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Source: Journal of Medical Entomology, 46(5):1176-1186.
Published By: Entomological Society of America
DOI: http://dx.doi.org/10.1603/033.046.0528
URL: http://www.bioone.org/doi/full/10.1603/033.046.0528

Patterns of *Aedes aegypti* (Diptera: Culicidae) Infestation and Container Productivity Measured Using Pupal and *Stegomyia* Indices in Northern Argentina

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J. Med. Entomol. 46(5): 1176–1186 (2009)

ABSTRACT A citywide control program of *Aedes aegypti* (L.) (Diptera: Culicidae) mainly based on the use of larvicides reduced infestations but failed to achieve the desired target levels in Clorinda, northeastern Argentina, over 5 yr of interventions. To understand the underlying causes of persistent infestations and to develop new control tactics adapted to the local context, we conducted two pupal surveys in a large neighborhood with ≈2,500 houses and recorded several variables for every container inspected in fall and spring 2007. In total, 4,076 lots and 4,267 containers were inspected over both surveys, and 8,391 *Ae. aegypti* pupae were collected. Large tanks used for potable water storage were the most abundant and the most productive type of container, accounting for 65–84% of all the pupae collected. Therefore, large tanks were key containers and candidates for improved targeted interventions. Multivariate analysis showed that containers located in the yard, at low sun exposure, unlidded, filled with rain water, and holding polluted water were all more likely to be infested by larvae or pupae. When only infested containers were considered, productivity of pupae was most closely associated with large tanks and rain water. A stochastic simulation model was developed to calculate the expected correlations between pupal and *Stegomyia* indices according to the characteristics of the distribution of larvae and pupae per container and the spatial scale at which the indices were computed. The correlation between pupal and *Stegomyia* indices is expected to increase as infestation levels decline.

KEY WORDS *Aedes aegypti*, dengue transmission risk, vector control, temephos, pupal survey

Dengue fever and its most severe forms, dengue hemorrhagic fever and dengue shock syndrome, are considered a major public health problem worldwide, with an estimated 50 million cases occurring each year (Farrar et al. 2007) and the most important arboviral disease with regards to its associated mortality and morbidity (Kourí et al. 2007). Efforts to reduce dengue transmission in the absence of a vaccine and clinical cures are generally centered upon the control of populations of its main vector, *Aedes aegypti* (L.) (Diptera: Culicidae). Control measures are usually focused on the mosquito's immature stages and rely on the reduction of developmental sites and/or chemical or biological treatment of the containers in which larvae or pupae are found.

New developments regarding entomological indices have been made during the last decade (Tun-Lin et al. 1996, Focks and Chadee 1997, Focks 2003). Before this, the traditional *Stegomyia* indices (house, container, and Breteau index) have been used as the standard measures of infestation (Pan American Health Organization 1994) and are still used in control programs around the world. These indices are based on qualitative observations, the registration of presence or absence of infestation. The house index is defined as the percentage of houses infested with *Ae. aegypti* larvae or pupae among the total number of houses examined at a given survey; the container index is defined as the percentage of containers found infested among the group of containers examined, and the Breteau index is defined as the number of containers infested per 100 houses inspected. Criticism has been raised against the *Stegomyia* indices and pupal indices are now favored for several purposes. Pupal indices are based on quantitative observations; they require counting the number of pupae in each developmental site examined and therefore are more informative. Pupae are easier to count than larvae and are better correlated with adult mosquito abundance (Focks 2003). It has been argued that conclusions drawn from pupal surveys can be used to scientifically develop targeted control strategies, and when coupled with demographic information (i.e., pupal/demographic surveys), they can be used to obtain dengue

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transmission thresholds (Tun-Lin et al. 1995, Focks and Alexander 2006).

Mosquito production of different containers is determined by multiple processes ranging from the scope of ecology to anthropology (Whiteford 1997, Barrera et al. 2006a, Phuanukoonnon et al. 2006). Factors related to these processes can be measured and the association between them and container productivity can be inferred through multivariate statistical analyses (Morrison et al. 2004, Barrera et al. 2006b, Hammond et al. 2007). Results obtained from pupal surveys are used as a proxy for adult production because pupal mortality is considered to be low and well characterized (Focks 2003).

Dengue incidence in America has shown a growing trend in the last decades (Guzmán et al. 2006), and *Ae. aegypti* has recolonized all the countries from where it had once been eradicated (Gubler 2005). In the city of Clorinda in northern Argentina, an intervention program mainly based on the use of larvicides (temephos), the destruction of potential developmental sites and occasional use of both truck and house-based ultralow volume (ULV) spraying has been conducted citywide since late 2002 by Fundación Mundo Sano (FMS), a nongovernmental private organization, in cooperation with the Ministry of Health (R.E.G. et al., unpublished). *Stegomyia* indices have been used to develop infestation maps for the city's neighborhoods and blocks. The program significantly reduced *Stegomyia* indices with respect to preintervention values, and the reported incidence of dengue fever cases in Clorinda has been null from 2002 to 2006 and remarkably lower than in neighboring Paraguay in 2007, where a major dengue outbreak occurred (Pan American Health Organization 2007). Despite sustained control interventions, indices remained above target levels (house index $<1\%$; Breteau index <5) every summer.

