Detection of and Monitoring for *Aedes albopictus* (Diptera: Culicidae) in Suburban and Sylvatic Habitats in North Central Florida Using Four Sampling Techniques

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ABSTRACT A sampling study using a BG-Sentinel trap baited with CO_2, a gravid trap baited with an oak-pine infusion, a human subject, and a vegetative aspirator was conducted to compare their reliability at detecting *Aedes albopictus* Skuse in suburban and sylvatic habitats. We collected 73,849 mosquitoes, representing 29 species from 11 genera over a 20-wk period. The BG-Sentinel trap accounted for over 85% of all *Ae. albopictus* captured and was significantly more effective at detecting the presence of *Ae. albopictus* compared with the other three techniques. Landing counts provided the fewest mosquito species ($n = 10$), yet provided a quick and effective weekly assessment of the major biting species and were the most effective method for sampling *Ae. albopictus* within a 10-min period. Fewer *Ae. albopictus* were sampled from sylvatic habitats compared with suburban ones. Sampling criteria advantageous for surveying *Ae. albopictus* and other mosquito species are discussed.

KEY WORDS surveillance, Asian tiger mosquito, BG-Sentinel, landing counts, aspirator

A variety of sampling methods and devices have been developed to detect and enumerate mosquito populations. Many of these collecting techniques are used to sample mosquitoes based on their biology and developmental state. Service (1993) describes a number of sampling methods used to survey adult mosquitoes, including light, colored patterns, and CO_2-baited traps for host-seeking mosquitoes; resting boxes and backpack aspirations for resting mosquitoes; and attractant-baited gravid traps (GTs) for ovipositing mosquitoes. However, all sampling devices used to survey mosquito populations often possess some degree of bias, as each one may be more selective to a particular mosquito species under certain environmental conditions (Huffaker and Back 1943). Breeding sites, altitude, faunal composition, and habitat type (urban or sylvatic) are a few known variables that influence mosquito diversity (Mendoza et al. 2008), and may, therefore, dictate the sampling method(s) used to target specific species. However, selecting a specific sampling method can be challenging when a particular species uses diverse habitats, especially those that reside within ecological interfaces.

The Asian tiger mosquito, *Aedes albopictus* (Skuse), believed to have originated from Southeast Asia, occupies urban, suburban, rural, and forest-edged environments and uses a range of larval habitats, including artificial and natural containers (Hawley 1988). Similarly, Florida offers a variety of environments that can sustain populations of *Ae. albopictus*, and has consequently been collected from tree holes in suburban and sylvatic areas (O'Meara et al. 1993, Obenauer et al. 2009). The rapid spread of *Ae. albopictus* throughout the state, as well as its invasion of over 28 countries in the past two decades (Benedict et al. 2007), is attributed not only to the used tire trade via ship transportation (Lounibos 2002), but also its ability to colonize a variety of habitats.

Because of *Ae. albopictus*'s diurnal feeding behavior, standard adult surveillance with the Center of Disease Control (CDC) mosquito light traps is ineffective (Service 1993). Therefore, adult surveillance of this species and population estimates of most other mosquitoes in the *Stegomyia* subgenus, such as *Aedes aegypti* L., primarily have relied on ovitraps, visual attractants, human landing counts (LC), sticky traps, and aspirator (ASP) collections (Focks 2003). Human LC are a significantly more effective method of surveying *Ae. aegypti* than traps specifically designed for diurnal mosquitoes (Jones et al. 2003, Schoeler et al. 2004). Numerous mosquito abatement districts have long used this surveillance technique to quickly ascertain mosquito abundance, species composition, and effectiveness of adulticides (Schmidt 1989). In

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addition, this technique is especially important when determining infection rates and vectorial capacity of a particular mosquito species (Service 1993). However, this proven and sensitive method to survey *Ae. aegypti* and *Ae. albopictus* can be labor intensive, expensive, and potentially dangerous for the collector, especially in endemic disease areas (Focks 2003). Furthermore, human attractiveness and collection efficiency can differ as a result of variability in carbon dioxide output, body temperature and size, making it difficult to develop repeatable standards for this technique.

Motorized traps that operate in the absence of personnel offer many advantages, including longer surveillance time and reduced impact of human presence on mosquito capture. The BG-Sentinel (BG) trap, which incorporates contrasting black and white colors with semiochemical attractants, has been used to collect host-seeking *Ae. albopictus* from suburban and sylvatic habitats (Bhalala and Arias 2009, Farajollahi et al. 2009, Obenauer et al. 2009). GTs have been primarily used to collect ovipositing *Culex* mosquitoes (Reiter 1983). However, *Ae. albopictus* have been recovered in GTs that were baited with leaf infusions as well (Burkett et al. 2004). Furthermore, GTs are an equally effective method for collecting *Ae. albopictus* as the commonly used CO_2-baited, CDC-style light traps (Burkett et al. 2004).

