PUBLIC HEALTH

Oviposition Activity of *Aedes aegypti* L. (Diptera: Culicidae) in Response to Different Organic Infusions

Eloína Santos[1], Juliana Correia[1], Luciana Muniz[1], Marcos Meiado[2], Cleide Albuquerque[1]

[1]*Depto de Zoologia, Centro de Ciências Biológicas, UFPE, Av Prof Nelson Chaves S/N, Cidade Universitária, 50570-420 Recife, PE, Brasil; eloina.santos@gmail.com; juliana.c.correia@gmail.com; luumuniz@gmail.com*
[2]*Depto de Botânica, Centro de Ciências Biológicas, UFPE, Av Prof Nelson Chaves S/N, Cidade Universitária, 50570-420 Recife, PE, Brasil; marcos_meiado@yahoo.com.br*

Edited by Fernando L Cônsoli – ESALQ/USP

Neotropical Entomology 39(2):299-302 (2010)

ABSTRACT - The present study investigates new sources of infusion as an oviposition attractant for *Aedes aegypti* L. Infusions with fetid and non-fetid odors were compared as an oviposition stimulant. Traps baited with infusions of dehydrated cashew leaves (*Anacardium occidentale*), potato peels (*Solanum tuberosum*) and graminea (*Panicum maximum*) were compared as attractants, and the effect of odor (fetid and unfetid) on attractiveness was tested. Oviposition activity changed significantly according to the concentration and type of infusion (F = 4.1279; gl = 2; P = 0.0231). A larger number of eggs were observed in cups containing 50% *A. occidentale* (non-fetid odor) and 30% *P. maximum* (fetid odor). When compared in the same cage, comparable oviposition was found between *A. occidentale* and *P. maximum*. Moreover, approximately 20% more eggs were recorded in the infusion without odor when compared to the grass infusion and water. These findings suggest *A. occidentale* as a new stimulant for use in ovitraps for *Aedes* surveillance and control, with the benefit of having an agreeable odor**.**

KEY WORDS: Attractive, mosquito, posture, ovitrap

Epidemiologically, gravid females are the most important component of the mosquito population and are targeted in mosquito reduction programs and in the active surveillance of disease for the early detection of epidemic events (Maciel-de-Freitas *et al* 2008a). Mosquito egg traps (ovitrap) are a current trend as integral components of surveillance efforts, contributing to control efforts and monitoring of mosquito populations in countries with endemic dengue fever. This method was proven effective in detecting early infestations of *Aedes aegypti* L., particularly when density is low and larval indices are not good indicators (Braga *et al* 2000, Morato *et al* 2005). It is also useful in indicating critical areas for control activities (Masuh *et al* 2008, Regis *et al* 2008).

There is a consensus regarding the use of organic infusions to achieve a better ovitrap performance. Most studies have used infusions of hay (Reiter *et al* 1991, Chadee *et al* 1993, Maciel-de-Freitas *et al* 2008b), white-oak leaf (Ponnusamy *et al* 2008, Trexler *et al* 1998), mixed grass (Regis *et al* 2008) and *Panicum maximum* (Sant'ana *et al* 2006, Gama *et al* 2007). The association of grass infusion, *Eleusine indica*, with the biological larvicide *Bacillus thuringiensis israelensis* in the field has also been used (Santos *et al* 2003), offering the advantage of a long-term duration of traps in the wild. Recent efforts have been made to identify potential synthetic oviposition attractants/repellents for mosquitoes using certain fatty acids and esters from grass infusions (Sant'ana *et al* 2006) and egg extracts of *A. aegypti* as oviposition attractants (Ganesan *et al* 2006). However, different types and concentrations of organic detritus lead to variations in the abundance and types of microorganisms (Yee & Juliano 2006, Murrel & Juliano 2008), which could, in turn, influence the potential attraction of the infusion. Thus, the most appropriate organic matter and its concentration remain the subject of study and controversy.

Anacardium occidentale originated in the eastern Americas and is widespread throughout tropical countries (Barros *et al* 1993). It belongs to the family Anacardiaceae and contains the medicinally important compound tannin in the leaves (Akinpelu 2001). The potential use of this plant infusion was investigated as an *A. aegypti* attractant, as this plant is very common throughout northeastern Brazil and preliminary studies in our laboratory have shown that an infusion of *A. occidentalis* leaves does not exhibit a fetid odor.

The aim of the present study was to search for new oviposition stimulant infusions for use in traps to collect eggs or females of *A. aegypti* and to compare egg density in recipients containing fetid and non-fetid infusions.

