Evaluation of BG-Sentinel Trap as a Management Tool to Reduce *Aedes albopictus* Nuisance in an Urban Environment in Italy

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The Asian tiger mosquito, *Aedes albopictus*, has spread from its Asian origin to 5 continents. It has now been detected in at least 38 countries and has become established in 28 (Benedict et al. 2007, Caminade et al. 2012). In Europe, *Ae. albopictus* was first detected in Albania in 1979 and Italy in 1990. Since then, it has been reported in 18 other European countries: Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, France (including Corsica), Germany, Greece, Malta, Monaco, Montenegro, the Netherlands, San Marino, Serbia, Slovenia, Spain, Switzerland, Turkey, and Vatican City (ECDC 2009, Medlock et al. 2012, ECDC 2014). Following its importation into Italy through Genoa in 1990 (Dalla Pozza and Majori 1992), *Ae. albopictus* has spread throughout 22 provinces, mainly in the northeastern part of the country (Scholte and Schaffner, 2007). Italy is now the highest incidence in the Veneto and Friuli-Venezia Giulia regions, large parts of Lombardia and Emilia–Romagna, and coastal areas of central Italy (ECDC 2009).

**Aedes albopictus** is not only an annoying daytime-biting mosquito, it is also a proven vector of disease pathogens. This species has been shown to be a competent vector of at least 22 arboviruses, including dengue and chikungunya (Gratz 2004, Bonizzoni et al. 2013). The risk for local transmission in Europe is not simply theoretical, as was shown by the outbreak of chikungunya in the Emilia–Romagna region of Italy in 2007, where at least 205 cases were identified between July 4 and September 27, 2007 (Rezza et al. 2007). *Aedes albopictus* is also a competent vector for both species of the dog heartworm, *Dirofilaria immitis* Leidy and *D. repens* Railliet and Henry (Cancrini et al. 2003; Cancrini et al. 2007).

Once *Ae. albopictus* becomes established, it is very difficult to eliminate (Enserink 2008). Even attempts instituted shortly after discovery are often unsuccessful (Peacock et al. 1988, Wheeler et al. 2009). The few successful eradication campaigns reported have involved quick reactions with adequate resources directed toward very focal introductions (Eads 1972, Jardina et al. 2009). The few successful eradication campaigns reported have involved quick reactions with adequate resources directed toward very focal introductions (Eads 1972, Jardina et al. 2009). The few successful eradication campaigns reported have involved quick reactions with adequate resources directed toward very focal introductions (Eads 1972, Jardina et al. 2009). The few successful eradication campaigns reported have involved quick reactions with adequate resources directed toward very focal introductions (Eads 1972, Jardina et al. 2009). The few successful eradication campaigns reported have involved quick reactions with adequate resources directed toward very focal introductions (Eads 1972, Jardina et al. 2009). The few successful eradication campaigns reported have involved quick reactions with adequate resources directed toward very focal introductions (Eads 1972, Jardina et al. 2009). The
problem local mosquito control agencies face with *Ae. albopictus* is that it lives and breeds in very heterogeneous peridomestic habitats: discarded automobile tires, trash, tree holes, catch basins, ornamental plant saucers, rain barrels, rain gutters, and corrugated extension spouts (Hawley 1988, Reiter 1998, Pena et al. 2003, Richards et al. 2008, Unlu et al. 2014). These various types of breeding sites are often difficult to locate. In the Brazilian municipality of Cosmopolis, strong efforts to eliminate breeding sites led to a significant reduction in populations of the main dengue vector, *Ae. aegypti* (L.), but *Ae. albopictus* was not significantly impacted (Gomes et al. 2005). Richards et al. (2008) showed that source reduction carried out at monthly intervals achieved temporary suppression of *Ae. albopictus* immature mosquitoes, but container habitats refilled by rainfall or residential water were rapidly repopulated by mosquitoes emerging from such habitats (tarps, bird baths, and plant pot receptacles) and were difficult to eliminate.