To understand the underlying causes of the high indices recorded and to develop new control tactics adapted to the local context, we conducted two pupal surveys to investigate the productivity of different container types and its association to selected factors in Clorinda in fall and spring 2007. To our knowledge, this is the first report of pupal surveys in the Southern Cone countries, excluding Brazil (Maciel-de-Freitas et al. 2007). Because counting pupae is much more laborious than recording the presence or absence of larvae or pupae, we compared results from qualitative and quantitative approaches and investigated under which circumstances the information they produced converged to a similar pattern by means of a stochastic simulation model.

Materials and Methods

Study Site. The city of Clorinda (latitude 25° 17′ S, longitude 57° 43′ W) is located in the Province of Formosa in northern Argentina on the border with Paraguay, 45 km away from Asunción. Clorinda had 47,240 inhabitants in 2001. Within the 38 identified neighborhoods, there were 10,752 houses and 15% of those were of mud-and-thatch construction with earthen floors (Instituto Nacional de Estadísticas y Censos 2001). An estimated 2,000–7,000 people walk across the border between Clorinda and Paraguay everyday for commercial purposes through two bridges.

A sustained vector control program centered upon the treatment with temephos of artificial containers has been conducted throughout the city since late 2002 by Fundación Mundo Sano. The program has aimed at complete coverage of the city roughly every 4 mo (R.E.G. et al., unpublished).

Our study was carried out in the neighborhood Primero de Mayo, which was selected for its rather high infestation levels relative to the rest of Clorinda over the previous 5 yr (house and Breteau indices averaging 10.7 and 13.7%, respectively). The neighborhood is almost completely residential and highly populated. On average, each block includes 20 lots and 90 residents. There are 123 populated blocks, making the neighborhood roughly 20% of the city (Fig. 1). The neighborhood has running potable water, but the service is deficient and intermittent, especially during summer. Houses are highly clumped within each block and are separated generally by a wired fence. Almost every house is surrounded by a yard where water-holding containers are found and animals are frequently kept. Every block in the neighborhood has been sketch-mapped and every lot was given a unique ID that identifies its exact location starting from the southeastern corner of each block. The position of each lot entrance was georeferenced using a global positioning system (Trimble GeoExplorer II; Trimble Navigation Ltd., Sunnyvale, CA), and a geographical information system was constructed using ArcGIS 8.1 (ESRI, Redlands, CA).

Entomological Surveys. Two surveys were carried out in 2007; one survey in the fall (between 26 March and 17 May) and another survey in spring (between 8 October and 29 November). In both cases, all 2,488 lots in the neighborhood were visited by four teams of three people each. The teams were constituted by experienced personnel of FMS, many of whom had been working in Clorinda since 2003, who were trained for the purpose of this investigation and supervised by the research team. Fieldwork was divided into morning and afternoon shifts, and each team was assigned one block per shift. Upon visiting a household, each team introduced itself to the household head and asked for permission to examine the premises. In the spring survey, if permission was not granted or if the residents were not present, the household was visited again on a different day but in a different shift. Upon entrance, the yard and the interior of the household were thoroughly inspected for containers. All water-holding containers found were examined for mosquito immature stages, taking samples of larvae and collecting and counting every pupa discovered. Containers were not inspected for temephos residues. Each container was scored for container type according to a previous classification (Table 2), location within the lot (inside or outside the

