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Resting and Energy Reserves of *Aedes albopictus* Collected in Common Landscaping Vegetation in St. Augustine, Florida

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Abstract

The resting behavior of *Aedes albopictus* was evaluated by aspirating diurnal resting mosquitoes from common landscape vegetation in residential communities in St. Augustine, FL. Energy reserves of the resting mosquitoes were analyzed to determine if there was a correlation between mosquito resting habitat and energy accumulation. Six species of plants were selected and 9 collections of resting mosquitoes were aspirated from each plant using a modified John W. Hock backpack aspirator during June and July 2012. Eight mosquito species were collected, with *Ae. albopictus* representing 74% of the overall collection. The number of *Ae. albopictus* collected varied significantly with the species of vegetation. When comparing the vegetation and abundance of resting mosquitoes, the highest percentages of *Ae. albopictus* were collected resting on *Ruellia brittoniana* (Mexican petunia), *Asplenium platyneuron* (fern), *Gibasis geniculate* (Tahitian bridal veil), followed by *Plumba goauriculata* (plumbago), *Setcreasea pallida* (purple heart), and *Hibiscus tiliaceus* (hibiscus). There were significant differences in lipid and glycogen accumulation based on type of vegetation *Ae. albopictus* was found resting in. Resting mosquitoes' sugar reserves were not influenced by species of vegetation. However, there was an overall correlation between vegetation that serves as a resting habitat and energy reserve accumulation. The results of our study demonstrate the potential to target specific vegetation for control of diurnal resting mosquitoes.

Keywords

Sugar feeding; mosquito control; landscaping; energy reserves; diurnal resting; *Aedes albopictus*

Introduction

The distribution of *Aedes albopictus* (Skuse) spatially and temporally has amplified the risk of arbovirus transmission and is a major public health concern (Lambrechts et al. 2010) as this species is considered the main vector in the global resurgence of dengue (Gubler 1998).

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Aedes albopictus is opportunistic and thrives in heavily vegetated habitats, making this species difficult to control (Hawley 1988, Braks et al. 2003). Combined with the reemergence of locally acquired dengue cases in Florida (Radke et al. 2012) and insufficient control methods, understanding the biology and behavior of this species is imperative for protection of public health.

Aedes albopictus needs regular sugar meals for nutrition and energy (Braks et al. 2006). Male and female mosquitoes ingest plant sugars in the form of floral and extrafloral nectar and fruit for flight energy and survival (Yuval 1992, Foster 1995, Müller et al. 2011). While sugar feeding preference of mosquitoes in nature is still largely unclear (Harada et al. 1975, 1976), this behavior more recently has been used to maximize vector control strategies (Xue et al. 2006, Müller et al. 2010a, Beier et al. 2012). Improving the knowledge of the diurnal resting and sugar feeding behavior of important vector or nuisance species can synergize with current control programs, providing more targeted application of insecticides. Therefore, we investigated the diurnal resting behavior of *Ae. albopictus* by aspirating resting mosquitoes from landscape vegetation found in residential communities in St. Augustine, FL. To better understand nonbloodfeeding behaviors of mosquitoes, the link between diurnal resting and sugar feeding on mosquito energy accumulation was analyzed.

Materials and Methods

Study area

We selected sites in and around residential communities in St. Augustine, FL, to sample diurnal resting mosquitoes from flowering and nonflowering plants commonly used in landscaping (Table 1). Landscape vegetation that dominated the yards and woods at the sample sites were palmetto (*Seronoa repens* Bartram), ornamental plants, live oak (*Quercus virginiana* Mill), as well as fern (*Polystichum munitum* Kaulfuss) and palm (*Livistona chinensis* Jacq.). Nine collections of resting mosquitoes from the 6 species of plants (Table 1) were conducted from mid-June to July, 2012.