An alternative approach to control local mosquito populations involves the use of attractive traps or insecticide-treated baits. This tactic has been shown to work well for *Hippelates* (eye gnats), tsetse flies, tabanids, and *Stomoxys* (stable flies) (Day and Sjogren 1994). Measures to control tsetse flies (Glossinidae) with traps or insecticide-treated targets have shown impressive results (Vale et al. 1986, Dransfield et al. 1990, Knols et al. 1993, Esterhuizen et al. 2006), but tsetse flies have an unusual life cycle compared with other Diptera and have an extremely low intrinsic rate of population increase (Hargrove 1988). Few studies have been conducted to assess the possibility of controlling mosquito populations by use of mosquito traps or insecticide-treated baits. In their controlled experiment in Brazil, Perich et al. (2003) demonstrated that the deployment of insecticide-treated ovitraps led to a significant reduction of larvae in known breeding sites of both *Ae. aegypti* and *Ae. albopictus*. In the USA, use of CO$_2$-baited Centers for Disease Control and Prevention (CDC) light traps led to significant reduction of the dark ricefield mosquito, *Psorophora columbiae* (Dyar and Knab) (Lothrop et al. 1993, Esterhuizen et al. 2006), but *Psorophora columbiae* seems to indicate that *Ae. albopictus* is a good candidate for a “control by traps” experiment. The BG-Sentinel mosquito trap, by virtue of its superior catch rates for Asian tiger mosquitoes of both sexes, as demonstrated in several studies (Kröckel et al. 2006, Meeraus et al. 2008, Drago et al. 2012), appears to be a promising tool for such an experiment. A preliminary study conducted in a greenhouse showed that the BG-Sentinel trap has the potential to eradicate an isolated population of *Ae. aegypti* (Almeida et al., 2010). The goal of our study was to determine if it is possible to reduce biting rates of *Ae. albopictus* and to reduce local populations in small defined areas with BG-Sentinel mosquito traps.

**MATERIALS AND METHODS**

**Study design, location, and duration**

In a prospective controlled experiment, we compared adult mosquito abundance by human landing collections (HLC) and ovitrap egg collections at 3 intervention sites (IS) and 3 matched control sites (CS). The study duration was 16 wk from the end of June to October 2008 (calendar weeks 26 to 41). All study sites were located in the municipality of Cesena, Emilia–Romagna region, Italy, and were therefore climatically comparable, with only modest variation in their altitude (23–45 m) above sea level. Intervention and control sites were matched for the criteria of urbanization and surface vegetation. Additionally, *Ae. albopictus* populations at all sites were well established based on ovitrap monitoring in previous years (C. Venturelli, unpublished data). Both sites of pair 1 were located on the periphery of Cesena, in districts characterized by single-family houses surrounded by gardens. The study sites of pair 2 were both situated in the urban cemetery of Cesena and separated by a distance of ~100 m. The paired cemetery sites were additionally separated from each other by a wall, measuring approximately 6 m high and 5 m wide. Sites of pair 3 were located closer to the center of Cesena in areas dominated by apartment houses. Figure 1 shows the location of the 3 paired sites in this study. All study sites were examined before the beginning of our investigation for potential or active *Ae. albopictus* breeding sites.

**Intervention**

Biogents BG-Sentinel mosquito traps (Biogents AG, Regensburg, Germany) were used as interventional traps. Based on the availability of electrical power, vegetation, and security, each trap was positioned to cover an area between 150 and 350 m$^2$. The BG-Sentinel is a collapsible white plastic cylindrical container with an open top,
which is covered and fitted with white gauze. Suspended in the center of the trap opening, there is a black cylinder with an attached catch bag. A12-V fan below the catch bag creates a draft that sucks in the mosquitoes. The air stream also creates an air current through and around the trap that is similar to convection currents generated by the human body. Each trap was equipped with a BG Lure (lactic acid, ammonia, and hexanoic acid). The lure has a lifetime of 5 months and produces an artificial cocktail of odor components that have been identified on human skin. No carbon dioxide was used in this study as a supplemental attractant. Intervention sites were supplied with 7 (IS1) or 8 (IS2 and IS3) BG-Sentinel mosquito traps. Interventional traps were continuously active during the entire study period and were placed around houses (IS1 and IS3), or in a network between graves at the cemetery (IS2), with intertrap distances between 5 and 10 m. Intervention traps were emptied daily, and the mosquitoes collected were identified, sexed, and counted. The matched control sites received no intervention measures.

