Characterization of *Aedes aegypti* (Diptera: Culcidae) Production Sites in Urban Nicaragua

SAMANTHA N. HAMMOND,[1] AUBREE L. GORDON,[1] EMPERATRIZ DEL C. LUGO,[2] GILBERTO MORENO,[3] GUILLERMINA M. KUAN,[4] MARÍA M. LÓPEZ,[2] JOSEFA D. LÓPEZ,[2] MARCO A. DELGADO,[2] SONIA I. VALLE,[2] PERLA M. ESPINOZA,[2] AND EVA HARRIS[1,5]

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ABSTRACT To characterize the production patterns of the dengue virus vector *Aedes aegypti* (L.) (Diptera: Culcidae), pupal surveys were conducted in selected neighborhoods of two major cities in Nicaragua. In León, 833 houses were visited in July and September 2003, corresponding to the beginning and middle of the dengue season; in Managua, 1,365 homes were visited in July 2003. In total, 7,607 containers were characterized, of which 11% were positive for *Ae. aegypti* larvae and 4% for pupae. In addition to barrels, potted plants and superficial water on tarps and in puddles were identified as highly productive sites. Univariate and multivariate analysis revealed frequency of container use, use of a lid, and rainwater filling as key variables affecting pupal positivity. Importantly, this survey demonstrated the risk associated with the presence of lids, the limited temporal efficacy of temephos, and the lack of association of water availability with risky water storage practices. Finally, we introduce the concept of an efficiency value and an accompanying graphical display system that can facilitate development of targeted pupal control strategies. These data underscore the importance of entomological surveillance of pupal productivity to gather information from which to derive streamlined, efficient, and effective vector control measures to reduce the density of *Aedes* mosquito larvae and pupae and thus the risk for dengue.

KEY WORDS *Aedes aegypti*, pupal survey, productivity, dengue, Nicaragua

Dengue is the most prevalent mosquito-borne viral illness in the world, and its impact continues to increase as the virus and its vector expand into more tropical and subtropical regions. Dengue is caused by four serotypes of the dengue flavivirus (DENV1–4), and ranges from asymptomatic infection to classic dengue fever, an acute febrile disease with headache, muscle ache, and joint pain, to dengue hemorrhagic fever, a severe illness characterized by increased vascular permeability, which may lead to shock and death if appropriate treatment is not received in a timely manner. Supportive treatment for the disease has improved measurably in the past two decades, significantly reducing mortality due to dengue; most fatalities are currently caused either by mismanagement or failure to implement supportive therapy early enough in the illness (Monath 1994, Kalayanarooj and Nimmannitya 2004). The economic and social cost of dengue is very high, with disability adjusted life years (DALYs) equivalent to 420–658 per 1,000,000 habitants per year (Meltzer et al. 1998, Shepard et al. 2004, Clark et al. 2005); this impact is exacerbated by the resource-poor developing country setting of the majority of endemic areas (Gubler and Meltzer 1999, DeRoeck et al. 2003, Shepard et al. 2004).

Aedes aegypti (L.) (Diptera: Culicidae), a domestic daytime-biting mosquito, is the principal vector for dengue virus (family *Flaviviridae*, genus *Flavivirus*, DENV) in the Americas, and it is a major DENV vector in most Southeast Asian countries. The mosquito is characterized by its biting pattern, which consists of multiple bloodmeals during each egg-laying cycle, and the ability of its immature stages to grow in clean water (Gubler 1988, Scott et al. 2000). These features make it an ideal vector for DENV transmission, especially in large urban areas, where there are high human population densities and numerous artificial containers where the aquatic stages of *Ae. aegypti* flourish (Tun-Lin et al. 1995).

Mosquito control is currently the only way to curb the spread of dengue, and vector control will remain

[1] Division of Infectious Diseases, School of Public Health, University of California, Berkeley, CA 94720–7360.
[2] Dirección de Entomología Médica, Centro Nacional de Diagnóstico y Referencia, Ministerio de Salud, Managua, Nicaragua.
[3] Dirección de Salud Ambiental y Epidemiología, SILAIS León, Ministerio de Salud, León, Nicaragua.
[4] Centro de Salud Socrates Flores Vivas, Ministerio de Salud, Managua, Nicaragua.
[5] Corresponding author: e-mail: eharris@berkeley.edu.

integral to controlling the disease even after the eventual implementation of a safe and effective vaccine. Vector control has been principally based on insecticide treatments (including ultralow volume spraying to target the adult mosquito population and temephos application in breeding sites to impede the maturation of the aquatic stages of the mosquito), and it is presently evaluated using surveillance techniques based on larval container indices to determine risk and to guide mosquito control activities (PAHO 1994). However, recently there has been a movement toward pupal indices (Focks et al. 1993a, 2000; Focks and Chadee 1997; Getis et al. 2003; Morrison et al. 2004; Barrera et al. 2006b; Romero-Vivas et al. 2006) or monitoring of adult populations (Focks et al. 1993a, Tun-Lin et al. 1996, Getis et al. 2003), considering early instars as too immature to be representative of true mosquito productivity, because survival from early stage larvae to pupae is variable and a majority of larvae do not survive to adulthood. Furthermore, increased awareness of the lack of sustainability of vertical insecticide-based interventions is driving a shift to greener community-based vector control measures that focus on breeding site elimination or destruction of aquatic-phase *Aedes.* To maximize the efficiency of source reduction campaigns, it is critical to know which containers are most productive. Because productivity patterns of *Ae. aegypti* vary from country to country (Morrison et al. 2004, Barrera et al. 2006a, Romero-Vivas et al. 2006), we performed a cross-sectional entomological survey in the two major Nicaraguan urban centers to identify the most productive domestic and peridomestic containers and to evaluate larval versus pupal surveillance strategies in Nicaragua. This report, which is the first of its kind in Central America, highlights several issues that may help guide vector control strategies in the future.