Sweep nets and ASPs have long been used to collect resting adult mosquitoes (Service 1993). Although past studies document the successful use of the CDC backpack ASP to collect *Ae. aegypti* within indoor environments (Schoeler et al. 2004), few studies exist demonstrating its use in sampling *Ae. albopictus* in an outdoor environment. Ponlawat and Harrington (2005) successfully collected *Ae. albopictus* from vegetation around the perimeter of homes in Thailand using a large custom-made ASP. Their study also demonstrated the use of ASPs for successful collection of blood-fed *Ae. albopictus*, a task for which few alternatives exist.

We evaluated the efficacy of the BG trap, a GT, human LC, and an ASP directed at vegetation to detect adult *Ae. albopictus* in suburban and sylvatic habitats. Currently, no published study exists simultaneously comparing these commonly used methods in surveying for *Ae. albopictus*. Using these four methods, we compared their reliability in the detection of *Ae. albopictus* and their usefulness as a general surveillance tool. We also report on other common mosquitoes that were collected in the course of this experiment.

Materials and Methods

Site Selection. This study was conducted in four suburban and four sylvatic habitats from May to September 2008. Suburban habitats (sites) were four residences in and around Gainesville, FL. Residences were selected based on the number of residents complaining of being bitten during the daytime by mosquitoes and having properties ranging from 0.10 to 0.40 ha with one household per property. The majority of sites contained a mixture of shrubs and trees, namely azalea (*Rhododendron* spp.), oleander (*Nerium oleander*), Indian hawthorn (*Rahphiolepis indica*), live oak (*Quercus virginiana* P. Mill), water oak (*Quercus nigra* L.), laurel oak (*Quercus laurifolia* Michx.), and longleaf pine (*Pinus palustris* P. Mill). One site contained large numbers of tank bromeliads, namely *Aechmea fasciata* (Lindley) Baker, *Neoregelia spectabilis* (Moore), and *Bilbergia* spp.

Sylvatic habitats (N 29° 43.267′, W 82° 26.725′; N 29° 44.669′, W 82° 28.099′; N 29° 44.287′, W 82° 27.354′; N 29° 43.848′, W 82° 27.297′) were located throughout San Felasco Hammock Preserve State Park, Alachua County, FL. All four sites were separated by at least 0.8 km and contained a similar mixture of mature hardwood and pine trees found in suburban habitats. Sylvatic sites were selected from this remote 2,834-ha park, as it permitted all four sites to be separated by at least 0.8 km from known residences. In addition, active *Ae. albopictus* populations were previously trapped from this park (Obenauer et al. 2009).

Surveillance Techniques. The BG trap (BioGents, Regensburg, Germany) is a white, collapsible cylindrical-shaped trap with a mesh-like covered opening and contains a black plastic tube (12 × 12 cm) that is inserted at the top of the trap, which empties into a catch bag, as described in Obenauer et al. (2009). Mosquitoes are drawn into the trap by a 12-V DC fan. To lure diurnal mosquitoes, white and black colors are used as visual cues in combination with a lure that mimics skin secretions (Kröckel et al. 2006). The Agrisense BG-Mesh Lure consisted of 2 m of coiled 4.75-mm internal diameter silicon tubing (containing 15 ml of lactic acid), 50 cm of 0.4-mm internal diameter high-density polyethylene tubing (2 ml of caproic acid), and a slow release ammonia acrylic fibrous tablet, as described in Williams et al. (2006). Carbon dioxide was supplied from a 9-kg compressed gas cylinder with a flow rate of 500 ml/min using 6.4-mm-diameter black plastic tubing (Clarke Mosquito Control, Roselle, IL). The tubing was placed inside the trap with the opening placed near the lure pocket. The CO_2 discharge was verified at every trap rotation using a Gilmont Accucal flowmeter (Gilmont Instrument Company, Barrington, IL). The BG traps were suspended at 1 m using a nylon cord attached to a shepherds hook after attaching an aluminum pan 30 cm above the trap entrance to prevent rain or other debris from damaging the motor components (Obenauer et al. 2009).

The CDC GT model 1712 (John Hock, Gainesville, FL) was used to lure gravid *Ae. albopictus*. As described in Reiter (1983), gravid mosquitoes are attracted to the trap, which contains an oviposition medium in the pan. This trap uses a 6-V, 12 ampere-hour battery (Battery Wholesale Distributors, Georgetown, TX) to power the motor. To maximize visual attractiveness, green Rubbermaid 439 pans (22 cm wide × 34 cm long × 17 cm deep; Rubbermaid Commercial Products, Winchester, VA) were spray painted with black gloss Krylon Fusion paint (Krylon Products Group, Cleveland, OH). To remove any paint odors or contaminants, trap pans were preconditioned and aged by filling them with well water and letting them sit for 2 wk in a semishaded environment before the study (Burkett et al. 2004). To prevent rains from flooding the trap, 0.60-cm holes were drilled into either side of the trap pan, ≈6 cm from the bottom.

