleigh in Wake County, NC (76.6° W, 35.8° N). Raleigh, the capital city of NC, is

located in the central Piedmont portion of the state. There are approximately 300,000 residents in the city. The eight neighborhoods were composed of 20 – 42 single-family dwellings of comparable age and structure with an average lot size ranging from 0.09 – 0.33 ha (Table 1). Vegetation consisted of a mixture of deciduous and evergreen trees and an array of ornamental shrubbery around homes. Study sites were chosen because they were separated from surrounding areas by major roads, woodlands, and other barriers, minimizing mosquito immigration from surrounding untreated areas.

Study Design. An incomplete block design (Neter et al. 1996) was used to evaluate the impacts of LO, SR, and the combination of LO with SR (LO+SR) on abundance of *Ae. albopictus* immatures. Each neighborhood represented a block and one treatment was assigned to each neighborhood. Our study was carried out from May through October in four neighborhoods in 2002 and was expanded to eight neighborhoods in 2003 to allow for the addition of SR treatments. Two neighborhoods each received LOs (N-1 and N-2), SR (N-5 and N-7), SR in conjunction with LOs (LO+SR) (N-6, N-8), or no treatment (No-TRT) (N-3, N-4). Monthly container surveys were completed by a field crew of 4-5 people, searching the home grounds of each premise for containers, which were examined for mosquitoes as described below. Properties were thoroughly canvassed or searched out to a distance of 50 m from the primary dwelling if the property was large. In LO and LO+SR neighborhoods, LOs were placed at each residence as described below.

Container Surveys. The content of each wet container was poured out into a white enameled pan and examined for mosquitoes. If mosquitoes were obscured by dark water or debris, tap water was added to the pan to improve visibility of mosquitoes. This process was repeated until the entire container was emptied. All pupae and a sample of approximately 10

larvae were transferred to Whirl-Pak sample bags (Fisher Scientific, Pittsburgh, PA) and samples were labeled with date of collection, house identification code, and type of container. If the volume of water in the container required what was considered an excessive sampling time, then the container was sampled for a standard time of five minutes. In No-TRT and LO neighborhoods, the contents were returned to each container after it was sampled. In SR and LO+SR neighborhoods, container contents were discarded, and the container was overturned so that it would not collect water. Wet containers that could not be emptied were treated with a granular formulation of the insect growth regulator methoprene (1.5% Pre Strike™, Wellmark, Schumburg, IL).

After collection, samples were placed on wet ice and transported to the lab where mosquitoes were killed in hot water and transferred to labeled vials filled with ethanol. Later, the larvae and pupae in each sample were identified to species using standard keys (Stojanovich 1962; Slaff and Apperson 1989). *Aedes albopictus* pupae were differentiated from pupae of other species by the presence of a fringe of hairs on the edge of the anal paddle (personal communication, Bruce Harrison). Once identified, pupae were counted and the counts, along with the container type, added to a database for subsequent statistical analyses. A GIS was created with ArcMap and these data were added to an attribute table within the GIS for each neighborhood so that the numbers of pupae per container could be linked to each residence.

Lethal Ovitrap (LO). The LO is a patented invention (US patent no. 5,983,557) for control of *Ae. aegypti* (Zeichner and Perich 1999). We evaluated the trap against *Ae. albopictus* populations in suburban landscapes. The LO is a modified version of a commonly used ovitrap (Fay and Eliason 1966) and consists of a black cup (500 ml nominal) half filled

with water and fitted with an oviposition strip (2.54 x 11 cm) of red velour paper impregnated with 1.0 mg of deltamethrin. Each cup contained three drainage holes about 4 cm from the rim. This device was designed to kill gravid females that attempted to oviposit in the trap by use of a fast acting insecticide, thereby reducing abundance of gravid females and diminishing the reproductive success of the population.

In 2002, cups were filled with water that contained sodium phosphate buffering salts to stabilize the pH of the water at neutrality. However, midway in the 2003 mosquito season, we substituted oak leaf infusion (Trexler et al. 1998) in an attempt to increase the amount of time that *Ae. albopictus* spent inside LOs. Oak leaf infusion contains chemo-stimulants that arrest gravid females and stimulate egg laying (Trexler et al. 1998), and our reasoning was that adding oak leaf infusion would increase the exposure time of *Ae. albopictus* to toxic strips. In LO and LO+SR neighborhoods, five LOs were placed in vegetated areas on the grounds of each residence. The number of LOs chosen for placement was based on recommendations made by a person who was experienced in using the lethal ovitrap (personal communication, Michael Perich). Each week, LOs were inspected and buffered water or oak leaf infusion was added as needed. We replaced the insecticide-treated oviposition strips with fresh strips once a month in 2002. In the 2003 mosquito season, strips were changed twice monthly to ensure that oak leaf infusion did not reduce activity of the toxic strips. Sithiprasasna et al. (2003) used similar methods where monthly placement of LOs resulted in fungal growth on strips, consequently leading to bimonthly strip replacement.

Demographic information. A door-to-door census was completed in 2003 to determine the numbers of people per residence in each neighborhood (Table 1). These data were analyzed later to determine if the numbers of people in each residence was associated

with the mean standing crop of pupae per container. Ratios of pupae to people were also compared between treatment areas to estimate mosquito nuisance levels as explained below.

Geographic Information System. A GIS was constructed so that maps of each neighborhood could be created and georeferenced data points could be used in spatial regression analyses. Shapefiles for property boundaries for all study sites were downloaded from the Wake County government GIS website (http://www.wakegov.com/county/propertyandmapping/gisdigitaldata.htm) and imported into ArcMap (ESRI ®, Redlands, California, 2002). The coordinate system used was Universal Transverse Mercator, North American Datum 1983. These shapefiles contained information on the size of each residential property for analyses described later. We used the '*Feature Analyst*' extension (ver. 3.4, Visual Learning Systems, Inc., Missoula, MT) of ArcMap to place a centroid in the geographic center of each property boundary of each residence to represent the average location for all containers on the property because containers were not georeferenced during surveys. If there was more than one household on a parcel of land, the same centroid was used for both houses. Once a centroid shapefile had been created, a unique alphanumeric code for each residence was added to the attribute table containing the corresponding centroid.

Data analysis. Mosquito production variables calculated from counts of pupae and containers are described in Table 2. We used box plots to visually examine mosquito production variables for each neighborhood for normality. Lack of normality was verified with Kolmogorov-Smirnov tests (PROC UNIVARIATE, SAS Institute 2000). To meet assumptions of normality, mosquito production variables (mean pupal standing crop per container per residence, mean total pupal standing crop per residence, mean numbers of

pupae-positive containers per residence, mean numbers of pupae- or larvae-positive containers per residence, mean numbers of pupae per person per residence, and mean numbers of pupae per hectare per residence) were $\log_{10}(x + 1)$ transformed before statistical analyses.

Mean total pupal standing crop per residence produced by 12 types of containers were compared by container type, treatment (No-TRT, LO, SR, LO+SR), and mosquito season (2002 and 2003).

Treatment effects on mosquito production. We used one-way analysis of variance (ANOVA) to determine if the level of mosquito suppression achieved by LO, SR, or LO+SR was different (PROC GLM, SAS Institute 2000). In these analyses, the dependent mosquito production variables were mean pupal standing crop per container per residence, mean total pupal standing crop per residence, mean numbers of pupae-positive containers per residence, mean numbers of pupae- or larvae-positive containers per residence, mean numbers of pupae per person per residence, and mean numbers of pupae per hectare per residence between treatment areas.

We compared the mean numbers of pupae per person per residence between treatment neighborhoods to determine the potential mosquito nuisance level and epidemiological risk. Neighborhoods producing high numbers of pupae in relation to people would have a higher potential for contact between mosquitoes and people.

Container type, household, and treatment were classification variables and analyses were completed for each monthly survey and mosquito season (2002 and 2003). Comparisons made between treatment areas were standardized on a 'per residence' basis to allow for differences in numbers of residences per neighborhood. If significant differences in

mosquito production variables between treatments and mosquito seasons resulted from ANOVA, Bonferroni *t*-tests were used to determine which means were significantly different.

Impact of treatments on pupal standing crop. The percentage of mean pupal standing crops per container per residence in LO, SR, LO+SR, and No-TRT neighborhoods as a function of the mean standing crops per container per residence in June of 2002 and May of 2003 was calculated across all container types each month and plotted (PROC GPLOT, SAS Institute 2000) to illustrate temporal differences in abundance of pupae between treatments.

For each treatment area in each mosquito season, we classified mean pupal standing crops per container per residence, mean total pupal standing crops per residence, mean numbers of pupae-positive containers per residence, and mean numbers of pupae- or larvae-positive containers into three mosquito abundance categories and created frequency distributions on a household basis (PROC FREQ, SAS Institute 2000). The abundance categories used for mean numbers of pupae-positive containers per residence and mean numbers of pupae- or larvae-positive containers per residence were 1-5, 6-10, and > 10 containers per household. For mean pupal standing crops per container per residence, the abundance categories we used were 1-15, 16-30, and > 30. Finally, the abundance categories constructed for mean total pupal standing crops per residence were 1-75, 76-150, and > 150.

To determine how the relationship between mean total pupal standing crops per residence and mean numbers of pupae-positive containers changed between treatments, we carried out Pearson Product Moment correlation analyses for these two variables within each treatment area (PROC CORR, SAS Institute 2000).

To test the null hypothesis that mean total pupal standing crops per residence and mean numbers of pupae-positive containers per residence were not concordant, we ranked these two variables by container type within neighborhoods blocked by treatment and compared them between treatments using Kendall's tau-*b* correlation (PROC CORR, SAS Institute 2000).

Key container analysis. The significance of each type of container to neighborhood mosquito production is a function of its density in the neighborhood landscape and its mean pupal standing crop. Consequently, we calculated an '*Index of Container Importance*' (*ICI*) for each container type within each treatment area by multiplying the mean pupal standing crop per container per residence by container density (mean number of pupae-positive containers per residence per hectare). We determined if *ICI*s in different treatment areas (LO, SR, LO+SR, No-TRT) were significantly different by ANOVA using a general linear model (PROC GLM, SAS Institute 2000). If there were significant differences in *ICI*s between treatments, Bonferroni *t*-tests were used to determine which treatments for particular container types were significantly different.

Key premise analysis. The spatial distribution of containers would potentially affect the performance of the LO. We modified the procedure of Chadee (2004) to identify '*key premises*' where *Ae. albopictus* production occurred. Households that had ≥ 3 *Ae. albopictus* pupae-positive containers in 2 or more survey periods were identified as '*Container Key Premises*' (*CKP*). Based on a preliminary review of results, we also completed a separate analysis where we classified households that had ≥ 3 *Ae. albopictus* pupae-positive containers in 3 or more survey periods as *CKP*s. We supplemented the *CKP* analysis with a '*Pupae Key Premise*' (*PKP*) index using the mean total pupal standing crop per residence for

each neighborhood as the cutoff value for classifying residences. In the 2002 mosquito season, residences in No-TRT and LO areas where > 43 and 46 total pupae, respectively, were collected during the entire season were considered *PKP*s. In the 2003 season, residences with > 79, 71, 50, and 50 total pupae collected in LO, SR, LO+SR, and No-TRT areas, respectively, were considered *PKP*s. We also completed separate *PKP* analyses for households with a mean total standing crop of > 100 and > 200 pupae per residence.

These retrospective analyses allowed us to calculate the percentage of mosquitoes that could have been eliminated by complete source reduction only at these '*key premises*'. We compared the percentage of households that were '*key premises*' between areas that were blocked by different treatments.

Spatial regression analyses. Since standard regression analysis does not take into account spatial dependence, we used spatial linear regression (R Development Core Team, Austria, 2004) to model mean pupal standing crops per container per residence and mean total pupal standing crops per residence in relation to mean numbers of mosquito-positive containers per residence within each neighborhood. For each neighborhood, the degree of association between the aforementioned variables was estimated, incorporating the spatial association of observed data values as an additional parameter. These analyses were completed separately for each neighborhood and mosquito season and only included residences where either larvae or pupae were collected. We used Restricted Maximum Likelihood Estimates of $\beta \pm$ standard error (RMLE of $\beta \pm$ SE) to estimate the association between covariables, based on the concurrent spatial patterns of data points observed in each neighborhood. The magnitude of the RMLE of β represented the strength of association between spatial regression covariables and provided a means to compare the spatial

association of mosquito production variables between neighborhoods receiving different treatments. If no significant spatial association was observed between covariables, we assumed that there was a large degree of variation between covariables at residences within the neighborhood. We fit the data using exponential variogram models and the spatial linear regression model equation we used was: $V_i = \beta_0 + \beta_1 X_i + \beta_2 Y_i + \beta_3 Z_i + \varepsilon_i$ where, at location i, V_i represented either mean or total pupal standing crop, X_i and Y_i were latitude and longitude coordinates of property boundary centroids, Z_i was either the number of pupae-positive containers or the number of pupae- or larvae- positive containers, ε_i was the spatially correlated error, and β_x were regression coefficients to be estimated. In our regression analyses, the dependent variables were mean pupal standing crops per container per residence and mean total pupal standing crops per residence and the independent variables were mean numbers of pupae-positive containers and mean numbers of pupae- or larvae-positive containers at each residence.

RESULTS

Container abundance. Four surveys (June – September) during the 2002 mosquito season and six surveys (May – October) during the 2003 mosquito season were completed. In surveys of 56 houses in No-TRT neighborhoods in the 2002 and 2003 mosquito seasons, 315 and 1,161 wet containers were sampled, respectively, including 306 (97 %) and 511 (44 %) containers with either larvae or pupae of *Ae. albopictus*. From 69 houses in LO neighborhoods in the 2002 and 2003 seasons, 641 and 1,841 wet containers were sampled, respectively, including 372 (58 %) and 856 (46 %) containers positive for *Ae. albopictus* larvae or pupae. In two SR neighborhoods in the 2003 mosquito season, from 44 houses, 1,263 wet containers were sampled, including 624 (49 %) containers positive for *Ae.*

albopictus immatures. In LO+SR neighborhoods in the 2003 mosquito season, from 54 houses, 1,636 wet containers were sampled including 778 (48 %) containers positive with *Ae. albopictus* immatures. A larger number of containers were sampled in 2003 compared to 2002 because of two additional surveys, four additional neighborhoods, and higher rainfall totals in 2003 compared to 2002 (Figure 1).

Treatment effects on mosquito production. Differences in mosquito production between treatment neighborhoods within each mosquito season were not statistically significant ($P > 0.05$) (Table 3). Production of mosquitoes was, generally, highest in neighborhoods where LOs were placed. Mean total pupal standing crops per residence were highest in neighborhoods receiving LO treatments during the 2002 (mean ± SE = 45.8 ± 11.4) and 2003 (79.3 ± 14.5) mosquito seasons (Table 3). Similarly, mean pupal standing crop per container was highest in neighborhoods receiving LO treatments during the 2002 (10.2 ± 1.6) and 2003 (8.8 ± 0.8) mosquito seasons (Table 3). Season-long mean numbers of pupae-positive containers per residence were highest in source reduction areas (9.3 ± 1.7) and mean numbers of larvae- or pupae-positive containers per residence were highest in LO+SR (14.4 ± 2.8) areas relative to other treatment neighborhoods. However, there was no significant difference ($P > 0.05$) between any treatment means within mosquito seasons for these variables. Numbers of pupae per hectare were highest in LO areas (644.2 ± 115.6) during the 2003 mosquito season and was more than twice that of No-TRT areas (274.7 ± 63.9) in 2002 and LO+SR (300.8 ± 97.1) areas in 2003. LO treatment areas had the highest ratio of pupae per person (50.9 ± 12.8); regardless, there was no significant difference ($P > 0.05$) in pupae per person between treatment areas. Furthermore, there was no significant

association between mean numbers of people per residence and mean total pupal standing crop per residence ($P > 0.05$).

Mean total pupal standing crop per container per residence did not vary significantly between treatments among the 12 types of containers in either 2002 or 2003 ($P > 0.05$) (Table 4). Mean total pupal standing crop per residence is illustrated for each container type in each treatment area (No-TRT, LO, SR, SL) in Figure 2. In the 2002 mosquito season, highest mean total pupal standing crops were produced in equipment (30.0 ± 16.0, N = 2) and garbage containers (39.1 ± 11.7, N = 19) in No-TRT and LO areas, respectively. In contrast, in the 2003 mosquito season in No-TRT and LO+SR areas, birdbaths produced the highest mean total pupal standing crops (62.4 ± 43.6, N = 13 and 33.5 ± 12.6, N = 15, respectively). Among all treatments in 2003, production of pupae was highest in toys (77.3 ± 37.2, N = 20) in LO areas and in tarps (57 ± 21.3, N = 78) in SR areas.

During the 2002 mosquito season, significant differences in mosquito production variables between types of containers were only observed in mean numbers of pupae- or larvae-positive containers per residence (Table 4); however, significant differences were observed in all production variables (mean pupal standing crop per container per residence, mean total pupal standing crop per residence, mean numbers of pupae-positive containers per residence, mean numbers of pupae- or larvae-positive containers per residence) between container types during the 2003 season. Due to the large number of container types (N = 12), Bonferroni *t*-tests could not be used to determine which type of container was the most productive for mosquitoes.

For both mosquito seasons, there was a significant difference in mean total pupal standing crops per residence between months ($P < 0.05$) (Table 4). Consequently, we plotted

mean pupal standing crop per container per residence as a percentage of mosquito production in June of 2002 and May of 2003 to evaluate seasonal changes in effects of control methods (Figures 3a and 3b). As the season progressed, mosquito production was higher in LO areas in relation to other treatment areas. Mosquito production declined in LO and SR areas between the pupal surveys conducted in July and August in both mosquito seasons; however, as discussed previously, differences in mosquito production between treatment areas were not statistically significant.

Frequency of mosquito production. We classified frequency distributions of abundance of mean pupal standing crop per container per residence, mean total pupal standing crop per residence, mean numbers of pupae-positive containers per residence, and mean numbers of pupae- or larvae-positive containers per residence. Results from Chi-square analyses indicated no significant differences in frequency distributions of mosquito production variables between treatment areas ($P > 0.05$).

Relationship between mosquito production variables. We found a significant relationship between mean total pupal standing crop per residence and mean number of pupae-positive containers per residence in all treatment areas. These two mosquito production variables were significantly correlated in No-TRT ($r = 0.852$, $P < 0.0001$) and LO ($r = 0.889$, $P < 0.0001$) neighborhoods in 2002 and in No-TRT ($r = 0.815$, $P < 0.0001$), LO ($r = 0.941$, $P < 0.0001$), SR ($r = 0.920$, $P < 0.0001$), and LO+SR ($r = 0.939$, $P < 0.0001$) neighborhoods in 2003.

Mosquito production potential of containers. So that we could determine if the mosquito production potential of the types of containers that were sampled varied between treatment areas, we categorized containers and ranked them by their abundance in each

neighborhood. Next, we ranked the container types in each neighborhood according to their corresponding mean total pupal standing crops per residence of *Ae. albopictus* pupae. We found no significant association between the two ranks in No-TRT (r = -0.168, *P* = 0.449, no. pairs = 12) or LO (r = -0.092, *P* = 0.679, no. pairs = 12) treatment areas in the 2002 mosquito season. There was also no significant association between ranked mean numbers of pupae-positive containers per residence and mean total pupal standing crop per residence during the 2003 mosquito season in No-TRT (r = -0.123, *P* = 0.582, no. pairs = 12), LO (r = -0.168, *P* = 0.449, no. pairs = 12), SR (r = 0.075, *P* = 0.753, no. pairs = 11), or LO+SR (r = -0.046, *P* = 0.837, no. pairs = 12) areas.

The mosquito production potential of each type of container is a function of its density and mean pupal standing crop. Accordingly, we calculated an Index of Container Importance (ICI) by multiplying the mean number of pupae-positive containers per residence per hectare by the mean pupal standing crop per residence for each container type (Tables 5 and 6). In the 2002 mosquito season in No-TRT areas, mean pupal standing crop from plant pots was ranked intermediately; however, when evaluated in conjunction with its density, plant pots had the highest mosquito production potential (ICI = 35.7). Conversely, in the same season in LO areas, garbage containers had a high mean pupal standing crop and an intermediate density, resulting in a high mosquito production potential (ICI = 53.3). In the 2003 mosquito season, container types exhibiting highest mosquito production potential included toys (ICI = 52.3; No-TRT areas), garbage containers (ICI = 137.9; LO areas), tarps (ICI = 33.9; LO+SR areas), and plant pots (ICI = 87.0; SR areas). We used ANOVA to determine that there was no significant difference in mean ICIs between treatment areas over all container types and both mosquito seasons (df = 5, 66; *F* = 1.34; *P* = 0.259). However,

there was a significant difference in ICIs between container types over all treatment areas and both mosquito seasons (df =11, 60; $F = 3.38$; $P = 0.001$). The highest mosquito production potential of garbage containers (mean ICI ± S.E. = 52.3 ± 18.1) was higher relative to other containers; however, due to the large number of container categories, statistical significance was not determined.

Key premise analyses. The spatial distribution of key containers among residences would potentially influence the effectiveness of the LO. In addition to characterizing container types in terms of which were key containers for mosquito production, we also identified '*key premises*' for mosquito production based on the types and abundance of containers found during backyard surveys (Table 7). Based on the mean total standing crop of *Ae. albopictus* pupae per residence, orienting control efforts to 'pupae key premises' (PKPs) would potentially eliminate 80.6 % of mosquitoes in SR areas (13 PKPs) and 80.6 % of mosquitoes in LO+SR areas (16 PKPs). In general, orienting control efforts to CKPs with ≥ 3 pupae-positive containers in 2 or more surveys would be more effective in eliminating mosquitoes from CKPs with ≥ 3 pupae-positive containers in 3 or more surveys. However, there were fewer premises included when 3 or more surveys were considered (4-8 premises) as compared to 2 or more surveys (10-15 premises).

Spatial regression analyses. Spatial regression analysis based on RMLE of β was used to determine to what extent the spatial distribution of mean numbers of pupae-positive and mean numbers of pupae- or larvae-positive containers per residence could explain the clustering of mean pupal standing crops per container per residence and mean total pupal standing crops per residence (Table 8). No significant spatial relationship was observed between pupal production variables and mean numbers of pupae- or larvae-positive

containers so this covariate was excluded from the analyses. In each neighborhood during the 2002 and 2003 mosquito seasons, we found a significant spatial association between mean numbers of pupae-positive containers per residence and mean total pupal standing crops per residence ($P < 0.05$). In 2002, the strongest associations between mean numbers of pupae-positive containers per residence and mean total pupal standing crops per residence were seen in N-1 (LO; RMLE of $\beta = 1.79 \pm 0.28$; Z-value = 6.46; $P < 0.0001$) and N-4 (No-TRT; RMLE of $\beta = 1.86 \pm 0.25$; Z-value = 7.49; $P < 0.0001$); however, in 2003, the strongest associations were observed in N-1 (LO; RMLE of $\beta = 1.87 \pm 0.25$; Z-value = 7.50; $P < 0.0001$) and in N-3 (No-TRT; RMLE of $\beta = 1.62 \pm 0.20$; Z-value = 7.96; $P < 0.0001$) compared to other neighborhoods.

In the 2002 mosquito season, there were significant spatial associations between mean pupal standing crops per container per residence and mean numbers of pupae-positive containers per residence in three of four neighborhoods; the exception being N-3 (No-TRT). Similar to the above analysis, the strongest associations between regression covariables were seen in N-1 (LO; RMLE of $\beta = 0.84 \pm 0.29$; Z-value = 2.89; $P = 0.005$) and in N-4 (No-TRT; RMLE of $\beta = 0.75 \pm 0.25$; Z-value = 2.97; $P = 0.003$). In 2003, we observed significant associations ($P < 0.05$) between mean numbers of pupae-positive containers per residence and mean pupal standing crops per container per residence in N-1 (LO), N-3 (No-TRT), and N-8 (LO+SR) and a marginally significant association ($P = 0.056$) in N-2 (LO). Again, the strongest association was observed in N-1 (LO; RMLE of $\beta = 0.75 \pm 0.25$; Z-value = 2.97; $P = 0.003$).

DISCUSSION

In our investigation, placement of LOs around residences did not suppress *Ae. albopictus* populations. Our results differ from the findings of the laboratory study of Zeichner and Perich (1999) that LOs aged in the field > six months provided high (89 %) mortality of *Ae. aegypti*; however, the outcome of the study by Perich et al. (2003) is in partial agreement with results of our investigation. In study sites receiving targeted SR, the findings of Perich et al. (2003) indicated a high mortality rate of *Ae. aegypti* 30 days after placement of LOs; however, in study sites receiving only LOs as mosquito control, the presence of alternative oviposition sites resulted in inadequate and delayed control by LOs.

Container-inhabiting *Aedes* exhibit a wide dispersal range that can exceed 800 m from their point of origin (Reiter et al. 1995; Honorio et al. 2003). Movement of gravid container-inhabiting *Aedes* is strongly influenced by the availability of oviposition sites (Edman et al. 1998); consequently, we reasoned that removal of peri-domestic containers would eliminate alternative oviposition sites forcing gravid mosquitoes to LOs.

The lack of control achieved by the LO supports the equivocal results reported by Sithiprasasna et al. (2003) for the LO that was evaluated against *Ae. aegypti* in Thailand. Since the highest pupal standing crops were found in LO areas, we speculate that LOs did kill some gravid females, reducing oviposition in surrounding containers and alleviating density dependent larval mortality. A reduction of intraspecific competition in containers enhanced survivorship of larvae and led to a corresponding increase in pupal standing crops. Intraspecific competition for resources leading to increased larval mortality has been demonstrated in previous research (Mori 1979; Gomez et al. 1995; Lord 1998). Furthermore, our monthly regimen of neighborhood-wide SR was labor-intensive and obviously not

sufficient to minimize the availability of alternative container habitats that were continually created through rainfall events and human activity. Perich et al. (2003) recommended using the LO as an element in a complete control plan, however, our research results suggest that further studies are needed to improve the activity of the lethal oviposition trap against *Ae. albopictus* populations.

Focks et al. (1993, 2000) reported that pupal densities in containers could be used to accurately index abundance of container-inhabiting mosquitoes, improving the precision of source reduction efforts. Studies performed on *Ae. aegypti* in Trinidad by Focks and Chadee (1997) and in Peru by Morrison et al. (2004ab) indicated that a large portion of the standing crop of pupae was produced by outdoor water storage containers such as: outdoor drums, laundry tubs, buckets, and small containers. An Australian study found that underground habitats, such as wells, service pits, and septic tanks, comprised 15 % of total oviposition sites for *Ae. aegypti* and produced 85 % of the total mosquitoes (Russell et al. 1996). Another study in Australia provided evidence of significant *Ae. aegypti* production in roof gutters (Montgomery and Ritchie 2002). *Aedes albopictus* inhabits a multitude of oviposition sites (Hawley 1988; Moore 1999). However, in our study of *Ae. albopictus* in suburban landscapes of Raleigh, we found mosquitoes were produced in a variety of containers, but that a significant portion of the mean total pupal standing crop per residence (49 %) was produced by birdbaths, toys, tarps, and garbage containers.

Our data indicated that mean total pupal standing crops per residence and mean pupal standing crops per container per residence varied significantly between types of containers within and between treatments and, standing crops were not necessarily correlated with mean numbers of pupae-positive containers per residence. A study performed on *Ae. aegypti* by

Southwood et al. (1972) in Thailand found a 23-fold difference in container productivity between some types of containers. In our study of *Ae. albopictus*, we found up to a 77-fold difference in production of mean total pupae per residence between the least (appliance) and most (toy) productive types of containers. In characterizing production of *Ae. albopictus* in suburban landscapes, we used the concept developed by Focks and Chadee (1997) that the *Ae. aegypti* production potential of each type of container is a function of its local abundance and pupal standing crop. Our *Index of Container Importance* varied between neighborhoods for specific types of containers. In neighborhoods receiving SR, difficult to reduce containers such as plant pots and tarps were most important. Based on ICI values that we calculated using container densities and estimates of pupal standing crop, the mosquito production potential of the types of containers in our study varied by almost 140-fold.

Focks and Chadee (1997) and Focks et al. (2000) recommended the use of numbers of pupae per person and per hectare to analyze the epidemiological risk posed by *Ae. aegypti*. While there were no significant differences in these two variables between treatments in our study, we noted that numbers of pupae per person and per hectare in LO study sites were consistently higher than in other areas. The high number of pupae with respect to humans and hectares in LO areas suggests the potential for contact between humans and mosquitoes would actually be higher in areas receiving lethal oviposition taps relative to other treatment areas.

Tun-Lin (1995a) and Chadee (2004) discussed an alternative to complete neighborhood SR, and recommended only eliminating containers at 'key premises' for mosquito production. Based on parameters for determining '*key premises*', our retrospective analyses indicate that a significant reduction in total pupal standing crops would occur after

focusing management efforts to *PKP*s or *CKP*s. We determined that about 60 % of the total pupal standing crop of *Ae. albopictus* was produced at approximately 20 % of the residences in our study neighborhoods. Consequently, placement of the same number of lethal oviposition traps at each residence and use of a uniform pattern of trap coverage across neighborhoods could explain the lack of significant impacts of the LO on mosquito populations in our investigation. The activity of the LO might be improved if the number of traps placed around each residence were proportional to the number of alternative oviposition sites. In addition, the effectiveness of the LO may be improved by orienting the spatial placement of LOs adjacent to areas where other oviposition containers are located. Similarly, environmental sanitation did not significantly reduce mosquito production when carried out on a monthly basis. Completion of SR at least bi-monthly or within several days following rain events may improve mosquito suppression when using the '*key premise*' approach to mosquito control. Additional studies would be needed to evaluate these recommendations.

Since mosquito production varies spatially (Focks and Chadee 1997), we can adopt Tobler's First Law of Geography (Tobler 1979) that 'everything is related to everything else, but near things are more related than distant things' to our study and hypothesize that there is a stronger association between mosquito production at adjacent households than at distant households. Consequently, spatial regression is an appropriate method for analyzing the relationship between the spatial distribution and abundance of mosquitoes and mosquito-positive containers. Contrary to traditional regression, spatial regression takes into account the irregular nature of spatial data and makes use of both the locations and data values of collection sites in the observation space (Cressie 1993; Getis 1999; Schabenberger and

Gotway 2005). Only a few studies have researched insects of public health importance utilizing this type of analysis as described by Robinson (2000). Significant spatial associations between mean total pupal standing crops per residence and mean numbers of pupae-positive containers per residence were found in all treatment neighborhoods in both mosquito seasons (2002 and 2003), suggesting that *Ae. albopictus* is not likely to disperse if there are suitable oviposition sites and that reducing the number of containers distributed in the landscape would decrease the total *Ae. albopictus* population. In the 2003 season, the lack of association between mean pupal standing crop per container per residence and mean numbers of pupae-positive containers per residence in SR and LO+SR areas probably resulted because containers supporting high levels of mosquito production (e.g. plant pot receptacles) were reduced, causing a shift in oviposition to containers that could not be easily eliminated or source reduced. This shift caused a high degree of variation in mean pupal standing crop per container and mean numbers of pupae-positive containers per residence between residences in SR and LO+SR areas. The large numbers of mosquito samples acquired from tarps in SR areas support this conclusion. In one LO+SR neighborhood (N-8) where there was a significant association between mean pupal standing crop per container and mean numbers of pupae-positive containers per residence, containers that were difficult to source reduce were abundant and spatially widespread, increasing the likelihood of functional association between the numbers of pupae-positive containers and mosquito abundance.

Despite our equivocal results, we feel that the LO concept is novel and offers promise as a management tool, because it is based on the biology and behavior of container-inhabiting mosquitoes. Modification in the design through the addition of a lure that contains

a volatile oviposition semio-chemical may improve the activity of the trap in suburban landscapes where there are alternative oviposition sites. Additional studies should also be conducted to determine the optimal ratio of LOs to alternative oviposition sites.

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Table 1. Demographic information for neighborhoods in Raleigh, NC that were used to investigate the impact of source reduction and lethal ovitraps on the abundance of *Aedes albopictus* immatures.

Neighborhood	Treatment	Mean lot size (ha) (± SE)	No. Houses	Mean no. people per residence (± SE)	Mean no. people per hectare (± SE)
N-1	Lethal ovitrap	0.09 (± 0.01)	27	2.3 (0.3)	29.5 (3.6)
N-2	Lethal ovitrap	0.17 (± 0.01)	42	2.1 (0.2)	14.7 (1.9)
N-3	No treatment	0.24 (± 0.03)	30	2.1 (0.3)	9.9 (1.3)
N-4	No treatment	0.16 (± 0.02)	26	2.5 (0.2)	19.5 (5.4)
N-5	Source reduction	0.17 (± 0.03)	20	2.2 (0.4)	13.9 (1.7)
N-6	Source reduction + lethal ovitrap	0.24 (± 0.06)	24	1.8 (0.2)	13.9 (3.3)
N-7	Source reduction	0.25 (± 0.02)	24	2.0 (0.2)	9.4 (1.4)
N-8	Source reduction + lethal ovitrap	0.33 (± 0.05)	30	2.1 (0.2)	8.2 (1.0)

Table 2. Mosquito production variables analyzed at the neighborhood and household level over the period of each container survey and over the entire mosquito season.