Materials and Methods

Study Site. León and Managua, with populations of ≈151,000 and 1.4 million persons, respectively, are the largest urban centers in Nicaragua. The two cities are located 80 km from each other on the Pacific side of the country. León, spanning 22 km^2, is located at an altitude of 109 m above sea level, with geographic coordinates of 12° N, 86° W. Approximately 80 km to the south of León, Managua is situated at an altitude of 80 m above sea level and encompasses 400 km^2. The site of the study in District II, Managua, was comprised of 18 neighborhoods with a population of 62,398 persons, in which 1,373 homes participated in the study. The area covered by the study in León had a population of 78,677, in which 833 houses participated in the study. Ambient temperatures in both cities range from 18 to 40°C, averaging 27–28°C. Both cities experience distinct rainy (May–January) and dry (February–April) seasons, with an average annual precipitation of 1,260 mm. Potable tap water in León is available 24 h/d in both the rainy and the dry seasons, whereas tap water availability in District II of Managua is limited to ≈10 h/d, with less during the dry season. Homes with built-in water tanks that act as individual reservoirs are unusual in the District II neighborhoods included in the study. Both León and Managua are considered areas of high dengue transmission.

Household Selection and Time Line. In June 2003, an entomological survey was conducted in 833 houses in León, and 1,373 houses in District II of Managua. Participating households were at least three houses apart, and they had child residents; in addition, the occupants agreed to participate in an accompanying study on the incidence and prevalence of DENV infection in children. This systematic sampling design was chosen 1) because the Nicaraguan Ministry of Health traditionally performs entomological surveys on 33% of homes in a given area, which translates roughly to every third house; and 2) to maximize the geographical coverage within neighborhoods and districts, given the constraints of the study size and resources. Sections of 20 barrios in León and 18 barrios in Managua were included, represented by 1,373 homes in Managua and 833 homes in León. Six hundred and one of the original 833 houses in León participated in a repeated survey in September 2003. The houses that were sampled once in León did not differ significantly during the first visit from the houses that were sampled twice in terms of the average number of pupae per house, the percentage of houses positive for pupae, and the average number of containers per house, as determined by two-sided *t*-test or chi-square test.

Entomological Inspection and Questionnaire. An entomologist visited each household to conduct the entomological surveys, along with a study nurse who collected samples for the accompanying serological survey. All potential breeding sites—containers or surfaces containing stationary water—were identified during each house visit. Detailed characteristics were documented for all containers, including: inside or outside location, sun exposure, location under a roof, size, method of filling (manual or rain-filled), frequency of filling, frequency of use, presence of fish (biological control agents), and presence and condition of a lid. At the first visit, a household questionnaire was administered that assessed the following: need for water storage, material of home construction, type of sewage system, access to trash collection, acceptance and use of Ministry of Health (MOH) mosquito source-reduction methods, and self-reported cases of dengue within the previous 6 mo. At the following visits, a questionnaire was administered to document self-reported cases of dengue since the last survey, although this measure is clearly subjective and prone to error.

Laboratory Methods. All larvae and pupae were transported to the entomology laboratories located at either the Centro Nacional de Diagnóstico y Referencia (CNDR) of the MOH in Managua or at the Sistema Local de Atención Integral de Salud (SILAIS) in León for species identification by morphological features under stereoscope and microscope. Relative numbers of larvae in each container were estimated in the field in units of 1–10, 10–100, and ≥100, and aliquots of larvae from each container were removed for morphological analysis and speciation. Pupae were left to emerge as adults to identify and enumerate by species and gender. Samples of larvae and pupae are deposited at the National Center for Diagnostics and Reference, Nicaraguan Ministry of Health, Managua.

Measurements. The house index is defined as the percentage of houses infested with *Ae. aegytpi* larvae and/or pupae, the container index is defined as the percentage of water-holding containers infested with *Ae. aegytpi* larvae and/or pupae, and the Breteau index is defined as the number of larvae- or pupae-positive containers per 100 houses (PAHO 1994). Productivity of a container type is defined as the number of pupae in each container type divided by the total number of pupae in all containers (Focks and Chadee 1997, Morrison et al. 2004, Barrera et al. 2006a). Prevalence of a container type is defined as the total number of the container type identified divided by the total number of all containers. Efficiency of a container type is defined as the pupal productivity of a container type divided by the prevalence of that container type. It is used to determine effectiveness of source reduction targeted to particular container types.

Containers were categorized in the following manner: a barrel is a 55-gallon metal drum; a wash basin (pila) is a concrete sink that often has no drain. Buckets refer to two types of plastic water storage containers: narrow, tall cylindrical containers (baldes), or shorter wider ones (tinas). Bowls to transfer water (panas) are used to pour water from a water storage container or a wash basin over an item to wash it or fill it with water. Additional containers and their respective local terms are tires (llantas), cooking pots (casuelas), metal paint cans (potes, latas), and potted plants (maceteras). Natural reservoirs and superficial water were also scored. The "other" group consisted of bottles (botellas), flower vases (floreros), children's toys, and assorted items.