The infusion used in the GTs was developed by collecting fallen dry leaves of water oak and longleaf pine needles, free of foreign organic matter, from the grounds at the University of Florida (Gainesville, FL). The infusion was prepared by fermenting 60 g of oak leaves, 60 g of pine needles, 7 g of brewer's yeast (MP Biomedicals, Solon, OH), and 7 g of lactalbumin (Sigma-Aldrich, St. Louis, MO) in 12 liters of well water and subsequently held at ambient temperature between 25 and 27°C in a sealed plastic bucket (Allan and Kline 1995). After 10 d, the infusion was passed through gauze netting to remove larger particulate matter, and 1.5 liters of infusion was transferred to 2-liter plastic bottles and frozen until needed. To each 1.5-liter infusion, 0.5 liter of deionized water was added, generating a 75% infusion concentration.

A large ASP originally designed and built by David Evans to sample salt-marsh mosquitoes in the Everglades (G. F. O'Meara, personal communication), and later modified by L.A. Harrington (Ponlawat and Harrington 2005), was used to collect resting mosquitoes. The ASP was powered by a 12-V, 12-ampere-hour battery, which enables a large fan to funnel mosquitoes through the ASP and into a mesh catch bag. Habitat at each site was sampled continuously for 10 min, paying special attention to tree holes, tree stumps, vegetation, artificial containers, and other ground debris.

LC were performed by collecting mosquitoes landing on the author using a mechanical flashlight ASP (Hausherr's Machine Works, Toms River, NJ). Collections were conducted for 5 min at two locations within each site to reduce bias. Before collecting mosquitoes, the surrounding vegetation was stirred up while the author exhaled vigorously, as prescribed by Schmidt (1989). To attract mosquitoes, the author sat in a collapsible chair, rolled up his pant legs ≈5 cm above the knee, and lowered his socks below the ankles, as these regions are most attractive to *Ae. albopictus* (Shirai et al. 2002). With the exception of the hands, face, and portions of the legs and ankles, all other extremities were covered by a Bugout mosquito jacket (Rattlers Brand, Osceola, IA).

Surveillance and Collection Scheme. Surveillance at all sites ($n = 8$) could not be conducted simultaneously because of the number of traps and time constraints. Therefore, surveillance was conducted twice weekly between 0800 and 1100, for consecutive 2 wk (four sampling dates) at each habitat (sylvatic or suburban), at which time sampling shifted to the other habitat and the process repeated. At each site, four surveillance methods were used in the following order at the start of the 48-h trap operation period: vegetation ASP, LC, and placement of the GT and BG traps. Traps were placed underneath trees in shaded areas and were set at least 20 m from each other and at least 3 m from any dwelling in suburban habitats. Traps were operated for 48 h (one trapping period = two trap nights), after which mosquitoes were collected. Adhesive tape was attached at the base of the GT catch bag and at the top of shepherd hooks to prevent ants from consuming captured mosquitoes. GT infusion and adhesive tape were replaced at the start of each trapping period.

Surveillance occurred over a total of five trials and 20 trapping periods (40 trap nights) per locale between 14 May and 27 September 2008. Temperature and precipitation were measured at the Department of Agronomy Forage Research Unit (Gainesville, FL), with data retrieved from the Florida Automated Weather Network, University of Florida. All mosquitoes collected were frozen (−20°C) and later identified to species using Darsie and Morris (2003).

Statistical Analysis. Differences between *Ae. albopictus* collection techniques were evaluated using a randomized complete block design with sites as the blocking effect. All data were analyzed in three ways and with combined captures of male and female mosquitoes as the response variable. Data were first analyzed by a presence/absence test using a binomial distribution as a measure to score the population to determine the most sensitive collection technique: that technique that documented the collection of at least one *Ae. albopictus* (male or female). Sample periods in which no *Ae. albopictus* were collected at a site, by any method, were excluded from this analysis. LC and vegetative aspirations were not conducted on days with periods of heavy rain. Lost data from these days were treated as missing values. On each sample date at each sampling site where either a male or female *Ae. albopictus* was captured with a sampling device, the data were scored as a "1," and if no *Ae. albopictus* were captured, a score of "0" was assigned. The mean of the four responses within a location (suburban or sylvatic) was obtained at each sample date. Before analysis, an arcsine-square-root transformation was conducted on the data. In the analysis of variance model described below, the percentage of positive sampling incidents was compared among the four sampling devices to identify the technique with the greatest sensitivity in capturing an *Ae. albopictus* when at least one of the four sampling techniques documented that they were present.

The second analysis examined surveillance tool efficacy over time. The LC and ASP procedures were conducted for 10-min time periods, whereas the traps were operated for 48 h; therefore, a time equalization data transformation was used. To standardize the trap collections to 10-min periods, mosquito captures from traps were divided by the value 144-min/trap period. This value was determined by the following formula: $TC_{10} = TP \times DLH \times DAY$, where TC_{10} = estimated trap capture in 10-min exposure. The variable $TP = 6$ reflected the six 10-min time periods in 1 h. $DLH = 12$ and represents the 12 daylight hours of diurnal activity for *Ae. albopictus,* which usually lasts from 0630 to 1830 (Ho et al. 1973). $DAY = 2$, which encompasses the two trapping days in a collection period.