**Outcome measurement**

The primary outcome of the study was the effect of BG-Sentinel mosquito traps on nuisance biting by *Ae. albopictus*, directly measured by HLC. The secondary outcome was measurement of the abundance of *Ae. albopictus* eggs through ovitraps. Human landing collections were initiated during wk 26 and continued through wk 41. Collections were performed once weekly at the beginning (wk 26–37) and twice weekly during the later course of the study (wk 38–41) for 1.5 h at each intervention and control site. A permanent HLC monitoring location that provided shade and wind protection was established at all 6 sites. In the intervention sites, the HLC monitoring location was established at a distance of at least 5 m from the nearest interventional trap. The same person performed all HLC measurements during late afternoon and early evening between 4 and 7 p.m. The order in which HLC measurements were performed at the 6 sites was randomized each week to avoid any time-of-day bias in biting activity. Mosquitoes landing on the investigator’s lower legs were collected in acrylic glass tubes and removed from the study site for identification and processing. Only mosquitoes actually caught were counted in the totals. Ovitraps for monitoring *Ae. albopticus* in Italy have been described by Bellini et al. (1996) and are in common and widespread use for detecting container-breeding mosquitoes, especially those in subgenus *Stegomyia*. Ovitraps were initiated during calendar wk 27 and continued through wk 41. Two ovitraps were placed at each of the 6 experimental sites and
monitored on a weekly basis for eggs of *Ae. albopictus*. The ovitraps were placed at ground level in shaded positions close to vegetation and separated by a distance of at least 10 m. Figure 2 illustrates IS1 with locations of the BG-Sentinel traps, ovitraps, and the position where HLC measurements were performed.

Statistical analyses: Statistical analyses were carried out with R, version 2.14.0 (R Development Core Team 2011). Considering the non-normal distribution of data from both the human landing and ovitrap collections (assessed visually by density curves plotted with R and statistically by Shapiro and Wilk’s W test for normality; Shapiro and Wilk 1965 [not shown]), nonparametric, one-sided Wilcoxon rank sum and signed rank tests (Wilcoxon 1945; Mann and Whitney 1947) were performed to examine statistically significant differences between intervention and control sites. To ascertain seasonal changes during the course of this investigation, the study duration was divided into 4 phases that were considered individually in addition to the overall data for analysis. An alpha level of 0.05 was chosen for significance, and to correct for multiple testing, the Benjamini and Hochberg false discovery rate (FDR) method (Benjamini and Hochberg 1995) was applied. Plots were prepared with R by use of the Plotrix library (Lemon 2006).

**Ethical considerations**

Human landing collections were conducted with the awareness of potential disease transmission by *Ae. albopictus* bites. The human collector was fully informed of potential risks, gave consent, and was in constant contact with local health authorities. During the course of this study, no transmission of mosquito-borne diseases was reported by local authorities for the area in which the study was conducted.

**RESULTS**

In total, 8,471 female and 4,973 male *Ae. albopictus* mosquitoes were caught in intervention traps. Figure 3 shows the weekly number of *Ae. albopictus* females removed from the population by traps at the intervention sites over the 16-wk study. Large numbers of *Culex* mosquitoes were also collected, especially during wk 26 through 32.