Statistical Analysis. Two-sided *t*-tests and chi-square tests were used to assess the significance of differences, as appropriate. Odds ratios (OR) and their Cornfield 95% confidence intervals were estimated. Correlations were calculated using the Pearson correlation coefficient. Univariate and multivariate logistic regression analysis were performed in a model based on presence or absence of pupae per container. Multivariate models were constructed using backward-stepwise construction with a P value of <0.20 as the cut-off for significance. Variables and correlations were assessed before the construction of the model. Maximum likelihood tests were further used to assess the significance of variables before removal from the model. Interactions were assessed in the multivariate models. Analyses were conducted in STATA, version 8 (Stata Corporation, College Station, TX). To assess the validity of combining the two sites (Managua and León), which differ in a number of aspects, including access to running water, the sites were analyzed separately and compared. No statistically significant trend was observed between the availability of these services and the number of potential breeding sites, nor with the number of larvae or pupae. In addition, no significant differences were observed in the container prevalence/production patterns in the two sites. Therefore, all further analysis was performed using the combined data set. To confirm the assumption that the data from the two cities could be combined, a site variable was included in the final regression model and a P value of 0.63 was obtained. A maximum likelihood test was also performed; the results indicated that location is not statistically significant. Finally, addition of the site variable did not produce a significant effect ($<5\%$ change) on the coefficients of the other variables.

Results

A combined total of 2,807 houses were surveyed in Managua in June 2003 and León in June and September of 2003, corresponding to the start and middle of the rainy season. Seroprevalence in children in the study area increased by age and ranged from 29 to 83% and from 26 to 93% in 2–14 yr-old children in León and Managua, respectively, as determined by inhibition enzyme-linked immunosorbent assay (Harris et al. 2000, Balmaseda et al. 2006; A. Balmaseda, S.N.H., and E.H., unpublished data), indicating high endemicity of DENV. Of the 2,807 houses investigated in both León and Managua, 9.6% of homes were positive for *Ae. aegypti* pupae, and each home contained an average of 2.7 potential breeding sites, or water-filled containers (range 0–18). Seven thousand six-hundred and seven potential breeding sites were analyzed, of which 872 (11.4%) were positive for *Ae. aegypti* larvae and 328 (4.3%) contained pupae. Four thousand five hundred and eleven *Ae. aegypti* pupae were identified from the 328 containers, with an average of 13.8 pupae per pupae-positive container (PPC). The most commonly collected larvae were *Ae. aegypti,* followed by *Toxorhynchites theobaldi* (Dyar and Knab), *Aedes albopictus* (Skuse), *Culex nigripalpus* (Theobald), *Culex corniger* (Theobald), and *Aedes* (Howardina) (Lugo et al. 2005).

Houses in León, which have running water 100% of the time, contained an average of 3.1 potential breeding sites, 0.50 water storage containers, and 0.24 barrels per house; Managua, which has running water only several hours a day, had 2.4 potential breeding sites per house, 0.41 water storage containers, and 0.22 barrels per house. The average overall property size and average number of persons per home were also comparable: 552 m^2 and 6.6 persons per house in León and 302 m^2 and 6.9 persons per house in Managua. In addition, both cities had a similar number of neighborhoods with limited trash collection services and no piped water within the house perimeter.

Principal Breeding Sites. The most productive breeding sites were found to be barrels, wash basins, natural reservoirs, tires, buckets, and superficial water, making up 29, 10, 7, 7, 7, and 6% of all pupae identified, respectively (Table 1). Additional container types, each producing 3–4% of all pupae, were bowls, large tin cans, cooking pots, and potted plants. The "other"

Table 1. Productivity and efficiency of principal water-containing receptacles

Receptacle type	Pupae			Containers (n)	Productivity[a]	Prevalence of container[b]	Efficiency[c]
	n	Avg[d] (SD)	Max[e]				
Superficial water	273	21 (53)	215	100	6.2	1.3	4.7
Potted plants	132	33 (41)	92	51	3.0	0.7	4.5
Barrels	1,296	22 (31)	175	651	29.4	8.5	3.5
Natural reservoirs	312	24 (42)	135	213	7.1	2.8	2.5
Tin cans	198	18 (45)	153	165	4.5	2.2	2.1
Cooking pot	135	9 (10)	24	119	3.1	1.6	2.0
Animal water dish	135	27 (39)	95	149	3.1	2.0	1.6
Tires	301	7 (8)	41	353	6.8	4.6	1.5
Bowls to transfer water	150	10 (20)	82	307	3.4	4.0	0.84
Buckets	286	11 (23)	119	622	6.5	8.2	0.79
Other	744	8 (10)	46	1,719	16.9	22.5	0.75
Wash basins	448	16 (27)	140	3,179	10.2	41.7	0.24
Total	4,412	14 (26)	215	7,628	100	100	1

[a] Productivity = pupae no. × 100/all pupae.
[b] Prevalence of container = container no. × 100/all containers.
[c] Efficiency = productivity/prevalence of container.
[d] Average no. of pupae.
[e] Maximum no. of pupae in an individual container.

category contained 17% of all pupae (Table 1), with each item producing <1% of pupae, and included flower vases and bottles.