Collections

A battery-modified Centers for Disease Control and Prevention backpack aspirator (Model 1412; John W. Hock Company, Gainesville, FL) was used to collect resting adult mosquitoes from vegetation. Mosquitoes were collected between 08:00 and 10:00 a.m. Before collecting the sample, a volunteer conducted a 1-min landing rate count (LRC) to determine the relative abundance of mosquitoes. The LRC provided information on mosquito populations and their distributions relative to plant vegetation. Mosquito aspirations were conducted if after the 1-min LRC mosquitoes were noticed flying or landing. A collection cup was inserted into the aspirating end of the suction vacuum and replaced with a new cup every time a new plant was sampled. The vegetation was aspirated for 5 min. After the collection, the cup was closed before turning the aspirator off. The collected mosquitoes were then brought back to the laboratory on dry ice, enumerated, sexed, and identified to species. The mosquitoes were saved in individual micro-centrifuge tubes at −70°C for later detection of energy reserves.

Laboratory sample preparation

The mosquitoes were transferred to 15-ml glass centrifuge tubes (Fisher Scientific, Pittsburg, PA) for extraction of sugar (fructose), glycogen, and lipid fractions to determine if the resting mosquitoes collected had energy reserves. Male and female mosquitoes were crushed individually with a pellet pestle motor in 0.2 ml of 2% sodium sulfate ($NaSO_4$) solution and then 1.5 ml of a 1:2 chloroform–methanol solution was added and mixed vigorously. Next, the samples were centrifuged at 14,000 × *g* for 5 min, and equal volumes of supernatant were divided into 16- × 100-mm glass tubes for lipid and fructose analysis. The remaining precipitate was used for glycogen determination. At the respective wavelength for detection, each sample had a single lipid, sugar, and glycogen value as determined using the anthrone–sulfuric acid and vanillin–phosphoric reagent test (Van Handel 1985a, 1985b; Van Handel and Day 1988; Kaufmann and Brown 2008). The absorbance was read using a spectrophotometer that determined the micrograms per mosquito set at specific wavelengths (Jenway Model 6700, London, United Kingdom).

Statistical analysis

The number of resting *Ae. albopictus* was analyzed with a generalized linear model, using a negative binomial link. Planned comparisons were made among the various plants. The amount of glycogen, lipids, and fructose detected in the mosquitoes was highly variable. Therefore, we used a generalized linear model to fit a negative binomial model to the data. This provided a better fit to the data than a general linear model. The data from this analysis are presented as means and standard errors. The 0.05 significance level was used to determine statistical significance using SAS 9.3 (SAS Institute, Inc. 2012) for all analyses.

Results

Mosquito collections

Eight mosquito species were collected, with *Ae. albopictus* representing 74% of the overall collection (122/164). The other mosquito species were collected in low numbers, but *Ae. taeniorhynchus* (Wiedemann) was found resting in 4 of the 6 plants sampled and represented 10% of the collection (Table 2). Females represented 74.4% of the collections, with 65.0% being *Ae. albopictus*. All of the male mosquitoes collected were *Ae. albopictus* with the exception of 1 *Culex quinquefasciatus* (Say) male found resting in the Mexican petunia (*Ruellia brittoniana* Leonard). Although the females were collected twice as often as males, the collection of males and females were significantly different only in the fern (*Asplenium platyneuron* L.) (t = 2.88, $P < 0.013$; Fig. 1).

The number of resting *Ae. albopictus* collected varied significantly with the type of vegetation (F = 2.47, $df_{1,2}$ = 5, 48; P = 0.045). There were significantly more *Ae. albopictus* resting in the Mexican petunia and the fern, followed by the Tahitian bridal veil (*Gibasis geniculate* Jacq.), plumbago (*Plumbago auriculata* Lam), purple heart (*Setcreasea pallida* Rose), and hibiscus (*Hibiscus tiliaceus* L.).

