Effect of Common Ornamental Plants on the Survivorship and Fecundity of the Aedes albopictus (Diptera: Culicidae)

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Effect of common ornamental plants on the survivorship and fecundity of the *Aedes albopictus* (Diptera: Culicidae)

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Abstract

Globally, *Aedes albopictus* Skuse (Diptera: Culicidae) has considerably expanded its habitat from rural areas to urban areas as a result of increased urbanization. In some urban areas, this expansion has resulted in this invasive species being elevated as an important vector of dengue virus. Ornamental plants are often a feature of the urban landscape that may provide harborage for mosquitoes. Because adult mosquitoes require carbohydrates for subsistence, landscape vegetation may provide natural sugar sources to meet those needs. The aim of our study was to determine whether feeding on different ornamental plants from urban areas affects the survivorship and fecundity of *Ae. albopictus*. Newly emerged mosquitoes were given access to 11 ornamental plant species (6 flowering, 5 nonflowering) as sugar sources under laboratory conditions. Generally, survivorship was greater significantly when mosquitoes fed upon the ornamentals compared with those that were offered only water, whereas survivorship was shortened when individuals fed only on sucrose ($P < 0.05$). Mosquitoes that fed on nonflowering plants laid fewer eggs compared with those exposed to flowering plants. No significant difference was observed in egg hatch from females feeding on any of the plant species. Our findings provide insight into the potential influence that urban ornamental plants may have on the ecology of adult *Ae. albopictus*. The results of this study provide new avenues for integrated mosquito management in urban landscaped areas by planting ornamental plant species that contribute to lower survivorship and fecundity of peridomestically produced mosquitoes.

Key Words: Asian tiger mosquito; vegetation; sugar feeding; biology; management

Resumen

Globalmente, *Aedes albopictus* Skuse (Diptera: Culicidae) ha ampliado considerablemente su hábitat de las zonas rurales a las áreas urbanas como resultado de una mayor urbanización. En algunas áreas urbanas, esta expansión ha resultado en que esta especie invasora se elevó como un vector importante del virus del dengue. Las plantas ornamentales son a menudo una característica de los lugares urbanos que puede proporcionar refugios para los mosquitos. Debido a que los mosquitos adultos requieren carbohidratos para la subsistencia, la vegetación presente en estos ambientes puede proporcionar fuentes naturales de azúcar para satisfacer esas necesidades. El objetivo de nuestro estudio fue determinar si la alimentación sobre diferentes plantas ornamentales en las áreas urbanas pueda afectar la sobrevivencia y la fecundidad de *Ae. albopictus*. Los mosquitos recién emergidos tuvieron acceso a 11 especies de plantas ornamentales (6 que florecen y 5 de plantas que no florecen) como fuentes de azúcar en condiciones de laboratorio. En general, la sobrevivencia fue significativamente mayor cuando los mosquitos se alimentaron sobre plantas ornamentales en comparación con los que solamente se les ofrecieron agua, mientras que la sobrevivencia fue mas corta cuando los individuos se alimentaron con solamente sacarosa ($P < 0.05$). Los mosquitos que se alimentaban de plantas sin flores pusieron menos huevos en comparación con los expuestos a las plantas con flores. No se observó una diferencia significativa en la eclosión de huevos de las hembras que se alimentaban sobre las varias especies de plantas. Nuestros hallazgos proporcionan una idea del potencial que las plantas ornamentales urbanas pueden tener para influenciar la ecología de los adultos de *Ae. albopictus*. Los resultados de este estudio proporcionan nuevas vias para el manejo integrado de mosquitos en jardines urbanos mediante la plantación de especies de plantas ornamentales que contribuyan a una menor sobrevivencia y fecundidad de los mosquitos producidos peridómicamente.