Table 1. Total mosquitoes collected by four surveillance methods in suburban and sylvatic habitats, Gainesville, FL, May–September 2008

Mosquito species	Surveillance method							
	BG trap		Gravid trap		Landing counts		Aspirator	
	Suburban	Sylvatic	Suburban	Sylvatic	Suburban	Sylvatic	Suburban	Sylvatic
Aedes albopictus	4,273	72	167	47	319	6	176	6
Ae. vexans	3,317	2,132	0	1	11	2	347	813
Ae. canadensis	1,125	149	0	0	5	6	30	8
Ae. fulvus pallens	0	1	0	0	0	0	0	1
Ae. infirmatus	15,245	9,113	38	9	388	249	1,391	493
Ae. triseriatus	88	242	2	52	2	1	9	70
Ae. sollicitans	7	0	0	0	0	0	11	0
Anopheles barberi	0	6	0	2	0	0	0	0
An. crucians	43	166	0	1	0	0	1	4
An. punctipennis	5	51	0	0	0	0	0	0
An. quadrimaculatus	81	689	2	23	0	1	1	2
Coquillettida perturbans	166	44	0	0	0	0	2	0
Culiseta inornata	1	0	0	0	0	0	0	0
Culex coronator	15	2	0	0	0	0	0	2
Cx. erraticus	42	61	13	0	0	0	28	17
Cx. nigripalpus	6,352	5,523	48	133	1	2	92	128
Cx. restuans	14	0	14	0	0	0	3	2
Cx. salinarius	11	0	0	0	0	0	0	0
Cx. quinquefasciatus	1,140	6	2,587	11	0	0	15	3
Mansonia titilans	2	15	0	0	0	1	0	0
Orthopodomyia signifera	0	0	0	9	0	0	0	1
Psorophora ciliata	18	17	0	0	0	0	0	1
Ps. columbiae	11	0	0	0	0	0	2	2
Ps. ferox	7,361	6,749	2	0	133	34	228	58
Ps. howardii	40	120	0	0	0	0	1	2
Toxorhynchites rutilus	6	13	1	1	0	0	0	0
Uranotaenia sapphirina	0	0	0	0	0	0	0	5
Wyeomyia mitchelii	942	0	5	0	20	0	9	0
Wy. smithii	60	0	0	0	0	0	0	0
Total mosquitoes/species	40,365/25	25,171/20	2,879/11	289/11	879/8	302/9	2,346/17	1,618/19

Surveillance methods included the BG-Sentinel trap baited with CO_2 at a flow rate of 500 ml/min and a BG-Mesh lure, CDC gravid trap = gravid trap baited with a 75% oak-pine infusion, human landing counts, and a vegetative aspirator. Total collection periods = 40 (48 h for traps; 10 min for landing counts and aspirations).

Data from these two analyses were examined using an analysis of variance model to identify differences between the fixed effects, locale (suburban or sylvatic) and collection method with the quantitative variable trial. The model also included the locale and collection method interaction. Where interactions were found to be significant, the interaction error term was used to calculate *P* values. Statistical analyses were conducted using PROC GLM (SAS Institute 2006). Multiple means comparisons were made with the Ryan-Einot-Gabriel-Welsh multiple range test (α = 0.05). LC and vegetative aspirations were not conducted on days with periods of heavy rain. Uncollected data from these days were treated as missing values.

In addition to *Ae. albopictus,* we also analyzed five of the most commonly collected mosquitoes using paired Student's *t* test (α = 0.05) to identify differences in the collection efficiency between the two time-comparable traps examined, the BG and GT, whereas a separate analysis was conducted on the LC and ASP collections. Data were analyzed using this procedure because collection time periods were either long or short in duration. Only sites that contained paired samples within the collections were analyzed.

Results

A total of 73,849 mosquitoes, representing 29 species from 11 genera, was captured (Table 1). The following six species composed 93.7% of the total collection and were subsequently analyzed: *Ae. albopictus*, *Aedes vexans* (Meigen), *Culex nigripalpus* Say, *Culex quinquefasciatus* Say, *Aedes infirmatus* (Dyar and Knab), and *Psorophora ferox* (von Humboldt). More mosquito species were collected in suburban habitats than in sylvatic habitats, with 25 and 22 species, respectively. The number of mosquito species collected within a locale was dependent on the surveillance method used. The BG trap collected 25 mosquito species, compared with 22, 15, and 10 using the ASP, GT, and LC, respectively (Table 1). LC were specific to collecting daytime biting mosquitoes, as *Ae. albopictus, Ae. infirmatus, Ps. ferox,* and *Wyeomyia mitchellii* (Theobald) comprised over 97% of the catch (Table 1). Data from two trapping periods (18–23 August) were not collected as a result of flooding by Tropical Storm Fay.