Energy reserves

There were significant differences found in the glycogen reserves detected in resting *Ae. albopictus* (F = 5.16, $df_{1,2}$ = 5, 116; $P < 0.01$; Table 3). Glycogen accumulation was highest in *Ae. albopictus* found resting in hibiscus, plumbago, and Mexican petunia, followed by Tahitian bridal veil, the fern, and purple heart. There were significant differences found in the amount of lipid reserves detected in resting *Ae. albopictus* (F = 4.69, $df_{1,2}$ = 5, 116; P = 0.001; Table 3). Lipid accumulation was the highest in *Ae. albopictus* found resting in the Mexican petunia and hibiscus, followed by the fern, Tahitian bridal veil, plumbago, and purple heart. Fructose accumulation of *Ae. albopictus* was not significantly different (Table 3). A strong correlation was detected between accumulation of energy reserves and resting habitat of *Ae. albopictus* (r = 0.06, $P < 0.001$).

Discussion

This study demonstrates that *Ae. albopictus* will rest during the daytime in vegetation commonly found in and around residential areas, thus suggesting that landscaping can influence species distribution within an area. Certain plants provide a more suitable resting habitat as shown in this study in St. Augustine, FL. *Aedes albopictus* attack rates tend to increase as the proximity to plants increases, highlighting the importance of vegetation with vector–human contact (personal observations of the authors). Further identifying the importance of the mosquito–vegetation relationship, we found a correlation between vegetation that serves as a resting habitat and energy reserve accumulation. This suggests that targeting vegetation that serves as a resting site could increase control efficacy and reduce the potential that vegetation serves in increasing vector–human contact.

This is the 1st report of energy reserves detected in field-collected *Ae. albopictus*. Day-to-day activities of the mosquito require an energy budget, and studies have demonstrated that energy reserves accumulated by laboratory mosquitoes deplete rapidly (Nayer and Sauerman 1975). Since laboratory-reared mosquitoes generally have higher energy reserves than their field conspecifics, depletion of these reserves should be consistent with what has been observed in the laboratory if not more rapidly (Day and Van Handel 1986). These reserves are replenished after the mosquito locates and feeds on a sugar meal. Studies have demonstrated that mosquitoes have flowering preferences for sugar feeding (Grimstad and DeFoliart 1974, Müller and Schlein 2005, Schlein and Müller 2008, Gouagna et al. 2010, Müller et al. 2010b). In our study, we established resting preferences by *Ae. albopictus* and a correlation with energy accumulation. Manda et al. (2007) showed that female *Anopheles gambiae* Meigen preference for different plants correlated with the relative fitness-related benefits the mosquito derives from feeding on the selected plant species. These findings suggest that mosquitoes preferentially feed on plants with a high sugar content, which in turn will confer a greater fitness advantage. Interestingly, *Ae. albopictus* acquired the greatest amount of total energy from the same plant this species was collected in greatest abundance, which supports the assumption of Manda et al. (2007).

Although we do not know for certain that energy reserves were acquired on the plants sampled, resting and sugar feeding within the same vegetation would also allow for an increase in fitness through energy conservation. This conservation of energy could in turn be used for other behaviors such as host seeking and oviposition. Manda et al. (2007) suggested that mosquitoes land on plants only to acquire a sugar meal. Thus, we assume that the number of mosquitoes caught per plant reflects the preference of the mosquitoes to feed on them as individual sugar sources. Another option may be that certain vegetation provides a more suitable resting habitat, i.e., holds more moisture, provides more shade from ultraviolet exposure, etc., and is therefore more favorable for the conservation of energy reserves acquired by the mosquito during sugar feeding. Most likely in different environments there are different plant–mosquito associations that need to be explored to provide a better understanding of how these behaviors can be exploited for effective control.