Palabras Clave: mosquito tigre asiático; vegetación; alimentación de azúcar; biología; administración

Aedes albopictus Skuse (Diptera: Culicidae) is currently considered to be one of the most invasive human disease vectors globally, and has expanded its habitat from Southeast Asia and the Indian Ocean islands to eastern Asia, northeast Europe, the Middle East, and the Americas (Benedict et al. 2007; Futami et al. 2015; Christy et al. 2017). This species has adapted successfully from its natural habitats (e.g., tree holes, bamboo tubes, bromeliads) in rural areas to artificial habitats (e.g., tires, plastic buckets, flower pots, etc.) in suburban and urban areas, and has become one of the most important mosquito vectors in urban areas (Mitchell 1995; Enserink 2008; Delatte et al. 2013). The global trend of rapid urbanization, in addition to changing temperatures, has played a key role in the range expansion of *Ae. albopictus* (Peng et al. 2012; Rezza 2012). Indeed, Li et al. (2014) recently showed that urbanization has increased the larval habitats of this mosquito species.

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According to a United Nations report, the global population in urban areas is expected to rise from 3.2 billion to 5 billion in the next few years, 90% of which will occur in developing countries (Rana 2011). Ornamental plants often are a feature of the urban landscape that provide tangible benefits by providing favorable environments for human physical activities, public health advancement, and socialization of urban residents. Also, landscape vegetation can provide visual enhancement of public areas, air filtration, and removal of pollution (Li et al. 2005; Wolch et al. 2014). Unfortunately, ornamental plantings may provide harborage for mosquitoes. Because adult mosquitoes require carbohydrates for subsistence, landscape vegetation may provide natural sugar sources to meet those needs.

Mosquitoes can obtain sugars from leaves, honeydew, floral and extrafloral nectaries, as well as exudates from rotting and damaged fruits (Foster 1995; Muller & Schlein 2005; Qualls et al. 2013). A previous study indicated that the availability of flowering plants could affect the sugar feeding rates of female mosquitoes, and was modulated by nectar availability (Martinez-Ibarra et al. 1997). As urbanization increases globally, and urban greening gains attention, it is crucial to evaluate the impact that landscape plantings have on the ecology of *Ae. albopictus* in these areas (Xu et al. 2007, Wu et al. 2010). The objective of the present study was to determine whether ornamental plants can affect the survivorship and fecundity of this vector species. Understanding the influence of surrounding vegetation in urban habitats on the bionomics of adult *Ae. albopictus* could benefit the management of this species by incorporating certain ornamental plants that contribute to local reduction of mosquito populations.

Materials and Methods

MOSQUITOES

Aedes albopictus eggs were obtained from a laboratory colony maintained at the Urban Entomology facility, Institute of Insect Sciences, Zhejiang University, People's Republic of China. Mosquitoes were reared in an insectary at 26 ± 1 °C, 75% RH, and a 14:10 h (L:D) photoperiod. Adult mosquitoes were maintained in 30 × 30 × 30 cm mesh-covered cages and provided a 10% sucrose solution ad libitum. Twice daily, larvae were fed a diet of commercial rat food mixed with wheat flour, wheat bran, corn powder, soybean powder, fish meat powder, yeast, salt, and vitamins at a rate of 0.5 g per 100 larvae (Yu et al. 2016). Pupae were collected daily and transferred to water-filled cups in mesh-covered cages for adult emergence.

PLANT MATERIALS

All ornamental plants used in this study are widely used for urban landscaping in most cities in south China. Cuttings were collected from the green belt of Zhejiang University (120.1200°E, 30.1600°N), Hangzhou, People's Republic of China, from May to Jul 2016, and consisted of 6 flowering plants: *Ligustrum quihoui* Carrière (Oleaceae), *Abelia grandiflora* (Rovelli ex André) Rehder (Caprifoliaceae), *Pittosporum tobira* (Murray) Aiton fil. (Pittosporaceae), *Ophiopogon japonicas* (L.f.) Ker-Gawl. (Liliaceae), *Pyracantha fortuneana* (Maxim.) H.L. Li (Rosaceae), *Nandina domestica* Thunb. (Berberidaceae), and 5 nonflowering plants: *Photinia fraseri* Dress (Rosaceae), *Buxus microphylla* Sieb. & Zucc. (Buxaceae), *Euonymus japonicas* Thunb. (Celastraceae), *Loropetalum chinense* (R. Br.) Oliv. (Hamamelidaceae), and *Viburnum odoratissimum* Ker-Gawl. (Caprifoliaceae) (Table 1, Fig. 1). For flowering plants, flowers and leaves were used in evaluations, whereas for nonflowering plants, only leaves were used. Plant matter was collected from sites where pesticides had not been used, and was inspected to ensure that potential predators were not present. Fresh plant cuttings (20 cm in length) were collected from the field 30 min prior to use in the experiment. Care was taken to prevent damage of the plant materials during transport.