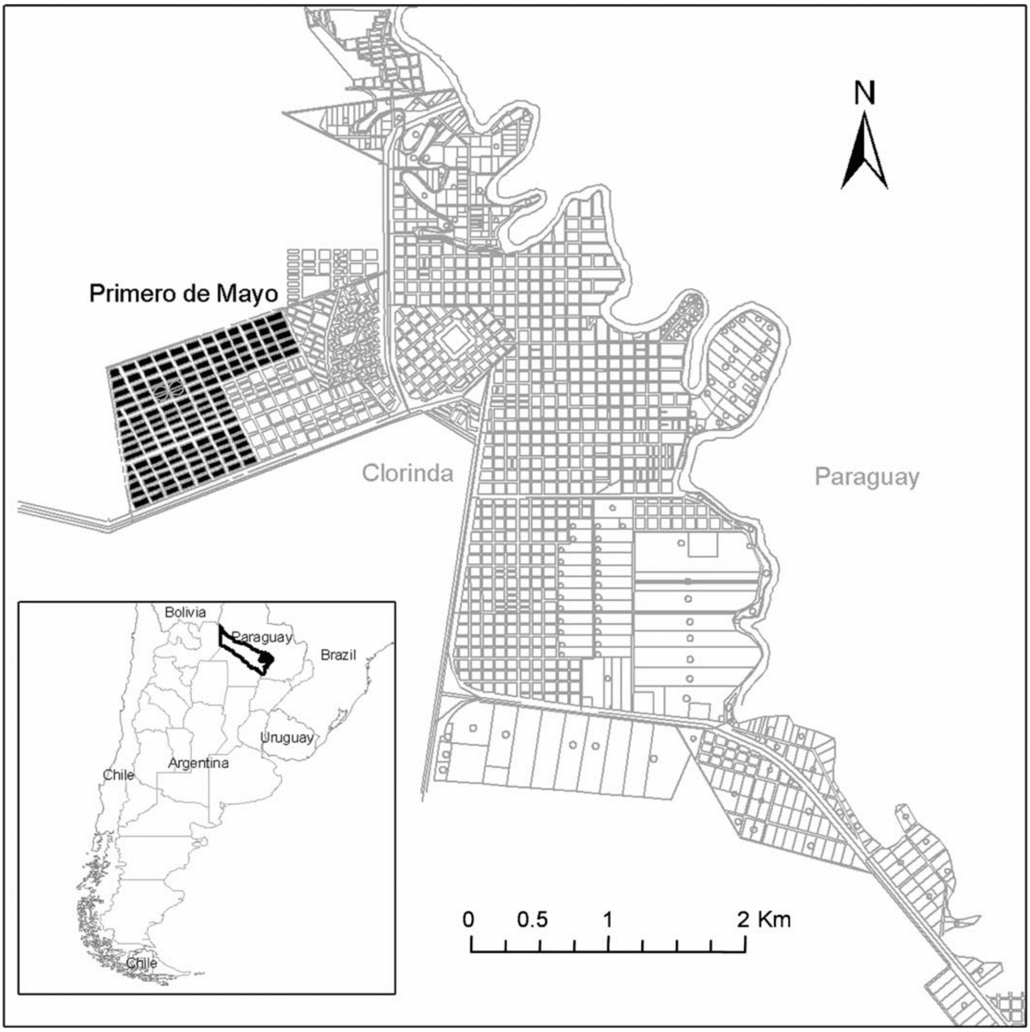

Fig. 1. Study area: Primero de Mayo, a neighborhood of the city of Clorinda, on the border with Paraguay. Inset shows location of Clorinda in the Province of Formosa, Argentina.

house), sun exposure (considered low if any structure such as a ceiling or a tree overshadowed the container, or high otherwise), lid status (fully lidded, partially lidded, or unlidded), water type (running water, rain or pump), water state (considered clean if it contained transparent water, or polluted otherwise), approximate container volume, and water volume within the container. These variables were selected because other researchers had found them associated with productivity and could be measured rather easily and rapidly. Houses also were searched for possible natural developmental sites, such as leaves of banana plants and bamboo canes. Each container was either turned upside down, treated with 1% temephos (Abate, BASF, Ludwigshafen, Germany) at 1 mg per liter applied as sand granules by using spoons or, more rarely, destroyed if permission was obtained from the residents. Animal drinking pots and natural containers were not treated with temephos because it is toxic to some of the animals commonly found in the neighborhood. No further vector control was applied between the two surveys. Demographic information was

Table 1. House index, container index, Breteau index, total pupae collected, and pupae per person in both surveys of Primero de Mayo, Clorinda, 2007

Survey	House index	Container index	Breteau index	Pupae collected	Pupae/ person
Fall (May–March)	19.4	30.2	27.0	6,198	0.83
Spring (Oct.–Dec.)	6.3	8.2	7.8	2,193	0.24

Table 2. Abundance, *Ae. aegypti* infestation, pupal productivity, container index, and efficiency of containers according to container type for both surveys, Clorinda 2007

	Container type[a]							
	A	B	C	D	E	F	H	Total
Abundance (%)								
March–May	189 (11)	909 (51)	72 (4)	13 (1)	312 (17)	97 (5)	191 (11)	1,783
Oct.–Dec.	305 (12)	1,382 (56)	68 (3)	7 (0)	242 (10)	118 (5)	362 (15)	2,484
No. infested (%)								
March–May	56 (10)	246 (45)	9 (2)	9 (2)	131 (24)	15 (3)	76 (14)	542 (100)
Oct.–Dec.	10 (5)	129 (64)	5 (2)	0 (0)	39 (19)	4 (2)	16 (8)	203 (100)
Pupae collected (%)								
March–May	483 (8)	4,058 (65)	13 (0)	56 (1)	821 (13)	75 (1)	692 (11)	6,198
Oct.–Dec.	53 (2)	1,839 (84)	13 (1)	0 (0)	188 (9)	43 (2)	57 (3)	2,193
Pupae/container								
March–May	2.56	4.5	0.2	4.3	2.6	0.8	3.6	3.5
Oct.–Dec.	0.2	1.3	0.2	0.0	0.8	0.4	0.2	0.9
Container index								
March–May	29.6	27.1	12.5	69.2	42.0	15.5	39.8	30.4
Oct.–Dec.	3.3	9.3	7.4	0.0	16.1	3.4	4.4	8.2
Efficiency								
March–May	0.74	1.28	0.05	1.24	0.76	0.22	1.04	
Oct.–Dec.	0.49	1.32	0.24	-	0.45	1.00	0.33	

[a] A, tires; B, large tanks, drums, or barrels; C, flower pots; D, construction materials and discarded vehicle parts; E, bottles, cans, and plastic goods; F, wells; and H, other.

obtained by asking each head of household for the number of people living in their home.

All pupae and larvae were collected with large-mouth pipettes and frequently the operators aided themselves with sieves to strain each container. Samples were placed in test tubes, labeled and transported to the laboratory for processing. Larvae were identified to species using an entomological magnifying glass and an illustrated key (Rossi and Almirón 2004). Pupae were kept in small water-filled plastic vials until emergence for accurate species identification and counted as adults. The proportion of *Ae. aegypti* pupae was estimated for each container. During the fall survey, 3% of the pupae did not survive to adulthood; in these few cases, the dead pupae were assumed to be *Ae. aegypti* according to the observed proportion of adult *Ae. aegypti* that emerged from the live pupae in each container.