Variable	Description
Mean total pupal standing crop per residence	Sum of the numbers of pupae collected in all containers or in each specific container type per residence.
Mean pupal standing crop per container per residence	Sum of the numbers of pupae collected in all containers / total numbers of all containers per residence.
Mean pupal standing crop per container type per residence	Sum of the numbers of pupae collected in each type of container / total numbers of each specific container per residence.
Pupae per person	Total numbers of pupae collected in all containers / number of people per residence.
Pupae per hectare	Total numbers of pupae collected in all containers / parcel size (ha) of the residential property.
% pupal standing crop	(Mean total pupal standing crop per residence in treatment areas / mean total pupal standing crop per residence in No-TRT areas) x 100.
Containers per hectare	Total numbers of pupa-positive containers / parcel size (ha) of the residential property.
Index of Container Importance	Mean pupal standing crop per container per residence x mean number of pupa-positive containers per residence per hectare.

Table 3. Household production (mean ± SE)* of *Aedes albopictus* over the 2002 and 2003 mosquito seasons in neighborhoods not receiving any treatment (No-TRT), and receiving lethal ovitraps (LO), source reduction (SR) and lethal ovitraps plus source reduction (LO+SR) treatments in Raleigh, NC.

Variable	No treatment		Lethal oviposition trap		Source reduction	Lethal oviposition trap + source reduction
	2002	2003	2002	2003	2003	2003
Mean total pupal standing crop per residence	42.7 (7.6)	49.2 (9.2)	45.8 (11.4)	79.3 (14.5)	71.4 (14.8)	50.4 (9.2)
Mean pupal standing crop per container per residence	9.0 (1.2)	7.8 (1.3)	10.2 (1.6)	8.8 (0.8)	8.2 (1.0)	6.1 (0.7)
Mean no. pupae-positive containers per residence	4.6 (0.8)	6.1 (0.8)	4.7 (0.8)	8.8 (1.6)	9.3 (1.7)	8.3 (1.6)
Mean no. larvae or pupae-positive containers per residence	11.5 (1.9)	10.2 (1.3)	11.6 (1.9)	13.4 (2.2)	14.2 (2.7)	14.4 (2.8)
Mean no. pupae per person	-	31.5 (7.4)	-	50.9 (12.8)	45.4 (9.2)	29.9 (6.3)
Mean no pupae per hectare	274.7 (63.9)	355.0 (76.6)	443.4 (95.5)	644.2 (115.6)	461.8 (105.3)	300.8 (97.1)

*Variable means within a mosquito season were not significantly different at $P < 0.05$ by ANOVA.

Table 4. ANOVA results for difference in mosquito production variables in neighborhoods not receiving any treatment (No-TRT), and receiving lethal ovitraps (LO), source reduction (SR) and lethal ovitraps plus source reduction (LO+SR) treatments in Raleigh, NC.

	2002			2003		
Source	df (model, error)	*F*	*P*	df (model, error)	*F*	*P*
			Mean pupal standing crop per container per residence			
Treatment	1, 100	0.03	0.875	3, 575	1.77	0.151
Month	3, 301	1.19	0.116	5, 1005	3.87	0.002
Treatment x Month	7, 297	2.68	0.011	23, 987	2.09	0.002
Container type	11, 214	1.53	0.108	11, 567	4.85	< 0.0001
Treatment x Cont. type	27, 200	1.62	0.034	46, 532	2.12	< 0.0001
			Mean total pupal standing crop per residence			
Treatment	1, 100	0.00	0.983	3, 190	1.77	0.154
Month	3, 192	3.13	0.027	5, 551	6.38	< 0.0001
Treatment x Month	7, 188	3.69	0.001	23, 533	2.58	< 0.0001
Container type	11, 210	0.98	0.467	11, 567	2.66	0.002
Treatment x Cont. type	23, 198	1.39	0.121	46, 532	1.94	0.0003
			Mean no. pupa-positive containers per residence			
Treatment	1, 100	0.00	0.949	3, 190	0.54	0.653
Month	3, 192	2.80	0.039	5, 551	7.31	< 0.0001
Treatment x Month	7, 188	1.76	0.098	23, 533	2.47	0.0002
Container type	11, 210	1.49	0.139	11, 567	2.90	0.001
Treatment x Cont. type	23, 198	1.48	0.079	46, 532	1.46	0.029
			Mean no. pupa- or larva-positive containers per residence			
Treatment	1, 112	0.12	0.732	3, 208	0.12	0.949
Month	3, 261	0.99	0.398	5, 722	10.21	< 0.0001
Treatment x Month	7, 257	0.54	0.802	23, 704	2.90	< 0.0001
Container type	11, 283	2.57	0.004	11, 746	4.05	< 0.0001
Treatment x Cont. type	23, 271	2.09	0.003	46, 711	1.53	0.015

Table 5. Household production of *Aedes albopictus* pupae in containers in neighborhoods receiving lethal ovitraps (LO) and not receiving any treatment (No-TRT) in Raleigh, NC during the 2002 mosquito season.

Container Type	No-TRT-2002			LO-2002		
	Mean pupal standing crop	Containers per ha	Index of Container Importance	Mean pupal standing crop	Containers per ha	Index of Container Importance
Plant pot	8.7	4.1	35.7	6.1	7.4	45.1
Bucket	5.7	4.0	22.8	8.4	1.5	12.6
Tire	3.0	0.8	2.4	9.5	2.6	24.7
Bird bath	16.5	1.3	21.5	8.3	0.6	5.0
Tarp	14.7	0.7	10.3	6.2	1.3	8.1
Toy	5.8	1.0	5.8	30.7	0.3	9.2
Cup/Bottle	7.8	3.1	24.2	6.3	4.9	30.9
Garbage container	6.2	2.1	13.0	21.3	2.5	53.3
Tray/Pan	20.0	1.3	26.0	16.0	2.3	36.8
Equipment	30.0	0.2	6.0	12.0	0.3	3.6
Appliance	10.1	0.4	4.0	6.0	0.3	1.8
Miscellaneous	13.8	0.7	9.7	5.1	0.8	4.1

Table 6. Household production of *Aedes albopictus* pupae in containers in neighborhoods receiving lethal ovitraps (LO), source reduction (SR), source reduction plus lethal ovitraps (SR+LO) and not receiving any treatment (No-TRT) in Raleigh, NC during the 2003 mosquito season. ICI = Index of Container Importance

	No-TRT-2003			LO-2003			LO+SR-2003			SR-2003		
Container Type	Mean pupal standing crop	Mean no. containers per ha	ICI	Mean pupal standing crop	Mean no. containers per ha	ICI	Mean pupal standing crop	Mean no. containers per ha	ICI	Mean pupal standing crop	Mean no. containers per ha	ICI
Plant pot	5.7	6.5	37.1	4.7	10.2	47.9	4.0	7.6	30.4	7.7	11.3	87.0
Bucket	9.2	2.4	22.1	9.5	2.6	24.7	7.9	2.4	19.0	5.5	1.3	7.2
Tire	2.0	0.6	1.2	5.3	5.1	27.0	6.7	4.6	30.8	7.8	2.2	17.2
Bird bath	18.9	1.1	20.8	11.3	0.8	9.0	18.1	1.3	23.5	16.6	2.7	44.8
Tarp	9.8	1.8	17.6	6.5	5.1	33.2	8.7	3.9	33.9	9.9	8.5	84.2
Toy	32.7	1.6	52.3	21.7	2.1	45.6	5.1	0.9	4.6	8.3	0.3	2.5
Cup/Bottle	5.8	3.6	20.9	6.6	10.5	69.3	3.2	5.2	16.6	4.5	3.3	14.9
Garb. cont.	7.3	3.9	28.5	12.2	11.3	137.9	8.2	3.9	32.0	9.8	5.0	49.0
Tray/Pan	8.3	1.3	10.8	10.1	1.4	14.1	5.5	0.5	2.8	3.3	0.3	1.0
Equipment	3.0	0.1	0.3	10.2	0.5	5.1	12.5	0.8	10.0	9.0	0.3	2.7
Appliance	8.6	0.6	5.2	12.4	1.5	18.6	3.5	0.4	1.4	0	0	0
Miscellaneous	9.6	1.8	17.2	6.9	2.5	17.3	6.1	3.4	20.7	3.1	2.1	6.5

Table 7. Container key premise and pupae key premise analysis in neighborhoods receiving lethal ovitraps (LO), source reduction (SR), source reduction plus lethal ovitraps (LO+SR) and not receiving any treatment (No-TRT) in Raleigh, NC during the 2002 and 2003 mosquito seasons.

Treatment area	No. pupae-positive premises (% total premises inspected)	Container Key Premise		Pupae Key Premise		
		≥ 3 pupae-positive containers in 2 or more surveys	≥ 3 pupae-positive containers in 3 or more surveys	> mean total pupal standing crop per premise*+	> 100 mean total pupal standing crop per premise	> 200 mean total pupal standing crop per premise
		No. premises (% of total pupal standing crop)				
		2002 mosquito season				
No-TRT	50 (89.3)	5 (34.5)	2 (22.5)	16 (77.7)	6 (49.6)	1 (13.4)
LO	50 (72.5)	9 (49.1)	3 (29.8)	17 (79.3)	3 (35.3)	1 (23.0)
		2003 mosquito season				
No-TRT	49 (87.5)	11 (55.0)	4 (26.1)	13 (75.1)	9 (62.5)	2 (22.0)
LO	58 (84.1)	15 (66.2)	8 (44.9)	19 (78.7)	13 (66.6)	6 (44.9)
SR	37 (84.1)	10 (69.5)	8 (51.8)	13 (80.6)	10 (71.3)	2 (28.4)
LO+SR	50 (92.6)	14 (68.2)	6 (43.0)	16 (80.6)	10 (63.6)	2 (20.5)

Mean total pupal standing crop per premise cut-off values: *2002 - No-TRT: > 43; LO: > 46; +2003 - No-TRT: > 50; LO: > 79; SR: > 71; LO+SR: > 50

Table 8. Spatial regression using Restricted Maximum Likelihood Estimation (RMLE) for describing household level spatial associations between dependent variables mean total pupal standing crops per residence and mean pupal standing crops per container per residence and independent variable mean numbers of pupae-positive containers in eight neighborhoods in the City of Raleigh, Wake County, NC during the 2002 and 2003 mosquito seasons.

Neighbor-hood	RMLE of β	± S.E.	*Z* - value	Two-tailed *P* – value	RMLE of β	± S.E.	*Z* - value	Two-tailed *P* – value
	Mean total pupal standing crop per residence				Mean pupal standing crop per container per residence			
				2002 Season				
N-1	1.79	0.28	6.46	< 0.001	0.84	0.29	2.84	0.005
N-2	1.52	0.19	8.03	< 0.001	0.43	0.20	2.15	0.032
N-3	1.43	0.32	4.49	< 0.001	0.35	0.33	1.08	0.280
N-4	1.86	0.25	7.49	< 0.001	0.75	0.25	2.97	0.003
				2003 Season				
N-1	1.87	0.25	7.50	< 0.001	0.75	0.25	2.97	0.003
N-2	1.48	0.15	9.73	< 0.001	0.27	0.14	1.91	0.056
N-3	1.62	0.20	7.96	< 0.001	0.39	0.18	2.15	0.032
N-4	1.28	0.26	4.94	< 0.001	0.01	0.24	0.36	0.719
N-5	1.20	0.16	7.62	< 0.001	0.004	0.02	-0.02	0.982
N-6	1.44	0.24	5.98	< 0.001	0.24	0.21	1.12	0.263
N-7	1.16	0.14	8.14	< 0.001	-0.02	0.14	-0.13	0.897
N-8	1.40	0.12	11.71	< 0.001	0.22	0.10	2.10	0.036

FIGURE CAPTIONS

Fig. 1. Mean daily temperature (^{o} C) and total rainfall (cm) per month in Raleigh, NC during the period from May - October in 2002 and 2003 (Lake Wheeler Rd. Field Lab, State Climate Office of NC).

Fig. 2. Mean total *Aedes albopictus* pupal standing crop per residence for each container type in neighborhoods receiving no treatment (No-TRT), lethal ovitraps (LO), source reduction (SR), and source reduction plus lethal ovitraps (LO+SR) for the 2002 and 2003 mosquito seasons. Mean values were calculated using the numbers of houses containing each type of pupae-positive container. Bars are 1 SEM with numbers (N) of each pupae-positive container sampled given above.

Fig. 3. a. Effects of lethal ovitraps (LO) and no treatment (No-TRT) in 2002 shown as a function of percent of the mean of *Ae. albopictus* pupal standing crop per container per month in June. b. Effects of lethal ovitraps (LO), source reduction (SR), source reduction plus lethal ovitraps (LO+SR), and no treatment (No-TRT) in 2003 shown as a function of percent of the mean of *Ae. albopictus* total pupal standing crop per container per month in May.

Figure 1

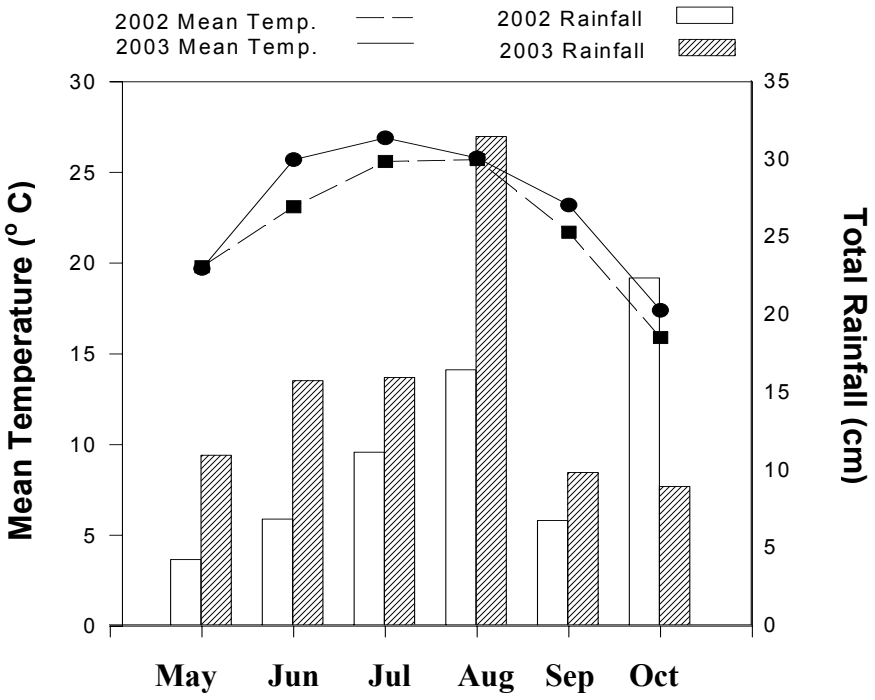
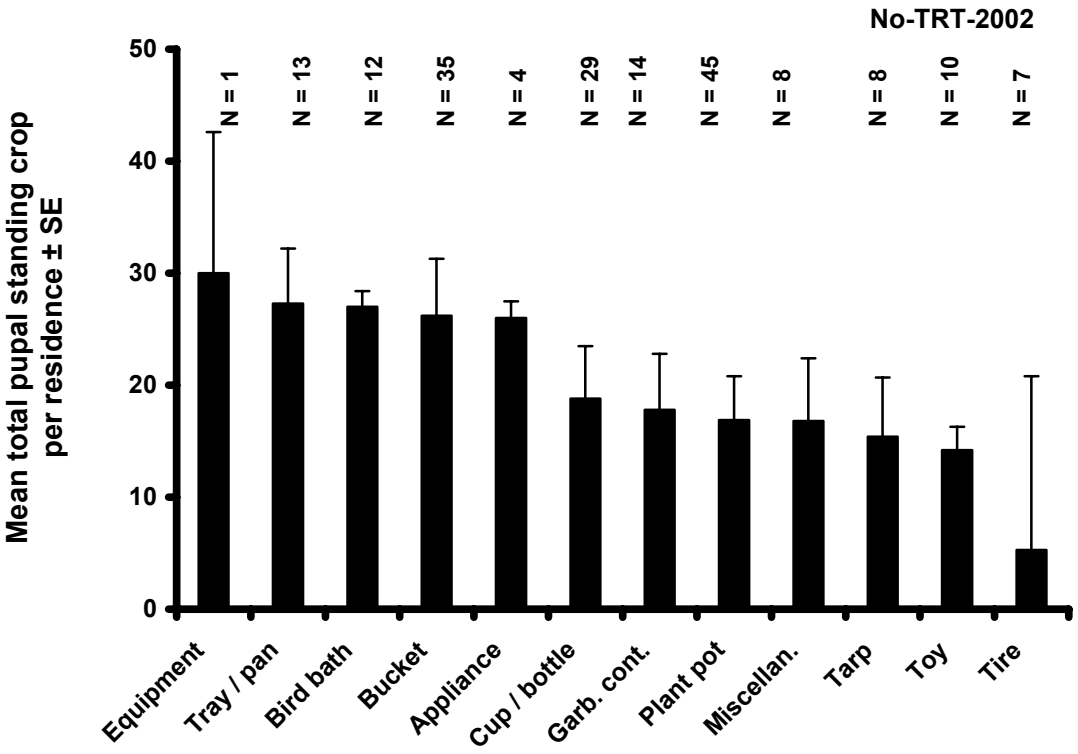

2002 Mean Temp.
2003 Mean Temp.
2002 Rainfall
2003 Rainfall
Mean Temperature (° C)
Total Rainfall (cm)
30
25
20
15
10
5
0
35
30
25
20
15
10
5
0
May
Jun
Jul
Aug
Sep
Oct

Figure 2


No-TRT-2002
Mean total pupal standing crop per residence ± SE
50
40
30
20
10
0
N = 1
N = 13
N = 12
N = 35
N = 4
N = 29
N = 14
N = 45
N = 8
N = 8
N = 10
N = 7
Equipment
Tray / pan
Bird bath
Bucket
Appliance
Cup / bottle
Garb. cont.
Plant pot
Miscellan.
Tarp
Toy
Tire

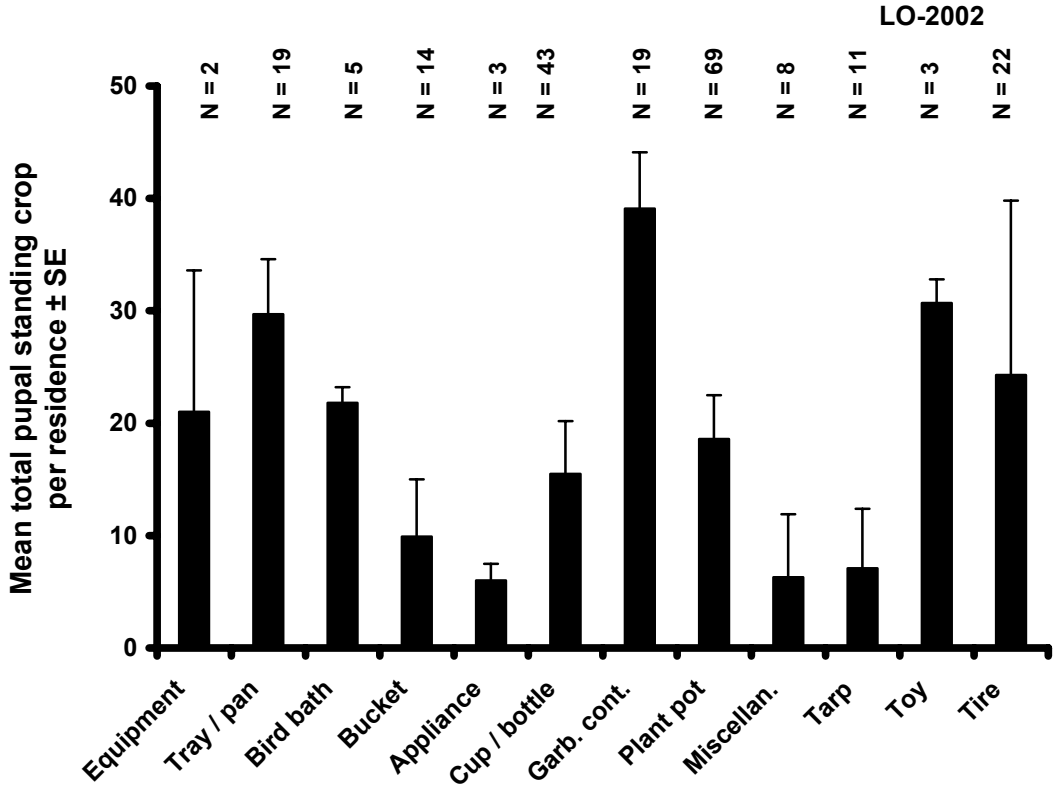

LO-2002
Mean total pupal standing crop per residence ± SE
50
40
30
20
10
0
N = 2
N = 19
N = 5
N = 14
N = 3
N = 43
N = 19
N = 69
N = 8
N = 11
N = 3
N = 22
Equipment
Tray / pan
Bird bath
Bucket
Appliance
Cup / bottle
Garb. cont.
Plant pot
Miscellan.
Tarp
Toy
Tire

Figure 2 (continued)

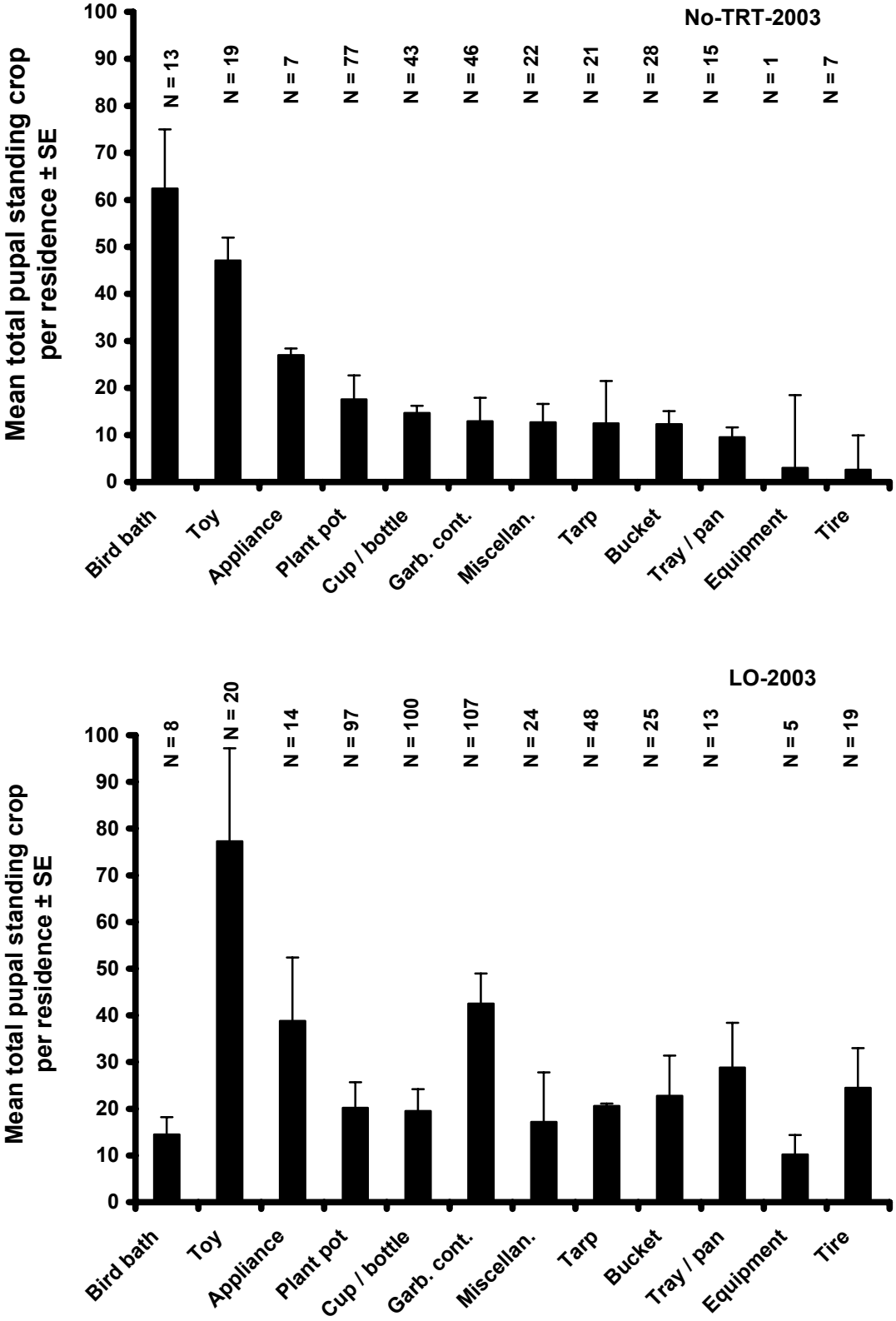


Figure 2 (continued)

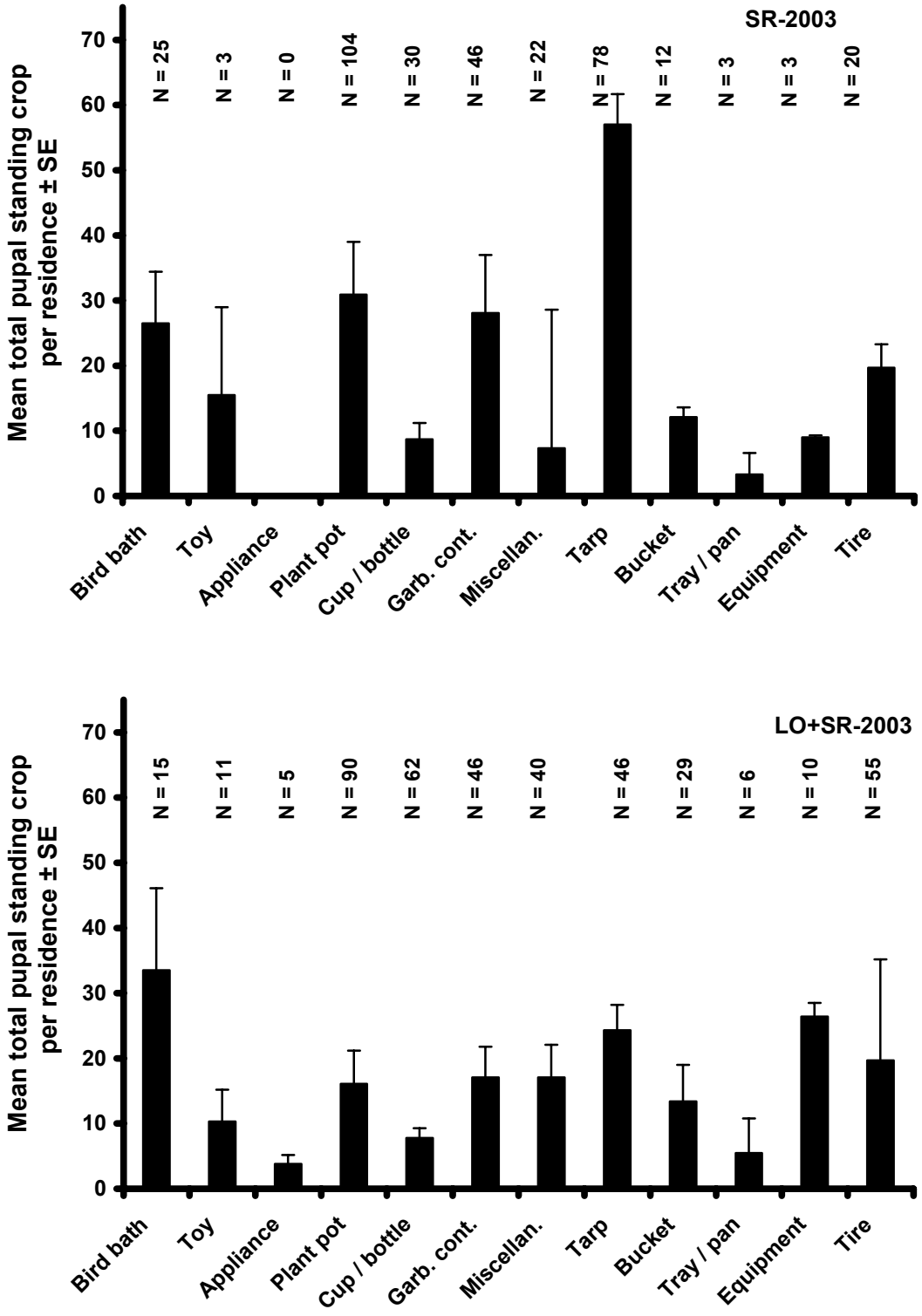
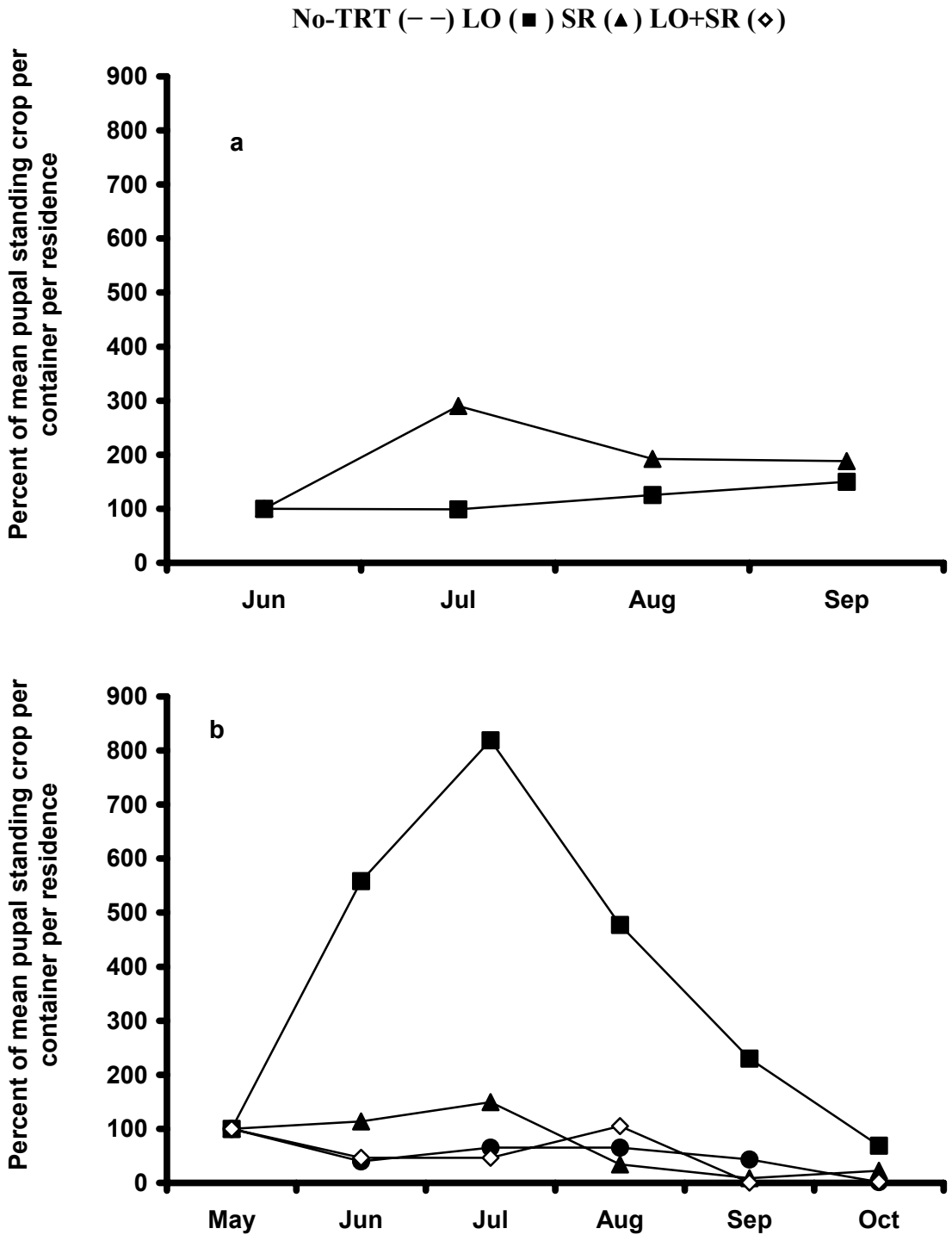


Figure 3


No-TRT (– –) LO (■) SR (▲) LO+SR (◇)
Percent of mean pupal standing crop per container per residence
a
900
800
700
600
500
400
300
200
100
0
Jun
Jul
Aug
Sep
Percent of mean pupal standing crop per container per residence
b
900
800
700
600
500
400
300
200
100
0
May
Jun
Jul
Aug
Sep
Oct

IMPACT OF SOURCE REDUCTION ON THE SPATIAL DISTRIBUTION OF LARVAL AND PUPAL PRODUCTION OF *AEDES ALBOPICTUS* (SKUSE) (DIPTERA: CULICIDAE) IN SUBURBAN NEIGHBORHOODS OF A PIEDMONT COMMUNITY IN NORTH CAROLINA

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ABSTRACT

We used spatial analyses to evaluate impacts of environmental management achieved through source reduction (SR) of man-made, water-holding containers on the spatial structure of the population of *Aedes albopictus* (Skuse) immatures during the 2003 mosquito season in four suburban neighborhoods of the City of Raleigh, NC. Back yard container surveys were conducted on a monthly basis in four study neighborhoods. All *Ae. albopictus* pupae and a sample of larvae were collected from each container deposited around residences. After mosquito collections, SR was carried out in two neighborhoods by emptying contents of all wet containers or treating containers that could not be emptied with an insect growth regulator, methoprene. In two control (No-TRT) neighborhoods, SR was not implemented following mosquito collections and container contents were replaced. The spatial distribution of *Ae. albopictus* immatures was examined using spatial statistical methods (Variography, G*i**, *K*-function). Analyses using pupal standing crops and numbers of mosquito-positive containers on a per residence basis indicated that containers producing immatures were not clustered over adjacent residences; however, at least one residence in all neighborhoods had numerous containers with pupae, relative to other households. K-function analyses that considered only the presence or absence of pupae indicated that residences with at least one pupa tended to be dispersed throughout SR areas and clustered throughout No-TRT areas, indicating that SR had an effect on the spatial distribution of pupae. Conversely, spatial analyses based on the presence or absence of larvae, as well as pupae, showed that mosquito production was randomly distributed among residences in both No-TRT and SR neighborhoods, indicating that *Ae. albopictus* rapidly re-colonized neighborhoods after SR was implemented.