Material and Methods

Mosquitoes. *Aedes aegypti* females from a laboratory colony established in 2004 from eggs collected in ovitraps from the

campus of the Universidade Federal de Pernambuco – Recife (8º04'03'' S and 34º55'00'' W) were used in this study. Mosquitoes were maintained at 27 ± 4ºC, 66 ± 10% RH and 12h light/dark photoperiod. Adults were given a 10% sucrose solution and females were weekly allowed to feed on a restrained mouse in order to produce eggs.

Infusion preparation. Three types of organic material were used: young cashew leaves (*Anacardium occidentale*) as a non-fetid infusion; and potato (*Solanum tuberosum*) peels and grass (*Panicum maximum*) as fetid attractants. Infusions were prepared from 30 g of dehydrated cutted material (24h at 100ºC), placed in bottles containing 2 L of distilled water for seven days in anaerobic conditions. The resulting infusion was diluted to 30% and 50% in distilled water. Experimental trials were performed comparing infusions of *A. occidental, S. tuberosum* and *P. maximum* at 100%, 50% and 30% concentrations to determine the most attractive infusion, using distilled water as a control. The highest oviposition rates of *A. aegypti* were found at concentration of 50% of *A. occidental* and *S. tuberosum* and 30% of *P. maximum,* which were then used in further experiments. *Panicum maximum* has been shown to be attractive to *A. aegypti* females (Sant'ana *et al* 2006), and was therefore used as a positive control in the present study.

Experimental design. Infusions of *A. occidental* (50%), *S. tuberosum* (50%) and *P. maximum* (30%) were simultaneously compared to determine their attractiveness to ovipositing *A. aegypti*. Blood-fed females (n = 30) were placed in cages (30 x 30 x 30 cm) containing three black plastic cups (50 ml) arranged triangularly and spaced 20 cm from each other. Each container received 25 ml of infusion and two wooden pallets (3 x 2 cm), which served as an egg-laying support. The containers were rotated 45º clockwise daily. After seven days, the pallets were removed and left to dry, and the eggs were counted. Oviposition sites were also investigated for the presence of eggs on the infusion surface. Infusion attractiveness was determined based on the number of eggs collected, larger the number of eggs, higher the infusion attractiveness.

As a result of the above experiments, the fetid (*P. maximum*) and non-fetid infusions (*A. occidentale*) proved most attractive. In order to simulate field conditions, these infusions were compared by placing 25 females in cages (1 m^2) containing three traps (*A. occidentale*, *P. maximum* and water as attractants). Ovitraps were made from plastic recycling bottles (500 ml) and pallets (12 x 5 cm). Three traps were placed in a triangular manner approximately 45 cm apart from each other and received 400 ml of attractant (infusions). The other test conditions were similar to those earlier described. Three replicates of this experiment were performed.

Statistical analyses. Comparisons between the best concentrations and oviposition rate between fetid and non-fetid infusions were performed using the one-factor ANOVA test, with Tukey's *post hoc* test. The normality of the data was determined using the Shapiro-Wilk test and the homogeneity of the variations was determined using Levene's test. All analyses were carried out using the STATISTICA 7.1 program, with statistical significance set at 0.05.

Results

Comparison of the oviposition rate among the most attractive infusions of cashew leaves, potato peel and grass. Overall, the average number of eggs laid varied significantly when infusions of 50% cashew leaves, 50% potato peels and 30% grass were compared (F = 4.1279; gl = 2; P = 0.0231). A similar attractiveness was found between infusions of 50% cashew leaves (non-fetid odor) and 30% grass (fetid odor) (Fig 1), for which the average number of eggs did not differ (P = 0.9858). In contrast, a significant reduction in the number of eggs was obtained using the 50% potato infusion when compared with the grass (P = 0.0396). A significant increase in egg number was found in the pallets from infusions of 50% cashew leaves compared to the infusion from potato peels at the same concentration (P = 0.0485).