Data Analysis. The data obtained were entered in an Access database, edited, and checked against the original forms by two people. The database was used to calculate the numbers of containers, of infested containers, of pupae collected for each type of container, and of infested containers found for every category of the variables measured. The efficiency of each container type was calculated as the proportion of pupae collected from each type divided by its relative abundance (Hammond et al. 2007).

To assess the association between the variables measured and the infestation status of individual containers, a technique devised by Burnham and Anderson (2002) to estimate the relative importance (RI) of variables was used. Given a set of models, the Akaike weight is computed for each model. For every variable considered, the sum of the Akaike weights of the models in which the variable is present is computed, obtaining a RI ranging from 0 to 1. If a variable is important in relation to the rest, it should be present in the best models of the set (i.e., those with the highest Akaike weight), therefore scoring a high RI. This allows a hierarchical ordering of all variables based on their association with the response variable, while taking into account all the models in the set and model selection uncertainty (Burnham and Anderson 2002).

We assessed the RI of the variables measured in relation with infestation (presence or absence of pupae or larvae) for every container, and in relation with productivity of pupae for the subset of containers found to be infested. Multiple logistic regressions were run in the former case, and multiple negative binomial regressions in the latter. The predictors used were type, location, sun exposure, lid status, water type, water state, container volume, and water volume. Because predictors were selected a priori based on existing knowledge, the analysis was conducted on every subset of models possible. Given that results were consistent between surveys, the data were pooled together and a dummy variable representing survey was included. A uniform random variable (from 0 to 1) was included to represent lack of association. Therefore, the number of factors considered was 10 and the number of models in each analysis (infestation or pupal productivity) was 1,024. Akaike weights were computed using Akaike Information Criterion with a correction for small samples (AIC_c).

The influence of categorical and continuous variables on the response variables (infestation and productivity) was assessed by their parameter estimates conditional on the full set of models as described by Burnham and Anderson (2002). These model-averaged parameter estimates were computed as the average of the parameters in every model weighted by the Akaike weight of the respective model. When variables were not present in a model, the respective parameters were computed as zero. Due to accessibility problems found when examining containers categorized as wells, they were excluded from these analyses. All calculations were done using R 2.5.0 software (R Development Core Team 2007).

The constancy of the infestation status of individual sites over surveys was evaluated at the lot and the container level by calculating the number of lots (or containers) with each of the four possible combinations of infestation status over both surveys and the number of pupae collected from them in both surveys. To do so we kept track of individual lots (based on their IDs) and large tanks (based on lot ID, tank volume, and location within the lot) from one survey to the next.

Stochastic Simulation Model. To investigate the relationship between larval and pupal indices, a stochastic simulation model was developed using MatLab 5.3 (Mathworks Inc. 1999). The objective of the model was to calculate the expected correlations between indices based on quantitative and qualitative observations according to characteristics of the distribution of infestation and productivity per container and the spatial scale at which the indices were computed. In particular, the relationship between the container index and the number of pupae per group of containers was examined.

Based on the highly clumped pattern reported worldwide (Focks and Alexander 2006), the distribution of pupae per infested container was modeled through the negative binomial distribution truncated at zero, which accounts for zero-inflated data (Martin et al. 2005). The negative binomial distribution has two parameters, r and P; if the distribution is interpreted to model the number of successes before a specified number of failures, r represents the number of failures and P the probability of success. In the model, r was set arbitrarily to 1.0 and P was left to vary freely representing the degree of clumping of pupae.

At initialization, the model randomly sorts which of *N* containers are not infested according to probability I. From the infested subset of containers, those that harbor larvae but not pupae are sorted according to probability L. From the subset of containers harboring pupae, the number of pupae is assigned randomly according to the truncated negative binomial distribution to the remaining subset of containers. Containers are then grouped to calculate the container index and the number of pupae in each group. The size of the groups is determined by parameter n which represents the spatial scale; n could represent the computation of indices at a neighborhood scale or at a smaller scale, such as a block, according to its value. Finally, the correlation between the two indices is obtained for the set of groups. A high correlation value would indicate that both indices stratify the risk similarly whereas a low value would be indicative of discrepancies in risk stratification between indices.

The model was explored numerically using N = 2,000 containers and varying P between 0.08 and 0.8, I between 0.3 and 0.9, L between 0 and 0.5, and n between 20 and 200. Fifty realizations were run for every parameter combination. The median and variance of the correlations between the container index and the number of pupae per group of containers were computed. Therefore, the effects on the correlation of the degree of infestation and clumping of pupae (parameters I and P), the number of containers infested but without pupae (parameter L) and the spatial scale (parameter n) were investigated by means of simulations.

Pearson's correlation coefficients between each of the observed Stegomyia indices and the number of pupae computed for each block were calculated. To compare the results of the model with the observed data, it was parameterized with the field data collected in Clorinda. For both parameterizations (one for each survey), 1,000 realizations were run and 95% confidence envelopes were calculated.

Results

In total, 4,076 lots and 4,267 water-holding containers were inspected during both surveys, and 8,391 pupae of *Ae. aegypti* were collected. Data were obtained from 71 and 93% of the lots in the fall and spring surveys, respectively. In the fall survey, 30.4% of the containers were found positive for either larvae or pupae, and 6,198 pupae were collected (Table 1). In the spring survey, 8.2% of the containers were positive and 2,193 pupae were collected. Infestation levels were higher in the fall than in the spring survey for every index computed. The spring survey was conducted after an especially cold winter, when minima were below 5°C over several days. All pupae collected came from artificial containers and were mostly *Ae. aegypti* (79% in the fall survey and 67% in the spring survey). All potential natural containers inspected were either uninfested or dry.