INTRODUCTION

In the United States, the "Asian Tiger Mosquito", *Aedes albopictus* (Skuse), has been an important peri-domestic pest since its introduction in the mid-1980s (Hawley et al. 1987). Oviposition occurs in man-made containers that are readily available in suburban landscapes, which has facilitated the spread of this mosquito (Moore and Mitchell 1997). Conventional methods for estimating the abundance of container-inhabiting mosquitoes have focused primarily on estimating the mosquito production potential of different types of containers (Moore et al. 1978; Barker-Hudson et al. 1988; Chadee 2004). These analyses are appropriate for determining the importance of different types of containers to mosquito production; however, the distribution of containers that produce mosquitoes varies spatially in the landscape. Knowledge of spatial distribution of mosquito production sites within neighborhoods would be important to mosquito control programs seeking to improve the efficiency of control efforts.

Over the past decade, there has been an increasing recognition of the analytical power derived from combining geographic information systems (GIS) with spatial statistical methods of data analysis (Kitron 1998). A GIS can be used to associate geo-referenced entomological data with environmental factors, and spatial analyses can explain or predict patterns of mosquito populations and environmental co-variables over time and space (Kitron 1998). Within a GIS, variograms can be constructed to illustrate the extent of autocorrelation in mosquito populations separated by a distance in space (Ali et al. 2003). Past studies involving spatial analyses related to mosquito species generally have tracked the associations between vectors and vector-borne diseases. For example, with local and global spatial analysis, Kitron et al. (1997) identified ‘hot spots’ of La Crosse virus transmission in Illinois

and related the spatial distribution of cases to risk factors, such as the presence of man-made containers. Most spatial analytical research on container-inhabiting mosquitoes has been conducted on *Aedes aegypti* in countries other than the U.S. Ali et al. (2003) examined the spatial associations between human dengue cases and abundance of *Ae. aegypti* and *Ae. albopictus* in Bangladesh using spatial regression analyses. Getis et al. (2003) used spatial analysis to describe the distribution of *Ae. aegypti* and its container habitats in Iquitos, Peru. In a follow up investigation, Morrison et al. (2004a) constructed a GIS using point pattern analysis to evaluate survey strategies for *Ae. aegypti* in relation to dengue risk. The spatial distribution of oviposition containers in the landscape may also affect the nuisance potential of mosquito populations (Focks et al. 1999) and spatial analysis has been used to assess the efficacy of mosquito control efforts, such as insecticide applications and environmental sanitation of oviposition containers (Focks et al. 1999; Getis et al. 2003).

Aedes albopictus is distributed throughout North Carolina (Moore and Mitchell 1997) where man-made containers in suburban landscapes are utilized for egg laying and rearing progeny. The main objective in our investigation was to evaluate how altering mosquito production through source reduction (SR) of oviposition containers impacted the spatial structure of the population of *Ae. albopictus* immatures. Our principal hypothesis was that production of *Ae. albopictus* immatures would be clustered at a few premises within neighborhoods and that SR of containers would decrease spatial aggregation of containers with *Ae. albopictus* immatures. Accordingly, we estimated the spatial distribution of mosquitoes in container habitats on residential property, and used distance-based local and global geostatistical and point pattern analysis methods to compare the spatial distribution of immatures in SR and control neighborhoods.

MATERIALS AND METHODS

Study areas. Our investigation was carried out in the City of Raleigh (76.6° W, 35.8° N), Wake County, North Carolina. Wake County is 2,212 km^2 in size and is located in a transitional zone in central NC between the Piedmont and the Coastal Plain. Raleigh presently has approximately 300,000 residents. The four suburban neighborhoods used in our study were comprised mainly of single-family dwellings of similar age and size. These neighborhoods were separated by 4 - 15 km. Study areas were segregated from surrounding neighborhoods by large roadways, woodlands, or complexes of buildings to minimize mosquito immigration. There were from 19 to 28 houses in each neighborhood with an average of about 50 m between dwellings and mean lot size of 0.17 to 0.25 hectares. Landscapes in neighborhoods N-3, N-4, and N-7 consisted of a mixture of deciduous (maple, oak, and elm) and evergreen (pine, cedar) trees intermingled with an under-story of unidentified shrubby vegetation. In neighborhood N-5, residential landscapes consisted of open grassy areas and pine woodlands. Ornamental shrubbery was common around homes in all areas, as well as low ground cover such as English ivy.

Study design. SR was used as a method for experimentally manipulating production of *Ae. albopictus* immatures in container habitats. An incomplete block experimental design (Neter et al. 1996) was used. Experiments were blocked by neighborhood with one treatment assigned to each neighborhood. All residences within neighborhoods were surveyed each month for water-holding containers. Container surveys were initiated in May and terminated in October in two neighborhoods in 2002 and in four neighborhoods in 2003. In the 2003 season, the impacts of environmental management on the spatial distribution of *Ae.*

albopictus immatures were evaluated in two SR neighborhoods (N-5 and N-7) in comparison to two control (No-TRT) neighborhoods (N-3 and N-4).

When each container survey was carried out, a crew of 4 - 5 people searched the home grounds of each premise within each neighborhood. Each search was conducted over the entire property or out to a distance of approximately 25 - 50 m from the primary dwelling if the property was large. Containers were examined for presence of water and then carefully examined for mosquitoes as described below. Approximately 8 - 10 hours were required to complete a container survey in each neighborhood, depending on the numbers of homes and containers present. In general, all neighborhood surveys were completed in the same week.

Geographic Information System. Shapefiles for property boundaries, buildings, and street centerlines for all study sites were downloaded from the Wake County government GIS website (http://www.wakegov.com/county/propertyandmapping/gisdigitaldata.htm). All shapefiles and other "data layers" were imported into ArcMap using Universal Transverse Mercator coordinates and the North American Datum 1983 coordinate system (ESRI, Redlands, California, 2002). A centroid was placed in the geographic center of the land parcel of each residence to represent the average location for all containers on the property because containers were not georeferenced during surveys. For container analyses, if there was more than one household on a parcel of land, the same geographic position for the centroid was used for both houses. A shapefile containing these centroids was created with the '*Feature Analyst*' extension (ver. 3.4, Visual Learning Systems, Inc., Missoula, MT) of ArcMap. Once shapefiles had been created and imported into the GIS, a unique alphanumeric code for each residence was added to the attribute table containing the

corresponding centroid. The coordinates of the centroids were used for spatial analyses described later.

Container surveys. Within each mosquito season (2002 and 2003), the same survey crew conducted a house-to-house search in all neighborhoods. The areas surrounding each premise were systematically searched for containers. The kind and number of wet mosquito-negative and -positive containers found at each residence were recorded. To examine a wet container for mosquitoes, water was poured into shallow white enameled pans and all pupae and a sample of up to 10 larvae were removed and transferred to Whirl-Pak sample bags (Fisher Scientific, Pittsburgh, PA) and, labeled with the collection date, house identification code and container type. Containers, initially identified in the field according to their specific function (e.g. plastic cup, appliance), were subsequently placed in specific categories of a classification scheme. If the liquid in the containers was turbid or dark, tap water was added to the pans so mosquitoes could be seen. This process was repeated until the entire container was emptied. If the volume of water in the container required what was considered an excessive sampling time, then the container was sampled for a standard time of five minutes. In No-TRT neighborhoods, once the container was sampled, the contents were returned; however, in SR neighborhoods, any remaining contents were discarded and the container was turned over. Wet containers that could not be emptied were treated with a granular formulation of the insect growth regulator methoprene (1.5% Pre Strike™, Wellmark, Schumburg, IL).

After collection, samples were placed on wet ice and transported to the lab where mosquitoes were killed in hot water and transferred to labeled vials filled with ethanol. Later, the larvae and pupae in each sample were identified to species using standard keys

(Stojanovich 1962; Slaff and Apperson 1989). *Aedes albopictus* pupae were differentiated from pupae of other species by the presence of a fringe of hairs on the edge of the anal paddle (Harrison 2005). Once identified, pupae were counted and the counts, along with the container type, added to the attribute table for each neighborhood.

Mosquito production variables. Mosquito production variables (Table 1) were analyzed for each mosquito season (2002 and 2003). Planned comparisons were made between SR and No-TRT areas to evaluate how altering mosquito production changed the spatial distribution of *Ae. albopictus* immatures. The residence and container were used as base units of study and measures of mosquito production were calculated for SR and No-TRT neighborhoods over each mosquito season.

Spatial analysis. Spatial distribution of mean total pupal standing crops per container per residence, mean pupal standing crop per residence, mean numbers of pupae-positive containers per residence, and mean numbers of pupae- or larvae-positive containers per residence were visually examined in a GIS for patterns of clustering and geographic associations between variables. Some patterns of clustering were observed for all mosquito production variables so geostatistical methods were utilized to examine spatial correlations between residences. Our hypothesis was that mosquito production variables of *Ae. albopictus* at a residence were more comparable to levels of mosquito production at adjacent versus distant residences. Geostatistical analyses (R Development Core Team, Austria 2004) were performed for mosquito production variables associated with the georeferenced centroid of each residential land parcel. To determine if the autocorrelation among mosquito production variables was similar in different directions (isotrophic), the 'Geo-R' package in 'R' was used to create directional semivariograms at 0, 30, 45, and 60 degrees. Isotropy was

observed among mosquito production variables and, consequently, we created omnidirectional semivariograms to illustrate the degree of spatial autocorrelation of mosquito production variables between residences within the study neighborhoods (Ribeiro and Diggle 2001). Following common practices, we plotted semivariograms to a distance of approximately half the size of the study area (Liebhold et al. 1993). The semivariograms modeled the degree of similarity in mosquito production variables (mean pupal standing crop per container per residence, mean total pupal standing crop per residence, mean numbers of pupae-positive containers per residence, or mean numbers of pupae- or larvae-positive containers per residence) as a function of the separation distance of the centroid of the land parcel of the residences (Rossi et al. 1992). Exponential models were fit to experimental semivariances, $r(h)$, as a function of a lag distance between centroid coordinates of residences as described in the following equation,

$$r(h) = \frac{1}{2n(h)} \sum_{i=1}^{n(h)} [z(x_i) - z(x_i + h)]^2$$

, where $x_i = (x_{1i}, x_{2i})$ and x_{1i} and x_{2i} represent the respective latitude and longitude coordinates of the centroid of each residential property, $z(x_i)$ and $z(x_i + h)$ are values for either standing crops, or numbers of mosquito-positive containers for two residences separated by lag distance h, and $n(h)$ is the total number of pairs of pupal standing crop or mosquito-positive container values separated by distance h. Before semivariograms were created for standing crops of pupae or mosquito-positive containers, a few variogram models were evaluated based on the assumption that there was a decreasing trend in autocorrelation out to a certain distance, where autocorrelation is equal to zero (Isaaks and Srivastava 1989). The exponential model assumes a semivariance value that arrives at the range slowly and this type of model was utilized for the semivariograms in this study. The predictive power of the exponential model is greatest when the sum of the

squared distances from the best-fit line is minimized (ESRI 2001; Rossi et al. 1992). Accordingly, we evaluated our exponential models with the predicted residuals sum of squares (Press) statistic, which is based on the fit of the model (Isaaks and Srivastava 1989).

Residence (local) spatial characteristics of mean pupal standing crops per container per residence, mean total pupal standing crops per residence, mean numbers of pupae-positive containers per residence, and mean numbers of pupae- or larvae-positive containers per residence for each sampling period were analyzed using Bio-Medware Point Pattern Analysis software (Getis et al. 1998). Neighborhood (global) spatial characteristics of the same variables were analyzed with the *G-stat* package in *R* (Pebesma 2004).

The spatial distribution of mean pupal standing crops per container per residence, mean total pupal standing crops per residence, mean numbers of pupae-positive containers per residence, and mean numbers of pupae- or larvae-positive containers per residence at the residential level in SR and No-TRT neighborhoods was examined with the G*i** statistic, which measured clustering of similar values around a residence at a specified distance from that residence, relative to the entire neighborhood (Ord and Getis 1995). Our hypothesis was that incidence and intensity of either *Ae. albopictus* pupal standing crops or mosquito-positive containers at the household level would be clustered over multiple, adjacent residences. The analysis of local clustering to determine "hot spot" residences was carried out to a maximum distance of 125 m, because in all neighborhoods, there were no residences where centroids of neighboring properties were > 125 m away. Distances between neighbors of residences with significant clustering patterns were measured on a case-by-case basis using the measuring tool in ArcMap to determine if the residence was a stand-alone "hot spot" or the member of a cluster of "hot spots". The individuality of a residence exhibiting

clustering as determined by distance between centroids of residential land parcels varied between neighborhoods. For example, if a residence exhibited significant clustering up to 50 m and neighboring residences were > 50 m away, it was considered the member of a stand-alone cluster.

The traditional measure of global autocorrelation, Moran's *I* (Schabenberger and Gotway 2005), was modified to test the degree of clustering of mean pupal standing crops per container per residence, mean total pupal standing crops per residence, mean numbers of pupae-positive containers per residence, and mean numbers of pupae- or larvae-positive containers per residence over each neighborhood (Isaacs and Srivastava 1989; Ghosh 2005). Global (neighborhood) clustering was determined by concurrently weighting pairs of observations to a neighborhood standard (Robinson 2000) and analyses included seasonal data for mosquito production variables. In calculating neighborhood clustering, different weighting schemes were tested to determine the impact of distance on autocorrelation. Consequently, we evaluated different search radii with regard to centroids of land parcels and created a weight matrix that applied a reduction in weight with increasing distance.

The *K*-function statistic, *L(d),* was used to test the null hypothesis that larvae- or pupae-positive houses were distributed in a spatially random fashion. To determine to what extent key containers (highly productive types of containers) were contributing to clustering patterns, individual spatial analyses were performed including all containers and key containers, as well as excluding key containers. Accordingly, a second order *K*-function analysis was completed, using Bio-Medware Point Pattern Analysis software (Getis et al. 1998) for residences where mosquitoes were collected. The *K*-function analysis compared the observed locations of pairs of points with their expected locations, assuming complete

spatial randomness of points (Gatrell et al. 1996). A detailed description of the *K*-function analysis is presented in Getis et al. (1998). Briefly, observed and expected *L(d)* values, plotted against distance *(d)* between centroids of land parcels of residences, were surrounded by a 95 % confidence envelope in each neighborhood based on the null hypothesis of complete spatial randomness. The *L(d)* is a linear expression of the expected number of cases (*j*) occurring within a certain distance (*d*) of all cases (*i*). For our study, *L(d)* estimated the variance of the distribution of distances between mosquito-positive households. When $L(d) = d$, the null hypothesis of spatial randomness was accepted; however, if the observed *L(d)* fell outside of the confidence envelope at any distance, the null hypothesis of spatial randomness was rejected. If the observed *L(d)* fell below the envelope ($L(d) < d$), the points were dispersed at that distance. If the observed *L(d)* fell above the envelope ($L(d) > d$), the points were clustered at that distance. Following common practices, the maximum distance input into the analysis was approximately one half the length of the shortest side of the study area. For this analysis, the distance for which *L(d)* was calculated was 500 m since the average length of all study areas was approximately 1000 m.

Statistical Analysis. Box plots were constructed so that mosquito production variables for each neighborhood could be examined for normality. The general lack of normality was verified with Kolmogorov-Smirnov tests (PROC UNIVARIATE, SAS Institute 2000). To meet assumptions of normality, transformations [$\log(x+1)$] of mean pupal standing crops per container per residence, mean total pupal standing crops per residence, mean numbers of pupae-positive containers per residence, and mean numbers of pupae- or larvae-positive containers were carried out before analyses. Analyses were carried out separately for each neighborhood and mosquito season. Quantitative mosquito

production variables were associated with the centroid coordinates of the land parcel of each residence before analyses for residential (G*i**) and neighborhood clustering were carried out. Categorical (presence or absence) mosquito production variables were also associated with the centroid coordinates of the land parcel of each residence before *K*-function analyses were performed.

RESULTS

Impact of SR on spatial distribution of immature *Ae. albopictus*. Household and neighborhood spatial statistics were computed and semivariograms constructed to describe the distribution of mosquito production variables (Table 1) in all four neighborhoods for the 2002 and 2003 mosquito seasons. Our intent was to describe how the spatial distribution of *Ae. albopictus* was influenced by SR and not how mosquito abundance was affected by SR.

Neighborhood clustering of immatures. Semivariograms for each mosquito production variable were fit using an exponential model. In most neighborhoods, the semivariance for each mosquito production variable among residences increased with increasing distance between residences up to a maximum sill; however, neighborhood mosquito production variables exhibited no significant correlation ($P > 0.05$). In general, semivariograms for mosquito production variables in both SR and No-TRT neighborhoods began exhibiting unstable semivariance patterns at a distance of > 200 m.

Residential clustering of immatures. Even though none of the mosquito production variables exhibited significant neighborhood clustering, local clustering in mosquito production was found in at least one home in all areas as evidenced by significant G*i** values ($P < 0.05$). Values of G*i** ≥ 1.96 indicated residences that were clustered around high values of mosquito production variables, while values of $-1.96 <$ G*i** < 1.96 did not indicate

significant clustering. Neighborhood maps of significant G*i** values observed at residences for mosquito production variables illustrate clustering patterns for No-TRT and SR areas (Figures 1-6). Both SR and No-TRT areas had seasonally repeating "hot spots" for mosquito production variables. In fact, both neighborhoods N-3 and N-5 had at least one household that was a "hot spot" of mosquito-positive containers in all six surveys during the 2003 mosquito season.

Spatial distribution of mosquito-positive residences. In the 2002 and 2003 mosquito seasons, *K*-function analyses for the distribution of pupae-positive and pupae- or larvae-positive households was performed for each neighborhood (Figures 7-8). Initially, we included all container types at households positive for pupae; subsequently, we performed *K*-function analyses to simulate the impacts of SR on spatial distribution of *Ae. albopictus* when the most productive containers, birdbaths and tarps, were excluded (Figure 9). *K*-function analyses were also performed on individual container types in each neighborhood for households where mosquitoes were collected over the entire season.

Results from analyses carried out on data collected over each mosquito season, including households positive for all types of containers, indicated that pupae-positive houses in No-TRT areas were spatially clustered; however, households in SR areas were either located randomly or spatially dispersed. *K*-function analyses for households where pupae- or larvae-positive containers were found indicated that containers were located at random throughout both No-TRT and SR neighborhoods.

K-function analyses were carried out on mosquito-positive key containers; namely plant pots, tarps, and birdbaths (Figures 10-12). Plant pots were distributed randomly in all four neighborhoods. In neighborhoods where there were sufficient data to complete

analyses, tarps were spatially dispersed in No-TRT neighborhoods and randomly located in SR neighborhoods; however, birdbaths were clustered in No-TRT areas and randomly located in SR areas. *K*-function analyses performed separately on all other types of containers indicated that most containers were distributed randomly or dispersed within neighborhoods; however, buckets in N-4 were clustered during the 2003 mosquito season.

DISCUSSION

Spatial analytical research on the distribution of larvae and pupae of container-inhabiting mosquitoes has mainly focused on urban populations of *Ae. aegypti* (Getis et al. 2003; Morrison et al. 2004b). There is a lack of information on the spatial structure of *Ae. albopictus* populations. Likewise, the impacts of systematic SR of oviposition containers on the spatial structure of *Ae. albopictus* populations in suburban landscapes has not been previously investigated. Results from our study and those of Focks et al. (1999) show that the significance of a container in terms of mosquito production is a function of its spatial distribution. We used GIS and distance-based point pattern analysis methods to characterize the spatial distribution of *Ae. albopictus* larvae and pupae and their container habitats. In our study, spatial patterns of pupal standing crops and pupae-positive containers were either spatially dispersed or random in SR neighborhoods and clustered or random in No-TRT neighborhoods. In Iquitos, Peru, Getis et al. (2003) reported that houses with *Ae. aegypti* pupae were dispersed within a neighborhood and that pupae were clustered within houses. Our study partially supports the findings of Getis et al. (2003) since, in No-TRT areas, we found that households with pupae tended to be clustered within a neighborhood and that pupae were clustered within houses.

We concur with Getis et al. (2003) that local "hot spots" of mosquito production vary over time and that some residences are recurrent "hot spots". Previous studies have proposed targeting control measures at "key premises" of *Ae. aegypti* production (Tun-Lin et al. 1995; Chadee 2004) and our study provides a geostatistical method for identifying these premises with regard to *Ae. albopictus* production. Nevertheless, households having containers that were difficult to reduce, such as tarps and birdbaths, continued to exist as local "hot spots" of mosquito production for re-infesting neighborhoods. We were not surprised at the lack of global neighborhood autocorrelation because the few local "hot spots" were being overwhelmed in the context of the entire area. The incidence of clustering within residences and absence of neighborhood clustering of *Ae. albopictus* indicates that an effective mosquito control plan based on a focused approach may be attainable for this mosquito species.

Households having containers with either larvae or pupae were randomly distributed in both No-TRT and SR neighborhoods, reflecting the ability of this mosquito species to quickly re-colonize areas after environmental management. These results are consistent with research findings of Getis et al. (2003) on *Ae. aegypti* in urban areas of Iquitos, Peru where a lack of clustering of mosquito-positive containers was found. In general, our findings are also consistent with results of Edman et al. (1998) and Reiter et al. (1995) that dispersal of *Ae. aegypti* is dependent largely on the availability of oviposition sites. In our investigation, SR elicited a dispersion effect on container-inhabiting mosquitoes, as indicated by the decrease in neighborhood clustering of pupae where environmental sanitation was applied.

Our findings indicate that point pattern analyses can be used for describing the household and neighborhood spatial structure of *Ae. albopictus* populations in suburban landscapes. Additional investigations should be carried out to evaluate how source reducing

key containers or key residences would affect the abundance of *Ae. albopictus*. Future studies should assess the mosquito suppression achieved by source reducing containers at household "hot spots" of mosquito production identified with the G*i** statistic. Additional information on container importance could be gained by determining the level of mosquito suppression achieved by focusing management efforts on residential "hot spots" for different mosquito production variables. Richards et al. (2005) estimated the percent reduction in *Ae. albopictus* mosquito populations from focusing control efforts on key premises for mosquito production. In this study, it was estimated that up to 80 % of the mosquito population could have been eliminated by focusing control efforts on "pupae key premises". A residence was considered a 'pupae key premise' if its mean total pupal standing crop exceeded the neighborhood average. Richards et al. (2005) also reported that up to 68 % of *Ae. albopictus* pupae could have been eliminated if control efforts were directed at "container key premises". "Container key premises" were identified based on the number and frequency of occurrence of pupae-positive containers at each residence. As suggested by Morrison et al. (2004b), some research is warranted to investigate the behavioral changes in mosquitoes resulting from removing oviposition sites. Our spatial analyses were based on a household average location of containers and not specific locations of containers; however, georeferencing each container in the field would allow for more precise analyses of the spatial distribution of containers to be carried out.

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TABLES

Table 1. Mosquito production variables analyzed at the neighborhood and household level over the period of each container survey and over the entire mosquito season.

Variable	Description
Mean total pupal standing crop per residence	Sum of the numbers of pupae collected in all containers or in each specific container type per residence.
Mean pupal standing crop per container per residence	Sum of the numbers of pupae collected in all containers / total numbers of all containers per residence.
Mean pupal standing crop per container type per residence	Sum of the numbers of pupae collected in each type of container / total numbers of each specific container per residence.
Mean numbers of pupae-positive containers per residence	Sum of the numbers of pupae-positive containers / total numbers of residences.
Mean numbers of pupae- or larvae-positive containers per residence	Sum of the numbers of pupae- or larvae-positive containers / total numbers of residences.

FIGURE CAPTIONS

Fig. 1. G*i** maps of *Ae. albopictus* mean total pupal standing crop per residence (A), mean pupal standing crop per container per residence (B), mean numbers of pupae-positive containers per residence (C), and mean numbers of pupae- or larvae-positive containers per residence (D) in N-3 in 2002.

Fig. 2. G*i** maps of *Ae. albopictus* mean total pupal standing crop per residence (A), mean pupal standing crop per container per residence (B), mean numbers of pupae-positive containers per residence (C), and mean numbers of pupae- or larvae-positive containers per residence (D) in N-4 in 2002.

Fig. 3. G*i** maps of *Ae. albopictus* mean total pupal standing crop per residence (A), mean pupal standing crop per container per residence (B), mean numbers of pupae-positive containers per residence (C), and mean numbers of pupae- or larvae-positive containers per residence (D) in N-3 in 2003.

Fig. 4. G*i** maps of *Ae. albopictus* mean total pupal standing crop per residence (A), mean pupal standing crop per container per residence (B), mean numbers of pupae-positive containers per residence (C), and mean numbers of pupae- or larvae-positive containers per residence (D) in N-4 in 2003.

Fig. 5. G*i** maps of *Ae. albopictus* mean total pupal standing crop per residence (A), mean pupal standing crop per container per residence (B), mean numbers of pupae-positive containers per residence (C), and mean numbers of pupae- or larvae-positive containers per residence (D) in N-5 in 2003.

Fig. 6. G*i** maps of *Ae. albopictus* mean total pupal standing crop per residence (A), mean pupal standing crop per container per residence (B), mean numbers of pupae-

positive containers per residence (C), and mean numbers of pupae- or larvae-positive containers per residence (D) in N-7 in 2003.

Fig. 7. Results of *K*-function (second order) analysis of the spatial distribution of *Ae. albopictus* pupae in No-TRT neighborhoods in 2002 and 2003 and SR neighborhoods in 2003.

Fig. 8. Results of *K*-function (second order) analysis of the spatial distribution of *Ae. albopictus* pupae AND larvae in No-TRT neighborhoods in 2002 and 2003 and SR neighborhoods in 2003.

Fig. 9. Results of *K*-function (second order) analysis of the presence of *Ae. albopictus* pupae in all containers EXCEPT tarps and bird baths by neighborhood in No-TRT neighborhoods in 2002 and 2003 and SR neighborhoods in 2003.

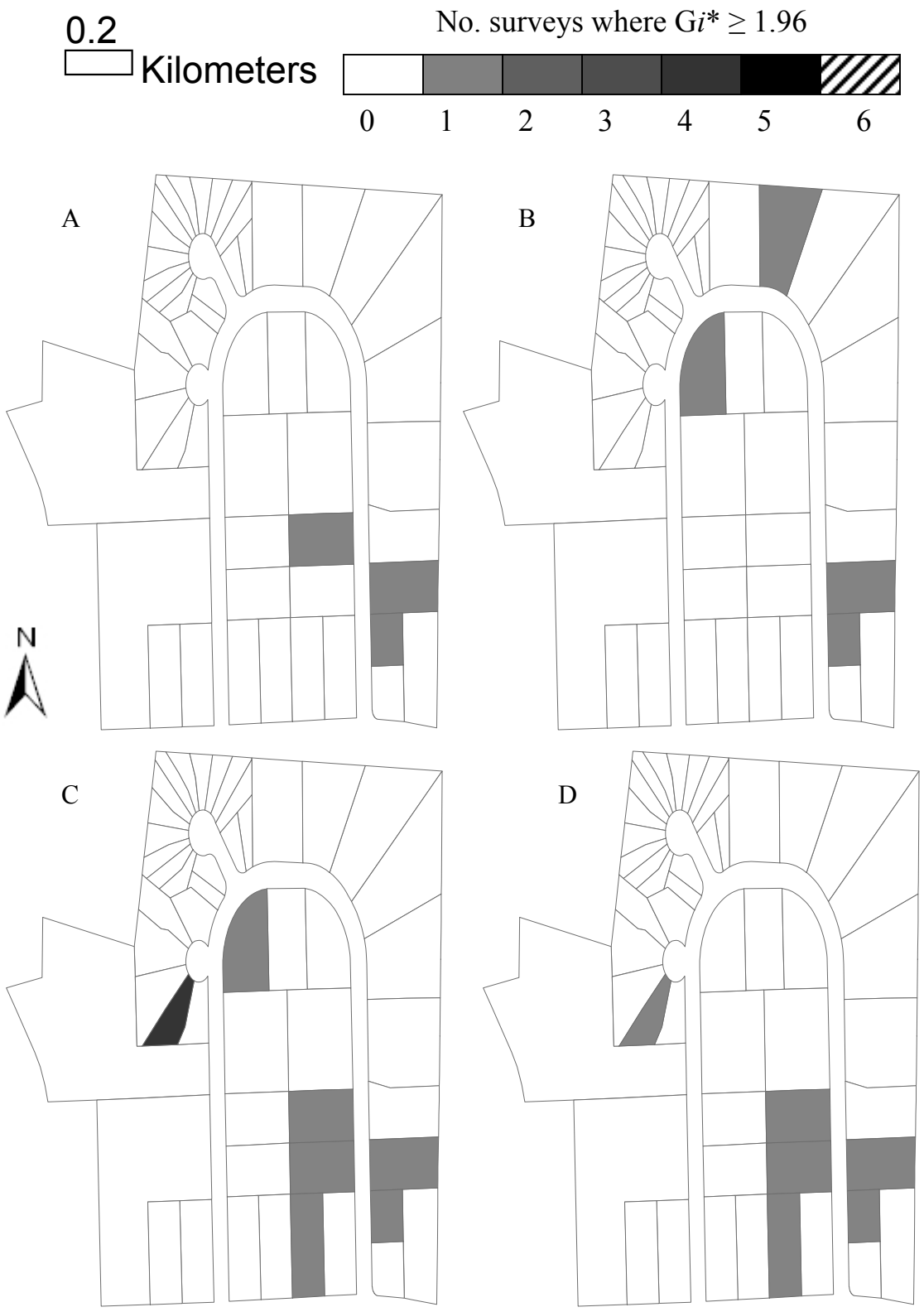
Fig. 10. Results of *K*-function (second order) analyses of the spatial distribution of PLANT POTS with *Ae. albopictus* pupae in No-TRT neighborhoods in 2002 and 2003 and SR neighborhoods in 2003.

Fig. 11. Results of *K*-function (second order) analyses of the presence of TARPS with *Ae. albopictus* pupae in No-TRT neighborhoods in 2002 and 2003 and SR neighborhoods in 2003. There were insufficient data to complete this analysis for N-3 in 2002.

Fig. 12. Results of *K*-function (second order) analyses of the presence of BIRD BATHS with *Ae. albopictus* pupae in No-TRT neighborhoods in 2002 and 2003 and SR neighborhoods in 2003. There were insufficient data to complete this analysis for N-4 in 2002 or N-3 and N-4 in 2003.