Furthermore, our findings support the targeting of diurnal resting behavior of mosquitoes. Based on our observations, mosquitoes were collected resting more often in thick, dense vegetation as seen with the Mexican petunia, fern, and Tahitian bridal veil. Barrier application of pyrethroids or attractive toxic sugar baits (ATSB) to the preferred resting vegetation of *Ae. albopictus* could be a viable option for this difficult-to-control species. The ATSB application for *Ae. albopictus* control has been successful in residential communities in St. Augustine, FL (Naranjo et al. 2013). Based on the current study findings, targeting nonflowering vegetation, like the fern, with combinations of barrier applications and spot target treatments that employ an attract-and kill strategy could synergize by targeting 2 specific nonbloodfeeding behaviors of mosquitoes. Thus, reducing area-wide applications of insecticides to control host-seeking mosquitoes overall will provide some cost benefit to control programs (Xue 2006, Qualls et al. 2012).

The resting behavior of *Ae. albopictus* deserves further research, as new approaches for integrated vector management for this species are urgently needed (Townson et al. 2005). Current control strategies of adulticiding and larviciding are not efficacious against this species as seen by its spread within the USA (Reiter and Gubler 1997). Comparable studies of mosquito resting behavior in other ecosystems are needed to determine how barrier treatment and ATSB applications can be maximized by targeting the diurnal resting mosquitoes.

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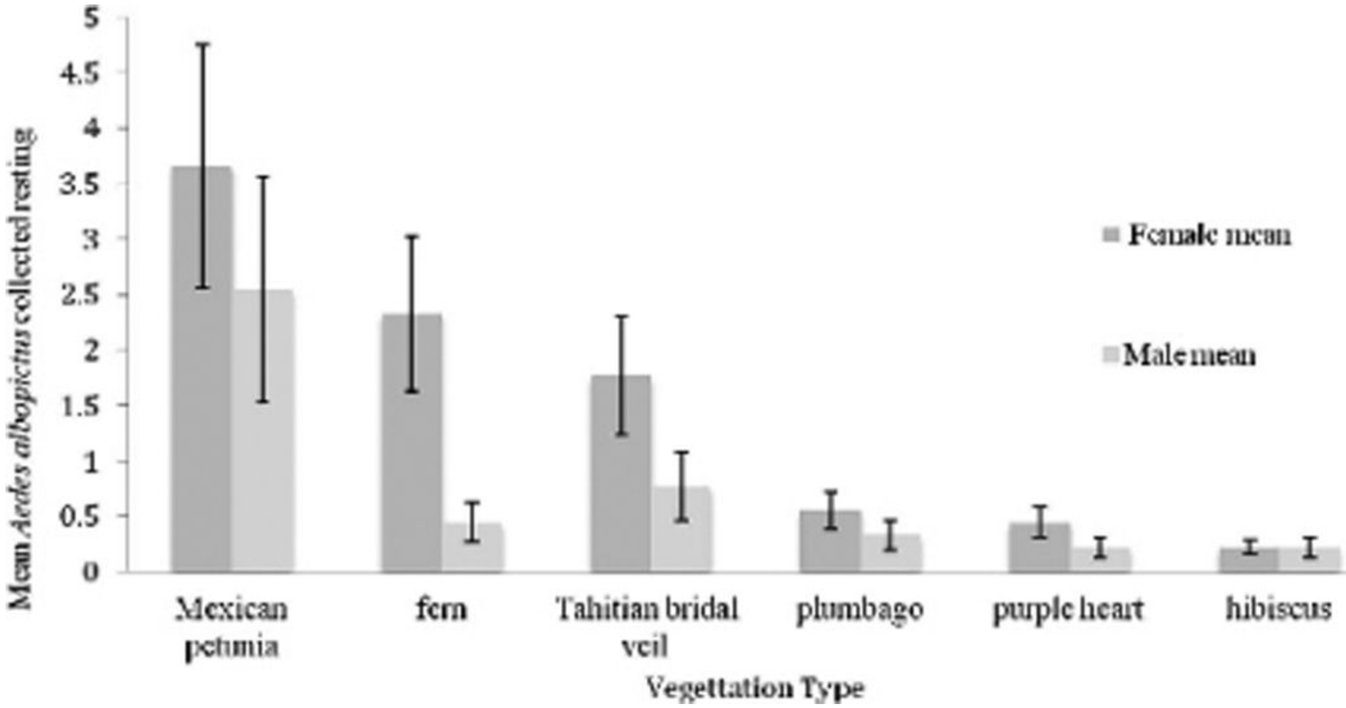

Fig. 1.