Large tanks, drums, or barrels (type B containers) were the most important containers in both surveys, based on container abundance (51–56%), infestation (45–64%), pupae per container (4.5–1.3), and efficiency (1.28–1.32) (Table 2). These containers accounted for 65 and 84% (mean, 70%) of the pupae collected in each survey. Bottles, cans, and plastic goods (type E containers) was the second most productive type, closely followed by the category "other" (i.e., type H containers, mainly ceramic pots, tin cans, pieces of canvas, canvas pools, and broken or unused appliances such as refrigerators or washing machines). When the *Stegomyia* indices were used, differences in the relative importance of container types were smaller. The second most infested containers in each survey were bottles, cans, and plastic goods (24 and 19%) (Table 2).

The distribution of pupae per container was highly clumped (Fig. 2). Some containers were superproductive compared with the others; three containers in the fall survey and one container in the spring survey exceeded 300 pupae. The number of water-holding containers per lot was also clumped in both surveys (variance to mean ratio = 1.67–1.75; χ^2, df = 1,764–2,310; $P < 0.001$). The average number of water-hold-

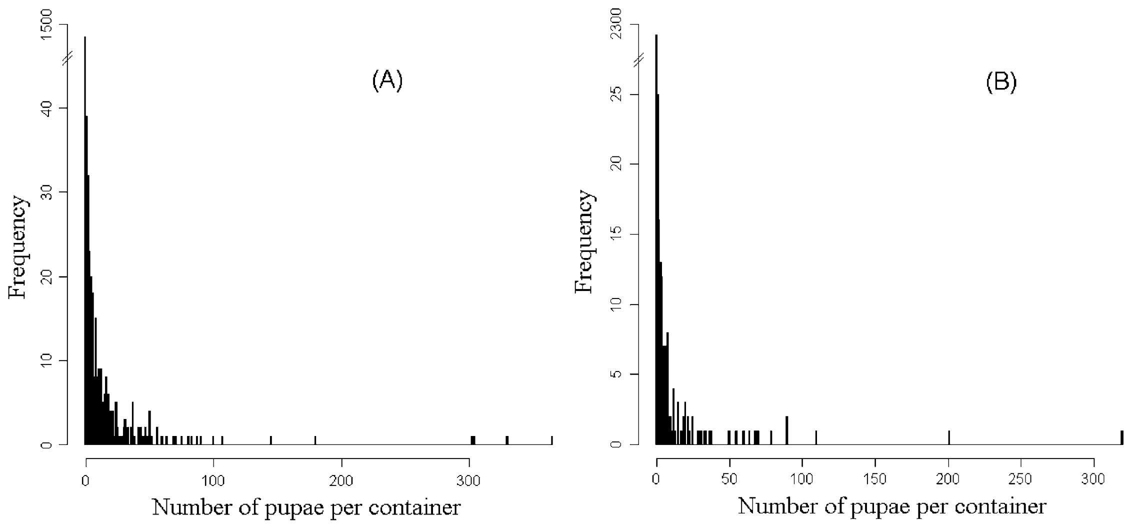

Fig. 2. Frequency distribution of pupae per container. (A) Spring survey. (B) Fall survey, Clorinda 2007.

ing containers per lot was very close to 1, and ≈40% of the lots inspected lacked water-holding containers.

Most containers (91%) were found outdoors in the yards, and only 4% of the pupae were collected from containers located indoors (Table 3). Containers were frequently found unlidded (71%) and only 31% of the large tanks were found fully lidded. Running water was as frequently reported as rain water (48%), yet large tanks were more often filled with running water.

The most important variables associated with infestation were container type, sun exposure, lid status, and water type, all of which scored an RI of 1.0, closely followed by water state and location (Table 4). Container volume was less important, and water volume had a RI close to that of the random variable. Tires were less likely to be infested than the rest of the containers. Large tanks and construction materials and discarded vehicle parts (type D containers) were the most likely to be infested (Table 5). Containers found outside, at low sun exposure, filled with rain water, not fully lidded and with polluted water were all more likely to be infested (i.e., their parameter estimates were all positive). Survey occasion was an important variable; containers examined in the spring survey were less likely to be infested than those inspected in the fall survey.

Table 3. Percentage of water-holding containers of each type and of pupae collected, container index, and pupae per container for every variable measured and every type of container, Clorinda 2007

	Container type (%)								Index		
	A	B	C	D	E	F	H	Total	% pupae collected	Container index	Pupae per container
Location within the lot											
Inside	1	9	44	0	3	12	16	9	4	8.5	0.8
Outside	99	91	56	100	97	88	84	91	96	18.5	2.0
Sun exposure											
High	36	49	10	35	24	57	27	40	30	14.2	1.5
Low	64	51	90	65	76	43	73	60	70	19.8	2.4
Lid status											
Unlidded	100	59	100	100	100	31	78	71	83	19.7	2.4
Partially lidded	0	10	0	0	0	22	1	7	9	26.8	2.6
Fully lidded	0	31	0	0	0	46	21	22	9	8.9	0.8
Type of water											
Pump	1	2	6	0	2	0	5	2	1	15.9	0.9
Rain	83	39	9	83	64	54	50	48	77	27.6	3.5
Running	16	57	86	17	34	40	44	48	20	10.7	0.9
Rain and running	0	2	0	0	0	5	1	2	3	18.3	3.6
Water state											
Clean	47	91	69	44	50	88	62	76	65	15.6	1.9
Polluted	53	9	31	56	50	12	38	24	35	30.7	3.2
Mean container vol (liters)	5	398	4	1	7	4,707	84	472			
Mean water vol (liters)	1	271	1	1	3	3,334	24	320			

[a] A, tires; B, large tanks, drums, or barrels; C, flower pots; D, construction materials and discarded vehicle parts; E, bottles, cans, and plastic goods; F, wells; and H, other.