SURVIVAL ASSAYS

To determine whether feeding on ornamental plants influenced *Ae. albopictus* survival, newly emerged mosquitoes (50 females, 50 males) were transferred to mesh-covered cages (30 × 30 × 30 cm) and offered cuttings from different plant species. Assays were conducted in an insectary controlled with a temperature of 26 ± 1 °C, 75% RH, and a photoperiod of 14:10 h (L:D). For each plant species, mosquitoes were given access to 50 g of fresh cuttings (25 g leaves for non-flowering plants, or 25 g flowers and leaves for flowering plants) and a water-soaked cotton pad. Cuttings were inserted into a 250 mL Erlenmeyer flask filled with water. The opening was sealed with Parafilm (Bemis Flexible Packaging, Neenah, Wisconsin, USA) to prevent mosquitoes from entering the flask. Control groups consisted of mosquitoes in cages with access to cotton pads saturated with 10% sucrose solution only (positive control) or distilled water only (negative control). All plant material, sucrose solutions, and cotton pads were replaced daily. During evaluations, survival of both sexes was recorded until all individuals died. Dead mosquitoes were removed daily from each cage at 8:00 PM. All of the experiments were replicated 3 times during the same time period.

FECUNDITY ASSAYS

In each trial of this experiment, 50 newly emerged mosquitoes (20 females, 30 males) were released into 1 cage and supplied with 1 of the

Table 1. List of ornamental plants and type of plant material used for *Aedes albopictus* feeding assays.

Species	Common name	Order: Family	Experimental use
Nandina domestica	Common nandina	Ranunculales: Berberidaceae	Flowers, leaves
Abelia grandiflora	Abelia	Dipsacales: Caprifoliaceae	Flowers, leaves
Ophiopogon japonicus	Radix ophiopogonis	Liliales: Liliaceae	Flowers, leaves
Ligustrum quihoui	Lobular privet	Scrophulariales: Oleaceae	Flowers, leaves
Pittosporum tobira	Pittosporum	Rosales: Pittosporaceae	Flowers, leaves
Pyracantha fortuneana	Firethorn	Rosales: Rosaceae	Flowers, leaves
Buxus microphylla	Lobular boxwood	Euphorbiales: Buxaceae	leaves
Euonymus japonicus	Ovatus aureus	Celastrales: Celastraceae	leaves
Viburnum odoratissimum	Japan arrowwood	Dipsacales: Caprifoliaceae	leaves
Loropetalum chinense	China loropetal	Hamamelidales: Hamamelidaceae	leaves
Photinia fraseri	Chinese photinia	Rosales: Rosaceae	leaves

Fig. 1. Common ornamental plants from urban areas used in survival and fecundity assays of adult *Aedes albopictus*.

plant species or 1 of the controls, as described earlier. More males than females were used to ensure that all females were inseminated. Female mosquitoes were provided with a blood meal from a restrained mouse for 30 min on the fifth d after emergence. Glass cups that contained 100 mL of distilled water and lined with filter paper on the interior wall surface were used as oviposition sites. Each group of blood-fed females was allowed to oviposit for 5 consecutive d in each cage. The number of mosquitoes that produced eggs and the total number of eggs per female for each plant species was recorded. During this assay all plant materials, sugar solutions, and water-soaked cotton pads were replaced every 2 d. The hatching rate of eggs also was recorded by placing them in dechlorinated tap water, and the emergent first instar larvae were counted daily. All assays were replicated 3 times during the same time period.

