Scientific Note

Tree holes as larval habitats for *Aedes aegypti* in public areas in Aguaray, Salta province, Argentina

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INTRODUCTION

Aedes aegypti L. is the main vector of dengue and urban yellow fever in the world (Gubler 2004). In Argentina, the largest dengue epidemic was recorded during 2009, affecting an estimated 26,000 people in 18 provinces (Torres 2010). Indigenous dengue cases have been recently reported in the city of Aguaray in northern Salta province, also located on a principal terrestrial transport and commercial route connecting northwest Argentina with Bolivia, the main route of introduction of dengue to northwest Argentina. Contact between Bolivian and northwestern Argentine *Ae. aegypti* populations is confirmed by a shared haplotype, and stresses the importance of passive transportation as a major factor favoring the mosquito reintroduction and dispersal in Argentina (Rondan Dueñas et al. 2009).

Aedes aegypti is highly adapted to the human environment, and artificial containers are the larval habitat most commonly used by this species (Forattini 2002). Nevertheless, in some countries in Africa, where the species might have originated, *Ae. aegypti* is a regular component of the tree hole fauna (Lounibos 1981, Anosike et al. 2007). Observations of *Ae. aegypti* developing in tree holes and other natural water containers are also reported from other regions of the world . In the Pacific Islands, larvae and pupae were found in tree holes and coconut shells, but none were collected in leaf axils (Burkot et al. 2007). In the Americas, prior to the eradication campaign of the 1950s and 60s, *Ae. aegypti* larvae were frequently found in human artifacts and sometimes also in tree holes along city streets and parks, bamboo stems, coconut shells, and damaged papaya trees (Soper 1967). Since then, there have been only a few reports from natural containers, such as tree holes, in South America (Tubaki et al. 2010). To the best of our knowledge there are no previous published reports of *Ae. aegypti* in tree holes or other phytotelmata in Argentina (Raúl Campos has observed *Ae. aegypti* larvae in a tree hole in Buenos Aires (*pers. com*.).

Even though mosquitoes whose aquatic stages occupy water-filled tree-holes include some of the most important transmitters of arboviruses, such as *Haemagogus janthinomys*, *Ae. aegypti, Aedes albopictus,* and *Sabethes chloropteurs* (Forattini 2002), studies of tree holes as mosquito larval habitats are scarce in South America. Knowing whether *Ae. aegypti* may use natural water containers in a region is important in the vector control framework, and also from an epidemiological point of view, because invasive mosquito species may alter the local mosquito fauna and pathogen transmission cycles (Bevins 2008). The objective of this study was to determine if *Ae. aegypti* and other mosquito species use tree holes as larval habitats in Aguaray, as part of a larger project on the influence of land cover change on mosquito populations.

Aguaray is located 20 km from the border with Bolivia (63° 44′W–21° 16′S), at 568 m above sea level. It has a population of approximately 13,500 covering an area of 2,265 km^2. The climate is subtropical, with an average summer temperature of 27.9° C and an average winter temperature of 13.4° C (http://www.portaldesalta.gov.ar/sanmartin01.htm). The annual rainfall is above 1,100 mm, occurring mostly during the warmer months (October to April, Figure 1) (http://smn.gov.ar). The area is included in the yunga subtropical montane moist forests (Brown et al. 2002) and has been subjected to ecological modifications related to human activities (urbanization, industrial development, agriculture, forestry, etc.).

We sampled trees along sidewalks and public access areas in 14 blocks selected at random. Tree species and the presence or absence of water holding tree holes were recorded. Once the tree holes were identified, the presence of water and larvae in each one was recorded. The larvae and pupae were collected using a siphon bottle (Müller and Marcondes 2006) during March and April, 2010. Immature stages were counted *in situ* and transported to the lab for further processing. Third and 4th instar larvae were killed and stored in 80% ethanol for taxonomic determination

Keyword Index: Mosquito, dengue vector, yellow fever, biological invasions, phytotelmata.

Table 1. Information about the tree species that were inspected for water holding holes and mosquito larvae (L) and/or pupae (P) in side walks and other public access areas in the locality of Aguaray, Salta province, Argentina. All larvae and pupae detected were *Ae. aegypti*.