Figure 1

Figure 2



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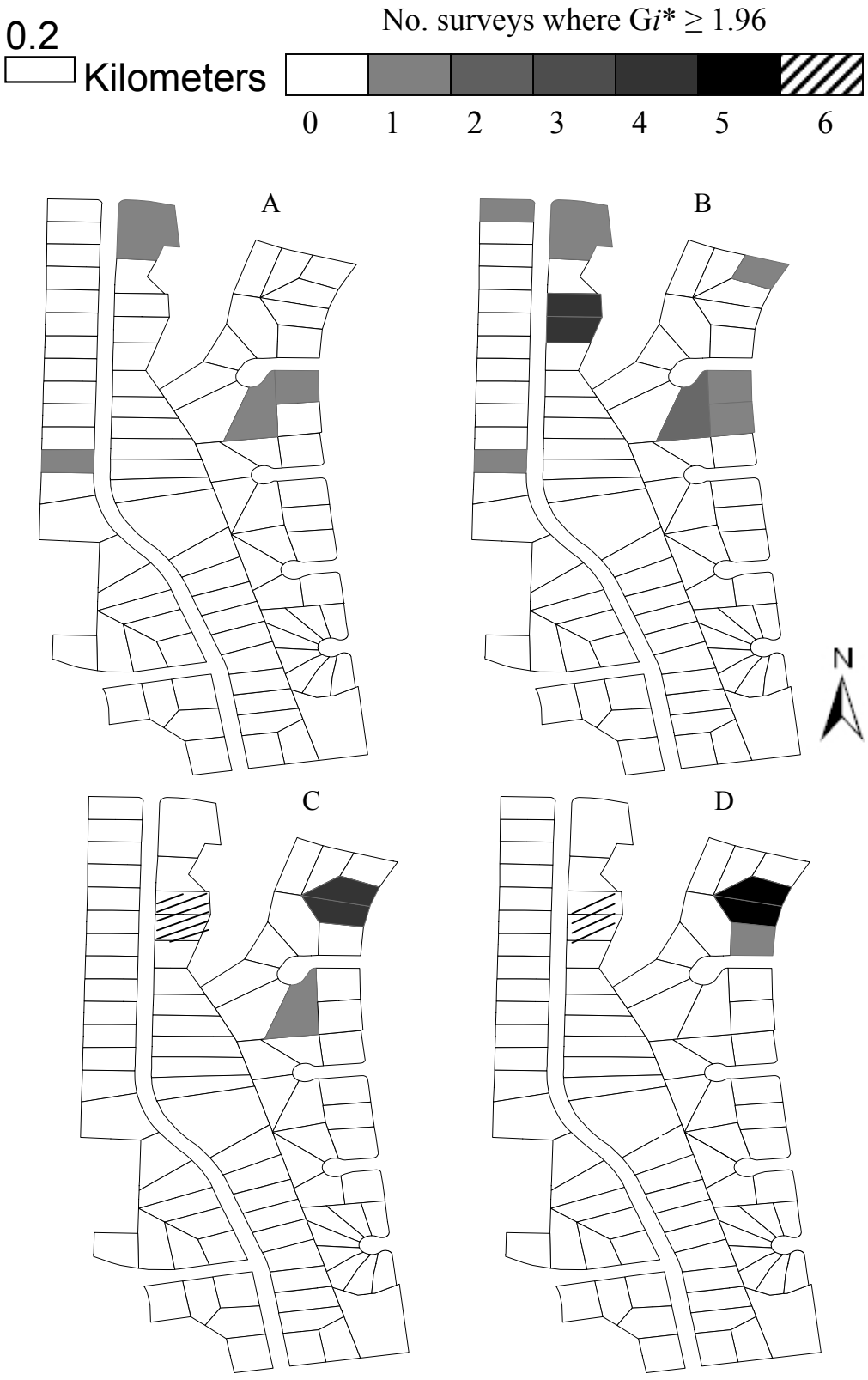
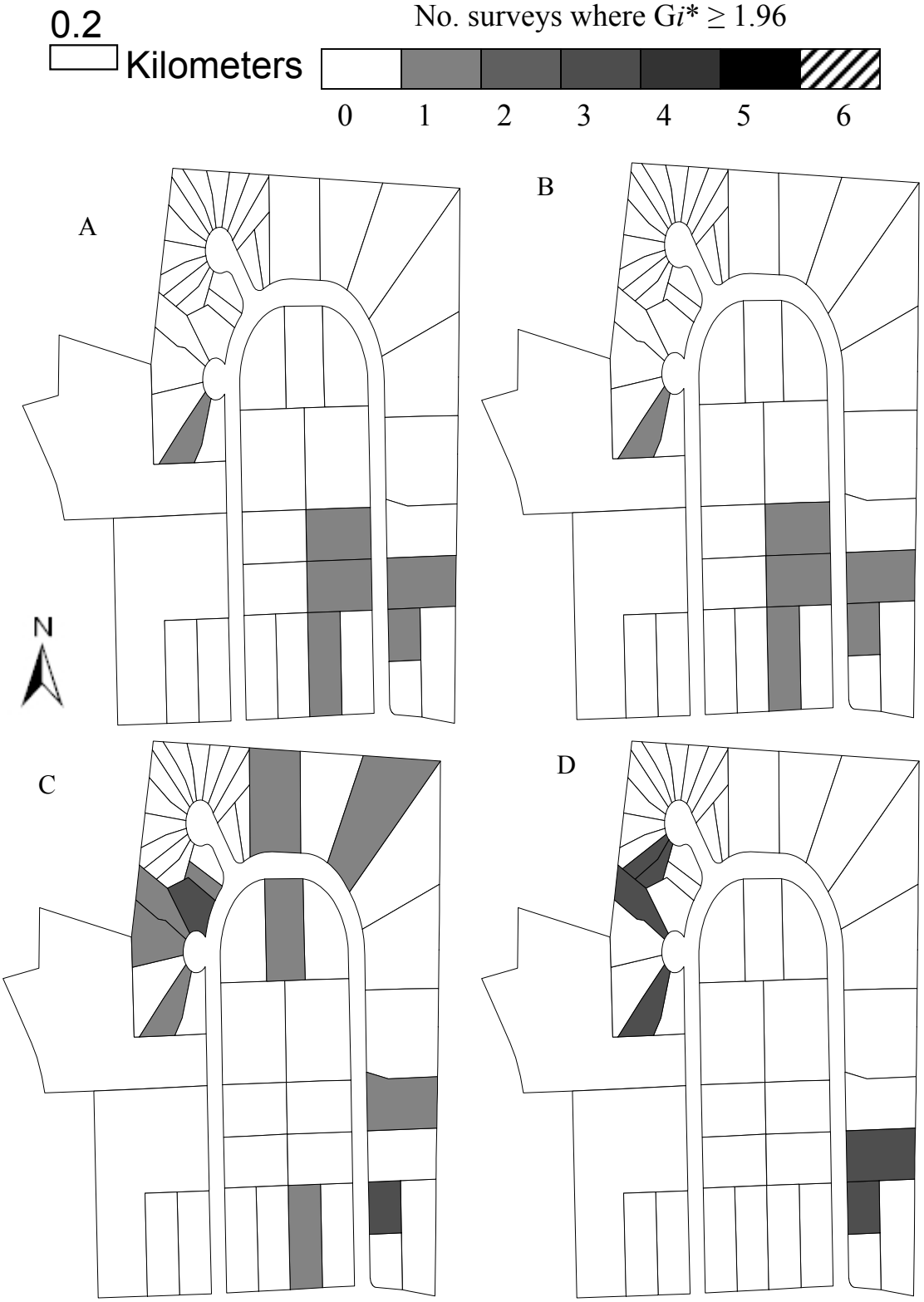


Figure 4



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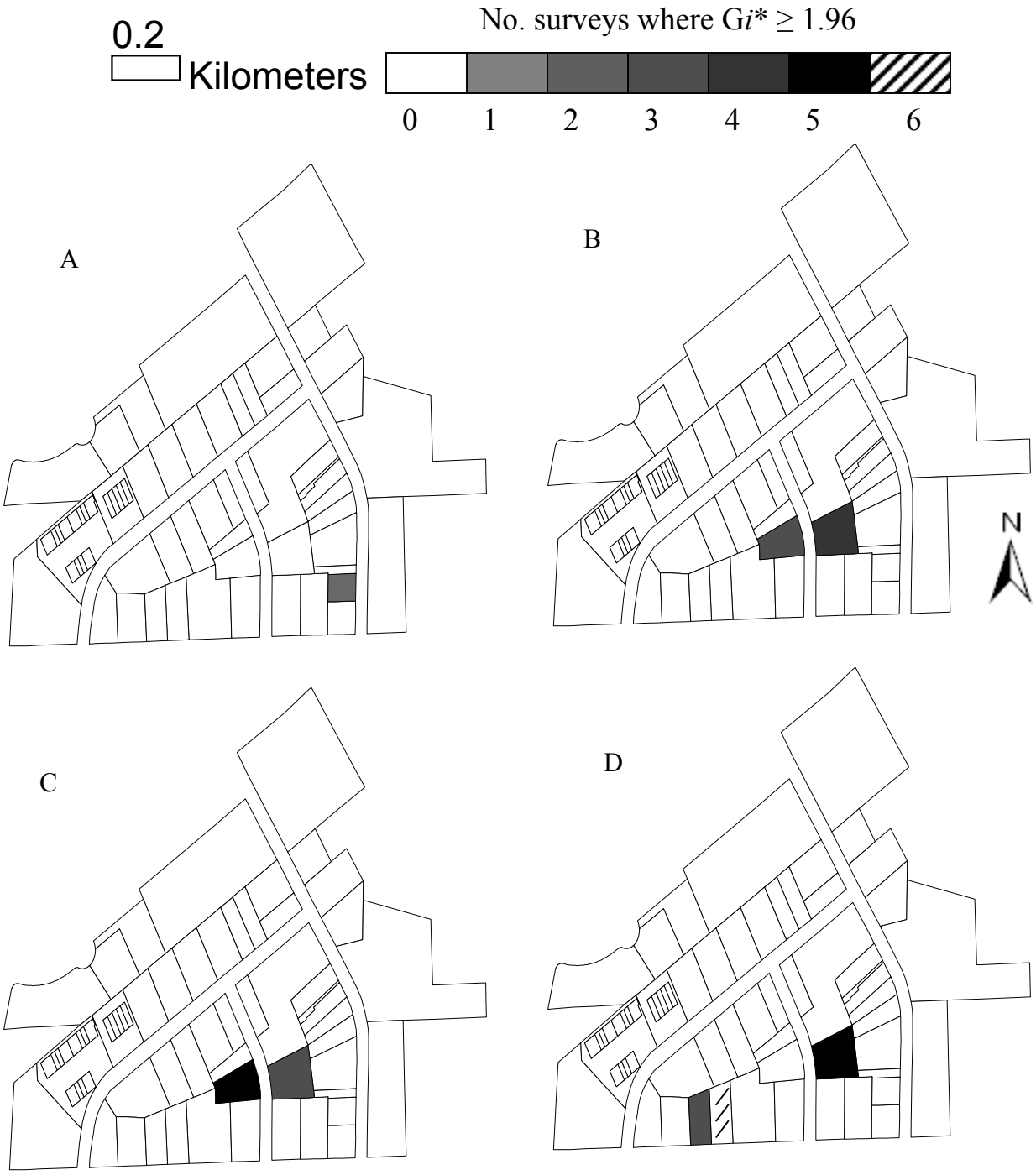

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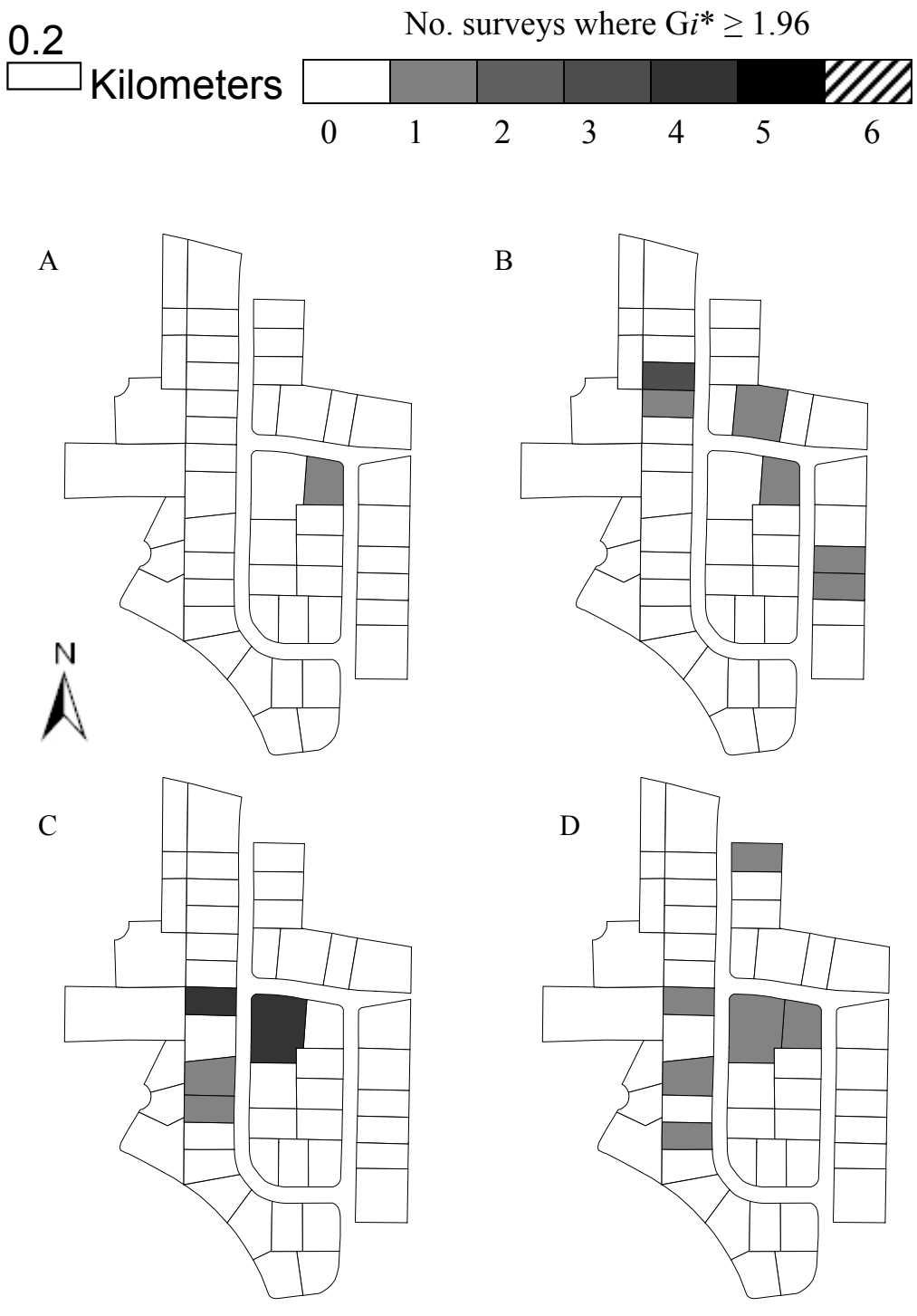

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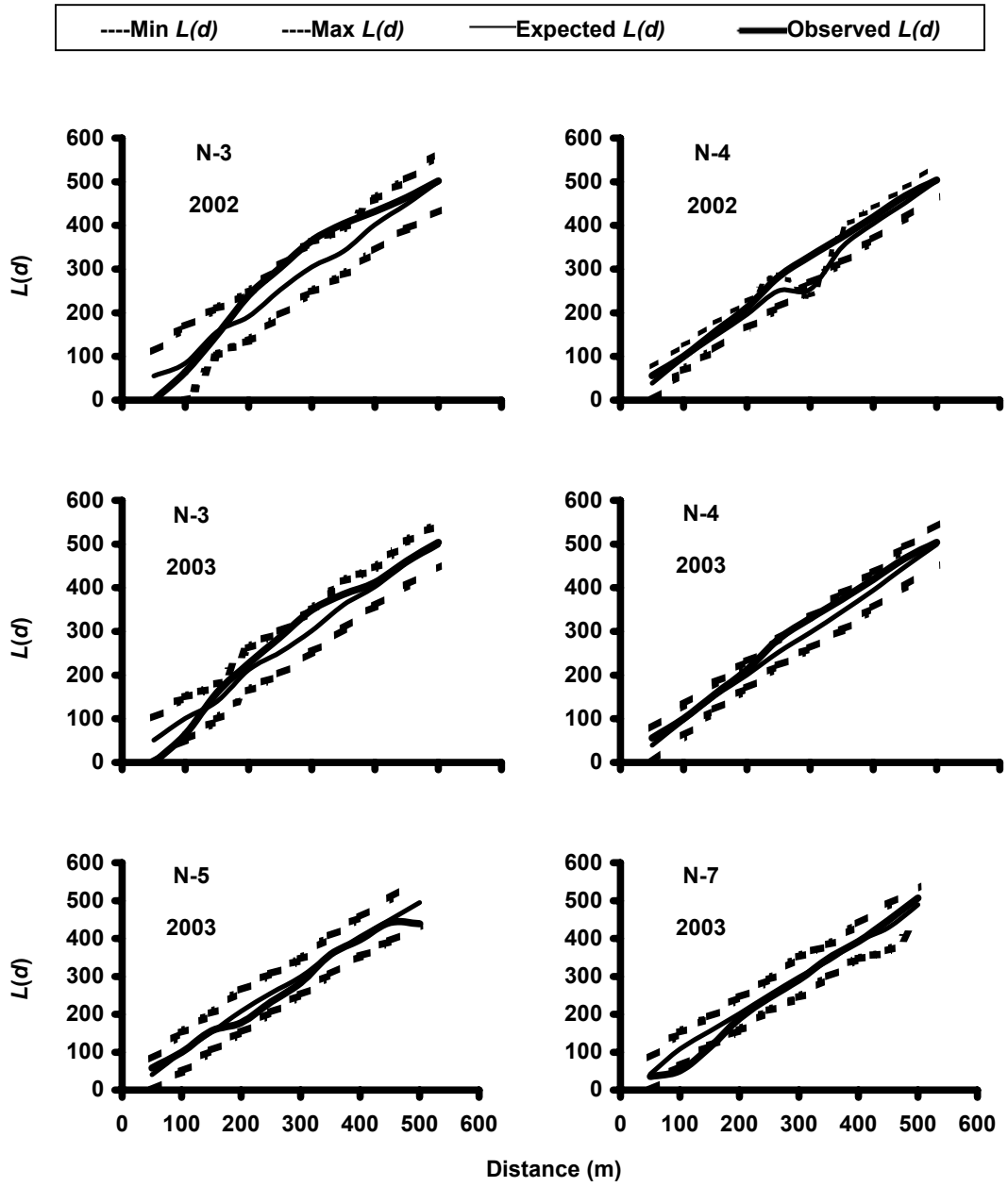
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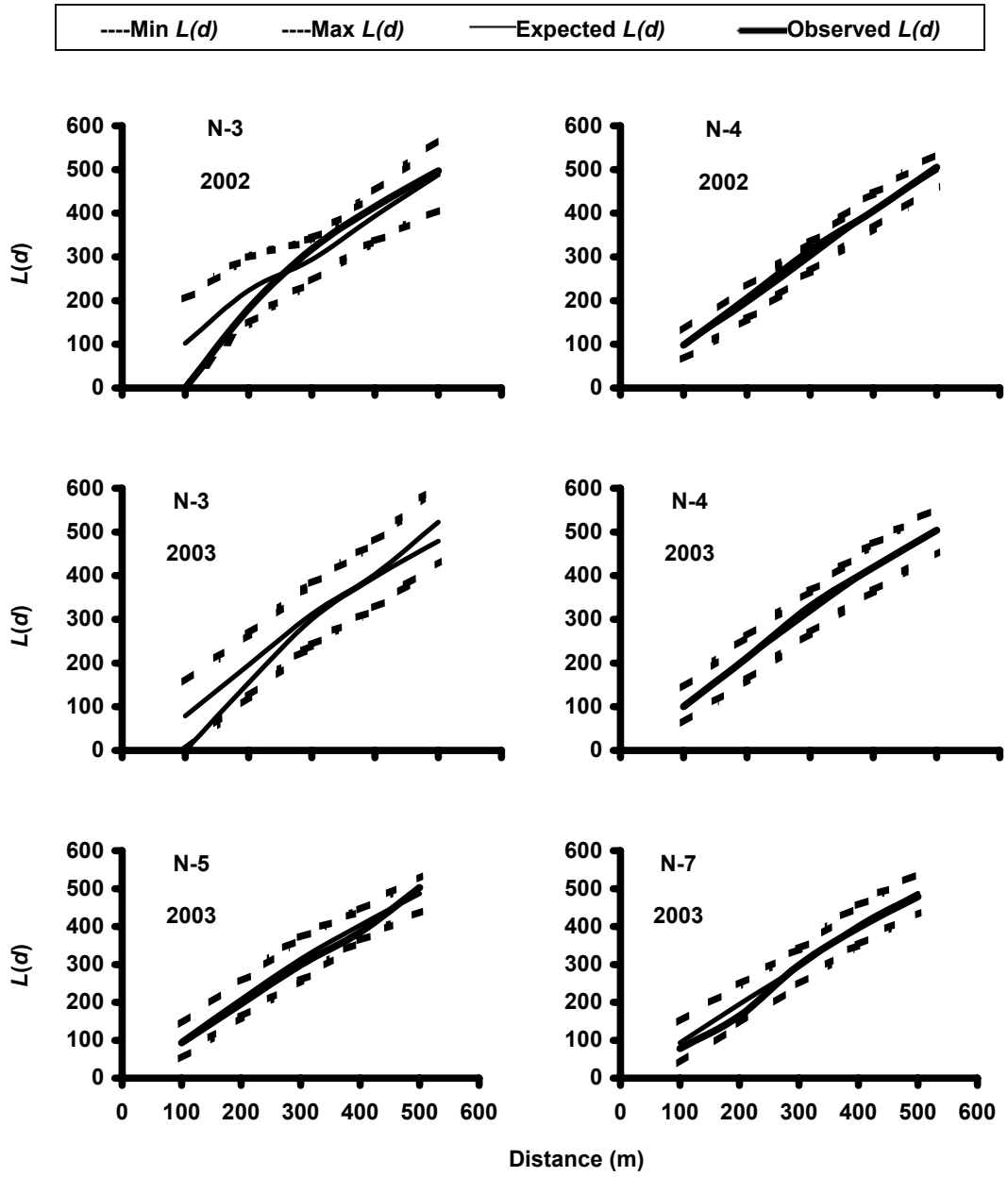
Expected L(d)
Observed L(d)
N-3
2002
N-4
2002
N-3
2003
N-4
2003
N-5
2003
N-7
2003
L(d)
Distance (m)
0
100
200
300
400
500
600

Figure 8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2002
N-4
2002
N-3
2003
N-4
2003
N-5
2003
N-7
2003
L(d)
Distance (m)
0
100
200
300
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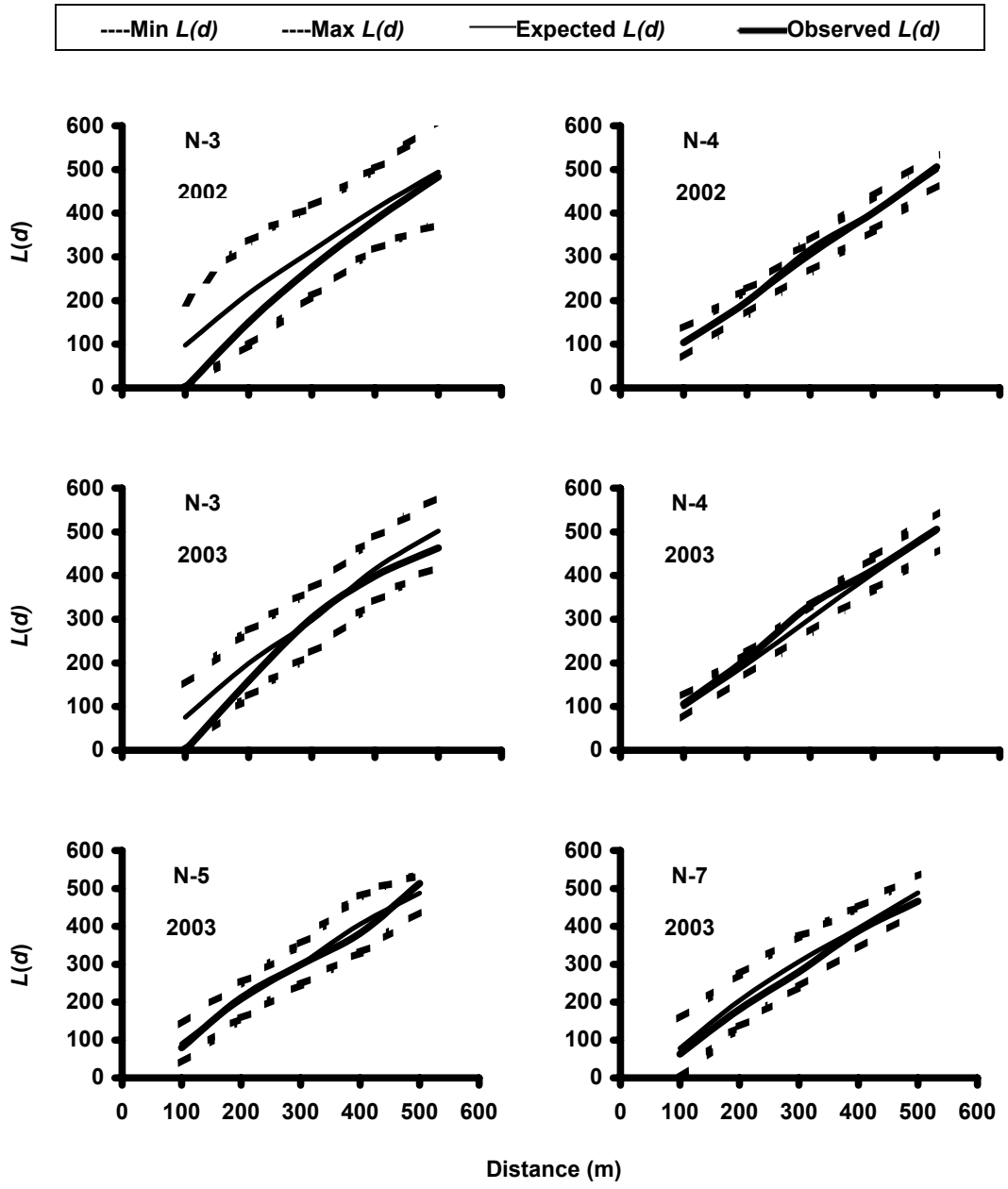
600

Figure 9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N-4
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N-3
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N-4
2003
N-5
2003
N-7
2003
L(d)
0
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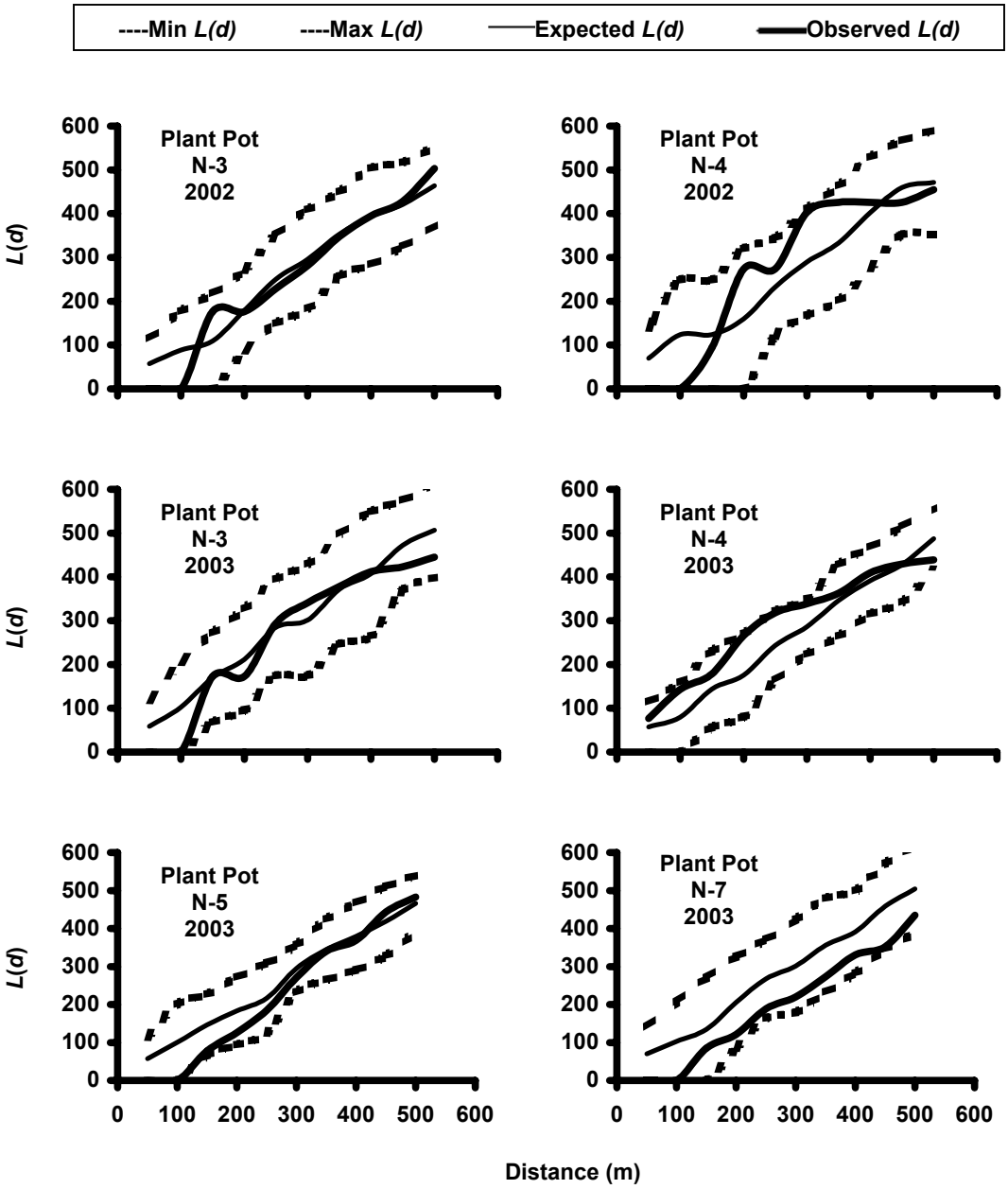
600
Distance (m)

Figure 10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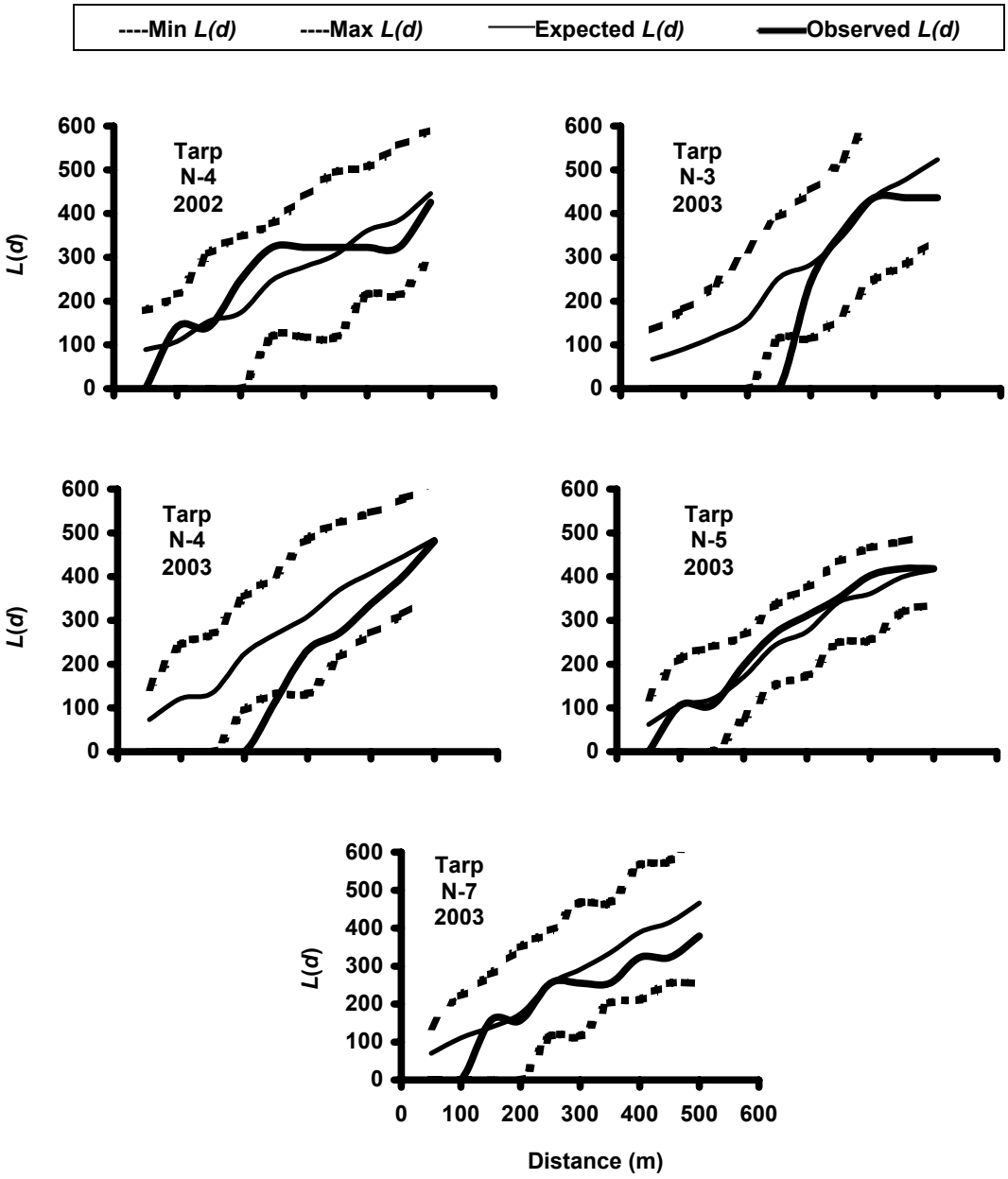
Plant Pot N-4 2002
Plant Pot N-3 2003
Plant Pot N-4 2003
Plant Pot N-5 2003
Plant Pot N-7 2003
L(d)
0
100
200
300
400
500
600
Distance (m)