Table 4. Relative importance of variables measured for every container in relation to infestation (presence or absence of larvae or pupae of *Ae. aegypti*) and productivity (number of pupae for the subset of infested containers) based on every subset of linear regression models possible, Clorinda 2007

Response variable	Random variable	Survey	Container type	Location	Sun exposure	Lid status	Container vol	Water vol	Water type	Water state
Infestation	0.291	1.000	1.000	0.993	1.000	1.000	0.467	0.301	1.000	0.998
Productivity	0.274	0.572	0.981	0.284	0.510	0.835	0.309	0.330	0.972	0.804

When only infested containers were considered, the number of pupae per container was most closely associated with container type and water type (RI > 0.97; Table 4). Lid status and water state were second in importance, and sun exposure and survey were not as important as in the infestation analysis (RI = 0.51–0.57). Container volume, water volume, and location had RIs close to random expectation. Large tanks were found positively associated with productivity and so did rain water (Table 5). Although less important, polluted water and absence of lid were also associated with productivity.

In total, 1,588 lots was examined twice, once in each survey; this group corresponds to 90% of the lots inspected in the fall survey and 70% of those examined in the spring survey. Only 57 lots (4%) were infested in both surveys (Table 6). This small subset of lots (15% of the fall-infested lots) provided 62% of the pupae collected in the next spring, yet only 17% of those collected in the fall. At the container level, 741 (94% of the total) large tanks were reidentified and examined twice, once in each survey. Only 27 (4%) of them were found infested in both surveys and accounted for 51 and 19% of the pupae collected from the reidentified tanks in the spring and fall surveys, respectively.

Table 5. Parameter estimates conditional on the full set of linear regression models of the variables measured for every container in relation to infestation (presence or absence of larvae or pupae of *Ae. aegypti*) and productivity (number of pupae for the subset of infested containers), Clorinda 2007

	Response variable	
	Infestation	Productivity
Container type[a]		
B	1.26	1.00
C	0.38	−1.10
D	1.62	−0.05
E	0.93	−0.19
H	0.96	−0.04
Location		
Outside	0.72	0.04
Sun exposure		
Low	0.77	0.13
Water type		
Running	−0.54	−0.39
Rain	0.45	0.26
Rain and running	0.38	0.61
Water state		
Polluted	0.48	0.36
Lid status		
Fully lidded	−0.88	−0.39
Partially lidded	0.37	−0.60
Survey		
Oct.–Dec.	−1.31	−0.17
Container vol	0.00	0.00
Water vol	0.00	0.00
Random	−0.05	−0.01

[a] B, large tanks, drums, or barrels; C, flower pots; D, construction materials and discarded vehicle parts; E, bottles, cans, and plastic goods; F, wells; and H, other.

Stochastic Simulation Model. The median of the correlation coefficients between the container index and the number of pupae per group of containers increased as the probability of not being infested (I) and the clumping of the infested containers (P) increased (Fig. 3). The probability of harboring larvae but not pupae given that a container is infested (L) was inversely related to the expected correlation. The variance of the correlation parameters increased with infestation (i.e., an increase in parameters P and a decrease in I) and with L. The expected median correlation showed almost no variations with the spatial scale parameter (n), though the variance increased as the scale increased.

The correlations between every pair of *Stegomyia* indices calculated for each block were very high ($r > 0.85$) and highly significant in both surveys ($P < 0.001$). However, the correlation between the container index and the number of pupae per block was much lower ($r = 0.42$ in the fall survey and $r = 0.39$ in the spring survey, $P < 0.001$). In the latter case, the correlation coefficient increased to 0.60 when an outlier data point (a highly productive block) was removed. These observed correlations are in qualitative agreement with those predicted by the model; because infestation was lower in the spring survey, a higher correlation was predicted and then observed when all data points but one were included in the analysis.

The model was parameterized with empirical values measured in both surveys. For the fall survey, the

Table 6. Distribution of infestation and pupae collected in lots and large tanks in the fall and spring surveys, Clorinda, 2007

	No. (%)	No. pupae collected in the fall (%)	No. pupae collected in the spring (%)
Lots			
Infested in both surveys	57 (4)	988 (17)	1,339 (62)
Only infested in the fall	322 (20)	4,826 (83)	0 (0)
Only infested in the spring	85 (5)	0 (0)	813 (38)
Negative in both surveys	1,124 (71)	0 (0)	0 (0)
Large tanks			
Infested in both surveys	27 (4)	603 (19)	794 (51)
Only infested in the fall	175 (24)	2,517 (81)	0 (0)
Only infested in the spring	56 (8)	0 (0)	769 (49)
Negative in both surveys	483 (65)	0 (0)	0 (0)