**Human landing collections**

A pronounced difference was observed in the number of mosquitoes landing per unit of time at the intervention sites compared to the control sites. The total number of female *Ae. albopictus* individuals collected in HLC over the course of the study at the intervention sites was 128 during 94.5 h of collection time, compared to 1,044 during 90.0 h at the control sites. This corresponds to an overall reduction in nuisance biting, directly measured by HLC, of 87%. At the beginning of the study, HLC measurements were only slightly different in both groups, but by wk 30, the HLC rates began to markedly increase in the control sites. Significantly fewer biting *Ae. albopictus* individuals were collected at all intervention sites during every phase of the study, with the exception of wk 26 to 29 in pair one, when no collections were made at the corresponding control site (CS1). Overall median numbers of biting females collected were 1 (range 0–14) and 11 (range 0–97) per 1.5 h at the intervention and control sites, respectively. The median numbers of HLC *Ae. albopictus* females for each of the paired sites for the 4 time phases and the combined number over the course of the study, with the corresponding and adjusted *P*-values, are shown in Table 1. Figure 4 shows the weekly median number (with interquartile range) of *Ae. albopictus* females captured in HLCs per 1.5 h collection period in intervention and control sites.
over the course of the study. Biting activity peaked during wk 36 at both the control and intervention sites. No collections were made during wk 37.

Ovitraps

In total, about 2,000 eggs were collected at the intervention sites, whereas about 6,000 eggs were collected at the control sites. This equates to a reduction of 64% in eggs deposited at sites with traps compared to sites without traps. Weekly collections of eggs deposited in ovitraps were divided into four phases: from wk 28 to 31, 32 to 35, 36 to 39, and 40 to 41 (Table 2). In the first phase, there were slightly more eggs collected at intervention sites compared to control sites (251 vs. 221). The median number of eggs collected at intervention sites was significantly lower compared to areas without traps in the 2nd and 4th phases and considerably lower, although not statistically significant, in the 3rd phase. Also, comparisons of the 2 cemetery sites (IS2 and CS2) with the lowest abundance yielded no statistically significant difference. Over the entire study, intervention sites collected significantly fewer *Ae. albopictus* eggs than control sites (2,147 vs. 6,002; *P* = 0.00009). The number of eggs laid in ovitraps peaked in wk 35 in the intervention sites and in wk 37 in the control sites. Figure 5 shows the weekly median number of *Ae. albopictus* eggs collected throughout the study.

**DISCUSSION**

This field study indicates that the use of efficient mosquito traps can reduce *Ae. albopictus* biting activity locally. A highly significant reduction of about 87% in HLCs was observed at intervention sites compared to sites where no traps were placed. This is a significant decrease in nuisance biting that could also potentially reduce exposure to vector-borne pathogens and lower the risk of disease transmission. Continued operation of traps throughout the mosquito season is critical to maintain this reduction in biting activity.
season might also have a long-term effect on the local mosquito population. At our intervention sites, the number of *Ae. albopictus* eggs laid in ovitraps was reduced by an average of 64% (range 49–66%) compared to control sites without traps. Considering the total numbers of eggs laid at intervention and control sites, the failure to show significance in single-site comparisons might be due to statistical-power issues. We hypothesize that improved trap performance could lower the number of traps needed to provide the desired protection. A recent study in Puerto Rico by Barrera et al. (2013) showed that by modifying the BG-Sentinel with a black outer covering and using a BG Lure, significantly more *Ae. aegypti*, *Ae. mediovittatus* (Coquillet), and *Culex quinquefasciatus* Say individuals were collected under field conditions than with the standard trap. The average trap density of 1 trap per 150–350 m² used by Barrera et al. (2013) was the same used in the present study. In our study, we also observed that trap placement is very important. Even in the small area of a typical Italian residence with garden, there were spots with higher mosquito densities and spots where mosquitoes occurred in lower numbers. It remains a challenge to define the common characteristics for optimum trap placement (Crepeau et al. 2013) under variable environmental conditions, and further studies are needed to determine the relationship between the number of traps deployed and the level of reduction in HLC. Randomization is an important strategy to control confounding variables in experiments. In our study, randomization of study sites was not possible due to infrastructural issues such as availability of electricity, access to properties, and acceptance by neighboring inhabitants. However, pairs of intervention and control sites were matched for selected characteristics to reduce the risk of baseline differences and to ensure comparability in effect-determining variables. Matching was performed on the basis of level of urbanization and attributed surface vegetation. Additionally, the study sites were all examined for active or potential breeding sites before our investigation began. At the cemetery sites, small