Ae. albopictus. A total of 5,066 *Ae. albopictus* was collected, with females comprising 68% of the capture. *Ae. albopictus* was the fifth most common mosquito collected and represented 6.9% of the entire mosquito

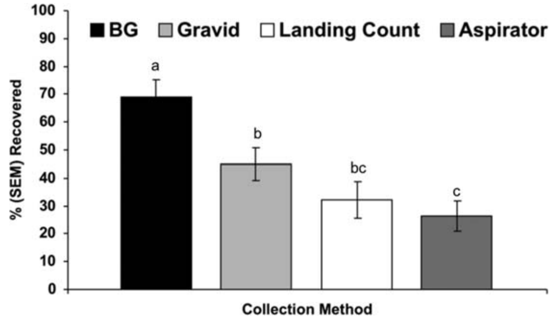

Fig. 1. Likelihood of detection of *Ae. albopictus* by each of four surveillance methods on dates when at least one *Ae. albopictus* was recovered by one of the sampling methods. Sampling occurred in suburban and sylvatic habitats between May and September 2008 in Gainesville, FL. Means with the same letter are not significantly different (Ryan-Einot-Gabriel-Welsh multiple range test). $\alpha = 0.05$. BG = BG-Sentinel trap baited with CO_2 at a flow rate of 500 ml/min and a BG-Mesh lure and operated for 48 h ($n = 148$); Gravid = CDC GT baited with a 75% oak-pine infusion and operated for 48 h ($n = 148$); Landing Count = human mosquito LC conducted for 10 min ($n = 144$); Aspirator = vegetative aspiration conducted for 10 min ($n = 145$).

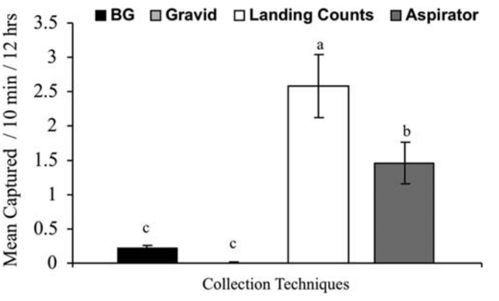

Fig. 2. Comparative efficiency of four sampling devices in capturing female *Ae. albopictus* in Gainesville, FL, suburban and sylvatic habitats between May and September 2008. Means with the same letter are not significantly different (Ryan-Einot-Gabriel-Welsh multiple range test). $\alpha = 0.05$. BG = BG-Sentinel trap baited with CO_2 at a flow rate of 500 ml/min and a BG-Mesh lure ($n = 129$); Gravid = CDC GT baited with a 75% oak-pine infusion ($n = 129$); Landing Counts = human mosquito LC ($n = 126$); Aspirator = vegetative aspiration ($n = 124$). Traps operated for 48 h = one trap period, whereas LC and aspirations over 10 min = one collection period. Traps were converted to 10-min comparatives by dividing total mosquito collection in one trap period by 144 (see text).

capture. Those collected in suburban habitats using all four sampling tools represented over 97% of the total *Ae. albopictus* captured, with a daily mean of 15.8 ± 2.27 in suburban habitats compared with 0.48 ± 0.07 in sylvatic habitats. One suburban site accounted for 47% (2,368) of *Ae. albopictus* captured. *Ae. albopictus* were collected in approximately equal numbers from the four sylvatic sites, ranging from 22 to 43 total specimens for the five trapping periods.

Ae. albopictus was the second most commonly collected mosquito from GT and LC surveillance techniques (Table 1). The BG trap accounted for over 85% of all *Ae. albopictus* captured and was significantly more effective at detecting the presence of *Ae. albopictus* as compared with the other three techniques ($F = 19.15$; df = 3, 143; $P < 0.0001$) (Fig. 1).

Locale was highly significant ($F = 82.96$; df = 1, 143; $P < 0.0001$), with nearly three times as many *Ae. albopictus* detections in suburban habitats (62.18% ± 4.22) as compared with sylvatic habitats (21.99% ± 3.63). Trial, which represents time of year, was also significant with more *Ae. albopictus* captured during trials three, four, and five compared with the two earlier trials ($F = 52.39$; df = 1, 143; $P < 0.0001$). All surveillance methods performed similarly in detecting a large *Ae. albopictus* population increase in mid-July, a peak in early August, and a decrease in late September.

After conversion to 10-min intervals, significantly more *Ae. albopictus* were collected with LC than the other methods ($F = 15.22$; df = 3, 496; $P < 0.0001$) (Fig. 2). In addition, an interaction effect was detected between sampling method and locale. Within the suburban locale, significantly more *Ae. albopictus* were captured using LC (4.14 ± 0.73) as compared with the ASP (2.32 ± 0.45), BG (0.38 ± 0.46), and GT methods (0.12 ± 0.01; $F = 24.43$; df = 3, 302; $P < 0.0001$). However, in the sylvatic environment, no differences were observed between sampling methods.

In suburban habitats, significantly more *Ae. albopictus* were collected with BG traps. (54.8 ± 7.79) as compared with gravid (2.01 ± 0.34) traps (paired Student's *t* test, $P \leq 0.0001$), whereas more mosquitoes were collected using LC (4.00 ± 0.71) than the ASP (2.3 ± 0.45) (paired Student's *t* test, $P = 0.0022$). No differences were detected between sampling methods in the sylvatic locale (Tables 2 and 3).