Tree species*	Trees examined	Trees with holes	Holes with water	Holes with L and/or P	L+P collected
Bahuinia sp.	8	3	1	0	0
Bougainvillea sp.	3	0	0	0	0
Citrus sinensis	7	1	1	1	1
E. contortisiliquum	4	1	1	1	4
Ficus sp.	9	2	0	0	0
Lagerstroemia indica	17	3	1	0	0
Melia azedarach	17	4	0	0	0
Morus alba	28	12	4	2	25
Salacia spp. 86	2	1	0	0	0
Thevetia nereifolia	11	4	2	1	4
Tipuana tipu	1	1	0	0	0

E. = *Enterolobium.* *Tree species that were examined but did not have tree holes include (number of trees in parenthesis): *Citrus limon (1), Citrus paradisi (3), Cotinus coggygria (1), Cupressus sp (5), Delonix regia (3), Eriobotrya japonica (4), Erythrina crista-galli (4), Eucalyptus sp. (3), Ficus benjamina (2), Fraxinus sp.(7), Grevillea sp (15), Hibiscus sp. (1), Jacaranda sp. (3), Manihot grahamii (1), Persea americana (4), Pinus sp. (2), Salacia spp. (2), Tabebuia sp. (11), undetermined (4), and Washingtonia filifera (3).*

(Darsie 1985), while 1st and 2nd instars and pupae were reared either to the 4th instar or to adult emergence. The monthly Breteau Index (BI: number of positive containers per 100 premises inspected) and Housing Index (HI: percentage number of houses infested per number of houses inspected) were provided by the Dr. L. Güemes Hospital of Aguaray (for index calculation, the city is divided in nine areas).

A total of 186 trees was examined, of which 33 (17.7 %) had holes, and 11 of the trees had holes that held water (Table 1). Larvae were found in five of 12 water holding holes, pupae were collected from three holes, and all of them were *Ae. aegypti* (Table 2). Tree holes held water for approximately a week after a rain event (Figure 1), enough time for some adults to emerge. The fact that pupae were collected from three of the five water-holding holes indicates that in Aguaray immature stages may complete their development in these larval habitats. From January to April, 2010 there were 11 suspected, but not laboratory confirmed, dengue cases in Aguaray (Dr. L. Güemes Hospital). Although with this study we cannot assess, nor do we expect, a relationship between the presence of larvae in tree holes and dengue virus transmission, the fact that pupae were detected highlights the potential role of these environments as reservoirs of the mosquito.

The number of larvae collected from the tree holes was comparable to collections reported from other areas, such as tropical rainforests in South-East Nigeria (ranging from three to ten larvae, Anosike et al. 2007), and American Samoan villages (16 larvae in 22 tree holes, Burkot et al. 2007). *Aedes aegypti* productivity from natural containers in general (leaf axils of plantain/banana, cocoyams, coconut shells) is usually low compared to artificial containers such as used tires (for example, Anosike et al. 2007, Burkot et al. 2007). In Brazil, fortnight collections for 11 months from 18 holes per tree (in an undetermined number of trees) yielded only seven *Ae. aegypti* larvae (Tubaki et al. 2010). On the other hand, in the Caribbean region calabash fruit and tree holes were the natural sites most frequently used by *Ae. aegypti* (Chadee et al. 1998).

In March, both BI and HI were higher in the city zones where larvae were detected in tree holes compared to areas with tree holes negative for larvae (BI: 32.6 ± 12.6 and 25.2 ± 6.2, respectively; HI: 18.6 ± 0.6 and 12.3 ± 3.2 for zones with tree holes with larvae and holes without larvae, respectively). However, in April both indexes were similar for areas with positive tree holes and tree holes without larvae (BI: 22.7 ± 14.2 vs. 23.8 ± 5.2; HI: 14.3 ± 6.3 vs. 14.67 ± 4.3). Further studies including longer time series and a larger number of tree holes are needed to assess whether the use of tree holes as larval habitats is related to use/availability of artificial containers (as estimated through Stegomyia indexes).

Chadee et al. (1998) propose that exploitation of artificial and natural containers may explain the limited success of past efforts to eradicate *Ae. aegypti*, because switching oviposition preferences from natural to artificial containers would increase the number of optimal breeding

Table 2. Information on the tree holes where *Aedes aegypti* larvae (L) and/or pupae (P) were collected in side walks and other public access areas in the locality of Aguaray, Salta province, Argentina.