<table>
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<tr>
<th>Pair</th>
<th>Weeks</th>
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<th>Eggs n</th>
<th>Median (range)</th>
<th>Eggs n</th>
<th>Raw P</th>
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<td>112</td>
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<td>36 (7–102)</td>
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<td>73</td>
<td>52–277</td>
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<tr>
<td></td>
<td>38–41</td>
<td>0 (0–42)</td>
<td>73</td>
<td>8</td>
<td>6.5</td>
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<td>30</td>
<td>73 (0–277)</td>
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<tr>
<td>Overall</td>
<td>12.5 (0–158)</td>
<td>2147</td>
<td>90</td>
<td>28 (0–522)</td>
<td>6,002</td>
<td>83</td>
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</tbody>
</table>

* Eggs, total number of eggs counted; n, number of ovitraps considered; raw P, P-value retrieved by u-test.

* Significant after correction for multiple testing.

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![Fig. 5](image_url) Median number of *Aedes albopictus* eggs collected per week in ovitraps from intervention and control sites. Error bars represent the interquartile range.

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Table 2. Median number of *Ae. albopictus* eggs collected from ovitraps at paired intervention and control sites.¹
water containers, such as flower vases, had been filled with sand during an anti-breeding site action initiated by the local authorities just prior to our arrival. However, during the study, active breeding sites (water drains) were detected near or within IS2 but not at CS2. At IS1 and the matched CS1, no active or potential breeding sites were identified. At both IS3 and CS3, active breeding sites were detected during the study: Drains at the road near IS3 and drains at a car park near CS3 produced moderate to high numbers of *Ae. albopictus* larvae. These breeding sites might explain the extraordinarily high landing rates (a maximum of 97 in 1.5 h) at CS3 and the larger variance observed in the median HLC values during wk 34–36.

Mosquito traps have been key components of many mosquito control programs; however, they have been almost exclusively used as surveillance tools (Kline 2006). Only a few field studies have been conducted to evaluate traps as a means of reducing mosquito populations, and the majority of these involved floodwater or saltmarsh species. In California, the deployment of CO2-baited CDC light traps caused a significant reduction of biting *Ps. columbiae* around a date palm grove (Lothrop and Husted 1997). Kitau et al. (2010) demonstrated the effectiveness of Mosquito Magnet® Liberty Plus traps under semifield conditions to reduce human biting rates of previously released *Anopheles gambiae* Giles and *Cx. quinquefasciatus* in experimental screen enclosures in Tanzania. The use of semifield enclosures and limited numbers of released mosquitoes could be considered analogous to isolated populations. Using Mosquito Magnet Liberty Plus traps at public recreational areas in British Columbia, Canada, Jackson et al. (2012) observed a significant reduction in nuisance *Ae. vexans* (Meigen) and *Ochlerotatus sticticus* (Meigen). In an earlier study, Henderson et al. (2006) also evaluated propane-powered traps to reduce biting pressure from *Ae. vexans* and *Oc. sticticus* in Manitoba, Canada. While over 2 million mosquitoes were collected in 4 traps over 2 years, they detected no reduction in biting activity in the area around the traps. However, *Ae. vexans* and *Oc. sticticus* are typical mass-breeding floodwater mosquito species with long flight ranges (Brust 1980, Bogojević et al. 2007). Whether control of these species by traps is possible seems to depend on how traps are arranged and how the nuisance factor is measured. Smith et al. (2010), using multiple Mosquito Magnet X (MM-X) traps baited with CO2 and octenol, also reported a failure to reduce populations of *An. crucians* Weidmann, *Oc. taeniorhynchus*, *Cx. salinarius* Coquillet, and *Cx. erraticus* Dyar and Knab in Florida. The MM-X traps did not significantly reduce mosquito numbers compared to control sites. Kline and Lemire (1998) evaluated a single line barrier consisting of 52 CDC-type traps baited with CO2 and octenol on Key Island, Florida. While they were unable to show a statistically significant reduction in mosquito numbers, there was a definite trend toward reduced mosquito abundance within the area designated for protection. Based on these studies, it would appear that more traps, a smaller treatment area, and/or a relatively isolated and low population density of the target mosquito species are key factors in the success of mass trapping to reduce mosquito populations.