Figure 11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Expected L(d)
Observed L(d)
Tarp N-4 2002
Tarp N-3 2003
Tarp N-4 2003
Tarp N-5 2003
Tarp N-7 2003
L(d)
Distance (m)
0
100
200
300
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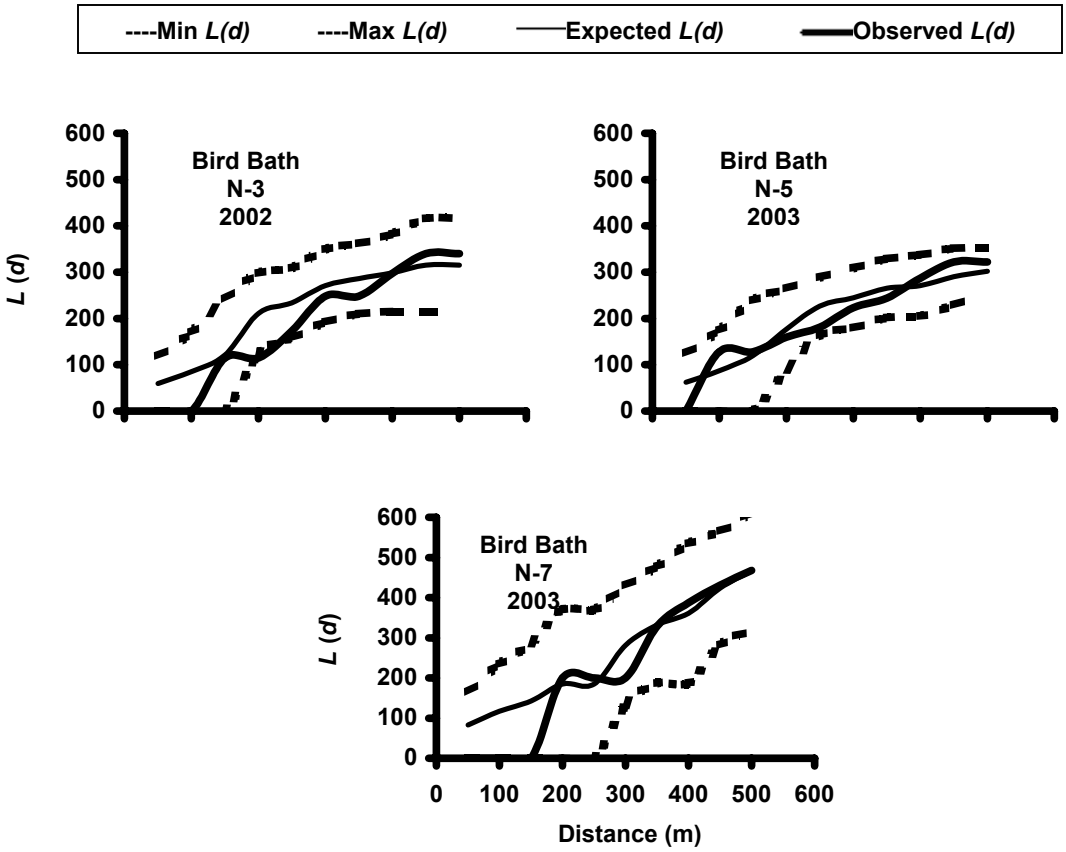
Figure 12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Expected L(d)
Observed L(d)
Bird Bath
N-3
2002
Bird Bath
N-5
2003
Bird Bath
N-7
2003
L (d)
Distance (m)
0
100
200
300
400
500
600

BLOOD FEEDING PATTERNS OF *AEDES ALBOPICTUS* (SKUSE) (DIPTERA: CULICIDAE) IN RELATION TO THE SPATIAL DISTRIBUTION AND TEMPORAL AVAILABILITY OF HUMAN AND DOMESTIC ANIMAL HOSTS IN SUBURBAN LANDSCAPES OF CENTRAL NORTH CAROLINA WITH NOTES ON BLOOD FEEDING PATTERNS OF OTHER MOSQUITO SPECIES

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ABSTRACT

We investigated effects of the spatio-temporal availability of human and domestic animal hosts on blood feeding patterns of *Aedes albopictus* during the 2002-2003 mosquito seasons in suburban neighborhoods in the City of Raleigh, Wake County, NC. On an approximately weekly basis, blood fed mosquitoes were aspirated from vegetation on home grounds in residential neighborhoods. Hosts of blood fed mosquitoes were determined with an Enzyme Linked Immunosorbent Assay, using anti-sera made in New Zealand white rabbits to the sera of animals that would commonly occur in peridomestic habitats. *Aedes albopictus* fed predominantly on mammalian hosts (83%). However, a notable proportion (7%) of blood meals was also taken from avian hosts. Mammalian hosts commonly fed upon included humans (24%), cats (21%), and dogs (14%). *Aedes albopictus* host feeding indices were calculated for human and domestic animal hosts based on the proportion of host specific blood-fed mosquitoes per collection in relation to the number of corresponding specific hosts per residence established from a door-to-door host survey in 2003. Estimates of the average amount of time that residents and pet animals (cats and dogs) spent out of doors were obtained. In general, humans were more abundant than pets. However, when host abundance was temporally weighted based on the number of hours of exposure out of doors, potential exposure to mosquitoes was higher for domestic animals. When host abundance was considered solely, host-feeding indices indicated that *Ae. albopictus* was more likely to feed on domestic animals. Conversely, when feeding indices included host abundance that were time-weighted based on potential exposure to mosquitoes, *Ae. albopictus* fed preferentially upon humans.

INTRODUCTION

Aedes albopictus is presently found in several geographic regions of the world where it is a vector of arboviruses (Shroyer 1986; Mitchell 1995; Gratz 2004). Its introduction and subsequent geographic range expansion in the United States since the 1980s (Sprenger and Wuithiranyagool 1986), coupled with its connection to arbovirus transmission cycles and propensity for anthropophilic blood feeding, has made the biology of this mosquito the subject of scrutiny in numerous research studies (e.g. Hawley et al. 1987; Niebylski et al 1994; Gomes et al. 2003). Patterns of mosquito blood feeding aid in understanding arbovirus transmission cycles since mosquitoes either acquire viruses from or transmit viruses to vertebrates during the course of a blood meal. Knowledge of host preference and biting frequency of potential vectors is critical for ranking a mosquito species' epidemiological significance because increased vector contact with hosts enhances the potential for spreading disease (Canyon et al. 1999; Gubler et al. 2001). *Aedes albopictus* is recognized as a significant nuisance species because it bites hosts aggressively (Gubler et al. 2001) and frequently takes more than one blood meal, even though it may not require it for egg development (Hawley 1988).

In the past, the "Human Blood Index", the proportion of human blood fed mosquitoes compared to other host-specific blood fed mosquitoes, was used by Garrett-Jones (1964) to estimate the epidemiological risk of mosquitoes to humans. Later, Kay et al. (1979) developed a 'Feeding Index' to evaluate the effect of host availability on mosquito host preference by taking into account the proportion of mosquitoes that fed upon one type of host in relation to other types of hosts. Host abundance is also a determinant of arbovirus transmission as demonstrated by a recent study on West Nile virus by Kilpatrick et al. (2005). Kilpatrick et al. (2005) created a virus transmission risk index based on human density, abundance of mosquitoes, proportion of

mammalian blood meals, and vector competence. It is widely known that *Ae. albopictus* opportunistically feeds on a variety of hosts including mammalian, avian, and reptilian species (Savage et al. 1993; Niebylski et al. 1994). However, while biting behavior is a significant element in disease transmission, seasonal abundance of potential vector populations, viral activity within reservoir hosts, and hosts of potential vectors must coincide if there is to be a significant epidemiological interaction (Savage et al. 1993; Niebylski et al. 1994). Previous studies have implicated *Ae. albopictus* as a vector of dengue and West Nile virus (WNV) in Asia (Hawley 1988; Mitchell 1991) and dog heartworm in Europe (Cancrini et al. 2003). Gratz (2004) recently evaluated the vector status of *Ae. albopictus* in the United States and lists eight arboviruses of public health importance that have been isolated from naturally occurring *Ae. albopictus* including: Eastern equine encephalitis, West Nile virus, Cache Valley, Potosi, Tensaw, Keystone, Jamestown Canyon, and La Crosse viruses. Evidence linking *Ae. albopictus* to transmission of these arboviruses to humans is lacking; however, its vector potential for these arboviruses, along with its avian and mammalian feeding habits, make it an important arbovirus transmission threat and *Ae. albopictus* has been definitively connected to a dengue outbreak in Hawaii in recent years (Gratz 2004). Because of its vector potential and high nuisance standing in the US and abroad, it is pertinent to study the host feeding preference of *Ae. albopictus* as it relates to host availability in suburban landscapes of North Carolina. The objective of our investigation was to evaluate the effects of spatio-temporal host availability on host-feeding patterns of *Ae. albopictus* in suburban landscapes of NC, providing insight into the vector potential of this species.

MATERIALS AND METHODS

Study areas. Our research was conducted in the City of Raleigh (76.6°W, 35.8°N) in Wake County, North Carolina where approximately 300,000 people currently reside. The suburban neighborhoods used in our study were composed primarily of single-family dwellings, but three neighborhoods also had an apartment complex. During the 2002 and 2003 mosquito seasons, four and eight neighborhoods, respectively, were included in our investigation. There were from 20 to 42 houses in each neighborhood with an average of about 50 m between dwellings and a mean lot size ranging between 0.09 and 0.33 hectares. Domestic animals, primarily dogs and cats, were present in most residences and a few residences in one neighborhood were in close proximity to farm animals (rabbits, horses, goats, chickens). The landscape of a typical residence consisted of a grass lawn in the front and back yards with shrubbery planted immediately adjacent to the dwelling. Along the edge of the property, there were woodlands composed of deciduous trees with undergrowth of grasses and low shrubs.

Host survey. For each neighborhood studied during the 2003 mosquito season, the abundance and spatial distribution of potential human and domestic animal hosts was evaluated through a door-to-door census. Information collected from the host survey included: 1) number of residents per house and their estimated weekly time spent outdoors in the summer months, 2) number and kind of domestic animals and their approximate weekly time spent outdoors in the summer months, 3) whether the domestic animals were kept indoors or outdoors, and 4) time of day (morning, afternoon, evening) residents and domestic animals were outdoors.

Collection and processing of mosquitoes. On a weekly basis in 2002 and a bimonthly basis in 2003, landscape vegetation on the grounds of ten residences in each neighborhood was aspirated for ten minutes each using a large-bore aspirator (Nasci 1981). Mosquitoes were

collected from shaded areas containing tall grass, herbaceous plants, or other knee-high vegetation. The aspirator was moved from side-to-side through the vegetation while the collector walked at a pace of approximately 0.5 m per second. Immediately after collection, each sample bag was placed in a cooler on wet ice and samples were transported to the laboratory for processing within three hours of collection. During both mosquito seasons, the same personnel were used to aspirate vegetation. Residences within each neighborhood were not selected randomly for sampling because we wanted to maximize the numbers of mosquitoes collected. Consequently, sampling was restricted to residences that had landscapes comprised of vegetation that we thought would be attractive to mosquitoes that were seeking resting cover.

In the laboratory, each sample bag was placed in a freezer to kill insects collected from foliage. Subsequently, mosquitoes were sorted from debris under 10X magnification against a white background, transferred to labeled vials, and stored in the freezer. Later, mosquitoes in each sample were identified to species (Slaff and Apperson 1989) and sex, and counted. In addition, females were examined to determine their gonotrophic status (unfed, blood fed, gravid). Blood fed mosquitoes were placed singly into labeled tubes and stored in a freezer for subsequent blood meal host identification, while unfed and gravid mosquitoes were discarded after they were identified and counted. The quality of each blood meal was scored based on the stage of blood digestion described by Sella (Detinova 1962). In 2002, all blood fed mosquitoes were analyzed for host source, regardless of the Sella stage; however, in 2003, a more selective approach was used with only "freshly" blood fed mosquitoes classified as Sella stage ≤ 3 tested. In this way, if a blood meal analysis was indeterminate, we could conclude that the specific antiserum for the blood meal host was not included in our test.

Blood meal analyses. Hosts of blood fed mosquitoes were identified using an indirect enzyme-linked immunosorbent assay (ELISA) described by Irby and Apperson (1988). Assays used host-specific anti-sera made in New Zealand white rabbits against vertebrate serum proteins (Irby and Apperson 1988). Anti-sera that we utilized included anti-human, -dog, -cat, -raccoon, -squirrel, -white-tailed deer, -horse, -cotton tail rabbit, -opossum, -frog, -turtle, and -bird.

Geographic information system. **A** geographic information system (GIS) was created for each neighborhood so that spatial patterns of blood feeding activity in relation to the abundance of humans and domestic animals could be visualized. Shapefiles for property boundaries, buildings, and street centerlines for all study sites were downloaded from the Wake County government GIS website (http://www.wakegov.com/county/propertyandmapping/gisdigitaldata.htm) and imported into ArcMap (ESRI, Redlands, California, 2002). Residential lot sizes were determined from data in attribute tables of property boundary shapefiles.

Data analyses. Variables related to host surveys and mosquito host preference were analyzed separately for humans, dogs, and cats for all households in each neighborhood and at households where host-specific blood meals were collected over the 2003 mosquito season (Table 1). With Kolmogorov-Smirnov tests, we determined that the numbers of hosts per residence and numbers of blood fed mosquitoes collected per sample were not normally distributed (PROC UNIVARIATE, SAS Institute 2000). Consequently, prior to analyzing data, the mean numbers of humans, dogs, and cats per residence and per hectare, as well as mean numbers of species-specific blood fed mosquitoes per aspiration sample, were log-transformed [log (x +1)] to normality. Each analysis described below was performed separately for humans,

dogs, and cats for all residences and for residences where host-specific blood fed mosquitoes were collected during the mosquito season of 2003.

Host-feeding patterns. The percentage of the total numbers of blood meals identified for different host groups (mammalian, avian, turtle, and frog) were determined for the 2002 and 2003 mosquito seasons for each mosquito species separately. Species-specific mammalian blood meals were further enumerated for each mosquito species.

Seasonal variations in blood feeding. We performed analyses on the three most commonly collected species *Ae. albopictus, Ae. vexans*, and *Oc. triseriatus.* To illustrate seasonal variation in mean numbers of blood-fed mosquitoes per aspiration sample for these species, we calculated mean numbers of blood fed mosquitoes per aspiration sample per month and created plots over calendar months for the 2002 and 2003 mosquito seasons.

We also performed One-way analysis of variance (ANOVA) (PROC GLM, SAS Institute 2000) to determine if differences between months and mosquito seasons for mean numbers of blood fed mosquitoes per aspiration sample for the most commonly collected species, *Ae. albopictus, Ae. vexans*, and *Oc. triseriatus*, were statistically significant. We used month and year as main effect variables and month x year as interaction effects. If significant differences were observed between main effect means, Tukey's studentized range test was used to determine which means were different.

Host-feeding indices. We examined the relationship between host abundance and the blood feeding frequency of *Ae. albopictus* through a Host-Feeding Index (*HFI*) modified from Kay et al. (1979). The index was calculated as:

$$HFI = \frac{N_x/N_y}{A_x/A_y}$$

where N_x and N_y are the mean numbers of blood meals taken from hosts x and y per residence or hectare, respectively; and A_x and A_y are the mean number per residence or hectare of hosts x and y, respectively.

Furthermore, we evaluated the host preference of *Ae. albopictus* by considering the concurrent availability of humans, dogs, and cats. Accordingly, we calculated separate *HFI*s to characterize host preference of dogs vs. humans, cats vs. humans, and cats vs. dogs. Additionally, we recalculated *HFI*s incorporating a temporal component of availability, to derive a time-weighted feeding index, HFI_{ET} as follows:

$$HFI_{ET} = \frac{N_x/N_y}{(A_xET_x)/(A_yET_y)}$$

where ET_x and ET_y are estimated time in hours (ET) spent outdoors per week for host x and host y, respectively.

If the *HFI* or HFI_{ET} was greater than one, this indicated that host x was preferentially fed upon, while a value of less than one indicated that host y was preferentially fed upon.

ANOVA (PROC GLM, SAS Institute 2000) was used to determine if there were significant differences in the mean *HFI*s and HFI_{ET}*s* between neighborhoods. Bonferroni t-tests were used to separate significantly different means.

Relationship between abundance of hosts and blood fed mosquitoes. Pearson Product Moment correlation analyses (PROC CORR, SAS Institute 2000) were completed for households where collections of host-specific mosquitoes were made during the 2003 mosquito season to determine if there was a significant relationship between A_x or A_y and total numbers of blood fed *Ae. albopictus* collected per residence and per hectare that had fed on host x and host y. Correlation analyses were repeated for A_xET_x and A_yET_y to determine effects of time-weighting host abundance on the host preference of *Ae. albopictus*.

RESULTS

Host-feeding patterns. In the mosquito seasons from 2002-2003, 3,065 blood fed mosquitoes of 15 species from six genera were tested for blood source. The three most commonly collected blood fed mosquito species were *Ae. albopictus* (n = 1,172; 38 % of total), *Ae. vexans* Meigen (n = 920; 30 % of total), and *Ochlerotatus triseriatus* Say (n = 601; 20 % of total) (Table 2). The majority (> 70 %) of the mosquitoes of each of these three species that were tested for host source had fed on mammals. The mammalian hosts fed upon most frequently by these three mosquitoes were humans, deer, and squirrels, respectively (Table 3); however, blood meals were taken frequently from other mammalian hosts, including dogs, cats, raccoons, horses, rabbits, and opossums, by these and other mosquito species. *Aedes albopictus* fed upon all 12 of the hosts for which we tested, but humans (18 %), cats (16 %), and dogs (11 %) were fed upon most frequently. Blood meals were commonly acquired from squirrels (9 %), rabbits (8 %), and birds (7 %). *Aedes albopictus* blood meals originating from more than one host (6% of total no. tested) included cats, rabbits, birds, humans, and squirrels.

Seasonality of commonly collected blood fed mosquitoes. To compare seasonal changes in abundance of the three most commonly collected blood fed mosquitoes, we plotted mean numbers of blood fed mosquitoes per aspiration sample over each calendar month and year (Figure 1). When both mosquito seasons were included in the respective analyses of the mean numbers of blood fed mosquitoes per aspiration sample there were significant differences between months for *Ae. albopictus* (df = 5, 791; $F = 19.63$; $P < 0.0001$) and *Oc. triseratus* (df = 5, 567; $F = 3.13$; $P = 0.008$); however, there was no significant differences ($P > 0.05$) in this variable between mosquito seasons for either species (Table 4). When both mosquito seasons were included in the analysis, mean numbers of blood fed *Ae. vexans* per aspiration sample were

significantly different between months (df = 5, 772; $F = 18.47$; $P < 0.0001$) and mosquito seasons (df = 1, 776; $F = 46.52$; $P < 0.0001$). In 2002, mean numbers of blood fed *Ae. albopictus* per aspiration sample were significantly higher ($P < 0.05$) in July and August, while in 2003, blood feeding activity was significantly highest in July. In 2002 and 2003, mean numbers of blood fed *Ae. vexans* per aspiration sample were significantly higher ($P < 0.05$) in September and July, respectively. Furthermore, there were significantly larger ($P < 0.05$) numbers of blood fed *Ae. vexans* per aspiration sample collected in 2003 in relation to 2002. For *Oc. triseriatus*, mean numbers of blood fed mosquitoes per aspiration sample were significantly larger ($P < 0.05$) in September in 2002 and in May and June of 2003.

Host survey of humans and domestic animals. Humans were most abundant among all hosts on a per hectare and per residence basis. The mean number of dogs and humans per residence was higher at residences where dog- and human-specific blood fed *Ae. albopictus* were collected, respectively. Conversely, the mean number of cats per residence was lower at residences where cat-specific blood fed *Ae. albopictus* were collected. However, on a per hectare basis, humans, dogs, and cats were more abundant at residences where human-, dog-, and cat-specific blood fed *Ae. albopictus* were collected, respectively.

Host feeding indices. When all residences in each neighborhood were included in analyses of *Ae. albopictus* feeding on humans relative to domestic animal hosts, we found a preference for feeding on dogs ($HFI_{dogs\ vs.\ humans}$ = mean ± SE = 4.4 ± 0.9) and cats ($HFI_{cats\ vs.\ humans}$ = 6.3 ± 1.3) relative to humans, and more feeding on cats relative to dogs ($HFI_{cats\ vs.\ dogs}$ = 1.0 ± 0.2). Subsequent analyses, including only residences in all neighborhoods where host-specific blood fed *Ae. albopictus* were collected, also indicated a greater feeding on dogs ($HFI_{dogs\ vs.\ humans}$ = 5.4 ± 1.1) and cats ($HFI_{cats\ vs.\ humans}$ = 4.6 ± 2.2) relative to humans; however

there was more feeding on dogs relative to cats ($HFI_{cats\ vs.\ dogs}$ = 0.4 ± 0.1). Additional analyses between neighborhoods revealed a significant difference in the means of the three feeding indices ($HFI_{dogs\ vs.\ humans}$, $HFI_{cats\ vs.\ humans}$, and $HFI_{cats\ vs.\ dogs}$) including all residences (df = 2, 21; F = 7.94; P = 0.003) and residences where host-specific blood fed *Ae. albopictus* were collected (df = 2, 21; F = 11.48; P = 0.0004). Furthermore, the mean $HFI_{cats\ vs.\ humans}$ was significantly higher ($P < 0.05$) compared to other *HFI*s when all residences were considered, while the mean $HFI_{dogs\ vs.\ humans}$ was significantly higher than other *HFI*s when residences where host-specific blood fed *Ae. albopictus* were collected were considered.

Effects of host availability on host feeding indices. Subsequent analyses were carried out incorporating estimates of the time spent out of doors in feeding indices. When all residences were collectively included in analyses, we found more feeding on humans relative to dogs ($HFI_{ETdogs\ vs.\ humans}$ = 0.7 ± 0.2) and cats ($HFI_{ETcats\ vs.\ humans}$ = 0.2 ± 0.04) and more feeding on dogs in relation to cats ($HFI_{ETcats\ vs.\ dogs}$ = 0.5 ± 0.1). Analyses exclusively including residences where host-specific blood fed *Ae. albopictus* were collected also indicated a greater host preference for feeding on humans relative to dogs ($HFI_{ETdogs\ vs.\ humans}$ = 0.7 ± 0.2) and cats ($HFI_{ETcats\ vs.\ humans}$ = 0.1 ± 0.02) and greater feeding on dogs in relation to cats ($HFI_{ETcats\ vs.\ dogs}$ = 0.1 ± 0.02). Additional analyses between neighborhoods revealed that differences in mean HFI_{ET}s including all residences were marginally significant (df = 2, 21; F = 3.30; P = 0.057); however, at residences where host-specific blood fed *Ae. albopictus* were collected, there was a significant difference between mean HFI_{ET}s (df = 2, 21; F = 16.63; $P < 0.0001$). Mean $HFI_{ETdogs\ vs.\ humans}$ were significantly higher than other HFI_{ET}s when residences where host-specific blood fed *Ae. albopictus* were collected were considered.

Relationship between the abundance of blood fed *Ae. albopictus* and the abundance of hosts. To determine if there was a relationship between $A_{x,y}$ for host-specific blood fed *Ae. albopictus* per residence and $A_{x,y}ET_{x,y}$ for hosts at residences where blood fed mosquitoes were collected, we conducted correlation analyses. This analysis indicated a marginally significant relationship between A_{dog} and $A_{dog}ET_{dog}$ for dog blood fed *Ae. albopictus* per residence ($r = 0.334$, $P = 0.053$); however, no relationship was observed between total numbers of human or cat blood fed *Ae. albopictus* and the corresponding $A_{humans,cats}$ or $ET_{humans,cats}$. There was also no statistically significant relationship ($P > 0.05$) between mean numbers of host-specific blood fed *Ae. albopictus* per aspiration sample and abundance of human, dog, or cat hosts per hectare.

DISCUSSION

Host-feeding patterns. *Aedes albopictus* has been previously reported to exhibit an opportunistic host-feeding pattern with a predilection to feed on mammalian hosts (Tempelis et al. 1970; Sullivan et al. 1971; Savage et al. 1993; Niebylski et al. 1994). Our results support these findings since > 80% of the blood fed *Ae. albopictus* that we tested had fed upon mammals. Furthermore, within mammalian hosts, most blood fed *Ae. albopictus* that we collected had fed upon humans, supporting previous research revealing the anthropophilic blood feeding nature of this mosquito. *Ochlerotatus triseriatus* fed primarily on mammals (71%), mostly squirrels (54%), and this contradicts Irby and Apperson (1988) who found this mosquito feeding on reptiles / amphibians (75%) in NC; however, the aforementioned study was carried out in woodlands of the coastal plain of NC where reptiles / amphibians would be expected to be more common hosts compared to suburban areas of central NC. Furthermore, a study carried out in the mountainous region of western NC (Szumlas et al. 1996) reported that *Oc. triseriatus* fed primarily on dogs (40%), while a study in Wisconsin found *Oc. triseriatus* feeding on deer

(65%), squirrels (16%), and chipmunks (8%), further evidencing the variation in host feeding patterns in different areas. A previous research study carried out in Texas by Hayes et al. (1973) found that *Ae. vexans* fed primarily on mammals (95%). We found that *Ae. vexans* fed chiefly on mammals (87%), such as deer (64%), and this supports the findings of Irby and Apperson (1988) who reported *Ae. vexans* feeding on mammals (83%) including deer. Similar results that *Ae. vexans* fed predominantly on deer were obtained from an investigation in Wisconsin (Burkot and Defoliant 1982). It is likely that the disparity in blood feeding patterns of these mosquitoes resulted from variation in host availability in different geographic regions.

We found that 7% of *Ae. albopictus* fed upon avian hosts and these results are in accordance with other studies reporting avian feeding of this mosquito in Missouri (17%) (Savage et al. 1993) and Hawaii (6%) (Tempelis et al. 1970). In addition, we found that multiple feeding (> one type of host) by a single *Ae. albopictus* occurred in 6% of our total samples, including the combination of avian and human hosts. Other studies have indicated that mosquitoes not feeding primarily on avian hosts would not be effective enzootic vectors of arbovirus cycles involving birds (Turell et al. 2001); however, the general feeding patterns of *Ae. albopictus*, including avian and mammalian hosts, makes it a potential bridge vector (Gubler et al. 2001) of bird-associated viruses, such as West Nile virus.

Host feeding indices. Even though past studies have elucidated the feeding patterns of *Ae. albopictus*, most have failed to provide insight into blood feeding frequency based on host availability. The feeding index proposed by Kay et al. (1979) provides a way to quantify mosquito host preference based on abundance of hosts and host-specific blood fed mosquitoes; however, this index does not incorporate the outdoor temporal availability of hosts. Our study supplements the *Ae. albopictus* feeding index by integrating a temporal component with host

abundance for the three most commonly fed upon hosts in suburban neighborhoods in Raleigh, NC; namely, humans, dogs, and cats. In general, the results from the HFI_{ET} analyses are in contrast to *HFI* results, indicating that time spent outdoors by some hosts does not increase the frequency of feeding of *Ae. albopictus* on these hosts. Our results for HFI_{ET} indicate that, even though the temporal availability of domestic animals was greater than humans, *Ae. albopictus* fed upon humans more frequently than on cats and dogs. Moreover, we established through *HFI* that domestic animals were fed upon more frequently when only abundance of potential hosts was considered. Regardless of host abundance or availability, a greater percentage of the mammalian blood fed *Ae. albopictus* came from humans (24%) when compared to cats (21%) and dogs (14%). These data support the findings of Gomes et al. (2003) in Brazil that *Ae. albopictus* fed upon humans more frequently than dogs. Kilpatrick et al. (2005) hypothesized that the density of humans influences the risk of West Nile virus transmission to humans and our results suggest that temporal host availability should also be considered in this analysis. We agree with Smith et al. (2004) that the distribution of mosquitoes and hosts are synchronous since mosquitoes require blood for the development of their eggs and that further research should address how the spatial distribution of hosts and host-seeking mosquitoes is related to the proliferation of mosquito-borne disease.

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Table 1. Host survey and blood feeding variables analyzed separately for humans, dogs, and cats for all households and for households where host-specific blood meals were collected over the 2003 mosquito season.

Variable	Description
Total no. of hosts	Total number of a specific host
Mean no. hosts per residence	Total number of a specific host at all residences / total number of residences.
Mean no. hosts per hectare	Total number of a specific host at each residence / parcel size (ha) of each residential property.
Time-weighted host abundance	Total number of a specific host x estimated time (hours) the specific host spent outdoors per week
Mean no. blood fed mosquitoes per aspiration sample	Total number of blood fed mosquitoes / total number of aspiration samples
Mean no. blood fed mosquitoes per aspiration sample per month	Total number of blood fed mosquitoes per month / total number of aspiration samples per month

Table 2. Host-feeding frequency of different mosquito species during the mosquito seasons of 2002 – 2003.

Mosquito Species	Total tested	No. identified (% of total tested)	Host Class (% of total identified)				
			Mammal	Bird	Frog	Turtle	> 1 host class
Ae. albopictus	1,172	1,094 (93)	909 (83)	82 (7)	20 (2)	17 (2)	56 (6)
Ae. vexans	920	896 (97)	782 (87)	51 (6)	9 (1)	10 (1)	44 (5)
An. punctipennis	2	2 (100)	2 (100)	-	-	-	-
Cq. perturbans	1	1 (100)	1 (100)	-	-	-	-
Cx. erraticus	2	2 (100)	1 (50)	1 (50)	-	-	-
Cx. pipiens	72	71 (99)	11 (15)	52 (73)	6 (8)	-	2 (4)
Cx. restuans	185	183 (99)	19 (10)	150 (83)	3 (1)	2 (1)	9 (5)
Cx. territans	8	8 (100)	5 (64)	1 (12)	1 (12)	-	1 (12)
Oc. atlanticus	10	10 (100)	6 (60)	3 (30)	-	1 (10)	-
Oc. canadensis	12	10 (83)	5 (50)	1 (10)	-	3 (30)	1 (10)
Oc. infirmatus	6	6 (100)	5 (83)	1 (17)	-	-	-
Oc. triseriatus	601	574 (96)	407 (71)	53 (9)	14 (2)	28 (5)	72 (13)
Ps. columbiae	1	1 (100)	1 (100)	-	-	-	-
Ps. ferox	67	62 (93)	50 (81)	7 (11)	-	-	5 (8)
Ps. howardii	3	3 (100)	2 (67)	-	-	1 (33)	-

Table 3. Mammalian host feeding frequency of different mosquito species during the mosquito seasons of 2002 – 2003.