Efficiency values of container types (pupal productivity of a container type divided by its prevalence) varied greatly, and they were not predicted by productivity. The range of efficiency values was 0.24 (wash basins) to 4.7 (superficial water) (Table 1), with 1.0 being the value if all containers were equally efficient. Of the six most productive breeding sites, only four had efficiency values >1. A graphical representation of container prevalence adjacent to productivity (in order of efficiency) demonstrates the potential impact of targeting a particular container type (Fig. 1). Ten percent of all containers produce almost 40% of all pupae, and conversely ≈40% of all containers produce 10% of all pupae. The importance of barrels as a target of intervention is clearly shown (Fig. 1), in that barrels have a high efficiency and are sufficient in number such that in theory, targeting by effective control measures would substantially reduce the number of pupae produced.

The high productivity but low efficiency of pupae production in washbasins led us to investigate whether targeting a specific feature could increase the efficiency of mosquito control efforts. As virtually all study houses (92%) had washbasins (Table 2), we investigated whether the number of washbasins in a house significantly affected the probability that the washbasin was positive. Houses with pupae-positive washbasins had on average 2.4 washbasins, whereas houses with pupae-negative washbasins had on average 1.3 washbasins. The data for larvae positivity were similar; houses with washbasins containing larvae or pupae had 2.0 washbasins per house, whereas homes with washbasins negative for larvae or pupae contained on average 1.2 washbasins. In the study, 497 (26%) houses contained more than one washbasin,

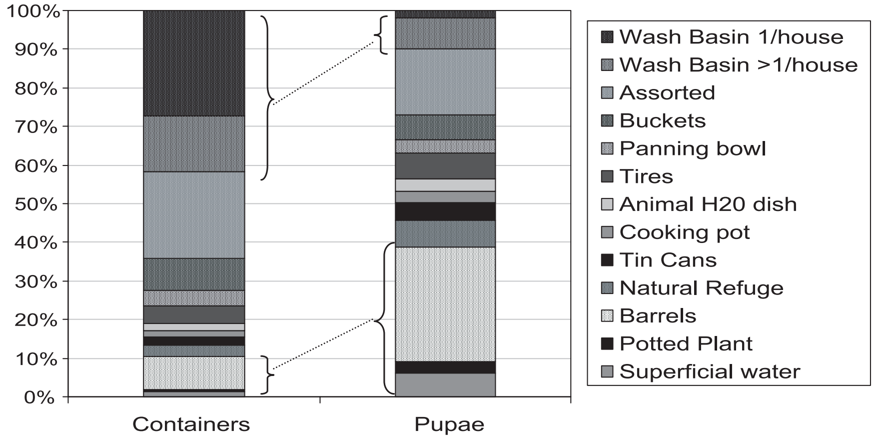

Fig. 1. Distribution of container number and respective pupae produced, in order of efficiency value (see Table 1). Bottom, 10% of all containers produce almost 40% of all pupae, and conversely 42% of all containers are wash basins which produce 10% of all pupae (top).

Table 2. Principal water-containing recipients positive for *Ae. aegypti* pupae and larvae

Water-containing recipient	Containers, *n* (%)	Houses with containers, *n* (%)	PPC,[a] *n* (% PPC) (% control)	LPC,[b] *n* (% LPC) (% control)	LPC:PPC
Wash basins	3,179 (42)	2,587 (92)	28 (9) (1)	100 (11) (3)	3.57:1
Barrels	651 (9)	532 (19)	59 (18) (9)	148 (17) (23)	2.51:1
Buckets	622 (8)	351 (12)	26 (8) (6)	55 (6) (12)	2.12:1
Tires	353 (5)	237 (8)	43 (13) (12)	35 (4) (21)	2.47:1
Bowl to transfer water	307 (4)	251 (9)	15 (5) (5)	51 (6) (17)	3.40:1
Natural reservoirs	213 (3)	115 (4)	13 (4) (6)	36 (4) (17)	2.77:1
Large tin cans	165 (2)	144 (5)	11 (3) (7)	35 (4) (21)	3.18:1
Animal water dish	149 (2)	123 (4)	5 (2) (3)	15 (2) (10)	3.00:1
Cooking pot	119 (2)	97 (3)	15 (5) (13)	27 (3) (23)	1.80:1
Superficial water	100 (1)	88 (3)	13 (4) (13)	26 (3) (26)	2.00:1
Potted plants	51 (1)	3 (0)	4 (1) (8)	11 (1) (22)	2.75:1
Other	1,719 (23)	1,105 (39)	93 (28) (5)	255 (29) (15)	2.71:1
Total	7,638 (100)	2,813 (100)	328 (100) (4)	872 (100) (11)	2.66:1

[a] PPC, pupae-positive container.
[b] LPC, larvae-positive container.

and they were at significantly higher risk (OR = 5.9; 95% CI, 2.35–15.2; $P < 0.001$) for presence of pupae in their washbasins than houses with only one washbasin (Fig. 1).