Numbers (mean ± SE) of female and male resting *Aedes albopictus* collected on the different vegetation sampled (*n* = 9).

Table 1

Information on the vegetation sampled for resting mosquitoes.

Scientific name and family	Common name	Plant type
Plumbago auriculata (Plumbaginaceae)	Blue Plumbago, Cape Plumbago, Cape Leadwort, Skyflower	Ornamental shrub, native to Tropical Africa Evergreen perennial shrub with vine-like habit
Hibiscus tiliaceus (Malvaceae)	Sea Hibiscus, Beach Hibiscus, Coastal (or Coast) Hibiscus, Coastal (or Coast) Cottonwood, Green Cottonwood, Native Hibiscus, Native Rosella, Cottonwood Hibiscus, Kurrajong, Sea Rosemallow	Native to the Old World tropics The leaves are alternate, ovate to lanceolate, often with a toothed or lobed margin. The flowers are large, trumpet-shaped, with 5 or more petals. The flowers of *H. tiliaceus* are bright yellow with a deep red center upon opening but, over the course of the day, gradually turns orange and finally red before falling
Ruellia brittoniana (Acanthaceae)	Mexican Petunia	Native of Mexico, the Caribbean, and South America. It has become a widespread invasive plant in Florida They have greenish-purple stems clad with sword-shaped green leaves. They thrive in a variety of conditions, ranging from wet pond margins to average garden soils
Setcreasea purpurea (Commelinaceae)	Purple Heart	Native to the Gulf Coast region of eastern Mexico Vibrant purple foliage with delicate lavender blooms and stems when grown in full sun
Asplenium platyneuron (Aspleniaceae)	Fern	This fern has erect, dark, evergreen fronds 6 to 20 in. tall. It is native throughout South Carolina Prefers some sun to light shade
Gibasis geniculate (Commelinaceae)	Tahitian Bridal Veil	Invasive in tropical areas A creeping plant with long, trailing stems that carry slender, lance-shaped leaves that are olive green on top and purple underneath. Small, airy, white flowers are held well out from the foliage and bloom from spring to fall

Table 2

Number of female mosquitoes by species collected resting in the different vegetation sampled in St. Augustine, FL.[1]

	Vegetation sampled				
Mosquito species	**Mexican petunia**	**Tahitian bridal veil**	**Hibiscus**	**Fern**	**Plumbago**
Aedes atlanticus	2				
Ae. infirmatus		3		7	
Ae. sollicitans			5		6
Ae. taeniorhynchus	1		6	4	6
Ae. vexans		2			

[1]All mosquitoes collected except for *Aedes albopictus*, 1 male *Culex quinquefasciatus* collected on the Mexican petunia, and 1 *Psorophora ferox* female collected resting in the hibiscus.

Table 3

Mean concentration (μg) ± SE of glycogen, lipid, and sugar reserves detected in collected resting *Aedes albopictus* on the different vegetation sampled.

		Glycogen		Lipids		Fructose	
Plant	***n***	**Mean[1]**	**SE**	**Mean[1]**	**SE**	**Mean[1]**	**SE**
Mexican petunia	56	33.2 a	5.0	33.2 a	4.7	28.0	3.4
Purple heart	6	9.6 b	4.1	10.0 b	4.4	11.9	4.7
Fern	25	13.0 b	3.0	13.0 b	3.0	19.7	4.1
Hibiscus	4	42.0 a	21.4	24.0 a	13.1	23.8	11.1
Tahitian bridal veil	23	15.0 b	3.0	15.0 b	3.2	18.4	3.4
Plumbago	8	35.3 a	13.0	11.1 b	4.2	20.0	7.1

[1]Columns with different letters indicate significant differences at $P < 0.05$ using a generalized linear model.