Mosquito species	No. tested	Mammalian Hosts (% of total mammalian hosts identified)								
		Human	Dog	Cat	Deer	Horse	Rabbit	Squirrel	Raccoon	Opossum
Ae. albopictus	909	219 (24)	124 (14)	193 (21)	25 (3)	40 (4)	88 (10)	104 (11)	53 (6)	63 (7)
Ae. vexans	782	23 (3)	14 (1)	45 (6)	494 (64)	62 (8)	77 (10)	36 (5)	24 (3)	7 (0.009)
An. punctipennis	2	-	-	-	2 (100)	-	-	-	-	-
Cq. perturbans	1	-	-	-	1 (100)	-	-	-	-	-
Cx. erraticus	1	-	-	-	-	-	-	-	1 (100)	-
Cx. pipiens	11				1 (9)	2 (18)		1 (9)	1 (9)	6 (55)
Cx. restuans	19	1 (5)	4 (21)	1 (5)	4 (21)	3 (16)	1 (5)	1 (5)	3 (16)	1 (5)
Cx. territans	5	-	-	1 (20)	-	-	4 (80)	-	-	-
Oc. atlanticus	6	-	-	-	2 (33)	-	-	2 (33)	2 (33)	-
Oc. canadensis	5	-	-	-	-	-	-	3 (60)	2 (40)	-
Oc. infirmatus	5	-	-	1 (20)	2 (40)	1 (20)	-	1 (20)	-	-
Oc. triseriatus	407	28 (8)	14 (3)	38 (9)	9 (2)	13 (3)	47 (12)	221 (54)	29 (7)	8 (2)
Ps. columbiae	1	-	1 (100)	-	-	-	-	-	-	-
Ps. ferox	50	1 (2)	9 (18)	18 (36)	9 (18)	-	7 (14)	2 (4)	1 (2)	3 (6)
Ps. howardii	2	-	-	2 (100)	-	-	-	-	-	-

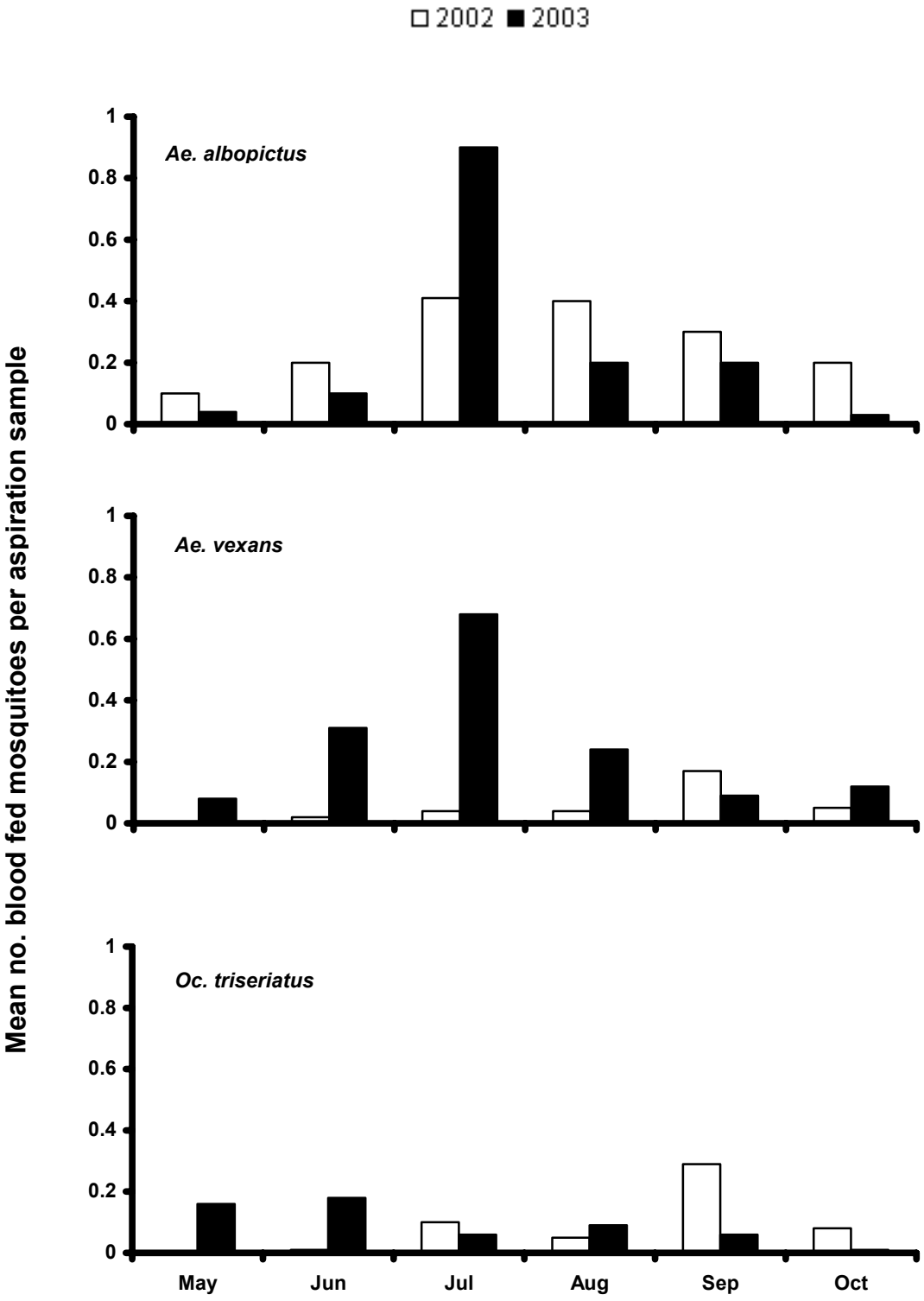
Table 4. One-way ANOVA for seasonal variation in mean numbers of blood fed *Ae. albopictus, Ae. vexans*, and *Oc. triseriatus* collected per aspiration sample.

Source	df (model, error)	*Mean square* (model, error)	*F*	*P > F*
		Ae. albopictus		
Month	5, 791	8.82, 0.44	11.78	< 0.0001
Year	1, 795	0.01, 0.50	0.02	0.876
Month x Year	11, 785	5.24, 0.44	12.01	< 0.0001
		Ae. vexans		
Month	5, 772	3.46, 0.19	18.47	< 0.0001
Year	1, 776	9.16, 0.20	46.52	<0.0001
Month x Year	11, 766	3.13, 0.17	18.80	< 0.0001
		Oc. triseriatus		
Month	5, 567	0.24, 0.08	3.13	0.008
Year	1, 571	0.001, 0.08	0.02	0.887
Month x Year	11, 561	0.39, 0.07	5.59	< 0.0001

FIGURE CAPTIONS

Figure 1. Mean numbers of blood fed *Ae. albopictus, Ae. vexans*, and *Oc. triseriatus* collected per aspiration sample during the 2002 and 2003 mosquito seasons.

Figure 1

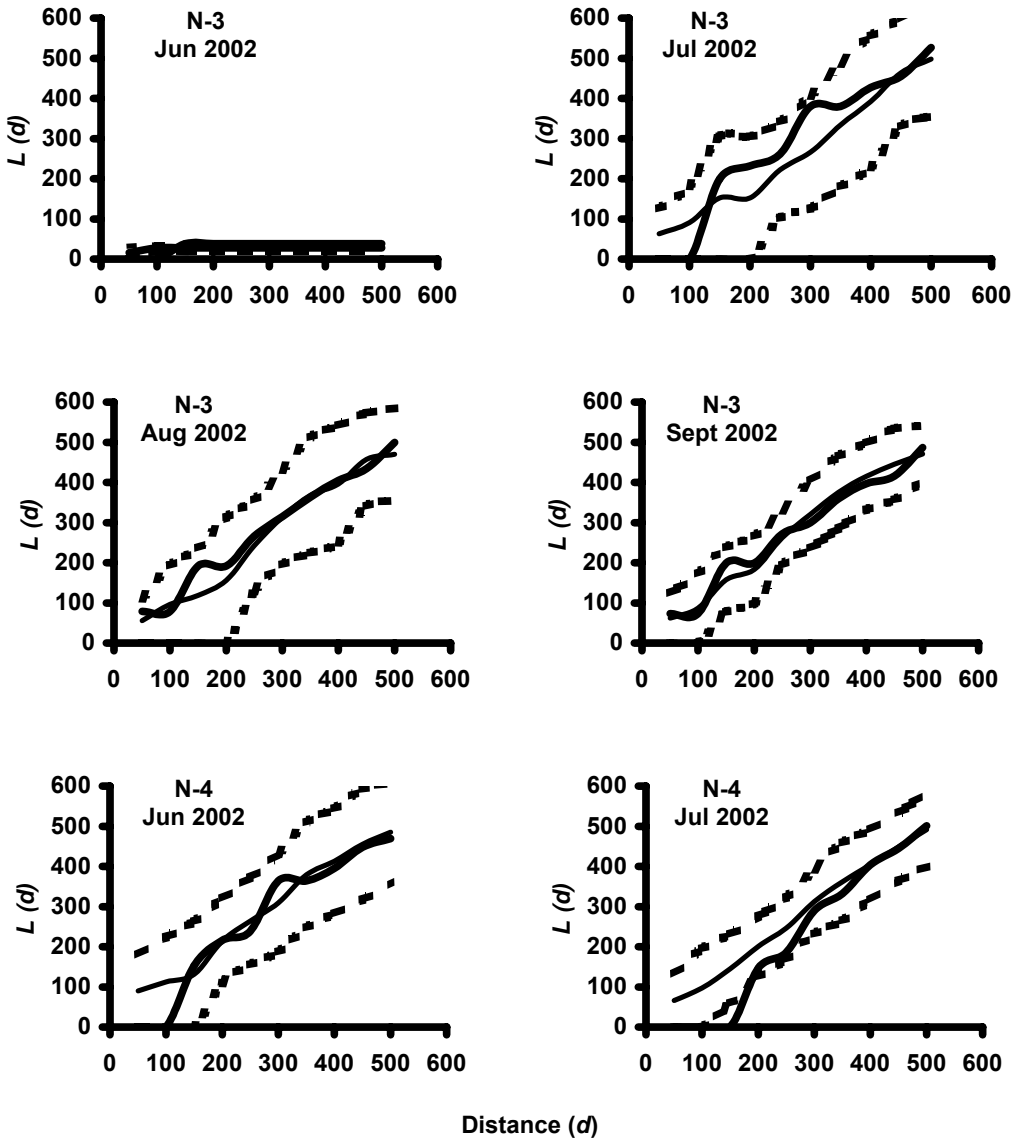

□ 2002 ■ 2003
Mean no. blood fed mosquitoes per aspiration sample
Ae. albopictus
Ae. vexans
Oc. triseriatus
0
0.2
0.4
0.6
0.8
1
May
Jun
Jul
Aug
Sep
Oct

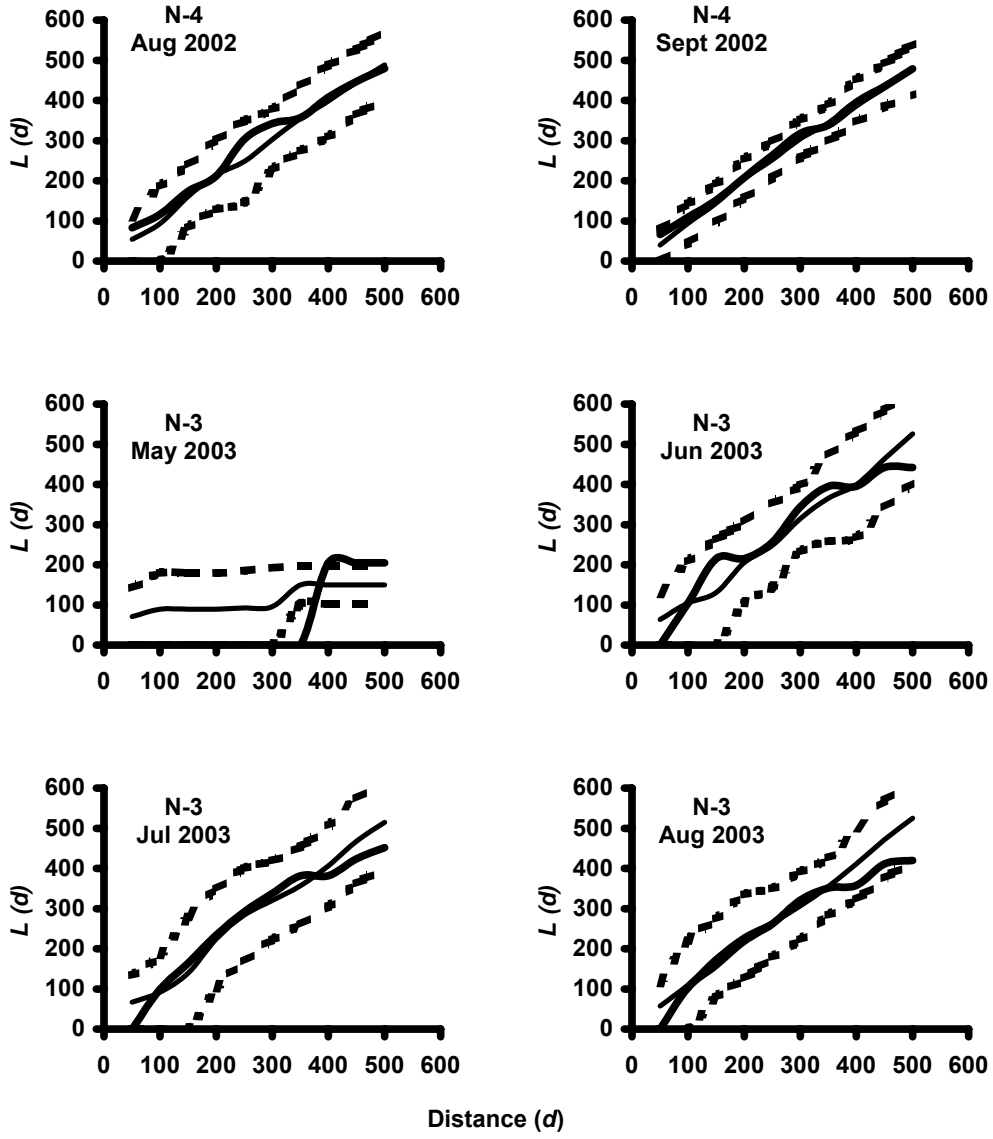
APPENDICES

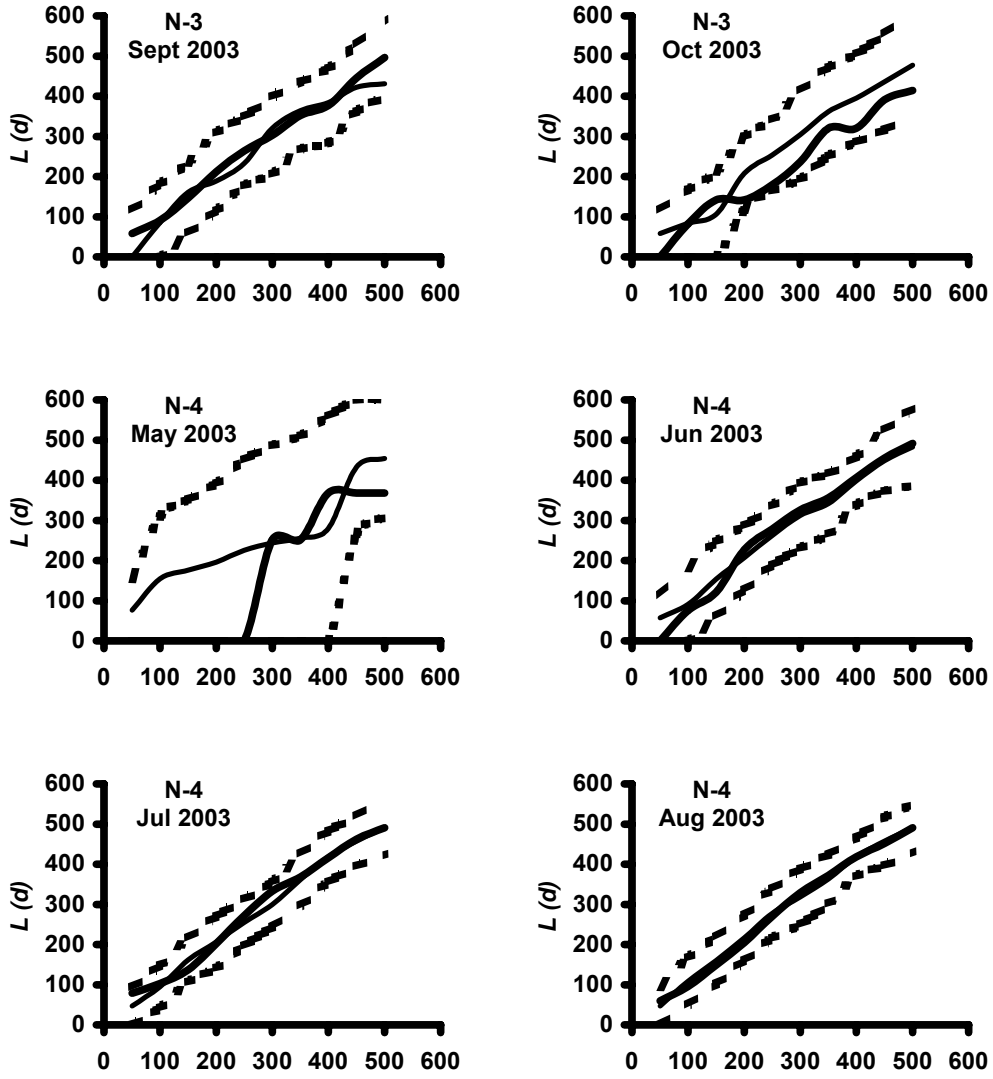
APPENDIX A

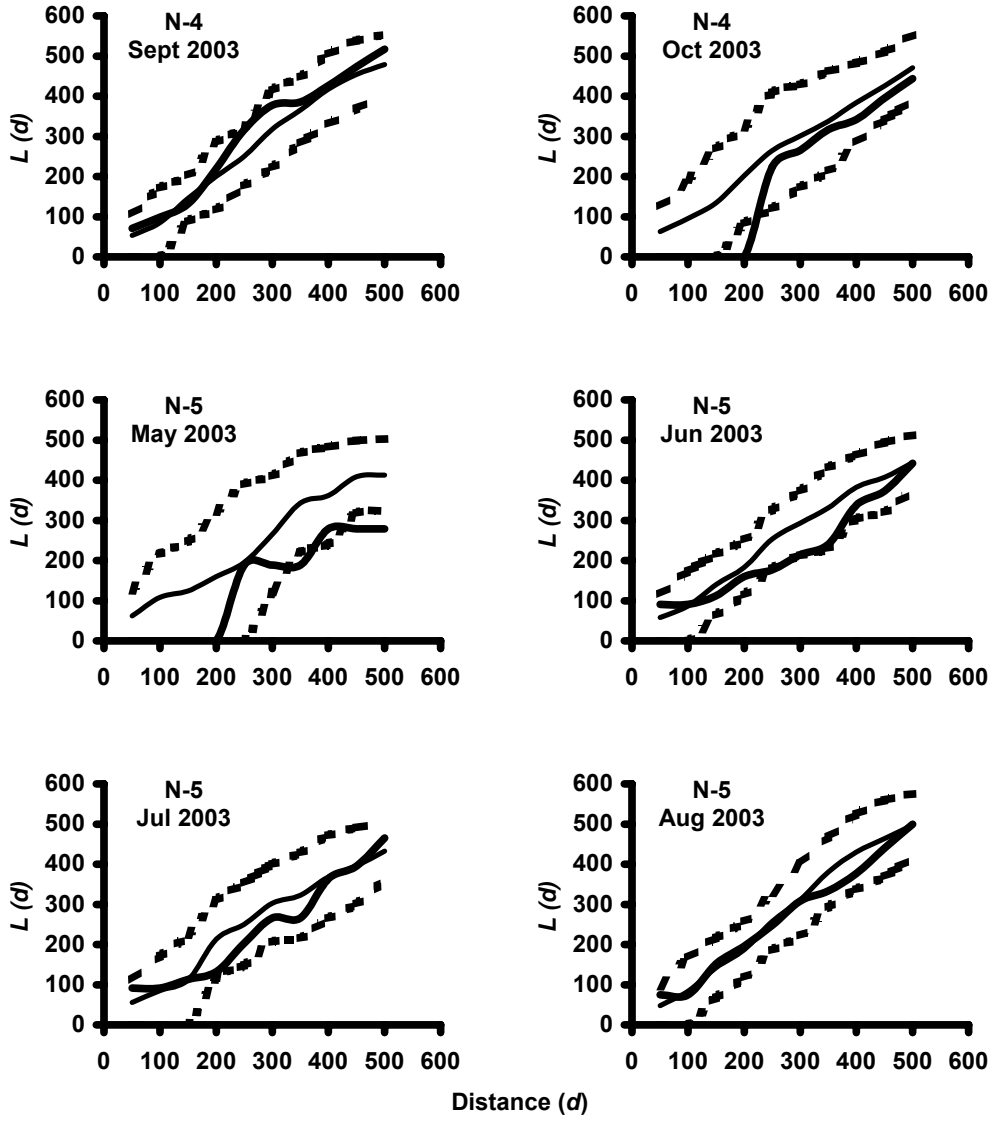
Fig. 1. Results of *K*-function analysis of the spatial distribution of *Ae. albopictus* pupae in neighborhoods receiving source reduction (N-5, N-7) and not receiving source reduction (N-3, N-4) by month for the 2002 and 2003 mosquito seasons.

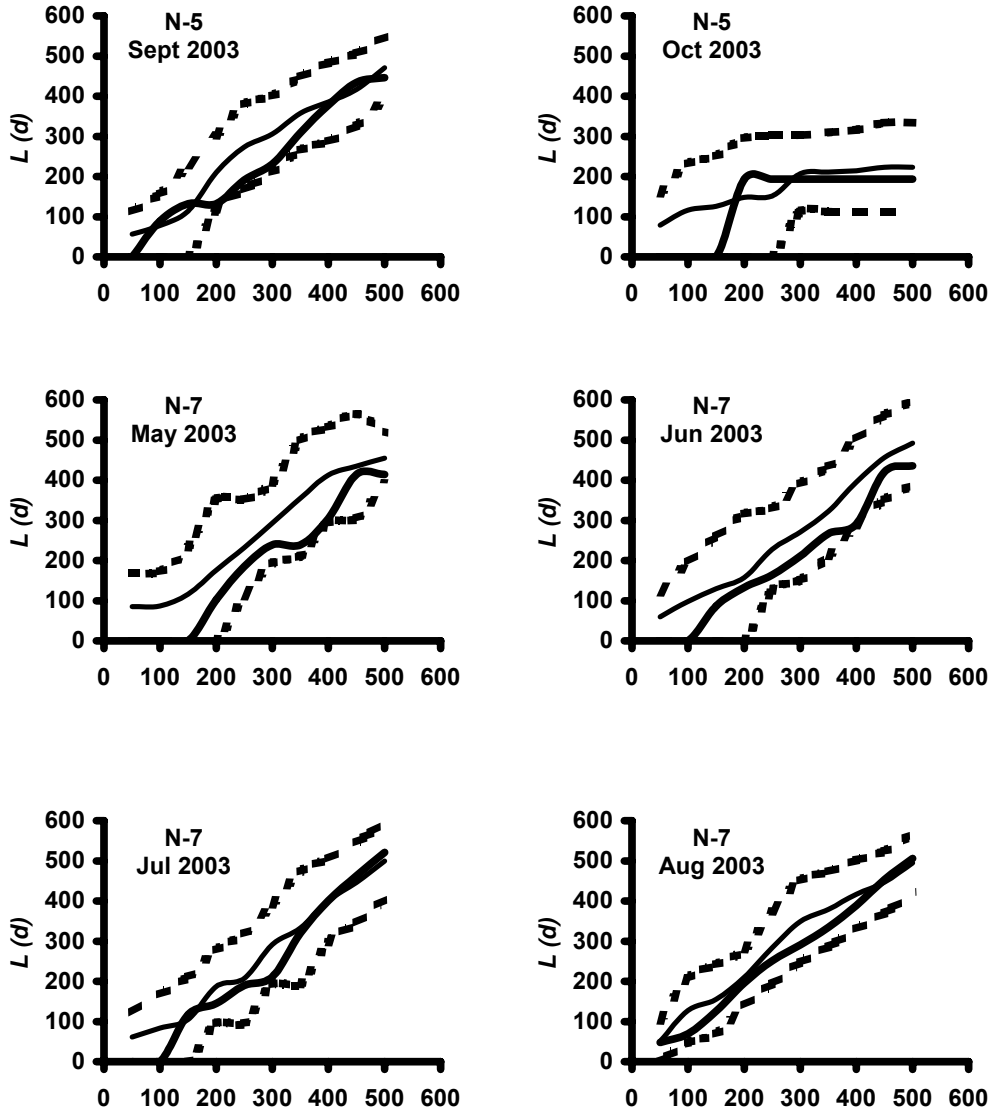
APPENDIX A

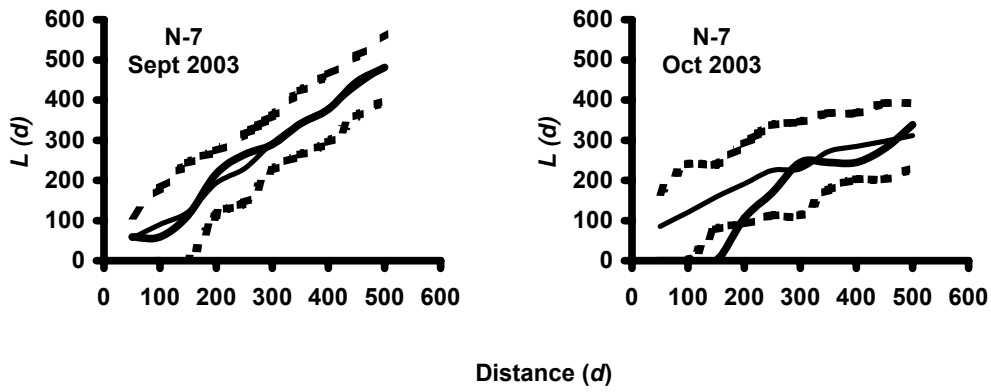

N-3
Jun 2002
N-3
Jul 2002
N-3
Aug 2002
N-3
Sept 2002
N-4
Jun 2002
N-4
Jul 2002
L (d)
0
100
200
300
400
500
600
Distance (d)


N-4
Aug 2002
N-4
Sept 2002
N-3
May 2003
N-3
Jun 2003
N-3
Jul 2003
N-3
Aug 2003
L (d)
0
100
200
300
400
500
600
Distance (d)


N-3
Sept 2003
N-3
Oct 2003
N-4
May 2003
N-4
Jun 2003
N-4
Jul 2003
N-4
Aug 2003
L (d)
0
100
200
300
400
500
600
Distance (d)


N-4
Sept 2003
N-4
Oct 2003
N-5
May 2003
N-5
Jun 2003
N-5
Jul 2003
N-5
Aug 2003
L (d)
0
100
200
300
400
500
600
Distance (d)

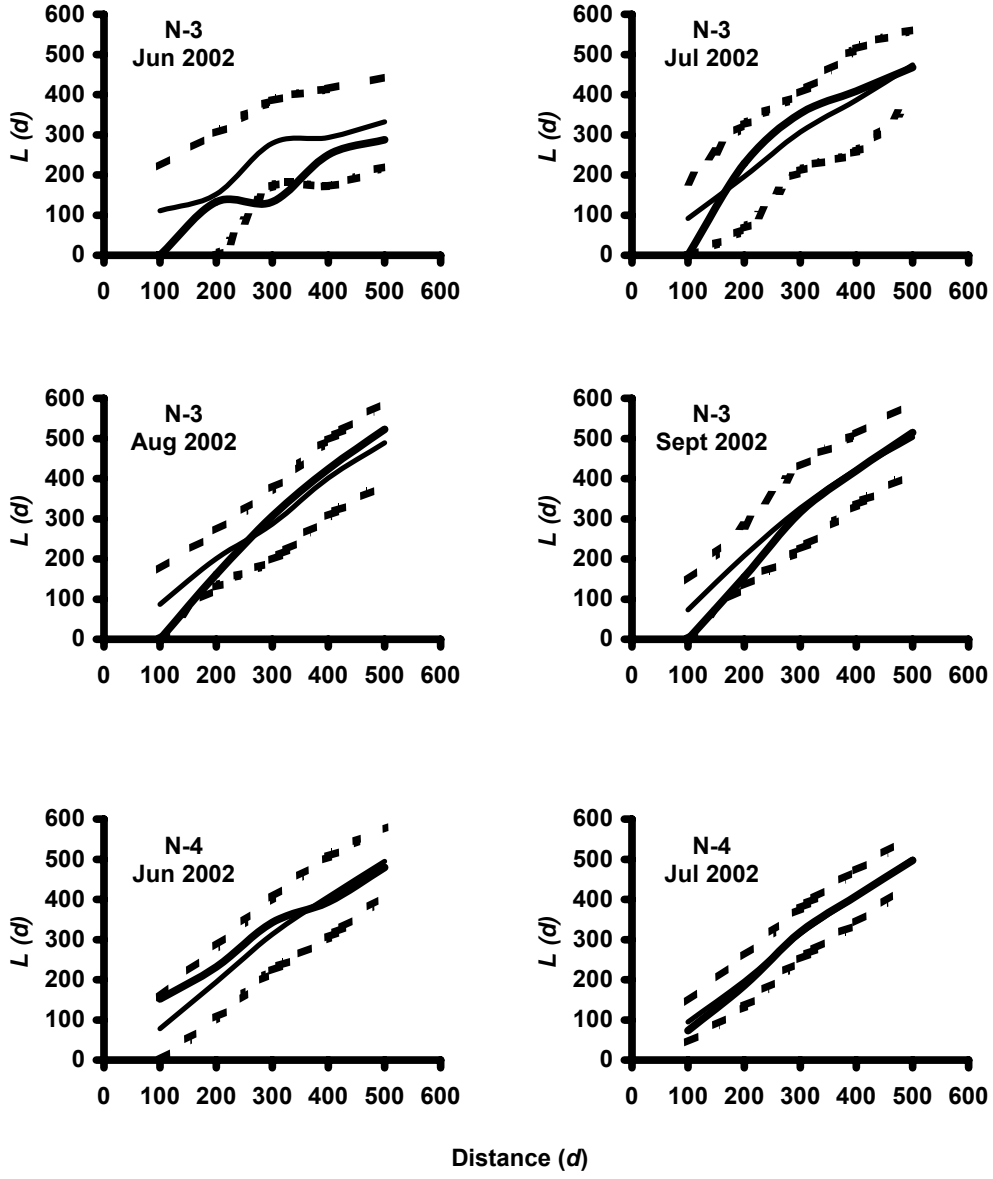

N-5
Sept 2003
N-5
Oct 2003
N-7
May 2003
N-7
Jun 2003
N-7
Jul 2003
N-7
Aug 2003
L (d)
0
100
200
300
400
500
600
Distance (d)

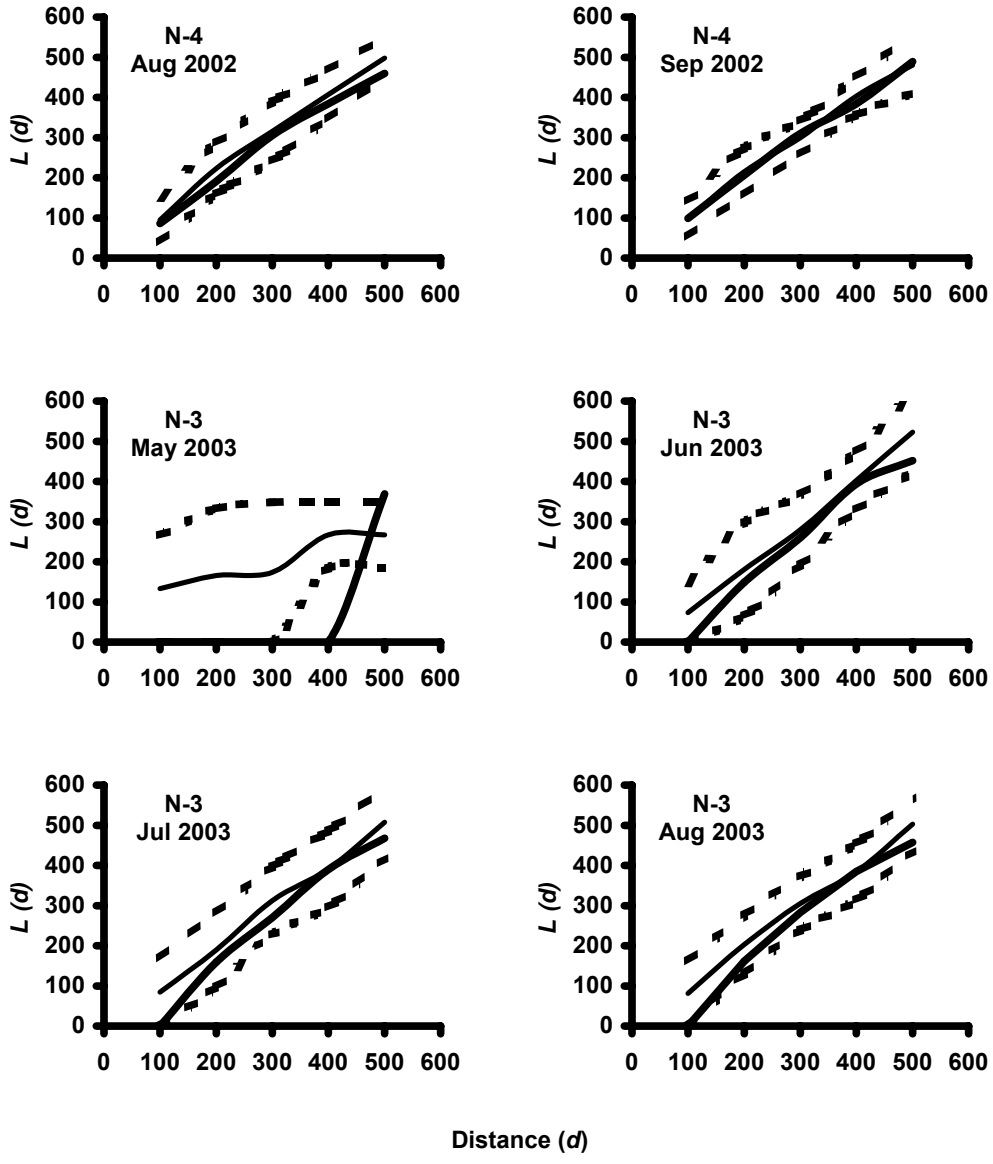

N-7
Sept 2003
N-7
Oct 2003
L (d)
600
500
400
300
200
100
0
0 100 200 300 400 500 600
Distance (d)