At the time of the entomological survey, barrels, which were present in one in five houses, were positive for pupae almost 10% of the time, could produce large numbers of pupae per barrel (maximum of 175 pupae found), were very productive (29%), and were highly efficient for generation of pupae (efficiency value of 3.5) (Fig. 1; Table 1). Buckets, which are used for water storage but are smaller and more manageable, were present in 12% of homes, were positive for pupae 6% of the time (Table 2), produced 7% of all pupae, and had an efficiency value of 0.79 (Table 1). Tires, cooking pots, and superficial water were the most frequently positive for pupae (Table 2). Furthermore, they had a low larvae to pupae ratio (Table 2), indicating that these receptacles contain the appropriate nutrients and environment for an egg to survive to the pupal stage.

Fifty-one potted plants with a superficial layer of water above the soil were present in only three (0.1%) houses (Table 2), yet they contributed 3% of all pupae present (Table 1). Two tree holes, breeding sites considered relevant for *Ae. albopictus* and classified here in the "natural reservoirs" category, were positive for both *Ae. albopictus* and *Ae. aegypti* larvae but not pupae (Lugo et al. 2005). Flower vases and bottles, although not a significant source of pupae, also were analyzed separately (in Tables 1 and 2, they are included in the "other" category). Flower vases made up 1.4% of all containers, had 1% of all pupae, were present in 3% of all homes, and were positive for pupae only 6% of the time, but were positive for larvae 22% of the time. Therefore, these receptacles had the highest ratio of larvae to pupae (3.43:1.0), and they are thus neither very productive nor efficient for pupae. Bottles, mentioned as a principal target in MOH literature, made up a significant number (3.4%) of water-filled containers, but they were rarely positive for larvae or pupae.

Breeding Site Characteristics

The following container characteristics were analyzed for association with presence of pupae in univariate and multivariate models: rain versus manual filling, frequency of use, presence of an adequate or inadequate (nonhermetically sealed) lid, sun exposure, roof coverage, and inside versus outside location. In all models, an inadequate top, various stages of disuse, and rain-filling were strong risk factors for the presence of pupae (Tables 3 and 4). Although <1% of containers had an inadequate lid, they were positive for pupae 16% of the time and contributed 5% of all pupae. An adequate lid, however, significantly diminished the likelihood of the presence of pupae when compared with containers without lids.

The majority (98%) of containers fell at the ends of the use spectrum, being used either at least once per week or not at all. The average frequency of use varied significantly according to type of container, with barrels, buckets and washbasins used weekly or more often, and the majority of all remaining containers considered not-in-use (Fig. 2A). Decreased frequency of use was strongly associated with pupal positivity and increased number of pupae (Tables 3 and 4); however, barrels in frequent use contributed 10% of all pupae, demonstrating that for barrels, frequent use does not diminish risk. Furthermore, barrels in use every 8–30 d were even more efficient producers of pupae (Fig. 2B). Washbasins and buckets that were not in use were the predominant source of pupae-positive containers and pupae (Fig. 2).

Rain-filled containers were between 2 and 4 times more likely to be positive for pupae than manually filled containers (Tables 3 and 4). Yet, manually filled containers produced many more pupae in one-third fewer containers than did rain-filled containers, and they were significantly more productive for pupae when positive: 28.7 pupae per manually filled container were identified versus 8.1 pupae per rain-filled container (Table 3). Manually filled containers consisted principally of barrels, buckets, and washbasins, which are among the largest of container types. Sun

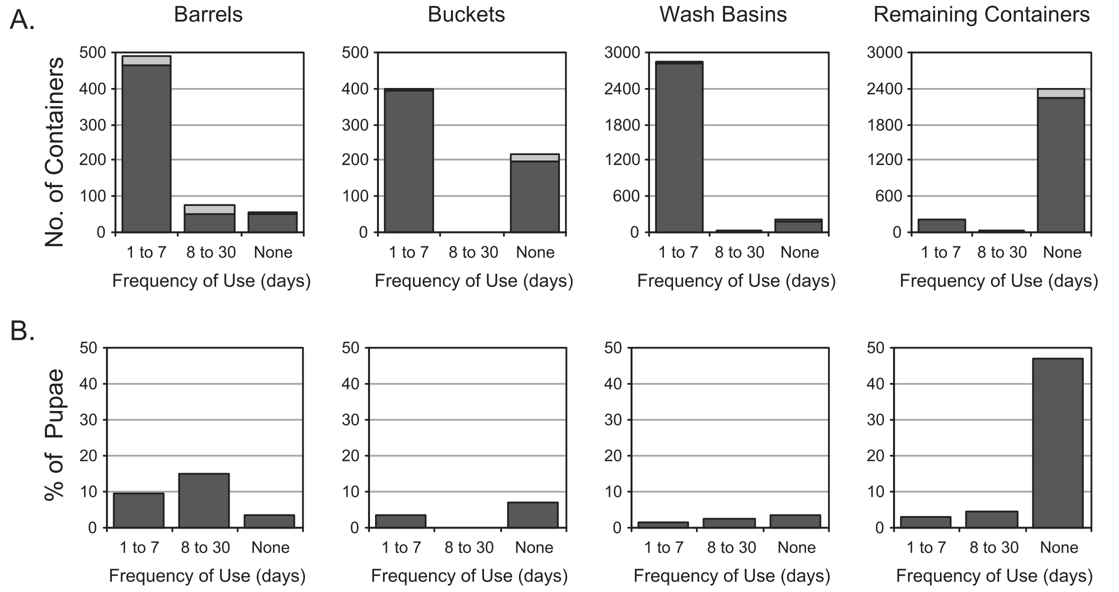

Fig. 2. Frequency of use. (A) Distribution of containers by frequency of use. Dark gray, negative containers; light gray, positive containers. (B) Distribution of pupae by frequency of use of container.

exposure, outside location, and presence under a roof were found to be correlated with each other. However, they were not colinear, and they were thus all assessed in the model. In addition, the association of container type with the presence of pupae in the model (Table 4) correlated well with the efficiency values calculated in Table 1.