The situations described in previous papers are markedly different from those encountered in areas infested by *Ae. albopictus*. Fonseca et al. (2013) noted that *Ae. albopictus* exhibited low autonomous dispersal in New Jersey and that populations can remain very local even across highly homogeneous urban environments. *Aedes albopictus* populations also never approach those of mass emergence floodwater/saltmarsh species. These characteristics could favor the concept of mass trapping. In a recent study conducted in Manaus, Brazil (Degener et al. 2014), mass trapping with BG-Sentinel traps deployed at an average density of one per 385 m2 significantly reduced the abundance of adult female *Ae. aegypti* during the first 5 rainy months of the study. No effect of mass trapping was observed in the subsequent dry season when populations were lower, and although fewer *Ae. aegypti* females were collected in the mass trapping areas compared to the control areas during the next rainy period, the differences were not significant.

The public continues to demand environmentally safer alternatives to broad-spectrum insecticides, and mass trapping could help satisfy this desire. For a mass trapping effort to be successful, traps should have the capability to attract large numbers and capture a high proportion of the attracted mosquitoes. Attractant lures should be optimized for blend composition and dose to maximize their attractiveness to the target species. Trap density should also be optimized to provide maximum coverage of areas designated for protection (El-Sayed et al. 2006).

Mass trapping with the BG-Sentinel as a lure and kill component could serve as the core of an IVM program. Source reduction has been a primary component of many *Ae. albopictus* control efforts (Abramides et al. 2011, Fonseca et al. 2013). In the Brazilian municipality of Cosmopolis, strong efforts to eliminate breeding sites led to a significant reduction in populations of the main dengue vector, *Ae. aegypti*, but *Ae. albopictus* was not significantly impacted (Gomes et al. 2005). In Italy, Caputo et al. (2012) tested the feasibility of using autodissemination stations treated with pyriproxifen to contaminate *Ae. albopictus* larval breeding sites (lure and contaminate) and prevent adult emergence. This technique
holds promise for controlling *Ae. albopictus* in cryptic locations that are difficult to identify and treat. Other researchers have recently developed traps targeting gravid female *Aedes* mosquitoes that also show promise for incorporation into an IVM program (Barrera et al. 2014, Eiras et al. 2014, Ritchie et al. 2014). These lure and kill traps have the added benefit that they do not require any external power or additional attractants. Combining effective traps with other innovative control techniques in an IVM program could increase the likelihood of reducing *Ae. albopictus* populations in urban and suburban environments.

In our study, we focused on “trap-control” of *Ae. albopictus*, utilizing the “gold standard” trap for that species in small, defined areas. We systematically assessed data over a period of 16 wk by 2 different methods, HLC and ovitraps. Data obtained by both methods were consistent. *Aedes albopictus* in Italy is not a mass occurrence species (Carrierri et al. 2011, Cianci et al. 2013). Thus, total numbers of *Ae. albopictus* females caught in interventional traps might appear low compared to numbers collected in other mass-trapping experiments on floodwater or saltmarsh species (Kline 2006, Henderson et al. 2006, Smith et al. 2010, Jackson et al. 2012).

We conclude that our trial reveals strong evidence that the continuous utilization of multiple BG-Sentinel traps is able to significantly reduce the nuisance of *Ae. albopictus* in localized suburban and urban environments.

**ACKNOWLEDGMENTS**

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