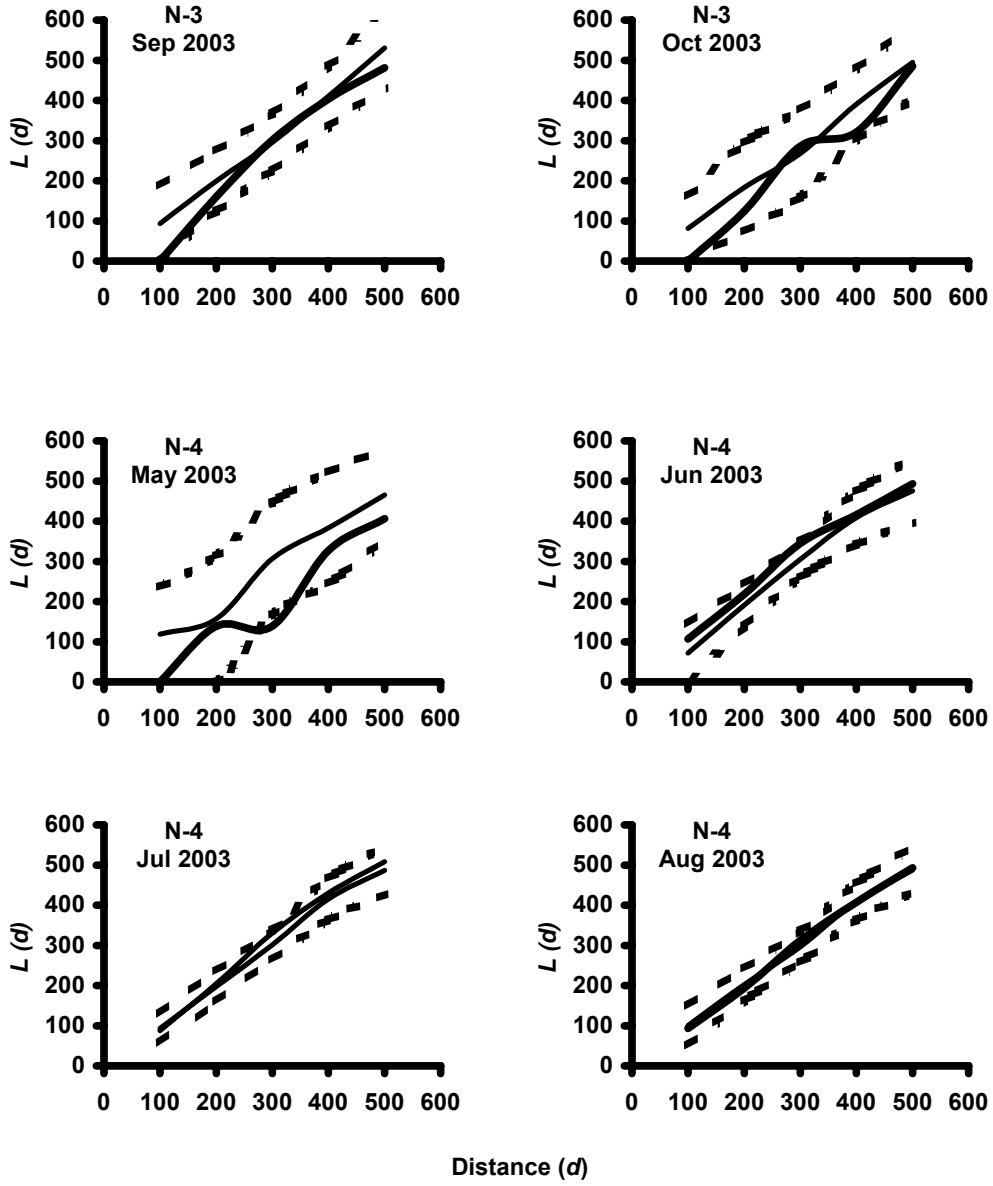
APPENDIX B

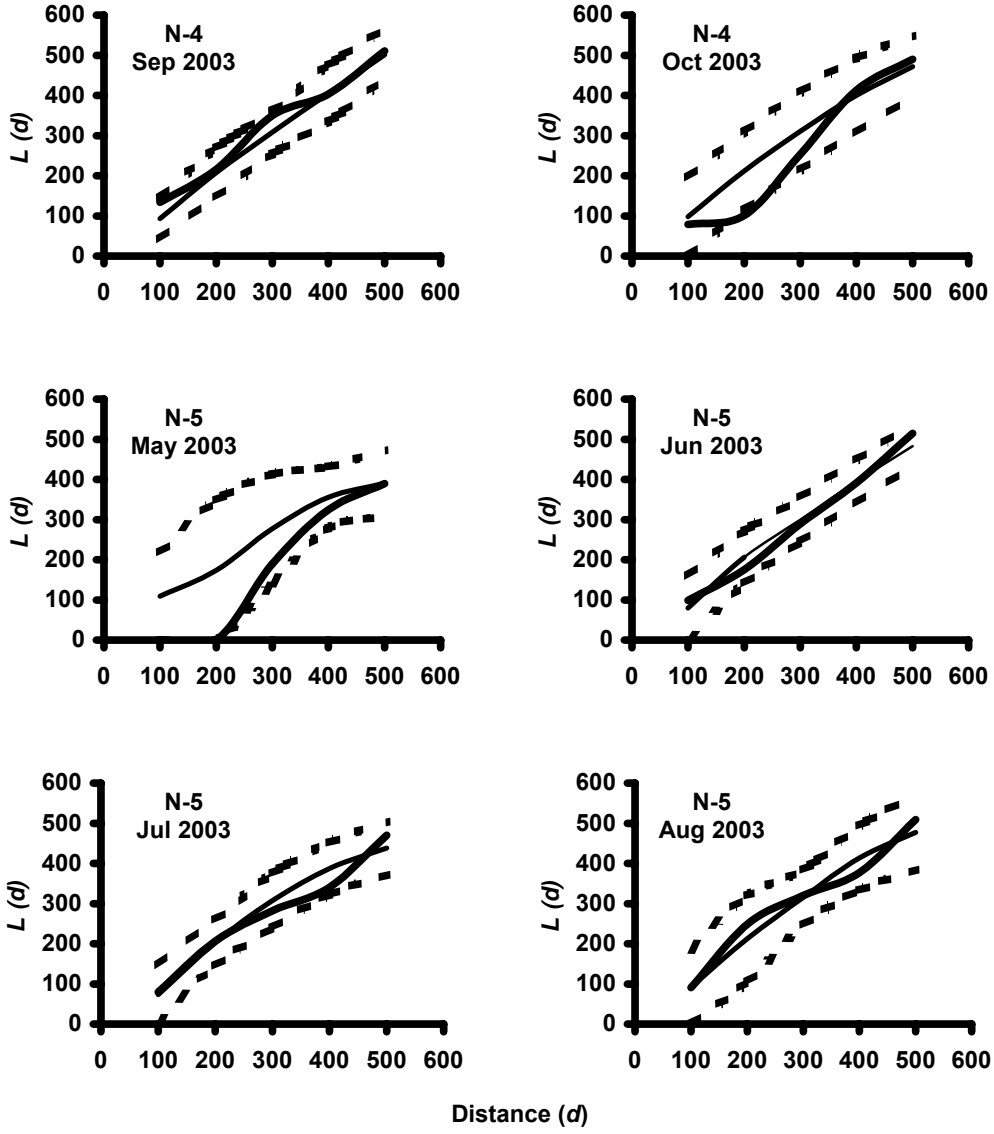
Fig. 2. Results of *K*-function analysis of the spatial distribution of *Ae. albopictus* larvae and pupae in neighborhoods receiving source reduction and not receiving source reduction by month for the 2002 and 2003 mosquito seasons.

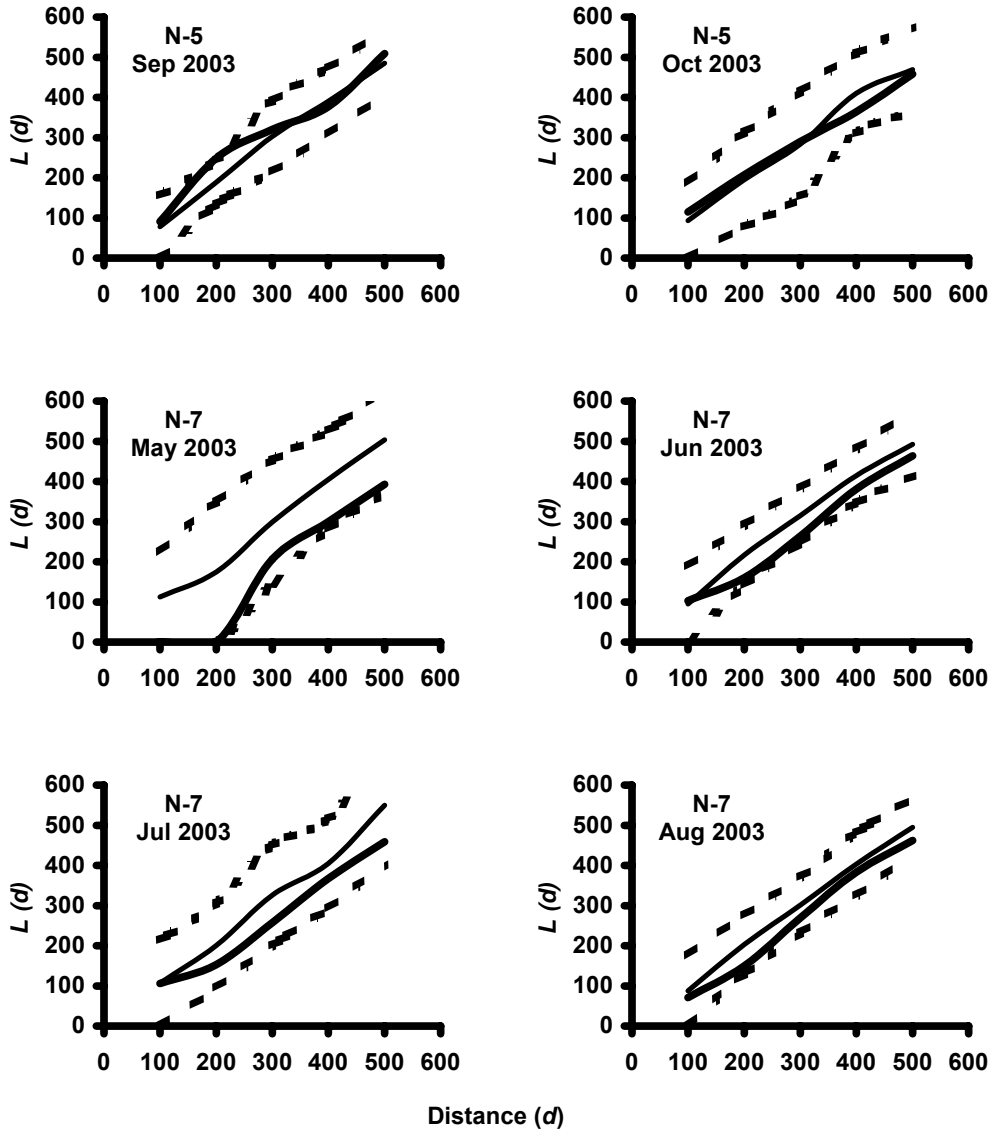
APPENDIX B

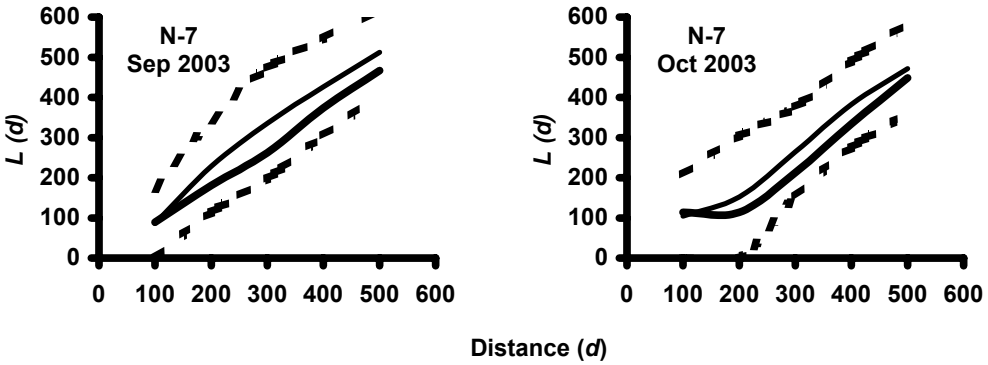

N-3
Jun 2002
N-3
Jul 2002
N-3
Aug 2002
N-3
Sept 2002
N-4
Jun 2002
N-4
Jul 2002
L (d)
0
100
200
300
400
500
600
Distance (d)


N-4
Aug 2002
N-4
Sep 2002
N-3
May 2003
N-3
Jun 2003
N-3
Jul 2003
N-3
Aug 2003
L (d)
0
100
200
300
400
500
600
Distance (d)


N-3
Sep 2003
N-3
Oct 2003
N-4
May 2003
N-4
Jun 2003
N-4
Jul 2003
N-4
Aug 2003
L (d)
0
100
200
300
400
500
600
Distance (d)


N-4
Sep 2003
N-4
Oct 2003
N-5
May 2003
N-5
Jun 2003
N-5
Jul 2003
N-5
Aug 2003
L (d)
0
100
200
300
400
500
600
Distance (d)

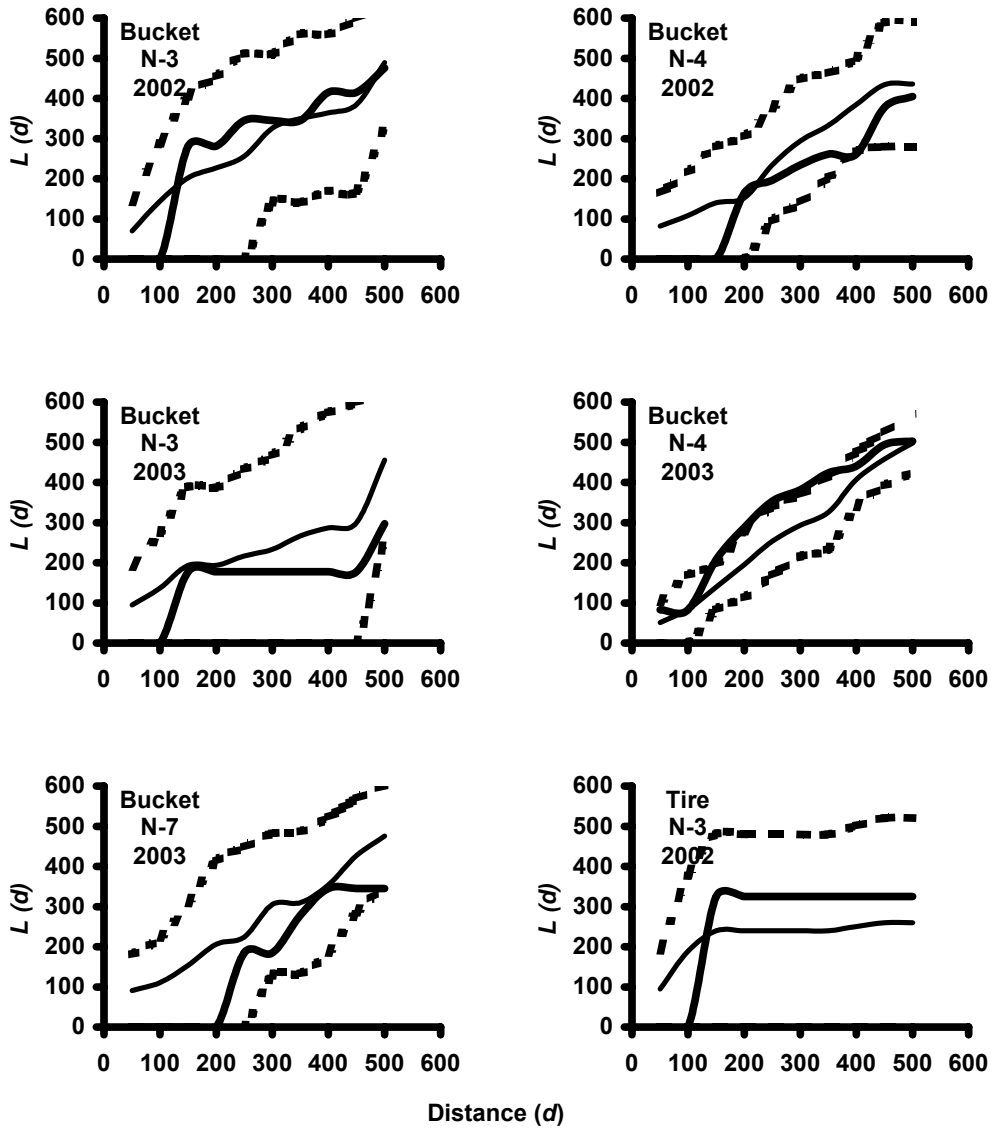

N-5
Sep 2003
N-5
Oct 2003
N-7
May 2003
N-7
Jun 2003
N-7
Jul 2003
N-7
Aug 2003
L (d)
0
100
200
300
400
500
600
Distance (d)

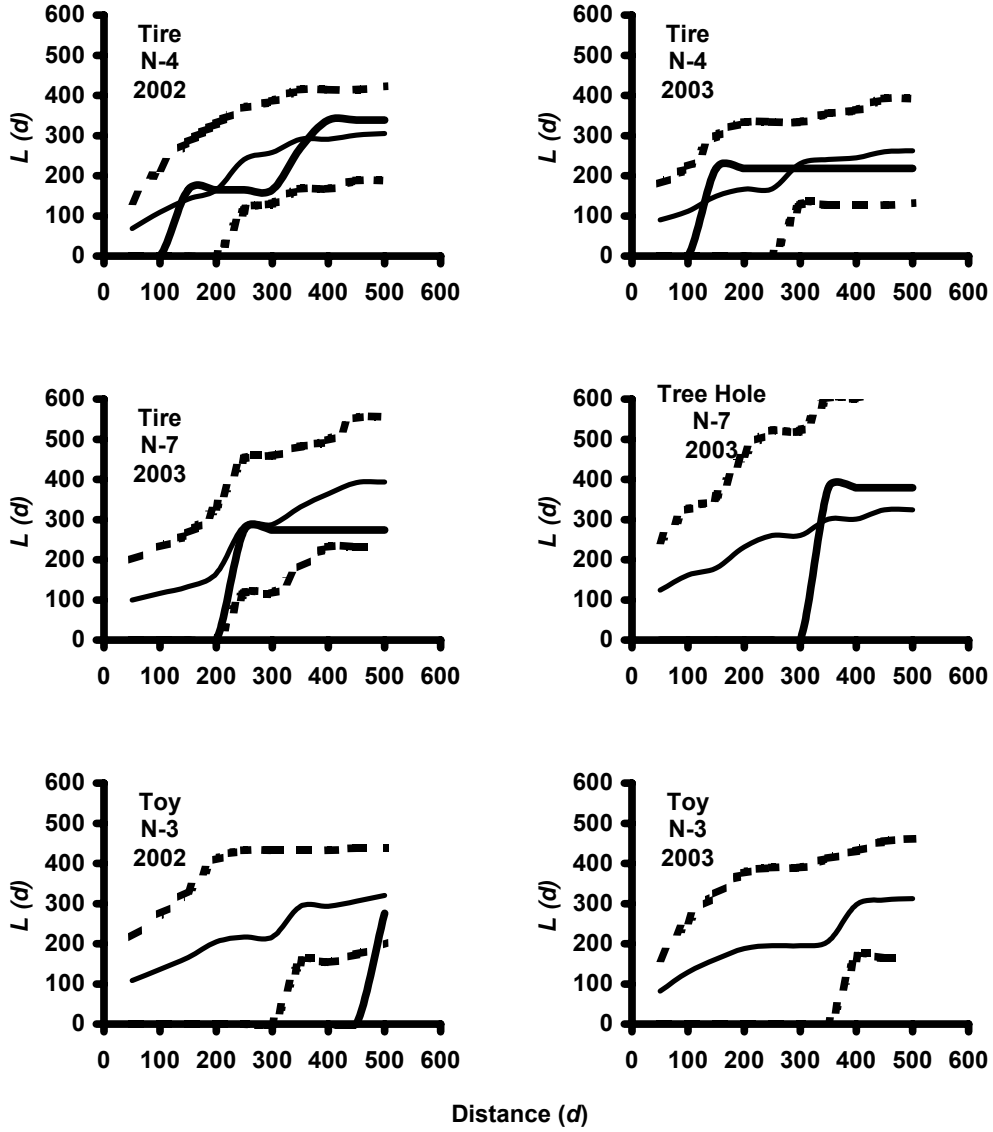

N-7
Sep 2003
N-7
Oct 2003
L (d)
600
500
400
300
200
100
0
0 100 200 300 400 500 600
Distance (d)

APPENDIX C

Fig. 3. Results of *K*-function analysis (second order) of the spatial distribution of *Ae. albopictus* pupae by container type in neighborhoods receiving source reduction (N-5, N-7) and not receiving source reduction (N-3, N-4) by month for the 2002 and 2003 mosquito seasons.

Appendix C


Bucket
N-3
2002
Bucket
N-4
2002
Bucket
N-3
2003
Bucket
N-4
2003
Bucket
N-7
2003
Tire
N-3
2002
L (d)
0
100
200
300
400
500
600
Distance (d)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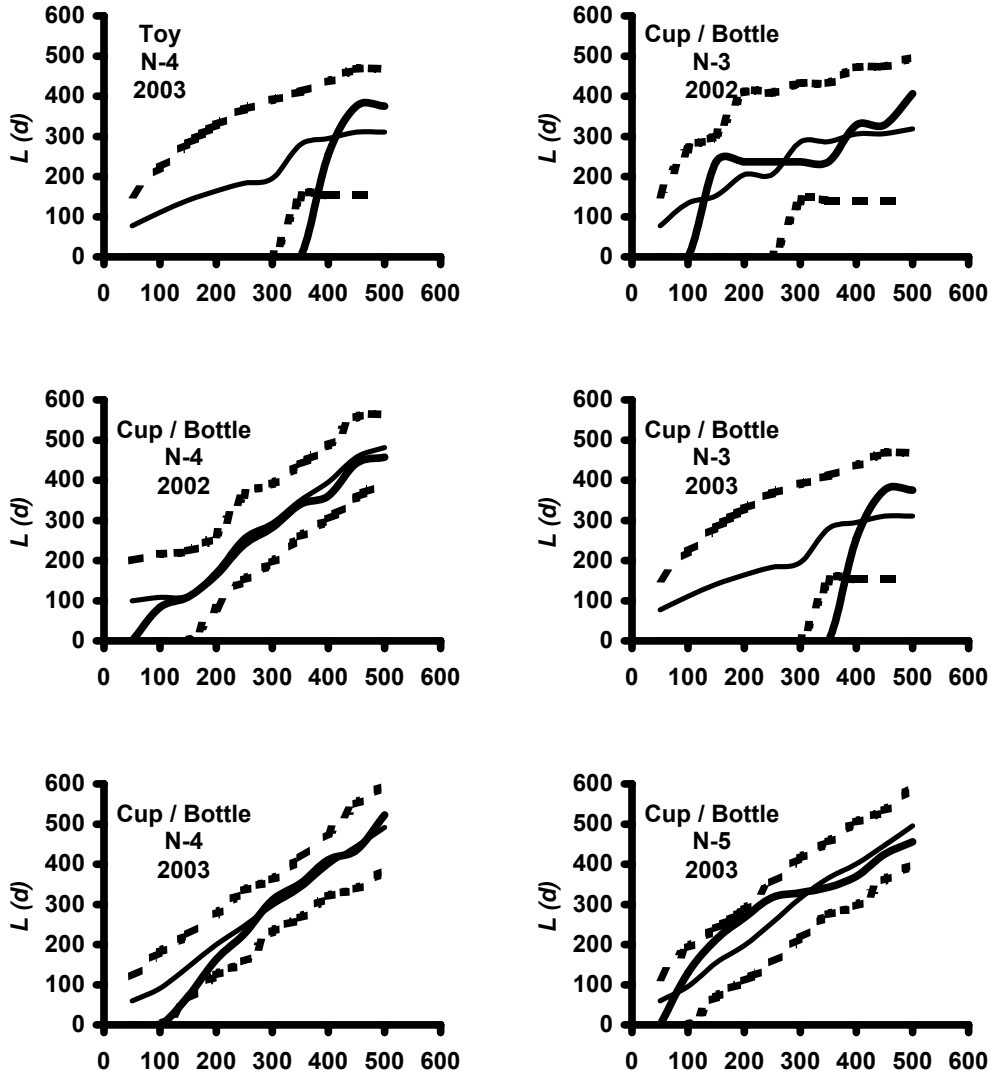
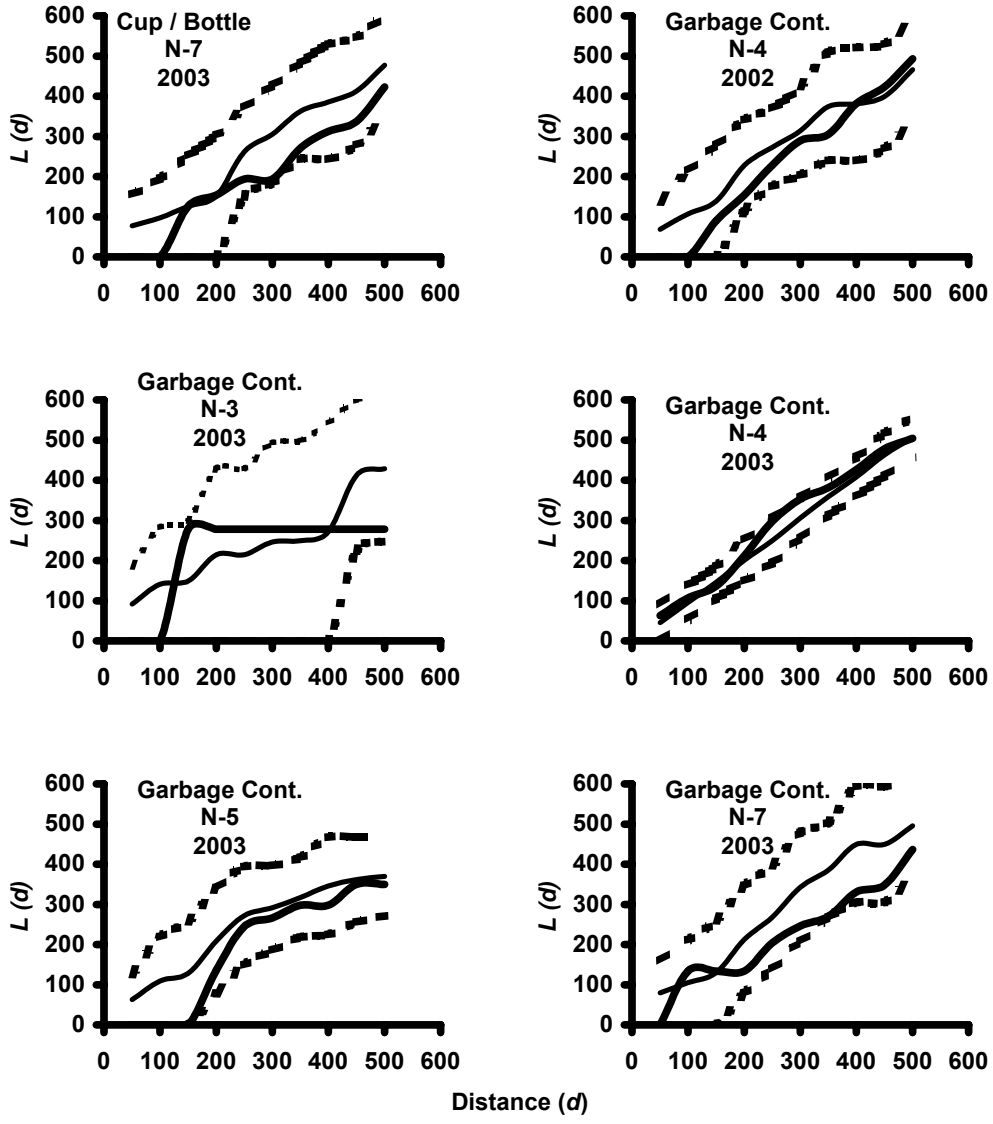
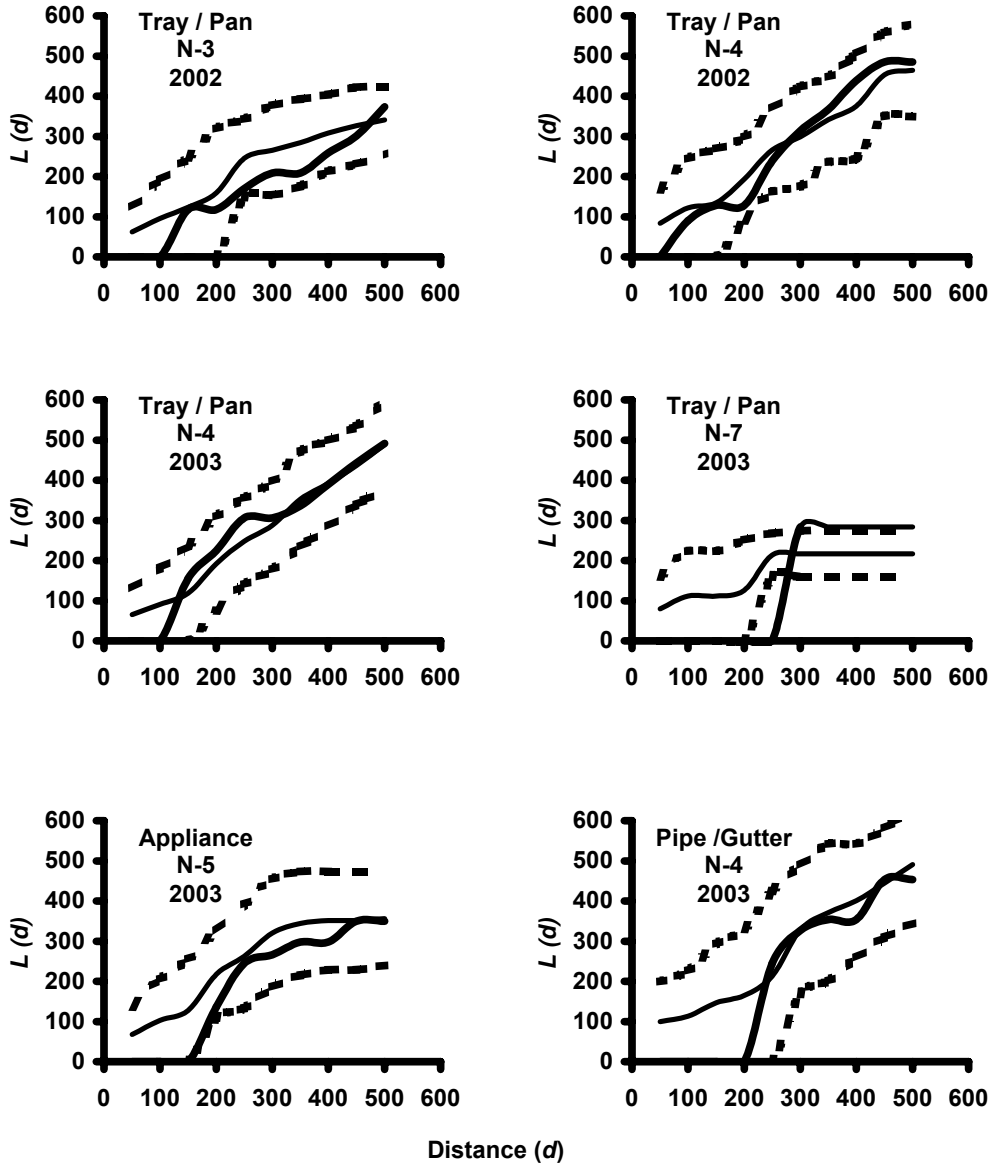
2002
Tire
N-4
2003
Tire
N-7
2003
Tree Hole
N-7
2003
Toy
N-3
2002
Toy
N-3
2003
L (d)
0
100
200
300
400
500
600
Distance (d)


Toy
N-4
2003
Cup / Bottle
N-3
2002
Cup / Bottle
N-4
2002
Cup / Bottle
N-3
2003
Cup / Bottle
N-4
2003
Cup / Bottle
N-5
2003
L (d)
Distance (d)
0
100
200
300
400
500
600


Cup / Bottle
N-7
2003
Garbage Cont.
N-4
2002
Garbage Cont.
N-3
2003
Garbage Cont.
N-4
2003
Garbage Cont.
N-5
2003
Garbage Cont.
N-7
2003
L (d)
Distance (d)
0
100
200
300
400
500
600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2002
Tray / Pan
N-4
2002
Tray / Pan
N-4
2003
Tray / Pan
N-7
2003
Appliance
N-5
2003
Pipe /Gutter
N-4
2003
L (d)
0
100
200
300
400
500
600
Distance (d)

APPENDIX D

Raw data summarized for each residence by treatment type, total pupal standing crop, numbers of pupae-positive containers, and numbers of larvae- or pupae-positive containers for *Ae. albopictus* in neighborhoods in 2002 (Table 1) and 2003 (Table 2).