Comparative oviposition rate between fetid and non-fetid infusions. The fetid odor infusion did not influence the oviposition rate of *A. aegypti* females, as there was no significant difference in the number of eggs recorded on the pallets collected in the containers with *A. occidentale* and *P. maximum* (P = 0.1832) (Fig 2). However, there was a tendency for a higher attractiveness to infusions with no fetid odor, which accounted for 20% more eggs than the other attractants (grass and water). Out of the 8,481 eggs counted, 5,100 were found on pallets soaked in the cashew leaf infusion. The two types of infusion tested achieved a significantly higher oviposition response than the distilled

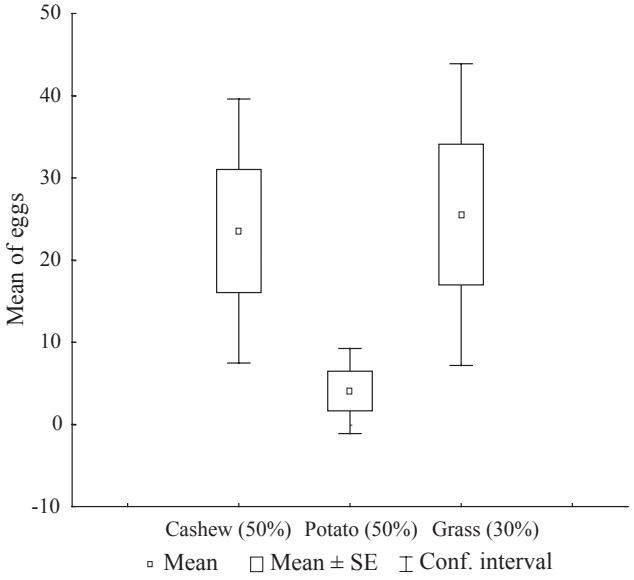

Fig 1 Mean number of eggs laid by *Aedes aegypti* females in cashew (*Anacardium occidentale*); potato peels (*Solanum tuberosum*) and graminea (*Panicum maximum*) infusion at different concentrations. Bars = confidence intervals.

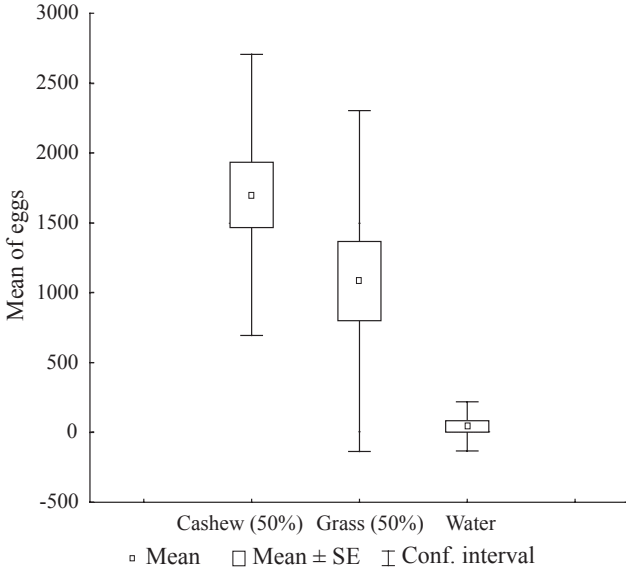

Fig 2 Mean number of eggs laid by *Aedes aegypti* females in non-fetid cashew (*Anacardium occidentale*) and fetid grass (*Panicum maximum*) infusions. Water was used as control. Bars = confidence intervals.

water (*A. occidentale*: P = 0.0039; *P. maximum*: P = 0.0318). There was no record of eggs attached to the recipient or on the water surface in any treatment.

Discussion

The use of organic infusions as attractants in ovitraps for gravid *A. aegypti* females has become the focus of interest in the last two decades and research on this subject has been extensively reported (Allan & Kline 1995, Trexler *et al* 1998, Santos *et al* 2003, Sant'Ana *et al* 2006). The potential attraction of infusions is highly influenced by the type (Le´Onard & Juliano 1995, Juliano 1998) and concentration (Reiter *et al* 1991, Allan & Kline 1995) of organic matter. The different oviposition rates in the present study confirm these concepts. Furthermore, the higher oviposition rates found for the cashew leaf and grass infusions differed significantly from distilled water, thereby lending support to the hypothesis tested by other authors that organic infusions are more attractive to *A. aegypti* than water (Santos *et al* 2003, Trexler *et al* 1998).

Unlike most organic infusions used as attractants in mosquito traps, which have an unpleasant odor, the data presented here indicate that the non-fetid *A. occidentale* infusions can attract *A. aegypti* females to oviposition sites in a manner comparable to the fetid *P. maximum* infusion. This represents a great advantage for the infusion traps placed in homes, as they would likely enjoy greater acceptance among the residents. The tannins in the cashew leaves have shown bactericidal and antifungal activities (Scalbert 1991), and tannins isolated from *Magonia pubescens* showed larvicidal activity against *A. aegypti* (Silva *et al* 2004). It is likely that the bactericidal activity attributed to acidic tannin is involved in the absence of fetid odor in the *A. occidentale* infusion due to the interruption of the fermentation process, thereby blocking the release of the volatile agents responsible for the fetid odor.