DATA ANALYSIS

Adult mean *Ae. albopictus* survivorship as affected by treatments was subjected to a 1-way ANOVA on transformed ($\log_{10}$) data. Survival time indices were calculated relative to the positive control (i.e., 10% sucrose solution) as follows: Survival Index = (positive control – treatment) / positive control (Manda et al. 2007; Yu et al. 2016). The Kaplan-Meier test, including a log rank test, was used to compare survival curves and test whether mean survival rate differed between different treatments or between sexes. Mean survival times were compared between treatments using a Student-Newman-Keuls (SNK) multiple range test (Manda et al. 2007; Yu et al. 2016).

Fecundity assay data were analyzed using the average number of *Ae. albopictus* eggs produced, and hatching rate of eggs laid by mosquitoes from different plant treatments. Mean number of eggs laid per female was $\log_{10}$ transformed, whereas the egg hatch data were arcsin-transformed prior to analysis. Transformed data were subjected to ANOVA. Effects of different treatments on fecundity were determined by the SNK test. For all assays, non-transformed means ± standard errors are reported in the text. All analyses were conducted using SPSS ver. 20 software (SPSS Inc., Chicago, Illinois, USA). Differences were considered significant at $P \leq 0.05$.

Results

SURVIVAL ASSAY

Mean survival of adult *Ae. albopictus* feeding on different plant cuttings were significantly different (F = 459.50; df = 12; P < 0.001). In addition, mean survivorship of mosquitoes given access to only 10% sucrose solution was 20.8 d, which was significantly greater than the mosquitoes that fed on plants (Table 2). However, mean survivorship of individual mosquitoes given access to *L. quihoui* was greater than those that fed on the other plant species or water alone (negative control). The lifespan of mosquitoes given access to *N. domestica* cuttings was significantly shorter than mosquitoes given access to other plant cuttings, compared with sucrose or only water.

The overall mortality rate of mosquitoes was influenced by the treatments (χ^2 = 4570; df = 12; P < 0.001) (Fig. 2). Approximately 90% of the individuals (pooled by sex) provided 10% sucrose (positive control) survived to about 15 d of age (Fig. 2A, B). Less than 10% of mosquitoes in the negative control survived beyond 5 d. Some individuals exposed to plants resulted in a faster rate of mortality compared with the positive control. Generally, 50% of mosquitoes were dead within the first 8 d regardless of the plant species to which they were exposed in cages, with the exception of those exposed to *L. quihoui* (Fig. 2C, D). The survival rate of mosquitoes feeding on this plant was consistently higher, with approximately 40% of mosquitoes still alive by d 20, and was even higher than the positive control after d 23.

Survival rates between female and male mosquitoes exposed to treatments were significantly different over time. Mean survival time of male mosquitoes fed on 10% sucrose was 19.4 d, which was 2.6 d shorter than females. In the treatment groups, most male mosquitoes died earlier than females with the exception of those exposed to caged *P. fraseri*, where no significant difference in mean survival was found between sexes (Table 3). The longest survivorship of females and males in treatment groups occurred with *L. quihoui*, where they lived 21.4 d and 15.8 d, respectively.

FECUNDITY ASSAYS

The number of eggs produced per female varied significantly among different treatment groups (F = 13.06; df = 9; P < 0.001). Mean number of eggs produced by mosquitoes exposed to *A. grandiflora* (71.3 ± 2.5) was similar to those produced by mosquitoes in the positive control (Table 4). Moreover, females in the above 2 groups produced significantly more eggs than females in the remaining treatments. The top 5 plant species that resulted in the most egg production were as follows: *A. grandiflora*, *L. chinense*, *V. odoratissimum*, *E. japonicas*, and *P. fraseri*. Mosquitoes exposed to *P. fortuneana*, *P. tobira*, and *L. quihoui* produced significantly fewer eggs per female than mosquitoes from other groups. Remarkably, we found that fewer eggs were oviposited by mosquitoes exposed to flowering plants compared with non-flowering plants, except flowering *A. grandiflora*, where mosquitoes that fed on this species produced the most eggs. No significant difference occurred in egg hatch from mosquitoes exposed to any of the plant species or sucrose (Table 4). Moreover, most mosquitoes exposed to caged *N. domestica*, *B. microphylla*, and water only were dead within the first 5 d; however, fecundity assays were not performed on those treatments.