Larval and Pupal Indices. Seven neighborhoods in Managua had a sufficient number of houses and containers sampled to calculate both a pupal house index and pupal container index to a 95% confidence level. The number of houses and containers needed to determine the pupal indices was significantly greater than that necessary for larval indices, because pupae were present in only 4% of containers versus 11% with larvae. Therefore, these seven neighborhoods were selected to assess whether the standard larval indices were comparable to the risk associated with the number of pupae per person (Focks et al. 1993a, 2000; Focks and Chadee 1997). These seven neighborhoods were analyzed using the house, container, and Breteau indices, based on either presence of pupae or presence of larvae. Little to no correlation was observed between these

Table 3. Breeding site characteristics

Category	Containers, *n* (%)	PPC, *n* (%)	LPC, *n* (%)	Pupae, *n* (%)	Univariate, OR (95% CI)
Use (d)					
1–7	3,949 (57)	59 (2)		707 (17)	Ref.
8–30	140 (2)	47 (34)		911 (22)	33.3 (21.1–52.7)*
Not used	2,878 (41)	215 (8)	587 (20)	2497 (61)	5.32 (3.9–7.2)*
Filled					
Rain filled	2,990 (40)	232 (8)	601 (20)	1,880 (42)	4.21 (3.3–5.4)*
Manually	4,549 (60)	89 (2)	261 (6)	2,555 (57)	Ref.
Lid					
None	7,179 (97)	303 (4)	817 (11)	4,027 (89)	5.02 (0.88–200.75)
Inadequate	97 (1)	15 (16)	32 (33)	220 (5)	20.85 (3.06–885.2)*
Adequate	115 (2)	1 (1)	4 (4)	1 (0)	Ref.
Location					
Outside	6,198 (82)	300 (5)	786 (13)	4,083 (91)	3.01 (2.0–4.6)*
Inside	1,384 (18)	23 (2)	77 (6)	412 (9)	Ref.
Under roof					
Yes	2,325 (31)	54 (2)	154 (7)	1,070 (24)	0.44 (0.3–0.6)*
No	5,273 (69)	271 (5)	717 (14)	3,433 (76)	Ref.
Sun exposure					
Yes	5,667 (75)	279 (5)	732 (13)	3,544 (79)	2.25 (1.6–3.1)*
No	1,912 (25)	43 (2)	131 (6)	920 (20)	Ref.

Ref., reference.
* $P < 0.05$.

Table 4. Multivariate logistic regression models of the association between container characteristics and pupae presence

Category	Multivariate, OR (95% CI)	Multivariate with container type, OR (95% CI)
Use (d)		
1–4	Ref.	Ref.
5–9	26.50 (15.61, 44.96)*	15.98 (9.10, 28.05)*
10–60	57.18 (26.8, 122.0)*	46.37 (20.56, 104.59)*
Not used	5.27 (2.97, 9.34)*	4.34 (2.33, 8.09)*
Filled		
Rain filled	1.99 (1.22, 3.22)*	2.11 (1.26, 3.52)*
Manually	Ref.	Ref.
Lid		
None	5.08 (0.67, 38.35)	6.99 (0.91, 53.72)
Inadequate	13.72 (1.68, 111.74)*	15.25 (1.84, 126.06)*
Adequate	Ref.	Ref.
Location		
Outside	1.55 (0.89, 2.67)	1.48 (0.85, 2.57)
Inside	Ref.	Ref.
Under roof		
Yes	—[a]	1.31 (0.87, 1.97)
No		Ref.
Container type		
Wash basins	NI[b]	Ref.
Water storage containers	NI	3.77 (2.25, 6.31)*
Potted plants	NI	1.94 (1.02, 3.69)*
Plastic containers	NI	2.49 (1.38, 4.48)*
Natural reservoirs	NI	1.18 (0.33, 4.18)
Superficial water	NI	2.45 (1.19, 5.02)*
Yard waste	NI	1.11 (0.63, 1.95)
Furniture and tools	NI	4.07 (1.85, 9.01)*
Glasses, cups and cans	NI	1.80 (0.93, 3.49)
Cooking pots	NI	2.20 (1.2, 4.02)*
Tires	NI	2.86 (1.58, 5.17)*

* $P < 0.05$.
[a] Not significant.
[b] Not included in model.

larval indices and number of pupae present per person (data not shown).

Effect of Abate Treatment on Larval and Pupal Indices. The date from the most recent temephos treatment was obtained in 1,903 of the homes in the study. It was assumed that all containers in the household were treated with temephos, because of the MOH policy. Temephos was most effective in controlling pupae up to 30 d postapplication (Table 5). No change was seen in mean number of potential breeding sites per house relative to time since treatment (data not shown), indicating that the effect of the treatment was not due to breeding site elimination, a vector control measure that often accompanies temephos application. Furthermore, no change in potential breeding site type was observed relative to time since temephos application. According to the questionnaire, 7, 39, and 36% of households discarded their MOH-applied temephos at 1, 14, and 30 d postapplication, respectively; only 18.5% of household were found to maintain their temephos for the MOH- specified 2 mo.