Other Mosquito Species. *Ae. infirmatus* was the most abundant species collected, comprising 36% of all mosquito specimens. The BG trap collected more *Ae. infirmatus* (202.8 ± 58.8) than all other techniques combined (Tables 2 and 3). The ASP collected significantly more *Ae. infirmatus* than the LC technique in suburban and sylvatic habitats (Table 3). *Ae. vexans* was collected with every sampling technique except the GT (Tables 2 and 3). Significantly more *Ae. vexans* were collected with the ASP than with LC in suburban (paired Student's *t* test, $P \leq 0.0001$) and sylvatic (paired Student's *t* test, $P \leq 0.0006$) habitats.

Over 96% of *Cx. nigripalpus* were collected with the BG trap. The ASP was significantly more effective than the LC at sampling their population in suburban (paired Student's *t* test, $P \leq 0.0521$) and sylvatic habitats (paired Student's *t* test, $P \leq 0.0153$). *Cx. quinquefasciatus* was the dominant species collected in GT, representing >80% of the total collection. Furthermore, 99% of *Cx. quinquefasciatus* were collected in suburban habitats. GTs (32.3 ± 4.50) placed in suburban areas captured significantly greater numbers of *Cx. quinquefasciatus* compared with the BG trap (15.0 ± 2.59) (paired Student's *t* test, $P \leq 0.0001$) (Table 2). *Ps. ferox* was the third most commonly

Table 2. Mean (SE) of the six most common mosquitoes collected using the BG Sentinel and CDC gravid traps in suburban and sylvatic habitats in Gainesville, FL

Mosquito species	Locale type	n	BG[a]	Gravid[a]	df	t	p
Aedes albopictus	Suburban	75	54.8 ± 7.79	2.01 ± 0.34	74	−6.920	<0.0001
	Sylvatic	60	1.0 ± 0.22	0.75 ± 0.21	59	−1.280	0.2057
Ae. vexans	Suburban	75	44.0 ± 16.8	0.00 ± 0.00	74	2.629	0.0104
	Sylvatic	60	35.2 ± 15.1	0.00 ± 0.00	59	2.320	0.0238
Aedes infirmatus	Suburban	75	202.8 ± 58.8	0.50 ± 0.25	74	3.442	0.0010
	Sylvatic	60	130.3 ± 35.2	0.10 ± 0.46	59	3.704	0.0005
Culex nigripalpus	Suburban	75	84.2 ± 32.6	0.62 ± 0.38	74	2.561	0.0125
	Sylvatic	60	73.4 ± 20.7	1.93 ± 0.57	59	3.487	0.0009
Cx. quinquefasciatus	Suburban	75	15.0 ± 2.59	32.32 ± 4.50	74	−4.756	<0.0001
	Sylvatic	60	0.1 ± 0.05	0.18 ± 0.07	59	−0.962	0.3402
Psorphora ferox	Suburban	75	97.8 ± 40.3	0.03 ± 0.03	74	2.425	0.0178
	Sylvatic	60	103.9 ± 30.3	0.00 ± 0.00	59	3.434	0.0011

Paired Student's t test, $\alpha = 0.05$.
[a] Traps were the BG-Sentinel trap baited with CO_2 at a flow rate of 500 ml/min and a BG-Mesh lure, CDC gravid trap = gravid trap baited with a 75% oak-pine infusion; n = no. of trapping periods (48 h each) between May and September 2008.

collected mosquito species. Significantly more *Ps. ferox* were collected in BG traps (97.8 ± 40.3; 103.9 ± 30.3) than in GTs (0.03 ± 0.03; 0.00 ± 0.00) in suburban and sylvatic habitats, respectively (Table 2).

Discussion

The rapid introduction of *Ae. albopictus* to many countries within the last 20 yr has driven considerable research efforts to develop effective surveillance tools for rapid and early detection of this invasive mosquito. *Ae. albopictus* is a potential health threat, even to countries that normally do not have endemic diseases. This was evident when it was recently incriminated as the primary vector responsible for chikungunya outbreaks in Italy (Rezza et al. 2007). *Ae. albopictus* is a versatile mosquito, feeding on a range of hosts, ovipositing in numerous types of natural and artificial containers, and occupying a number of diverse habitats (Hawley 1988). Therefore, its behavior and biology may vary dependent on habitat type, complicating traditional collection methods to survey adult populations.

Whereas many mosquito-trapping studies have compared and evaluated traps based on the quantity of mosquitoes collected, we compared four methods used to sample *Ae. albopictus* to determine the most appropriate method for detecting its presence. The BG trap and LC techniques targeted host-seeking *Ae. albopictus,* whereas GT technique collected females searching for oviposition sites. ASP collections of resting *Ae. albopictus* would seem to provide, perhaps, the most unbiased sampling method as recently eclosed, host-seeking, blood-fed, or gravid mosquitoes can be captured. However, previous collection studies using sweep nets or ASPs have recovered disproportionately more blood-fed females and nectar-engorged males compared with other sampling techniques (Huffaker and Back 1943, Bidlingmayer 1974). Therefore, comparisons between collection techniques as presented in the current study should be approached with caution, as the aim of this study was to detect the presence of *Ae. albopictus* and rate the techniques based on their performance in the sylvatic and suburban habitats.