Table 2. Mean ± SE daily survival of *Aedes albopictus* (males and females) exposed to different plant species, 10% sucrose, and water only under laboratory conditions.

Treatment regime	Number mosquitoes tested	Mean daily survival[a]	Range	Survival index[1]
10% sucrose	333	20.8 ± 0.3 a	3.0 – 33.0	0.00
L. quihoui	297	18.6 ± 0.6 b	4.0 – 45.0	0.14 a
P. tobira	317	11.8 ± 0.5 c	2.0 – 26.0	0.48 bc
L. chinense	295	10.5 ± 0.3 d	6.0 – 31.0	0.57 bc
P. fortuneana	298	8.1 ± 0.3 e	2.0 – 26.0	0.61 bcd
O. japonicus	306	7.5 ± 0.1 e	4.0 – 11.0	0.64 bcd
*A. grandiflo*ra	294	6.4 ± 0.1 f	4.0 – 12.0	0.69 cd
V. odoratissimum	302	6.2 ± 0.1 f	3.0 – 12.0	0.71 cd
E. japonicus	307	6.0 ± 0.1 f	4.0 – 11.0	0.76 cd
P. fraseri	297	6.0 ± 0.2 f	3.0 – 27.0	0.76 cd
B. microphylla	332	4.9 ± 0.1 g	2.0 – 6.0	0.77 cd
Water	295	3.9 ± 0.1 h	1.0 – 7.0	0.81 de
N. domestica	280	2.8 ± 0.1 i	2.0 – 6.0	0.86 e

[a]Means within a column followed by the same letter are significantly different from the reference group (i.e., 10% sucrose); Student-Newman-Keuls multiple range test, α < 0.05.
[1]Survival Index: (C-T)/C [(10% sucrose − treatment)/10% sucrose]

Discussion

In this study, we have shown that ornamental plants could influence survivorship and fecundity of *Ae. albopictus*. This information may provide insights into understanding the role of urban ornamental plants on this mosquito's ability to maintain populations in urban areas. Previous studies conducted by Wachira et al. (2014) and Silva et al. (2018) have shown that several characteristics of host plants, such as nutritional values and secondary chemical compounds, may affect the physiological processes of survivorship and fecundity of insects. It is well known that males of most mosquito species need to consume sugar from their local environment to support flight and mating performance. But sugar feeding is a common behavior of female mosquitoes when nectar sources are abundant, despite the presence of blood hosts. Qualls et al. (2013) reported that when *Ae. albopictus* was given feeding access to different indoor ornamental plants, the survival probability of both sexes increased significantly compared with water only. Generally, we found this to be true for the plant species we evaluated with the exception of *N. domestica* where survivorship was limited to 5 d. Iwasa et al. (2008) stated that this plant contained alkaloid compounds, such as jatrorrhizine, palmatine, and berberine, as well as some alkaloids.

Interestingly, we found that *Ae. albopictus* exposed to non-flowering plants produced more eggs than mosquitoes given access to most of the flowering plants. Mostowy and Foster (2004) suggested that egg-batch size is related to crop size and energy reserves. They found that blood-fed mosquitoes with full crops and low energy reserves produced the fewest eggs, whereas those with empty crops and high energy reserves produced the most eggs. We noticed that the crops of female mosquitoes exposed to non-flowering plants in our study were empty; this may be an indication that those individuals may have had difficulty in feeding on leaves. This situation allowed them to increase the volume of blood ingested during a blood meal that, in turn, contributed to higher egg-production.