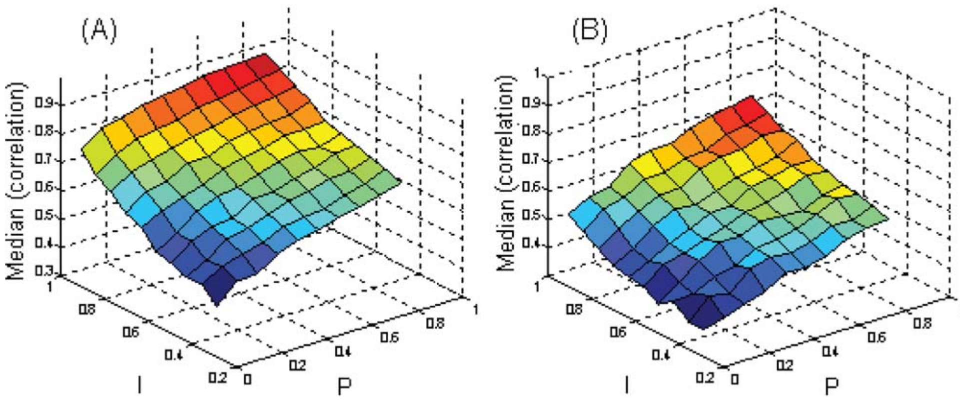

Fig. 3. Expected correlation coefficients between the container index and the number of pupae per group as a function of the degree of clumping (parameter P of the truncated negative binomial) and the proportion of containers uninfested (I) at two proportions of containers harboring larvae but not pupae (L). (A) L = 0. (B) L = 0.3.

negative binomial distribution was fitted to the number of pupae in positive containers, yielding $r = 0.655$ and $P = 0.033$. The rest of the parameters estimated were L = 0.388 and I = 0.696. The median of the correlations of the simulations using these parameters was 0.45 (95% confidence envelope, 0.29–0.60) and was in very close agreement with the observed value (0.42). For the spring survey, the model was parameterized with $r = 0.644$, $P = 0.042$, L = 0.339, and I = 0.918. The median of the correlation coefficients obtained was 0.53 and the 95% confidence envelope was 0.36–0.67, which includes the observed correlation coefficient ($r = 0.39$).

Discussion

Our study shows that large tanks were the key container type in a large neighborhood with a persistent infestation problem in the city of Clorinda in the context of a sustained temephos-based control campaign. They produced 70% of the pupae of *Ae. aegypti* despite the recurrent application of larvicides on water-holding containers of this type among others over the previous five years. Large tanks were widespread; almost 50% of the lots had at least one. These containers were either made of plastic or fibrocement, had usually 200–500 liters of potable water, and were therefore very valuable to householders. Although running water is available throughout the neighborhood at present, the service is discontinuous, especially during summer when the demand is high. Also, some householders reported a preference for drinking rain water and/or mistrusting running water.

Large containers for water storage have also been identified as the most important type elsewhere (Arredondo-Jiménez and Valdez-Delgado 2006, Bisset et al. 2006, Midega et al. 2006, Romero-Vivas et al. 2006, Maciel-de-Freitas et al. 2007). Unlike these studies, in our study area large tanks were also the most abundant type of container, as reflected in the small range and low absolute values of efficiencies found (Table 1). In Nicaragua, barrels (apparently equivalent to large tanks in our study) were the most productive type (30% of the pupae collected) but not very prevalent (8% relative abundance), thus scoring a high efficiency value (3.5) (Hammond et al. 2007). In our study area, large tanks scored the highest efficiency value but it was not as high (1.3) as in the Nicaragua study. Qualitatively different results were reported in some other locations. In nonresidential areas of Iquitos, no key container types were identified (Morrison et al. 2006). In Puerto Rico, seven different container types, including drums, were identified as the most productive containers; therefore, there was no single dominant key container type (Barrera et al. 2006b).

A very high proportion of the lots inspected (almost 40%) did not have water-holding containers. This fact explains the unusually high container indices in relation to the Breteau and house indices recorded; lots without containers contribute to reducing both Breteau and house indices but do not alter the container index.

Multivariate analysis revealed that container type was an important variable, and large tanks were associated with infestation and increased pupal productivity. Most of the containers were in the yard, and these were more likely to be infested than those found indoors. Containers with low exposure to the sun, unlidded, and filled with rain water were associated with higher infestation levels. Similar findings were recorded in the Peruvian Amazon (Morrison et al. 2004). It is noteworthy that containers with water scored as polluted had both increased probability of infestation and productivity, in opposition to the widely held view regarding *Ae. aegypti* as a mosquito breeding only in clean waters. This perhaps reflects that containers that are more likely to have their water dirtied by debris are unmanaged containers (i.e., those in which the turnover of water is low), which may be more suitable for mosquito breeding. In addition, the category polluted as scored here is broad and contains various degrees and forms of pollution. This category includes containers with high food supply, which could be associated with higher infestation. Leaf litter and algae were associated significantly with infestation in Puerto Rico, where food supply was identified as a limiting factor and septic tanks were discovered as large producers of *Ae aegypti* (Barrera et al. 2006a, 2008).