This study demonstrates that surveillance techniques used to sample *Ae. albopictus* were influenced by habitat type. For example, the BG trap was no more effective at collecting *Ae. albopictus* than GT in sylvatic habitats, whereas its performance drastically increased when used in suburban habitats. Perhaps

Table 3. Mean (SE) of the six most common mosquitoes collected using human landing counts and a vegetative aspirator in suburban and sylvatic habitats in Gainesville, FL

Mosquito species	Locale type	n	Landing counts[a]	Aspirator[a]	df	t	p
Aedes albopictus	Suburban	75	4.00 ± 0.71	2.30 ± 0.45	74	−3.172	0.0022
	Sylvatic	60	0.08 ± 0.04	0.08 ± 0.04	60	0.000	1.0000
Ae. vexans	Suburban	75	0.15 ± 0.06	4.59 ± 0.92	74	4.920	<0.0001
	Sylvatic	60	0.03 ± 0.02	12.40 ± 3.41	60	3.619	0.0006
Aedes infirmatus	Suburban	75	5.16 ± 1.39	18.50 ± 6.33	74	2.346	0.0217
	Sylvatic	60	3.40 ± 0.76	7.27 ± 2.10	60	2.625	0.0110
Culex nigripalpus	Suburban	75	0.01 ± 0.01	1.22 ± 0.61	74	1.974	0.0521
	Sylvatic	60	0.03 ± 0.03	1.83 ± 0.72	60	2.498	0.0153
Cx. quinquefasciatus	Suburban	75	0.00 ± 0.00	0.20 ± 0.07	74	2.922	0.0046
	Sylvatic	60	0.00 ± 0.00	0.05 ± 0.03	60	1.762	0.0832
Psorphora ferox	Suburban	75	1.77 ± 0.80	3.04 ± 1.30	74	1.055	0.2948
	Sylvatic	60	0.47 ± 0.15	0.90 ± 0.46	60	1.173	0.2453

Paired t-test, $\alpha = 0.05$.
[a] Techniques included human landing counts (landing counts) and a vegetative aspirator (aspirator), each performed for 10 min; n = collection periods (10 min) between May and September 2008.

lower *Ae. albopictus* populations in sylvatic habitats or unrecognized environmental competitive influences masked these differences. In addition, GTs placed in sylvatic habitats offered prime *Ae. albopictus* oviposition targets in an otherwise sparse environment, whereas availability of oviposition sites in suburban backyards was much greater. Low captures of *Ae. albopictus* with the GT compared with BG traps in suburban habitats (Table 2) were similar to results reported by Farajollahi et al. (2009), further supporting the idea that GTs are selective for *Culex* spp., whereas the BG trap is selective for *Ae. albopictus.*

Although sylvatic habitats contained a number of tree holes, many remained dry throughout the summer. Therefore, suburban habitats in our study, which used frequent irrigation, most likely provided more abundant and stable breeding areas for *Ae. albopictus*, resulting in decreased GT captures relative to the adult mosquito population size. For example, one residential suburban site accounted for nearly half of all *Ae. albopictus* captured. This site contained numerous water-holding tank bromeliads covering the ground, providing ideal breeding sites for *Ae. albopictus,* and most likely contributed to the high trap captures.

Ae. albopictus were most likely attracted to the visual and olfactory cues presented by GTs used in this study. Pans were shiny and black, a known color to be attractive for ovipositing *Ae. albopictus* (Yap et al. 1995). In addition, the odors from the oak-pine infusion may have increased the attraction of gravid females. Oak-pine infusion has been shown to be an effective *Ae. albopictus* oviposition attractant in preliminary laboratory trials by Obenauer et al. (2010). Similarly, Burkett et al. (2004) demonstrated that oak-baited infusions used with black GTs were attractive to *Ae. albopictus.*

Results from this *Ae. albopictus* collection comparison study are comparable to those reported in numerous studies for *Ae. aegypti* in that the BG trap specifically targets daytime-feeding mosquito species, whereas other traps were developed for crepuscular or nighttime-feeding species. In Thailand, researchers determined that LC were still more effective at collecting adult *Ae. aegypti* when compared with Omnidirectional Fay-Prince, sticky, or CDC Wilton traps (Jones et al. 2003). Schoeler et al. (2004) also determined that no trap tested was an acceptable alternative to backpack aspiration or human landing collections. Of the total mosquitoes collected in their study, 73% were collected via backpack ASP, followed by 23% with human landing methods. In contrast, the BG trap compared favorably to 10-min samplings conducted with a CDC backpack ASP in Australia (Williams et al. 2006). Although their study determined the BG trap collected significantly more female *Ae. aegypti* compared with the CDC ASP, both devices proved equally effective when males were included in the data set. However, unlike *Ae. aegypti, Ae. albopictus* is an exophilic mosquito, preferring to feed and rest outside of dwellings (Hawley 1988), potentially making collections more challenging because of various outdoor environmental influences. We demonstrated that *Ae. albopictus* can be successfully collected with an ASP in suburban and sylvatic habitats. Of all collection methods, the ASP was the second most effective sampling technique during 10-min intervals (Fig. 2).