The global trend of urbanization and the subsequent adaptation of *Ae. albopictus* to urban areas have undoubtedly provided additional factors that influence the ecology of this species. We found that certain ornamental plants in urban areas can have a strong impact on the survival and fecundity of *Ae. albopictus*. Indeed, the availability of plant sugar sources in urban areas may play a key role in the local spatial distribution of mosquitoes. Previous studies have reported that the absence of plant sugar sources caused a reduction of mosquito survivorship and insemination rates that con-

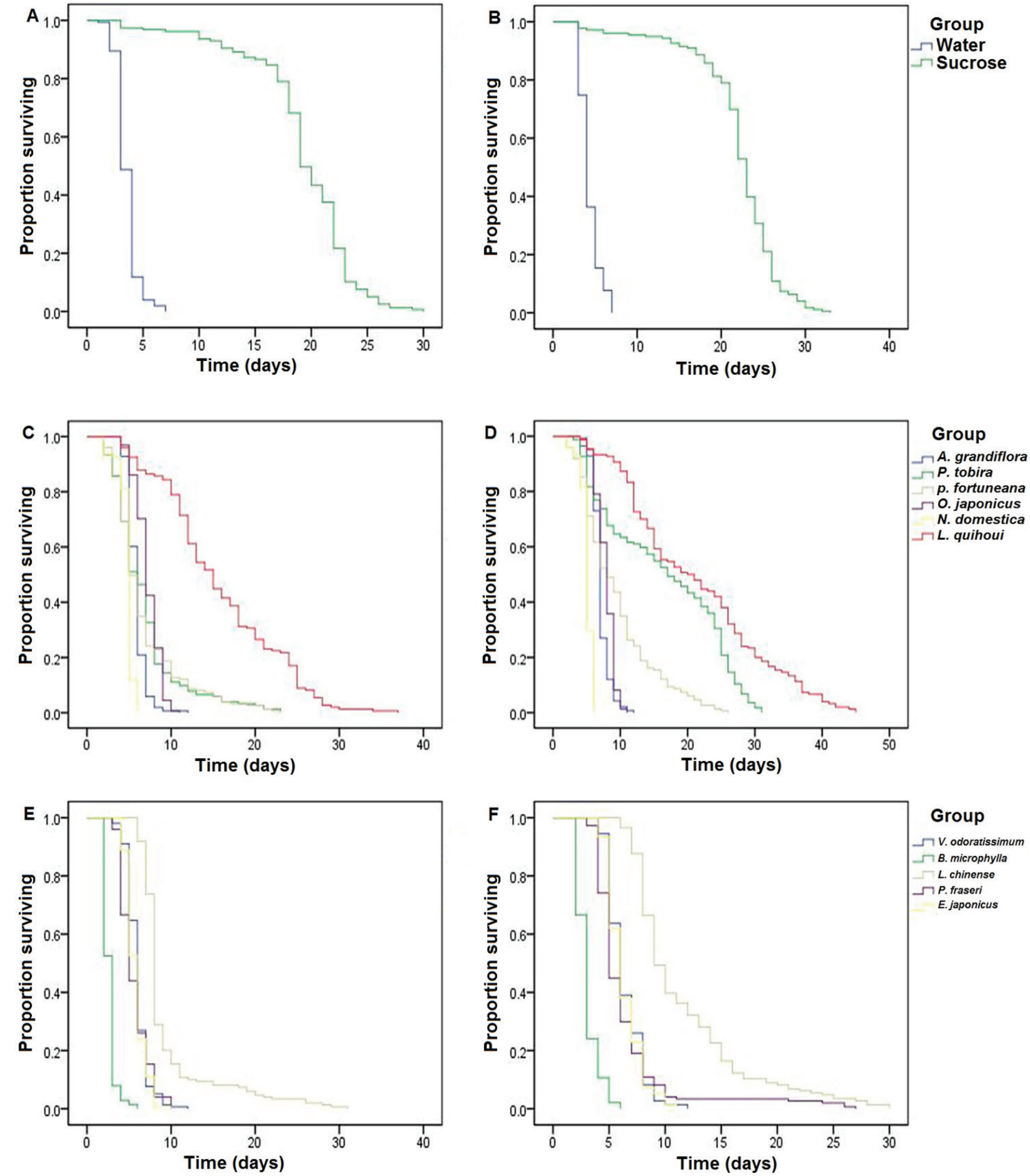

Fig. 2. Survival curves of male (A, C, E) and female (B, D, F) *Aedes albopictus* exposed to different plant species, 10% sucrose, or water only. A and B refer to control groups; C and D refer to flowering plants; E and F refer to nonflowering plants.