Raw data summarized for each residence by treatment type and mean numbers of *Ae. albopictus* eggs per ovitrap-week in neighborhoods in 2002 (Table 3) and 2003 (Table 4).

Table 1. Summary of *Ae. albopictus* pupal standing crop and numbers of pupae-positive and larvae- or pupae-positive containers in neighborhoods during the 2002 mosquito season.

Address	Treatment	Pupal Standing Crop	Pupae-positive Containers	Pupae- or Larvae-positive Containers
512 Kirby St.	LO	2	1	1
516 Kirby St.	LO	92	19	33
518 Kirby St.	LO	1	1	3
601 Kirby St.	LO	2	1	2
605 Kirby St.	LO	23	1	1
611 Kirby St.	LO	20	1	1
613 Kirby St.	LO	50	3	5
615 Kirby St.	LO	0	0	1
617 Kirby St.	LO	0	0	2
625 Kirby. St.	LO	11	2	4
627 Kirby St.	LO	2	2	3
601 Bilyeu St.	LO	12	1	2
606 Kirby St.	LO	1	1	3
610 Kirby St.	LO	1	1	2
614 Kirby St.	LO	7	3	4
519 Bilyeu St.	LO	0	0	6
517 Bilyeu St.	LO	0	0	3
515 Bilyeu St.	LO	69	11	21
511 Bilyeu St.	LO	1	1	1
525 Kirby	LO	24	2	2
900 Method Rd.	LO	27	6	14
904 Method Rd.	LO	43	2	3
908 Method Rd.	LO	1	1	3
4102 Reavis Rd.	LO	44	11	21
4104 Reavis Rd.	LO	96	5	6
4106 Reavis Rd.	LO	3	1	1
907 Chaney St.	LO	11	2	2
903 Chaney St.	LO	33	1	2
901 Chaney St.	LO	17	2	3
902 Chaney St.	LO	60	10	14
908 Chaney St.	LO	9	3	3
906 Chaney St.	LO	3	1	2
910 Chaney St.	LO	87	4	5
4200 Reavis Rd.	LO	151	15	28
4202 Reavis Rd.	LO	8	2	2
4204 Reavis Rd.	LO	3	1	2
4206 Reavis Rd.	LO	18	5	9
4210 Reavis Rd.	LO	0	0	1
907 Lorimer Rd.	LO	72	10	14
905 Lorimer Rd.	LO	6	1	3
903 Lorimer Rd.	LO	55	4	6
901 Lorimer Rd.	LO	18	2	3

1003 Lorimer Rd.	LO	28	1	2
Apt.	LO	38	9	12
4101 Reavis Rd.	LO	79	4	5
1005 Chaney St.	LO	0	0	2
4130 Western Blvd.	LO	67	8	11
4208 Western Blvd.	LO	511	26	29
4204 Western Blvd.	LO	59	2	2
4202 Western Blvd.	LO	86	9	17
4200 Western Blvd.	LO	54	8	18
1006 Chaney St.	LO	124	2	2
4201 Reavis Rd.	LO	31	3	7
4203 Reavis Rd.	LO	4	2	4
4211 Reavis Rd.	LO	0	0	4
4217 Reavis Rd.	LO	51	3	5
900 Chaney St.	LO	0	0	1
4206 Western Blvd.	LO	9	1	4
5201 Olive Rd.	NO-TRT	115	7	12
5200 Olive Rd.	NO-TRT	45	2	5
5202 Olive Rd.	NO-TRT	13	1	1
5204 Olive Rd.	NO-TRT	1	1	2
5208 Olive Rd.	NO-TRT	0	0	2
5316 Olive Rd.	NO-TRT	16	4	5
5224 Olive Rd.	NO-TRT	44	2	8
5228 Olive Rd.	NO-TRT	52	7	9
5300 Olive Rd.	NO-TRT	19	3	6
5302 Olive Rd.	NO-TRT	5	2	5
5304 Olive Rd.	NO-TRT	40	1	5
5305 Olive Rd.	NO-TRT	24	2	3
5231 Olive Rd.	NO-TRT	19	3	4
5221 Olive Rd.	NO-TRT	124	15	25
5207 Olive Rd.	NO-TRT	49	11	15
5317 Olive Rd.	NO-TRT	32	9	17
5329 Olive Rd.	NO-TRT	43	7	12
5327 Olive Rd.	NO-TRT	1	1	1
5328 Olive Rd.	NO-TRT	1	1	2
5229 Olive Rd.	NO-TRT	43	1	1
5337 Olive Rd.	NO-TRT	4	2	4
5222 Olive Rd.	NO-TRT	10	1	1
5321 Olive Rd.	NO-TRT	1	1	1
102 Pineland St.	NO-TRT	249	13	17
104 Pineland St.	NO-TRT	24	3	3
108 Pineland St.	NO-TRT	0	0	1
112 Pineland St.	NO-TRT	18	3	4
120 Pineland St.	NO-TRT	48	5	8
128 Pineland St.	NO-TRT	1	1	4
132 Pineland St.	NO-TRT	16	1	2
105 Pineland St.	NO-TRT	170	26	36

109 Pineland St.	NO-TRT	7	2	5
113 Pineland St.	NO-TRT	80	3	4
125 Pineland St.	NO-TRT	7	1	3
137 Pineland St.	NO-TRT	1	1	2
147 Pineland St.	NO-TRT	145	3	4
4406 Graceland St.	NO-TRT	11	1	1
4410 Graceland St.	NO-TRT	6	1	1
4411 Graceland St.	NO-TRT	32	5	7
4409 Graceland St.	NO-TRT	3	2	2
4405 Graceland St.	NO-TRT	13	1	2
4402 Bleeker St.	NO-TRT	13	2	8
4406 Bleeker St.	NO-TRT	1	1	1
4412 Bleeker St.	NO-TRT	47	5	6
4413 Bleeker St.	NO-TRT	122	4	5
4405 Bleeker St.	NO-TRT	1	1	1
4403 Bleeker St.	NO-TRT	74	6	8
157 Pineland St.	NO-TRT	5	1	1
5012 Western Blvd.	NO-TRT	28	3	7
5008 Western Blvd.	NO-TRT	8	1	3
4400 Graceland St.	NO-TRT	6	1	1
103 Pineland St.	NO-TRT	0	0	3
133 Pineland St.	NO-TRT	28	5	8
4401 Graceland St.	NO-TRT	0	0	2

Table 2. Summary of *Ae. albopictus* pupal standing crop and numbers of pupae-positive and larvae- or pupae-positive containers in neighborhoods during the 2003 mosquito season.

Address	Treatment	Pupal Standing Crop	Pupae-positive Containers	Pupae- or Larvae-positive Containers
512 Kirby St.	LO	63	10	11
516 Kirby St.	LO	67	14	30
518 Kirby St.	LO	38	2	4
520 Kirby St.	LO	1	1	3
605 Kirby St.	LO	25	4	4
609 Kirby St.	LO	3	1	2
611 Kirby St.	LO	61	4	6
613 Kirby St.	LO	10	4	19
615 Kirby St.	LO	115	7	12
617 Kirby St.	LO	80	25	56
619 Kirby St.	LO	4	1	4
621 Kirby St.	LO	7	2	7
625 Kirby St.	LO	24	6	10
627 Kirby St.	LO	271	16	28
601 Bilyeu St.	LO	44	3	5
603 Bilyeu St	LO	29	2	3
606 Kirby St.	LO	11	3	6
608 Kirby St.	LO	4	1	1
610 Kirby St.	LO	8	4	6
614 Kirby St.	LO	0	0	2
519 Bilyeu St.	LO	16	2	5
517 Bilyeu St.	LO	15	2	4
515 Bilyeu St.	LO	435	70	101
513 Bilyeu St.	LO	0	0	1
511 Bilyeu St.	LO	265	19	26
900 Method Rd.	LO	95	6	10
908 Method Rd.	LO	0	0	2
4100 Reavis Rd.	LO	23	3	6
4102 Reavis Rd.	LO	27	7	9
4104 Reavis Rd.	LO	97	6	9
4106 Reavis Rd.	LO	4	1	3
907 Chaney St.	LO	23	5	6
905 Chaney St.	LO	10	1	3
903 Chaney St.	LO	1	1	5
902 Chaney St.	LO	194	27	49
908 Chaney St.	LO	0	0	2
906 Chaney St.	LO	50	8	14
910 Chaney St.	LO	9	3	6
4200 Reavis Rd.	LO	113	12	23
4202 Reavis Rd.	LO	101	11	16
4206 Reavis Rd.	LO	1	1	2
4208 Reavis Rd.	LO	21	1	3

913 Lorimer Rd.	LO	9	2	4
907 Lorimer Rd.	LO	209	24	39
905 Lorimer Rd.	LO	89	6	11
903 Lorimer Rd.	LO	139	12	19
901 Lorimer Rd.	LO	185	21	26
1003 Lorimer Rd.	LO	151	5	7
Apt.	LO	599	58	75
4101 Reavis Rd.	LO	0	0	2
4111 Reavis Rd.	LO	67	5	5
1005 Chaney St.	LO	3	2	3
1011 Chaney St.	LO	48	4	6
4128 Western Blvd.	LO	7	1	3
4130 Western Blvd.	LO	63	11	17
4208 Western Blvd.	LO	98	15	24
4204 Western Blvd.	LO	0	0	2
4202 Western Blvd.	LO	66	7	17
4200 Western Blvd.	LO	97	5	8
1006 Chaney St.	LO	1	1	1
4201 Reavis Rd.	LO	286	13	19
4203 Reavis Rd.	LO	55	9	20
4211 Reavis Rd.	LO	42	10	17
4217 Reavis Rd.	LO	21	2	7
5304 Old Avent Ferry Rd.	NO-TRT	56	4	8
4711 Highland Rd.	NO-TRT	1	1	1
4707 Highland Rd.	NO-TRT	83	8	9
5212 Old Avent Ferry Rd.	NO-TRT	23	9	23
5208 Old Avent Ferry Rd.	NO-TRT	94	16	19
5204 Old Avent Ferry Rd.	NO-TRT	30	12	37
5200 Old Avent Ferry Rd.	NO-TRT	19	3	6
4705 Green Valley Rd.	NO-TRT	5	1	1
5201 Olive Rd.	NO-TRT	6	3	8
5200 Olive Rd.	NO-TRT	277	9	15
5206 Olive Rd.	NO-TRT	135	12	16
5208 Olive Rd.	NO-TRT	3	1	4
5216 Olive Rd.	NO-TRT	2	1	6
5224 Olive Rd.	NO-TRT	20	5	6
5300 Olive Rd.	NO-TRT	1	1	6
5302 Olive Rd.	NO-TRT	2	2	4
5304 Olive Rd.	NO-TRT	40	8	12
5305 Olive Rd.	NO-TRT	21	7	10
5235 Olive Rd.	NO-TRT	9	1	1
5221 Olive Rd.	NO-TRT	106	23	35
5207 Olive Rd.	NO-TRT	254	21	31
5329 Olive Rd.	NO-TRT	1	1	1
102 Pineland St.	NO-TRT	45	13	23
108 Pineland St.	NO-TRT	20	4	7
112 Pineland St.	NO-TRT	2	1	2

120 Pineland St.	NO-TRT	20	3	9
124 Pineland St.	NO-TRT	19	5	5
128 Pineland St.	NO-TRT	27	4	5
132 Pineland St.	NO-TRT	11	1	1
109 Pineland St.	NO-TRT	141	14	16
113 Pineland St.	NO-TRT	167	3	9
125 Pineland St.	NO-TRT	22	2	7
137 Pineland St.	NO-TRT	34	7	10
147 Pineland St.	NO-TRT	6	4	6
140 Pineland St.	NO-TRT	155	11	22
4402 Graceland St.	NO-TRT	16	1	1
4406 Graceland St.	NO-TRT	0	0	2
4410 Graceland St.	NO-TRT	120	6	7
4411 Graceland St.	NO-TRT	37	2	4
4409 Graceland St.	NO-TRT	4	2	2
4405 Graceland St.	NO-TRT	6	2	2
146 Pineland St.	NO-TRT	35	4	9
4402 Bleeker St.	NO-TRT	70	8	14
4406 Bleeker St.	NO-TRT	32	5	13
4412 Bleeker St.	NO-TRT	29	12	29
4413 Bleeker St.	NO-TRT	150	22	25
4403 Bleeker St.	NO-TRT	13	3	5
169 Pineland St.	NO-TRT	14	3	4
5012 Western Blvd.	NO-TRT	12	5	8
5008 Western Blvd.	NO-TRT	14	3	5
326 Powell Dr.	SR	74	12	23
318 Powell Dr.	SR	7	3	4
314 Powell Dr.	SR	9	2	2
304 Carolina Ave.	SR	0	0	3
306 Carolina Ave.	SR	16	4	5
411 Heather St.	SR	185	28	45
408 Heather St.	SR	125	7	9
406 Heather St.	SR	24	8	17
401 Carolina Ave.	SR	15	3	3
404 Carolina Ave.	SR	18	1	2
406 Carolina Ave.	SR	51	5	9
407 Carolina Ave.	SR	0	0	2
409 Cariolina Ave.	SR	0	0	1
415 Carolina Ave.	SR	25	5	10
5408 Western Blvd.	SR	21	3	4
5404 Western Blvd.	SR	10	1	3
5400 Western Blvd.	SR	15	3	13
5310 Western Blvd.	SR	137	23	48
5300 Western Blvd.	SR	13	3	3
5210 Western Blvd.	SR	5	1	1
100 Singleton Rd.	SR	28	5	12
108 Singleton Rd.	SR	4	1	2

116 Singleton Rd.	SR	37	1	2
120 Singleton Rd.	SR	156	39	66
200 Singleton Rd.	SR	26	3	6
212 Singleton Rd.	SR	29	4	6
216 Singleton Rd.	SR	78	9	15
220 Singleton Rd.	SR	360	34	63
224 Singleton Rd.	SR	108	18	33
226 Singleton Rd.	SR	0	0	1
240 Singleton Rd.	SR	55	8	17
239 Singleton Rd.	SR	29	5	11
233 Singleton Rd.	SR	8	2	3
223 Singleton Rd.	SR	5	2	5
217 Singleton Rd.	SR	14	2	2
213 Singleton Rd.	SR	136	16	37
5809 Kimbal St.	SR	128	27	40
5806 Kimbal St.	SR	95	5	9
5801 Kimbal St.	SR	158	17	32
5800 Kimbal St.	SR	48	5	7
213 Irelan St.	SR	389	28	45
217 Irelan St.	SR	0	0	1
225 Irelan St.	SR	0	0	1
40 Bashford Rd.	SR+LO	308	56	103
612 Ramona Rd.	SR+LO	3	2	5
613 Ramona Rd.	SR+LO	72	17	26
609 Ramona Rd.	SR+LO	7	2	2
6216 Myra Rd.	SR+LO	0	0	1
6220 Myra Rd.	SR+LO	4	4	7
6300 Myra Rd.	SR+LO	0	0	1
6302 Myra Rd.	SR+LO	4	1	4
605 Hurley St.	SR+LO	176	9	18
6403 Myra Rd.	SR+LO	4	3	4
511 Glosson St.	SR+LO	148	32	58
505 Glosson St.	SR+LO	22	6	16
508 Glosson St.	SR+LO	21	5	10
506 Glosson St.	SR+LO	127	10	15
405 Canal St.	SR+LO	138	22	35
409 Canal St.	SR+LO	29	6	8
6304 Garrett St.	SR+LO	17	5	6
6302 Garrett St.	SR+LO	25	4	5
6216 Garrett St.	SR+LO	9	1	6
6212 Garrett St.	SR+LO	10	5	6
6208 Garrett St.	SR+LO	65	7	15
6204 Garrett St.	SR+LO	33	6	12
6221 Garrett St.	SR+LO	41	10	23
6213 Garrett St.	SR+LO	90	18	38
6209 Garrett St.	SR+LO	13	4	5
6205 Garrett St.	SR+LO	5	2	8

501 Strother St.	SR+LO	208	31	56
507 Strother St.	SR+LO	160	30	54
511 Strother St.	SR+LO	1	1	2
515 Strother St.	SR+LO	4	1	1
5817 Hillsborough Rd.	SR+LO	57	8	10
14 Oakdale Rd.	SR+LO	8	2	8
19 Oakdale Rd.	SR+LO	2	1	3
23 Oakdale Rd.	SR+LO	16	2	5
25 Oakdale Rd.	SR+LO	5	1	3
31 Oakdale Rd.	SR+LO	39	11	17
5708 Waycross Rd.	SR+LO	1	1	2
5706 Waycross Rd.	SR+LO	57	4	8
5809 Waycross Rd.	SR+LO	17	4	14
5805 Waycross Rd.	SR+LO	17	2	3
5801 Waycross Rd.	SR+LO	0	0	1
5723 Waycross Rd.	SR+LO	130	40	83
5719 Waycross Rd.	SR+LO	106	6	9
5715 Waycross Rd.	SR+LO	13	2	3
5711 Waycross Rd.	SR+LO	35	3	7
5703 Waycross Rd.	SR+LO	2	1	3
24 Burton Rd.	SR+LO	0	0	3
20 Burton Rd.	SR+LO	1	1	5
18 Burton Rd.	SR+LO	46	2	5
16 Burton Rd.	SR+LO	89	9	16
27 Burton Rd.	SR+LO	8	1	2
25 Burton Rd.	SR+LO	102	8	10
23 Burton Rd.	SR+LO	25	5	7
5669 Hillsborough Rd.	SR+LO	1	1	1

Table 3. Mean number of *Ae. albopictus* eggs per ovitrap-week in neighborhoods during the 2002 mosquito season.

Address	Treatment	Mean no. Eggs per Ovitrap-week
512 Kirby St.	LO	32.63
516 Kirby St.	LO	49.58
518 Kirby St.	LO	42.01
520 Kirby St.	LO	74.45
522 Kirby St.	LO	91.77
601 Kirby St.	LO	63.62
605 Kirby St.	LO	98.41
609 Kirby St.	LO	34.10
611 Kirby St.	LO	42.31
613 Kirby St.	LO	94.69
615 Kirby St.	LO	38.54
617 Kirby St.	LO	39.88
619 Kirby St.	LO	12.98
621 Kirby St.	LO	64.92
625 Kirby St.	LO	73.83
627 Kirby St.	LO	52.10
601 Bilyeu St.	LO	52.92
603 Bilyeu St.	LO	10.29
606 Kirby St.	LO	113.76
608 Kirby St.	LO	57.03
610 Kirby St.	LO	99.24
614 Kirby St.	LO	105.57
519 Bilyeu St.	LO	3.67
517 Bilyeu St.	LO	31.19
515 Bilyeu St.	LO	18.63
513 Bilyeu St.	LO	42.92
621 Kirby St.	LO	175.00
900 Method Rd.	LO	67.78
904 Method Rd.	LO	38.47
908 Method Rd.	LO	58.35
912 Method Rd.	LO	57.51
4100 Reavis Rd.	LO	50.02
4102 Reavis Rd.	LO	70.11
4104 Reavis Rd.	LO	45.27
4106 Reavis Rd.	LO	87.26
907 Chaney St.	LO	41.26
905 Chaney St.	LO	53.37
903 Chaney St.	LO	39.57
901 Chaney St.	LO	71.46
902 Chaney St.	LO	62.01
904 Chaney St.	LO	76.18
908 Chaney St.	LO	86.67
906 Chaney St.	LO	50.88

910 Chaney St.	LO	45.99
4200 Reavis Rd.	LO	41.38
4202 Reavis Rd.	LO	71.79
4204 Reavis Rd.	LO	72.32
4206 Reavis Rd.	LO	85.49
4208 Reavis Rd.	LO	91.98
913 Lorimer Rd.	LO	51.69
909 Lorimer Rd.	LO	0.00
907 Lorimer Rd.	LO	115.29
905 Lorimer Rd.	LO	31.68
903 Lorimer Rd.	LO	25.65
901 Lorimer Rd.	LO	69.54
1003 Lorimer Rd.	LO	57.60
Apt.	LO	89.05
4101 Reavis Rd.	LO	21.45
4111 Reavis Rd.	LO	50.33
1005 Chaney St.	LO	35.33
1011 Chaney St.	LO	0.00
4128 Western Blvd.	LO	81.01
4130 Western Blvd.	LO	89.51
4208 Western Blvd.	LO	57.56
4204 Western Blvd.	LO	21.58
4202 Western Blvd.	LO	48.74
4200 Western Blvd.	LO	83.76
1006 Chaney St.	LO	86.55
4203 Reavis Rd.	LO	49.04
4211 Reavis Rd.	LO	32.71
4217 Reavis Rd.	LO	53.26
903 Method	LO	24.20
4212 Reavis Rd.	LO	0.00
5201 Olive Rd.	No-TRT	44.90
5200 Olive Rd.	NO-TRT	69.44
5204 Olive Rd.	NO-TRT	60.92
5208 Olive Rd.	NO-TRT	58.32
5224 Olive Rd.	NO-TRT	46.89
5228 Olive Rd.	NO-TRT	59.19
5300 Olive Rd.	NO-TRT	29.63
5302 Olive Rd.	NO-TRT	47.56
5304 Olive Rd.	NO-TRT	36.72
5305 Olive Rd.	NO-TRT	69.80
5231 Olive Rd.	NO-TRT	91.06
5221 Olive Rd.	NO-TRT	32.54
5207 Olive Rd.	NO-TRT	106.49
5317 Olive Rd.	NO-TRT	59.04
5329 Olive Rd.	NO-TRT	45.39
5328 Olive Rd.	NO-TRT	60.07
5337 Olive Rd.	NO-TRT	47.60

5401 Olive Rd.	NO-TRT	109.04
102 Pineland St.	NO-TRT	39.68
104 Pineland St.	NO-TRT	38.50
108 Pineland St.	NO-TRT	33.49
112 Pineland St.	NO-TRT	67.65
120 Pineland St.	NO-TRT	84.94
124 Pineland St.	NO-TRT	88.42
128 Pineland St.	NO-TRT	90.39
132 Pineland St.	NO-TRT	73.46
105 Pineland St.	NO-TRT	68.13
109 Pineland St.	NO-TRT	49.50
113 Pineland St.	NO-TRT	79.27
125 Pineland St.	NO-TRT	43.70
137 Pineland St.	NO-TRT	53.93
147 Pineland St.	NO-TRT	45.72
4402 Graceland St.	NO-TRT	34.38
4411 Graceland St.	NO-TRT	83.61
4405 Graceland St.	NO-TRT	87.78
4412 Bleeker St.	NO-TRT	60.74
4405 Bleeker St.	NO-TRT	86.14
4403 Bleeker St.	NO-TRT	35.52
157 Pineland St.	NO-TRT	73.10
169 Pineland St.	NO-TRT	72.68
5100 Western Blvd.	NO-TRT	61.51
5012 Western Blvd.	NO-TRT	62.66
5008 Western Blvd.	NO-TRT	58.63
5006 Western Blvd.	NO-TRT	67.50
4400 Graceland St.	NO-TRT	70.77
4401 Graceland St.	NO-TRT	58.97

Table 4. Mean number of *Ae. albopictus* eggs per ovitrap-week in neighborhoods during the 2003 mosquito season.

Address	Treatment	Mean no. eggs per ovitrap-week
512 Kirby St.	LO	14.06
516 Kirby St.	LO	64.63
518 Kirby St.	LO	19.79
520 Kirby St.	LO	77.43
522 Kirby St.	LO	45.40
601 Kirby St.	LO	37.54
605 Kirby St.	LO	124.43
609 Kirby St.	LO	121.34
611 Kirby St.	LO	145.09
613 Kirby St.	LO	88.57
615 Kirby St.	LO	40.89
617 Kirby St.	LO	31.66
619 Kirby St.	LO	16.66
621 Kirby St.	LO	30.63
625 Kirby St.	LO	79.44
627 Kirby St.	LO	51.54
601 Bilyeu St.	LO	78.64
603 Bilyeu	LO	15.52
606 Kirby St.	LO	118.71
608 Kirby St.	LO	121.00
610 Kirby St.	LO	104.54
614 Kirby St.	LO	32.77
519 Bilyeu St.	LO	62.35
517 Bilyeu St.	LO	63.68
515 Bilyeu St.	LO	56.92
513 Bilyeu St.	LO	91.50
511 Bilyeu	LO	28.69
900 Method Rd.	LO	55.35
904 Method Rd.	LO	62.10
908 Method Rd.	LO	56.04
912 Method Rd.	LO	65.38
4100 Reavis Rd.	LO	75.41
4102 Reavis Rd.	LO	46.51
4104 Reavis Rd.	LO	85.48
4106 Reavis Rd.	LO	50.85
907 Chaney St.	LO	46.62
905 Chaney St.	LO	41.64
903 Chaney St.	LO	91.51
902 Chaney St.	LO	3.82
904 Chaney St.	LO	49.73
908 Chaney St.	LO	97.99
906 Chaney St.	LO	48.38
910 Chaney St.	LO	26.29

4200 Reavis Rd.	LO	94.52
4202 Reavis Rd.	LO	61.19
4204 Reavis Rd.	LO	87.04
4206 Reavis Rd.	LO	53.01
913 Lorimer Rd.	LO	65.63
909 Lorimer Rd.	LO	102.01
907 Lorimer Rd.	LO	67.09
905 Lorimer Rd.	LO	36.45
903 Lorimer Rd.	LO	81.48
901 Lorimer Rd.	LO	64.18
1003 Lorimer Rd.	LO	38.75
Apt.	LO	89.40
4101 Reavis Rd.	LO	37.85
4111 Reavis Rd.	LO	84.35
5012 Western Blvd.	LO	38.81
5008 Western Blvd.	LO	34.44
4128 Western Blvd.	LO	68.32
4130 Western Blvd.	LO	93.12
4208 Western Blvd.	LO	90.64
4204 Western Blvd.	LO	73.44
4202 Western Blvd.	LO	89.40
4200 Western Blvd.	LO	64.12
1006 Chaney St.	LO	49.49
4203 Reavis Rd.	LO	73.90
4211 Reavis Rd.	LO	60.30
4217 Reavis Rd.	LO	52.58
5304 Old Avent Ferry Rd.	NO-TRT	25.29
4711 Highland Rd.	NO-TRT	47.04
4707 Highland Rd.	NO-TRT	46.21
5212 Old Avent Ferry Rd.	NO-TRT	88.16
5208 Old Avent Ferry Rd.	NO-TRT	66.21
5204 Old Avent Ferry Rd.	NO-TRT	37.77
5200 Old Avent Ferry Rd.	NO-TRT	165.41
5201 Olive Rd.	NO-TRT	73.41
5200 Olive Rd.	NO-TRT	56.25
5206 Olive Rd.	NO-TRT	74.91
5208 Olive Rd.	NO-TRT	22.26
5216 Olive Rd.	NO-TRT	83.30
5224 Olive Rd.	NO-TRT	89.54
5300 Olive Rd.	NO-TRT	118.17
5302 Olive Rd.	NO-TRT	85.83
5304 Olive Rd.	NO-TRT	66.17
5305 Olive Rd.	NO-TRT	23.33
5235 Olive Rd.	NO-TRT	25.37
5231 Olive Rd.	NO-TRT	69.12
5221 Olive Rd.	NO-TRT	49.50
5207 Olive Rd.	NO-TRT	84.03

102 Pineland St.	NO-TRT	46.09
104 Pineland St.	NO-TRT	48.55
108 Pineland St.	NO-TRT	45.82
112 Pineland St.	NO-TRT	83.39
120 Pineland St.	NO-TRT	51.74
124 Pineland St.	NO-TRT	78.79
128 Pineland St.	NO-TRT	56.84
132 Pineland St.	NO-TRT	57.45
109 Pineland St.	NO-TRT	54.08
113 Pineland St.	NO-TRT	56.31
125 Pineland St.	NO-TRT	61.19
137 Pineland St.	NO-TRT	44.18
147 Pineland St.	NO-TRT	24.93
140 Pineland St.	NO-TRT	42.55
4406 Graceland St.	NO-TRT	49.88
4410 Graceland St.	NO-TRT	24.10
4409 Graceland St.	NO-TRT	19.09
4405 Graceland St.	NO-TRT	56.97
4402 Bleeker St.	NO-TRT	28.77
4413 Bleeker St.	NO-TRT	45.29
4405 Bleeker St.	NO-TRT	61.27
4403 Bleeker St.	NO-TRT	48.16
157 Pineland St.	NO-TRT	70.28
169 Pineland St.	NO-TRT	69.82
5100 Western Blvd.	NO-TRT	47.92
5012 Western Blvd.	NO-TRT	54.33
5008 Western Blvd.	NO-TRT	73.22
5006 Western Blvd.	NO-TRT	70.63
326 Powell Dr.	SR	135.92
318 Powell Dr.	SR	38.09
314 Powell Dr.	SR	68.52
304 Carolina Ave.	SR	74.23
306 Carolina Ave.	SR	97.65
310 Carolina Ave.	SR	57.74
411 Heather St.	SR	93.12
408 Heather St.	SR	67.97
406 Heather St.	SR	76.15
401 Carolina Ave.	SR	82.16
404 Carolina Ave.	SR	57.46
406 Carolina Ave.	SR	51.93
407 Carolina Ave.	SR	72.60
409 Cariolina Ave.	SR	94.82
415 Carolina Ave.	SR	68.87
5408 Western Blvd.	SR	73.23
5404 Western Blvd.	SR	29.74
5400 Western Blvd.	SR	103.16
5310 Western Blvd.	SR	119.04

5308 Western Blvd.	SR	75.91
5300 Western Blvd.	SR	75.45
5214 Western Blvd.	SR	81.30
5210 Western Blvd.	SR	91.17
100 Singleton Rd.	SR	100.15
108 Singleton Rd.	SR	116.74
116 Singleton Rd.	SR	83.97
120 Singleton Rd.	SR	42.57
200 Singleton Rd.	SR	56.26
212 Singleton Rd.	SR	62.36
216 Singleton Rd.	SR	78.68
220 Singleton Rd.	SR	118.46
224 Singleton Rd.	SR	57.60
240 Singleton Rd.	SR	92.93
239 Singleton Rd.	SR	71.99
233 Singleton Rd.	SR	94.96
223 Singleton Rd.	SR	133.93
217 Singleton Rd.	SR	123.91
213 Singleton Rd.	SR	39.61
5809 Kimbal St.	SR	35.17
5806 Kimbal St.	SR	75.45
5801 Kimbal St.	SR	44.76
5800 Kimbal St.	SR	23.26
213 Irelan St.	SR	39.62
225 Irelan St.	SR	58.69
24 Burton Rd.	SR+LO	59.84
20 Burton Rd.	SR+LO	105.30
18 Burton Rd.	SR+LO	87.17
16 Burton Rd.	SR+LO	98.95
14 Burton Rd.	SR+LO	69.38
27 Burton Rd.	SR+LO	40.49
25 Burton Rd.	SR+LO	38.74
23 Burton Rd.	SR+LO	76.66
21 Burton Rd.	SR+LO	97.07
5669 Hillsborough Rd.	SR+LO	92.39
40 Bashford Rd.	SR+LO	103.64
612 Ramona Rd.	SR+LO	105.91
613 Ramona Rd.	SR+LO	50.91
609 Ramona Rd.	SR+LO	51.13
6216 Myra Rd.	SR+LO	40.54
6220 Myra Rd.	SR+LO	83.06
6302 Myra Rd.	SR+LO	48.94
605 Hurley St.	SR+LO	53.16
6403 Myra Rd.	SR+LO	34.13
515 Glosson St.	SR+LO	58.60
511 Glosson St.	SR+LO	43.88
505 Glosson St.	SR+LO	33.94

508 Glosson St.	SR+LO	39.24
506 Glosson St.	SR+LO	33.22
405 Canal St.	SR+LO	46.22
409 Canal St.	SR+LO	28.37
6304 Garrett St.	SR+LO	25.80
6302 Garrett St.	SR+LO	86.14
6216 Garrett St.	SR+LO	31.62
6212 Garrett St.	SR+LO	36.00
6208 Garrett St.	SR+LO	29.14
6204 Garrett St.	SR+LO	29.20
6221 Garrett St.	SR+LO	70.82
6213 Garrett St.	SR+LO	55.75
6209 Garrett St.	SR+LO	27.74
6205 Garrett St.	SR+LO	21.62
501 Strother St.	SR+LO	46.74
507 Strother St.	SR+LO	58.73
515 Strother St.	SR+LO	67.67