Tree species	Sampling date					Tree hole characteristics				
	18-Mar-10	26-Mar-10	2-Apr-10	7-Apr-10	30-Apr-10	Water volume	Hole depth (cm)	Orientation	Height in tree trunk (cm)	Tree-hole type
Thevetia neifolia	2L	D	0	D	1L+1P	<45 ml	4	N	66	BI
Morus alba	13L	D	0	6L	D	<45 ml	4	T	88	BI
Morus alba	D	D	D	0	4L+2P	<45 ml	2	T	28	BI
Citrus sinensis		D	1L	D	0	<45 ml	8	S	82	BI
E. contortisiliquum			0	1L	2L+1P	180 ml	6	W	131	RH
Bahuinia spp.	D	D	D	D	0	<45 ml	4	W	114	BI
Ficus benjamina	D	D	D	D	0	<45 ml	4	W	114	RH
Lag. indica	0	D	0	0	0	<45 ml	11	S	104	RH
Morus spp.	D	D	D	D	0	<45 ml	4	T	115	BI
Morus spp.	D	D	D	D	0	<45 ml	6	W	153	RH
Thevetia neifolia	D	D	D	D	0	<45 ml	2	N	106	BI

E. = *Enterolobium.* D = the hole was dry; Orientation of the hole opening: N = North, S = South, E = East, W = West, T = Top; tree hole types: BI = pan at branch intersection; RH = rot hole .

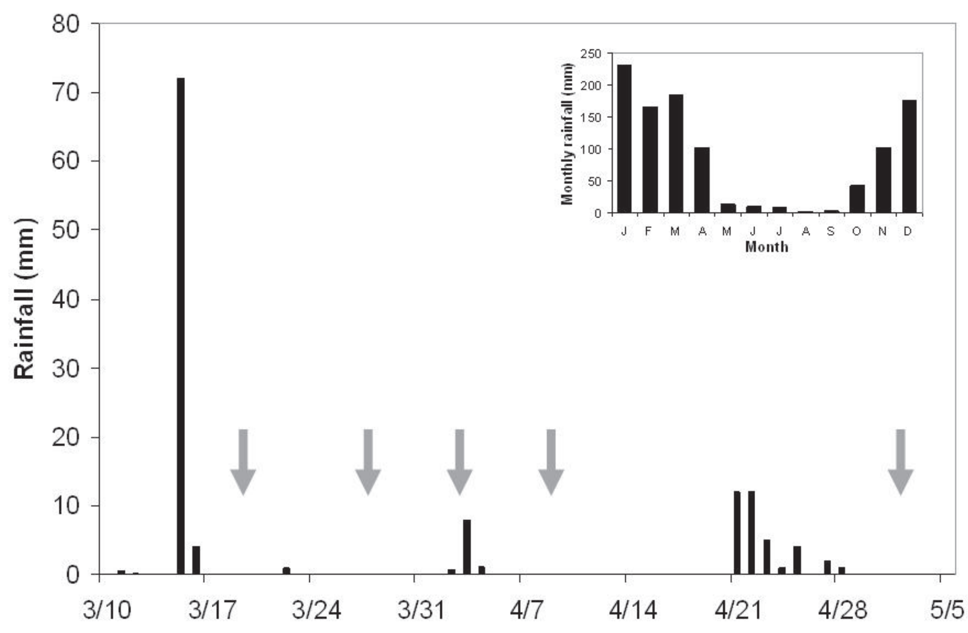

Figure 1. Rainfall (mm) events in the study area. Arrows indicate sampling dates. Inset shows average monthly rainfall (data provided by the National Meteorological Service).

sites available, thus increasing fitness by reducing density dependent factors, search time, and time dependent mortality.

Since it is assumed that *Ae. aegypti* breeds almost exclusively in artificial containers, potential larval habitats, such as tree holes, may be overlooked by vector control personnel. Even though in urban environments these natural habitats may appear to be comparatively scarce in relation to artificial containers, and their productivity may be low, they nevertheless may contribute to maintenance of populations of *Ae. aegypti* and become relevant as reinfestation sources if monitoring and control are not sustained.

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