tributed to eventual population decline (Stone et al. 2009, 2012). Moreover, Harrington et al. (2001) reported that the short-range movement of some mosquitoes indicated that their living spaces were circumscribed; hence, it is plausible that availability of plant sugar sources could impact local mosquito populations. Thus, our results could provide new directions for integrated mosquito man-

Table 3. Comparison of mean ± SE daily survival of *Aedes albopictus* (male and female) stratified by treatment regime (log-rank test).

Treatment regime	Males (N)	Females (N)	χ^2*	*P*
10 % sucrose	(156) 19.4 ± 0.4	(177) 22.0 ± 0.4	45.1	0.000
L. quihoui	(147) 15.8 ± 0.6	(150) 21.4 ± 0.9	34.5	0.000
P. tobira	(153) 6.8 ± 0.3	(164) 16.6 ± 0.7	128.6	0.000
L. chinense	(149) 7.5 ± 0.4	(146) 9.6 ± 0.4	14.7	0.000
P. fortuneana	(149) 6.8 ± 0.3	(149) 9.4 ± 0.5	22.6	0.000
O. japonicus	(158) 7.2 ± 0.1	(148) 7.8 ± 0.1	9.4	0.002
A. grandiflora	(156) 6.0 ± 0.1	(146) 6.4 ± 0.1	6.1	0.013
V. odoratissimum	(156) 6.0 ± 0.1	(146) 6.4 ± 0.1	6.1	0.013
E. japonicus	(154) 5.8 ± 0.1	(153) 6.3 ± 0.1	11.1	0.001
P. fraseri	(150) 5.6 ± 0.1	(147) 6.4 ± 0.3	2.7	0.098
B. microphylla	(190) 4.8 ± 0.1	(142) 5.0 ± 0.1	8.8	0.003
Water	(152) 3.55 ± 0.08	(143) 4.4 ± 0.1	33.0	0.000
N. domestica	(139) 2.7 ± 0.1	(141) 3.0 ± 0.1	12.0	0.001

*Test statistic for log rank analysis: survival distribution comparison between male and female mosquitoes in each respective treatment regime (row).

Table 4. Mean ± SE fecundity, and associated percent egg hatch, of single blood fed *Aedes albopictus* exposed to each treatment regime.

Treatment regime	N	Eggs laid	Hatching rate (%)
A. grandiflora	33	71.3 ± 2.5 a	55.0 ± 1.4 a
10 % sucrose	42	69.0 ± 0.8 ab	55.1 ± 2.2 a
L. chinense	20	64.6 ± 0.8 b	57.7 ± 2.8 a
V. odoratissimum	38	63.1 ± 0.1 b	53.7 ± 1.6 a
E. japonicus	47	62.9 ± 0.7 b	66.0 ± 6.3 a
P. fraseri	50	60.8 ± 1.6 bc	58.8 ± 10.6 a
O. japonicus	17	59.5 ± 2.2 bc	61.7 ± 2.0 a
L. quihoui	58	56.0 ± 3.0 cd	52.8 ± 6.0 a
P. tobira	23	55.2 ± 1.7 cd	62.1 ± 2.8 a
P. fortuneana	31	51.9 ± 0.8 d	68.7 ± 4.0 a

N = number of mosquitoes that oviposited. Any 2 means with different letters in the same column are significantly different; Student-Newman-Keuls multiple range test, $\alpha < 0.05$.

agement in urban areas by planting ornamental plant species that would contribute to lower survivorship and fecundity of mosquitoes, leading to a reduction of the local mosquito population.

ETHICAL STATEMENT

All applicable international, national, and institutional guidelines for the care and use of animals were followed. The study protocol received approval from the local health and administrative authorities (animal use approval protocol number: SYXK 2012-0178, Zhejiang University). These studies did not involve endangered or protected species.

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