Discussion

The *Ae. aegypti* mosquito is an effective urban vector for dengue virus due to its preferred sites of production and its biting habits. An entomological survey based on quantification of pupae and identification of container type characteristics, such as the one described in this study, can serve to provide critical information for vector control measures. In addition to productivity, a pupal efficiency value to direct targeting strategies was calculated. Barrels, in particular, were very productive and efficient, and they had unique characteristics permitting a targeted intervention. Interestingly, increased availability of running water did not lead to a reduction in either the number of water storage containers or water storage container productivity. Lids were more harmful than helpful in the reduction of pupae due to inappropriate use. Temephos application had a temporally limited but noticeable impact on immature stages. Excluding water storage containers, frequent container use was protective against the production of pupae, suggesting that simple measures can reduce pupae production and assist in targeting vector control interventions. Traditional larval container indices provided little correlation with the number of pupae per person. Despite certain similarities, a number of conclusions from this study differ from previous reports, emphasizing the importance of performing such studies in different environments.

In addition to the concept of productivity, we introduce the use of an efficiency value and an impact graph to facilitate more streamlined targeting of pupae. The productivity of a container has been described previously as the percentage of all pupae arising from particular container types, often illustrating the concept that most of the pupae derive from a few container types or containers with specific characteristics (Focks and Chadee 1997, Morrison et al. 2004, Arredondo-Jimenez and Valdez-Delgado 2006, Barrera et al. 2006a, Midega et al. 2006, Romero-Vivas et al. 2006). However, high productivity does not necessarily imply the most targeted strategy, because not all containers are in equal abundance. A measure of efficiency (as opposed to productivity) was defined to determine the relative efficiency of a container type per container, where one is the efficiency if all con-

Table 5. Effect of abate on entomological indices

Days since temephos application	Houses positive for larvae or pupae, *n* (%)	Houses positive for pupae, *n* (%)	Mean pupae per house	PPC[a] (% of all containers)
1–7	10 (11.1)	5 (5.6)	0.189	4 (1.4)
8–14	16 (10)	12 (7.5)	0.550	14 (2.8)
15–30	26 (7.6)	22 (6.5)	0.759	24 (2.3)
31–60	87 (12.3)	75 (10.6)	1.841	92 (4.7)
61–90	65 (10.7)	73 (12.0)	1.630	85 (5.6)

[a] PPC, pupae-positive container.

tainers are equal. The resulting 20-fold range of efficiency values (0.24–4.7) serves as a potential guide to assess which targeted containers may produce the greatest return on control efforts. The graphical representation of the number of pupae per container type and the numbers of that container type could assist vector control groups to develop the best strategy. An elegant tool to achieve similar goals is the flow chart proposed by Morrison et al. (2004), where the algorithm is based on container characteristics.

In urban Nicaragua, 60% of all pupae were produced by the five most productive container types, representing 67% of all wet receptacles. In contrast, the eight most efficient producers of pupae generated >62% of all pupae but consisted of only 25% of all wet containers. By either measure, barrels, which are the principal water storage container, score high, and they should be a principal target of any vector control measure in urban residential Nicaragua. Barrels and tires are already targeted by MOH activities; the other most efficient producers of pupae are not. However, tin cans could be discarded, water could be removed from potted plants and puddles, water could be changed more frequently or removed from animal drinking dishes and cooking pots, and natural reservoirs could be controlled with temephos or filling with earth.

Our analysis indicated that common containers, such as washbasins and buckets, that are not in use should be targeted by control programs. For example, >90% of homes contain a washbasin, a container type targeted by MOH vector control policies. In the study area, focusing on washbasins in infrequent use or washbasins in homes where two or more washbasins are present would narrow the targeted number of washbasins to 7 and 35% (together, 37%) of all washbasins, respectively, while capturing 96% of pupae-positive washbasins, and it would therefore conserve resources and energy in either an insecticide-based or green strategy for pupae control. Similarly, the majority of pupae develop in buckets that are not in use, unlike barrels, for which productivity was high regardless of use. Thus, targeting unused buckets would be the most efficient strategy.

To reduce the productivity of water storage containers, the Nicaraguan MOH, alongside World Health Organization (WHO)/Pan American Health Organization (PAHO), has promoted the use of lids. Our data demonstrate that placing lids on water storage containers can be both effective, when lids are well placed and hermetically sealed, and also counterproductive, due to an increased risk of pupae when inadequate lids are present, consistent with previous findings (Strickman and Kittayapong 1993). The overall presence of any lid increased the risk of pupal infestation, suggesting that future interventions in urban Nicaragua either need to more clearly define and promote the use of "adequate" lids, or seek interventions other than lids.

Interestingly, the difference in water availability between the two urban centers in the study allowed assessment of how water availability affected both the number and positivity of water storage containers, which contributed 40% of all pupae. León, with running water available all the time, and District II of Managua, with water availability ranging from 6 to 18 h a day, showed no significant difference in water storage container number or positivity for the aquatic phase of the vector. The data here provide evidence that storing water is based on factors beyond just potable water availability, a theory previously posited by Reiter and Gubler (1997), and supported by a recent study in Barranquilla, Columbia (Romero-Vivas et al. 2006). For example, in many places in Nicaragua, washing clothes often relies on water from barrels or buckets even when running water is available. Thus, although continuous availability of potable water is beneficial in general, it is unlikely that it will universally impact the entomological indices without other interventions targeted at the ways water is used.