Each surveillance technique evaluated in this study had advantages and disadvantages in sampling *Ae. albopictus.* The BG trap was the most effective tool at capturing a range of mosquito species, including large numbers of male and female *Ae. albopictus.* Furthermore, unlike LC and ASP collections, which can vary between operators, the BG trap is objective and could serve as a standard (Williams et al. 2006). However, traps were susceptible to periodic mechanical malfunctions and required batteries, lures, and CO_2 canisters. The CDC GT is easy to operate, can be transported to the field, and only requires a 6-V battery. However, the trap collected only 15 mosquito species compared with 25 and 22 with the BG and ASP, respectively. In addition, it was susceptible to periodic mechanical malfunctions and was occasionally vandalized, presumably by ground-dwelling mammals. Furthermore, preparation of large volumes of infusion required throughout the trapping season created additional weight and storage issues.

The ASP was quick and effective, aspirating 22 mosquito species from brush, tree holes, and various other containers. The ASP may provide an important tool for future studies that investigate host preference, as many of the *Ae. albopictus* collected by this method had recently blood fed (P. Obenauer). However, the ASP was cumbersome to operate, particularly in thickly wooded areas. Occasional mechanical problems and a 12-V battery were additional drawbacks.

LC provided the fewest collected mosquito species ($n = 10$), yet they were a quick and effective weekly assessment of the major biting species and were the most effective method for sampling *Ae. albopictus* within a 10-min period. Ritchie et al. (2006) also determined LC to be the most effective means at detecting the presence of *Ae. albopictus* in the Torres Strait of Australia.

This study demonstrated that the BG trap was an effective surveillance device in detecting *Ae. albopictus* (Fig. 1). The addition of CO_2 in this study was designed to maximize its effectiveness. However, despite these added host-seeking cues, it was still not as effective as LC when examined as equivalent 10-min sample periods (Fig. 2).

Techniques used to capture *Ae. albopictus* in this study provide a variety of research applications. For example, in the current study, nulliparous females were consistently collected using the BG trap and LC (data not shown). However, to test for infected mosquitoes or to conduct a blood meal analysis, vegetative aspiration and, to some extent, the GT would be a more effective technique, as they target previously blood-fed females. Similarly, Bidlingmayer (1974) demonstrated that ASPs collected a larger proportion of blood-fed mosquitoes.

Results of this study demonstrate that selecting a sampling device to survey *Ae. albopictus* populations should not only be based on the aim of a study, but also the habitat features. Sampling mosquito field populations are known to produce bias among collection techniques (Service 1977). When habitats were not considered, the BG trap was significantly more effective at detecting *Ae. albopictus* (69% of total collections) than other methods (Fig. 1). However, whereas significant differences were detected among surveillance methods in suburban habitats, neither technique was more effective at collecting *Ae. albopictus* in sylvatic habitat (Tables 2 and 3). Similarly, ASP collections demonstrated that *Ae. albopictus* could be detected with the same effectiveness as LC when habitat was not a consideration. Furthermore, the aim of ASP collections was to collect resting mosquitoes, one of the more challenging and time-consuming processes as a result of mosquito dispersal and preferences for specific habitats (Service 1977). We aspirated for mosquitoes from various containers found within sites (i.e., tree holes, bromeliads, vegetation, artificial containers, etc.) and did not standardize these resting sites based on type or dimensions. *Culex* and *Anopheles* species are known to select their resting sites based on size and shape (Burkett-Cadena et al. 2008). Therefore, future studies of *Ae. albopictus* resting sites are warranted to elucidate preferences for types of resting containers within habitats, thereby improving the collection of blood-fed specimens.

This study also demonstrated that collection method efficiency is often based on several variables. For instance, if surveillance was required to be conducted in a short time period, LC were the most time-efficient surveillance method for detecting *Ae. albopictus* (Fig. 2). However, this was strictly based on overall captures and did not compensate for habitat differences.

These results may affect the manner in which future *Ae. albopictus* surveillance is conducted, especially in areas where it has been recently introduced. Based on our results, the BG trap would most likely be the choice for detecting the presence of *Ae. albopictus* in suburban habitats. Our BG trap results are similar to those in Australia, demonstrating it as an effective sentinel device, capable of detecting *Ae. albopictus* populations, where other methods have failed (Ritchie et al. 2006). Although recent advances in mosquito attractants have been made, no attractant has worked as effectively as human baits for anthropophagic mosquito surveillance (Service 1993). Future studies are needed to further develop surveillance tools for detecting other daytime mosquitoes, especially invasive species that may otherwise go unnoticed using traditional tactics.

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