Broad-scale temporal variations between surveys were not detected by univariate or multivariate analyses except for overall infestation, which was much lower in the spring survey carried out after an especially cold winter. Infestation at a fine scale exhibited large changes between surveys. Although only 4% of the lots and of the large tanks examined were infested in both surveys, they were responsible for most of the pupae collected in the spring. This suggests the likely existence of a few persistently infested key premises and key containers, both of which may be the focus of targeted control measures. Key premises were also found in Trinidad in very similar proportion and under a similar temephos-based control strategy (Chadee 2004). Chadee (2004) defined "key premises" as premises with three or more infested containers during two or more consecutive surveys. Here, we use the term in a less restricted manner (i.e., infested during two consecutive surveys) because premises with three or more containers were very rare (only 0.7% of the total and none in both surveys).

The stochastic simulation model showed that the correlation between the container index, based on qualitative observations, and the number of pupae per group, based on quantitative observations, is expected to increase as infestation levels decline. Because the correlation between the *Stegomya* indices in Clorinda was very high, we believe that this result can be extended to the Breteau and the house index as well, at least in this location. In the extreme hypothetical situation in which every container is either uninfested or bears only one pupa, qualitative and quantitative observations are exactly equivalent. As infestation declines, the equivalence between indices is approached and the correlation is expected to increase. This could be taken to mean that the qualitative approach would be more cost-effective than the quantitative one in low-infestation scenarios. However, if pupae are not abundant, the quantitative approach would require very little extra labor; therefore, the use of *Stegomyia* indices would not be justified. We conclude that pupal indices are to be recommended in both low and high infestation scenarios, especially in the latter, where pupal and *Stegomyia* indices diverge the most.

The choice of an index depends on the objectives of the work. The *Stegomyia* indices were developed in the context of eradication campaigns in which the emphasis was on finding the presence of infestation (Scott and Morrison 2003) and a single larva was considered unacceptable (Soper 1963). In this scenario, the extra labor imposed by the quantitative nature of pupal indices would not be justified. Pupal demographic surveys seem more appropriate for other purposes in which the additional information they provide is valuable, such as investigating mosquito population dynamics and container productivity in the search for targeted cost-effective strategies.

The simulation model showed that as the probability of container infestation with larvae but without pupae increased, the expected correlation between the container index and the pupal index decreased. Containers with larvae but not pupae are considered uninfested in pupal surveys despite harboring larvae. The way this impacts on the usefulness of the index is unknown. It may confer an advantage to pupal indices if pupae are not found in such containers because they are not suitable for larval development and pupation, rendering them virtually a sink. However, it may be a shortcoming if pupae are not found due to a cohort effect, thereby causing productive containers to be overlooked. A cohort effect may also be problematic because it can produce large changes in the numbers of pupae collected depending on the particular day of the survey (Scott and Morrison 2003).

Our study has some limitations. Factors associated with productivity were identified through multivariate analysis, which only points to statistical associations between variables which may result from different underlying processes. Manipulative experiments are needed to distinguish between alternative processes. For example, differences in infestation according to type of water may be interpreted either as evidence for rain water being a better medium for mosquito breeding and development, or that water type reflects water-use practices affecting mosquito survival and insecticide residuality. No clear knowledge on how water management affects mosquito abundance is available. Processes related to water use may be especially relevant to account for infestation and design alternative control measures, because the water held by large tanks and other container types is of prime importance for householders and a part of their daily life. In addition, point observations may not necessarily reflect the history of a container (e.g., some containers were found infested despite the fact that they were found 100% lidded at the time of inspection).

Another limitation is the fact that the information gathered in this work is only entomological and could not be related to confirmed cases of dengue, which were not reported in the study neighborhood in 2007 (Ministerio de Salud de la Nación 2007). Predicting dengue transmission risk through entomological indices based on mosquito immatures is an important goal yet to be achieved. *Stegomya* indices are regarded as weak predictors of transmission risk and pupal surveys have not been validated yet (Morrison et al. 2008).

Larval indices especially in the fall survey were above target levels. Consequently, the risk of dengue transmission in the neighborhood and the city exists, especially considering that the disease is currently expanding in the Southern Cone region. Therefore, the search for improved vector control strategies is justified. Large tanks were the key containers in this study. An effective control strategy aiming at these containers would have a strong impact over the mosquito population, reducing the number of pupae by roughly 70% and, therefore, significantly diminishing the risk of dengue transmission.

Acknowledgments

For continuing support and advice, we thank Evangelina Aldama and the Clorinda team, Marcos Aguirre, Gustavo Caballero, Myriam Cardoso, Oscar Gómez, Gladys López, Zunilda Lovera, Marisabel Maldonado, Sonya Martínez, Carmen Ovando, Karina Paredes, Alba Pattenden, Alicia Roa, Juan Sancho, Gloria Vielma, Susana Zárate, and Cyntia Zárate. For help with the fieldwork, we thank Bárbara Vincenti. For helpful comments, we thank David Anderson, Amy Morrison, and Victoria Cardinal. The reported intervention program has been supported by Fundación Mundo Sano, and in part, by the Municipality of Clorinda through a welfare support program for unemployed heads of family. R.E.G. is member of Consejo Nacional de Investigaciones Científicas y Técnicas Researcher's Career, Argentina. The participation of R.E.G. was also supported by the National Institutes of Health/National Science Foundation Ecology of Disease program through National Institutes of Health research grant R01 TW05836 funded by the Fogarty International Center and the National Institute of Environmental Health Sciences (Uriel Kitron, to R.E.G.).

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Received 1 July 2008; accepted 3 November 2008.