The attractiveness of organic infusions is influenced by the process of bacterial growth, with subsequent secondary metabolite production, as observed both in the laboratory (Navarro *et al* 2003) and in the field (Mboera *et al* 1999). The significant variation in oviposition found within and between the different infusions tested may be also affected by specific active compounds in the infusions and considered another source of attractant. In previous studies on *P. maximum*, Sant'ana *et al* (2003) identified seven biologically active compounds for *Aedes* sp. (nonanal, decanal, benzothiazol, 3-methyl indole, p-cresol, limonene indole) using gas chromatography coupled to a mass spectrometer (GC/MS) and the GC coupled to electro-antennographic detection (GC-EAD).

In a recent study on the essential oil from steam distillation of fresh *A. occidentale* leaves, 182 compounds were identified, including hexadecanoic acid (Kossouoh *et al* 2008), which has been shown to be highly effective in inducing *A. aegypti* egg laying (Ponnusamy *et al* 2008). Although chemical analyses of the cashew leaf infusion were not performed, the presence of this carboxylic acid in the essential oil suggests its presence in the infusion, which may have an attractant potential for mosquito females.

Data on the effect of concentration on the attractiveness of infusions are controversial in the literature. Among the concentrations tested in the present study (100%, 50% and 30%), oviposition preference of gravid females was recorded for infusions of 50% *A. occidentale*, 50% *S. tuberosum* and 30% *P. maximum*. In contrast, Reiter *et al* (1991) recorded significantly more *A. aegypti* eggs in traps containing a lower concentration of hay infusion (10%) when compared to a 100% concentration, which were the opposite of those later reported by Chadee *et al* (1993). Regardless of the concentration used (10%, 20%, 25%, 50%, 60% and 80%), a similar number of eggs was recorded for the traps analyzed by these authors. These results may be attributed to the fact that organic infusions are a complex mixture of compounds affecting not only mosquito performance, population growth and the outcome of competition (Murrel & Juliano 2008), but also female oviposition site selection.

Further laboratory or field studies on the chemical and microbial properties of *A. occidentale* infusion could help to define the mechanistic relationship between *A. aegypti* females and substances that certainly serve as a stimulant for selecting traps as egg-laying sites. This type of infusion represents a new alternative for use in oviposition traps and offers the advantage of not having a fetid odor, which may expand its use in homes and, consequently, assist in the monitoring and control of *A. aegypti* populations.

Acknowledgments

We are especially grateful to the anonymous reviewers for their comments and very helpful suggestions to the manuscript. We also thank the Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE) for supporting this research.

References

Akinpelu D A (2001) Antimicrobial activity of *Anacardium occidentale* bark. Fitoterapia 72: 286-287.

Allan S A, Kline D L (1995) Evaluation of organic infusions and synthetic compounds mediating oviposition in *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). J Chem Ecol 21: 847-298.

Barros L M, Pimentel C R M, Correa M P F, Mesquita A L M (1993) Recomendações técnicas para a cultura do cajueiro-anão-precoce. Fortaleza, EMBRAPA-CNPAT, 65p.

Braga I A, Gomes A C, Nelson M, Mello R C G, Bergamaschi D P, Souza J M P (2000) Comparative study between larval surveys and ovitraps to monitor populations of *Aedes aegypti*. Rev Soc Bras Med Trop 33: 347-353.

Chadee D D, Lakhan A, Ramdath W R, Persad R C (1993) Oviposition response of *Aedes aegypti* to different concentrations of hay infusion in Trinidad, West Indies. J Am Mosq Control Assoc 9: 346-348.

Ganesan K, Mendki M J, Suryanarayana M V S, Prakash S, Malhotra R C (2006) Studies of *Aedes aegypti* (Diptera: Culicidae) ovipositional responses to newly identified semiochemicals from conspecific eggs. Aust J Entomol 45:75-80.

Juliano S A (1998) Species introduction and replacement among mosquitoes: interspecific resource competition or apparent competition? Ecology 79: 255-68.

Kossouoh C, Moudachirou M, Adjakidje V, Chalchat J C, Figueredo G (2008) Essential oil chemical composition of *Anacardium occidentale* L. leaves from Benin. J Essent Oil Res 20: 5-9.

Le´Onard P M, Juliano S A (1995) Effect of leaf litter and density on fitness and populations performance of the tree hole mosquito *Aedes triseriatus*. Ecol Entomol 20: 125-136.