Characteristics that were significantly associated with positivity of the principal *Ae. aegypti* breeding sites included frequency of use less often than once every 7 d, presence of a lid, and rain filling. In agreement with recent studies (Morrison et al. 2004, Barrera et al. 2006a), we find that superficial water such as puddles and plastic tarps are very productive and very efficient and that they contain appropriate characteristics for the maturation of pupae. Also in agreement with other studies (Morrison et al. 2004), we identified potted plants as being very productive and efficient, although their numbers were small. Neither potted plants nor superficial water are listed as *Ae. aegypti* breeding sites in the Nicaraguan MOH educational materials, nor are they emphasized for *Ae. aegypti* control in WHO/PAHO literature.

Interestingly, results of such entomological surveys vary significantly from country to country, giving rise to different targeting strategies. For example, a similar study conducted in Iquitos, Perú, found that unlidded, outdoor, rain-filled containers comprise 20% of all containers and 78% of *Ae. aegypti* productivity (Morrison et al. 2004); in Barranquilla Columbia, cement ground tanks and drums comprised 16.3% of containers and produced 78.2% of *Ae. aegypti* pupae (Romero-Vivas et al. 2006). In Salinas, Puerto Rico, 77% of all pupae derived from discarded containers, ornamental vessels, cover sheets and toys; however, these containers comprised 65% of containers, making a targeted container-specific strategy less efficient than general yard management (Barrera et al. 2006a). In urban southern Mexico, large cement washbasins, present in almost every household investigated, produced 84% of all *Aedes* pupae (Arredondo-Jimenez and Valdez-Delgado 2006). Two findings are fairly consistent among pupal productivity surveys: first, when water access and water storage (either when necessary or as a habit) has not been adequately addressed, water storage containers predominate as strategic targets, and second, a small number of often unusual containers produce a large percentage of all pupae (Morrison et al. 2004, Barrera et al. 2006a, Romero-Vivas et al. 2006).

The Nicaraguan MOH currently uses the container indices that have been promoted throughout the world for the past half century. Our data are in agreement with the increasing number of studies that maintain that the container and house larval-based (*Stegomyia*) indices are at best only weakly correlated with number of pupae per person (Focks et al. 1995, Focks and Chadee 1997, Arredondo-Jimenez and Valdez-Delgado 2006, Barrera et al. 2006a). Importantly, pupal productivity per person is a principal variable, along with ambient temperature, rainfall, and herd immunity, in models that assess the risk for dengue epidemics (Focks et al. 1993a, 1993b; Focks et al. 1995, 2000). As these models become more accessible, the importance of having pupal survey data will be magnified. Furthermore, there is a growing body of work that describes how to plan and conduct a statistically relevant pupal survey (Alexander et al. 2006) that would diminish costs and increase efficacy of pupal productivity surveys, which are inherently more labor-intensive than larval-based (*Stegomyia*) indices.

Temephos application, the mainstay of preventative vector control measures in Nicaragua since the mid-1980s, demonstrated a short-term but significant effect in controlling pupal indices. Our data reveal a noticeable decline in temephos effectiveness from 7 d to 1 mo postapplication and no significant effect 1 mo after application. This decline may be due to discarding the temephos; only 18.5% of household were found to maintain their temephos for the specified 2 mo. The protective effect of temephos was not attributable to source reduction, which frequently accompanies temephos application, because no change in the number of potential breeding sites was found. The results of this study suggest that temephos application in its current form is not effective as a long-term sustainable intervention (reapplication of temephos every 2–3 mo) but that application may be effectively used in emergency epidemic control. Overall, these data support the shift to greener community-based alternatives in the home, or alternative larvacide/insecticide presentations that accompany education for appropriate and sustainable use. It should be noted that the study survey occurred in homes and not businesses or public areas, where temephos application may provide better results and be an appropriate intervention measure.

This study did entail several limitations. First, the sample size was relatively small, due to the resource constraints of the study. Second, although two geographically distinct sites were included, no temporal analysis was conducted, and repeated measures models could not be constructed because the containers were not marked during the first visit. Third, collection of pupae is time-sensitive and pupal positivity depends on the timing of the survey, as in all cross-sectional studies. Fourth, only homes with children were included; this should not have caused bias in the study; however, no data are available to prove this. Last, in the analysis of the effect of temephos, it was assumed that all containers were treated, because of the MOH policy; however, information ascertaining this assumption is not available. Nonetheless, we do not think that these constraints mitigate the validity of the study's conclusions.

In this study, we identified that lids are a risky intervention, that temephos application has a limited real-life efficacy, and that increasing water availability alone does not lead to a change in water storage habits or *Ae. aegypti* productivity. Furthermore, we propose an efficiency index and graphical data display that helps target productive containers more strategically. Last, this detailed entomological survey is the first of its kind to be reported in Central America, and it provides data that not only reinforces the findings from other studies in Southeast Asia, South America, and the Caribbean but also identifies new information specific to the urban situation in Nicaragua and of potential use for vector control programs worldwide.

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