Maciel-de-Freitas R, Eiras A E, Lourenço-de-Oliveira R (2008a) Calculating the survival rate and estimated population density of gravid *Aedes aegypti* (Diptera, Culicidae) in Rio de Janeiro, Brazil. Cad Saúde Pública 24: 2747-2754.

Maciel-de-Freitas R, Peres R C, Alves F, Brandolini M B (2008b) Mosquito traps designed to capture *Aedes aegypti* (Diptera: Culicidae) females: preliminary comparison of Adultrap, MosquiTRAP and backpack aspirator efficiency in a dengue-endemic area of Brazil. Mem Inst Oswaldo Cruz 103: 602-605.

Masuh H, Seccacini E, Zerba E, Licastro S A (2008) *Aedes aegypti* (Diptera: Culicidae): monitoring of populations to improve control strategies in Argentina. Parasitol Res 103:167-170.

Mboera L E G, Mdira K Y, Salum F M, Takken W, Pickett J A (1999) Influence of synthetic oviposition pheromone and volatiles from soakage pits and grass infusions upon oviposition site-selection of *Culex* mosquitoes in Tanzania. J Chem Ecol 25: 1855-1867.

Morato V C G, Teixeira M G, Gomes A C, Bergamaschi D P, Barreto M (2005) Infestation of *Aedes aegypti* estimated by oviposition traps in Brazil. Rev Saúde Publ 39: 553-558.

Murrel E G, Juliano S A (2008) Detritus type alters the outcome of interspecific competition between *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae). J Med Entomol 45: 375-383.

Navarro D M A F, Oliveira P E S de, Potting R P J, Brito A C, Fital S J F, Sant'Ana A E G (2003) The potential attractant or repellent effects of different water types on oviposition in *Aedes aegypti* L. (Dipt., Culicidae). J Appl Entomol 127: 46-50.

Ponnusamy L, Xu N, Nojima S, Wesson D M, Schal C, Apperson C S (2008) Identification of bacteria and bacteria-associated chemical cues that mediate oviposition site preferences by *Aedes aegypti*. Proc Natl Acad Sci USA 105: 9262-9267.

Reiter P, Amador M A, Colon N (1991) Enhancement of the CDC ovitrap with hay infusion for daily monitoring of *Aedes aegypti* populations. J Am Mosq Control Assoc 7: 52-55.

Regis L, Monteiro A M, Melo-Santos M A V, Silveira Jr J C, Furtado A F, Acioli R V, Santos G M, Nakazawa M, Carvalho M S, Ribeiro Jr P J, Souza W V (2008) Developing new approaches for detecting and preventing *Aedes aegypti* population outbreaks: basis for surveillance, alert and control system. Mem Inst Oswaldo Cruz 103: 50-59.

Scalbert A (1991) Antimicrobial properties of tannins. Phytochemistry 30: 3875-3883.

Santos S R A, Santos-Melo M A V, Regis L, Albuquerque C M R (2003) Field evaluation of ovitraps consociated with grass infusion and *Bacillus thuringiensis* var. israelensis to determine oviposition rates of *Aedes aegypti*. Dengue Bull 27: 156- 162.

Sant′Ana A L (2003) Avaliação, extração, identificação e estudos eletrofisiológicos dos voláteis presentes em infusões de *Panicum maximum* que estimulam e/ou atraem fêmeas de *Aedes* (*Stegomyia*) *aegypti* Linnaeus, 1762 (Diptera: Culicidae) para oviposição. D. Sc. thesis. Belo Horizonte, Universidade Federal de Minas Gerais, 103p.

Sant'ana A L, Roque R A, Eiras A E (2006) Characteristics of grass infusions as oviposition attractants to *Aedes* (Stegomyia) (Diptera: culicidae) J Med Entomol 43: 214-220.

Silva H H G, Silva I G, Santos R M G, Rodrigues Filho E, Elias C N (2004) Atividade larvicida de taninos isolados de *Magonia pubescens* St. Hil. (Sapindaceae) sobre *Aedes aegypti* (Diptera, Culicidae). Rev Soc Bras Med Trop 37: 396-399.

Trexler J D, Apperson C S, Schal C (1998) Laboratory and field evaluation of oviposition responses of *Aedes albopictus* e *Aedes triseriatus* (Diptera: Culicidae) to oak leaf infusions. J Med Entomol 35: 967-976.

Yee D A, Juliano S A (2006) Consequences of detritus type in an aquatic microsystem: assessing water quality, micro-organisms, and the performance of the dominant consumer. Fresh Biol 51: 448-459.

Received 13/VIII/08. Accepted 08/I/10.