Revista Brasileira de Entomologia 62 (2018) 304–310

SOCIEDADE BRASILEIRA DE ENTOMOLOGIA
FUNDADA EM 1937

REVISTA BRASILEIRA DE
Entomologia
A Journal on Insect Diversity and Evolution

www.rbentomologia.com

Medical and Veterinary Entomology

Oviposition of *Aedes aegypti* Linnaeus, 1762 and *Aedes albopictus* Skuse, 1894 (Diptera: Culicidae) under laboratory and field conditions using ovitraps associated to different control agents, Manaus, Amazonas, Brazil

William Ribeiro da Silva[a], Joelma Soares-da-Silva[b], Francisco Augusto da Silva Ferreira[a], Iléa Brandão Rodrigues[a], Wanderli Pedro Tadei[a], João Antonio Cyrino Zequi[c,*]

[a] *Instituto Nacional de Pesquisas da Amazônia, Programa de Pós-Graduação em Entomologia, Laboratório de Controle Biológico e Biotecnologia da Malária e Dengue, Manaus, AM, Brazil*
[b] *Universidade Federal do Maranhão, Curso de Ciências Naturais, Campus VII, Maranhão, MA, Brazil*
[c] *Universidade Estadual de Londrina, Departamento de Biologia Animal e Vegetal, Laboratório de Entomologia Médica, Londrina, PR, Brazil*

ARTICLE INFO

Article history:
Received 26 April 2018
Accepted 10 August 2018
Available online 25 August 2018
Associate Editor: Ana Campos

Keywords:
Chikungunya
Control
Dengue
Insecticides
Zika

ABSTRACT

The aim of this study was to analyze the effectiveness of different control agents of *Aedes aegypti* and *Aedes albopictus* associated with ovitraps under laboratory and field conditions. Five treatments were used: grass infusion + *Bacillus thuringiensis israelensis* (gI + Bti), grass infusion + *Saccharopolyspora spinosa* (gI + Ss), grass infusion + Pyriproxyfen (gI + P), distilled water + *Toxorhynchites haemorrhoidalis* (dW + Th), and grass infusion (gI) (control). The highest mean number of eggs of both species were obtained with grass infusion in the laboratory. Among control agents, the lowest mean of *A. aegypti* eggs occurred with gI + Ss and the lowest mean of *A. albopictus* eggs occurred with dW + Th. There was no difference between treatments in *A. aegypti* ($P = 0.4320$) and *A. albopictus* ($P = 0.7179$). In the field, the highest mean number of eggs for both species were obtained with gI + Ss, and the lowest values were obtained with gI + P ($P = 0.0124$). The treatments can be applied to both the surveillance and the control, but ovitraps with biological larvicide Bti were more effective and safer considering the number of eggs laid and selectivity of pathogens for mosquitoes.

Introduction

Aedes (*Stegomyia*) *aegypti* Linnaeus, 1762 and *Aedes* (*Stegomyia*) *albopictus* Skuse, 1894 (Diptera: Culicidae) are particularly susceptible to several arboviruses that are threats to public health in several regions of the world (Alahmed, 2012; Forattini, 2002; Rossati et al., 2015). Both species have wide epidemiological importance in the Americas due to implications in the transmission of many arbovirus responsible for high hospitalization rates and deaths. They are distributed throughout several regions in the continent (PAHO, 2018). These mosquito-borne pathogens affect economies due to an impaired workforce and the need to treat sick people (Calvo et al., 2016; Gubler, 2005; Vega-Rúa et al., 2014).

Derived from Africa where there are wild and urban populations, *A. aegypti* is currently widespread and is found in all states and the Federal District of Brazil (Carvalho and Moreira, 2017). This species is the main vector of Dengue, Chikungunya, Zika, and Urban Yellow Fever viruses in the Americas. This anthropophile is adapted for urban environments, where it inhabits laundry tanks, containers, barrels, bottles, pots of plants and other containers (Pinheiro and Tadei, 2002; Soares-da-Silva et al., 2012).

These oviposition sites are different from those used by *A. albopictus*, which inhabits sites with native or secondary vegetation near human populations (Martins et al., 2013; Silva et al., 2006). Considering its epidemiological role, this species has potential for transmitting Urban Yellow Fever and Venezuelan Equine Encephalitis viruses; laboratory assays have checked this species susceptibility to more than 20 arboviruses including the Dengue virus (the primary vector in Asia) and the Chikungunya virus (Gubler et al., 2001; Moore and Mitchell, 1997; Vega-Rúa et al., 2014). The first record of this species in Brazil was in 1986 in the

* Corresponding author.
E-mail: joaozequi@gmail.com (J.A. Zequi).

https://doi.org/10.1016/j.rbe.2018.08.001

state of Rio de Janeiro; a few years later it had already spread to all states of the Southeastern region. It is currently distributed nationwide (Martins et al., 2013; Santos, 2003).

Vector control practices that are less damaging to the environment and are effective for mosquito larvae have increased over the last years. These include natural predators, bacteria, growth regulators, and more recently, fungi and plant extracts (Darbro et al., 2011; Ferreira et al., 2015; Lopes, 1999; Medeiros et al., 2013; Soares-da-Silva et al., 2015; Soares-da-Silva et al., 2017; Zequi et al., 2015). New attempts at mosquito control have advanced to suppress populations of medical importance including the use of transgenic mosquitoes (Beech et al., 2009; Carvalho et al., 2015) and the symbiotic bacterium *Wolbachia*, which inhibits the development of Dengue virus inside the insect thus blocking its transmission (Dutra et al., 2016).

Vector species monitoring is essential. Studying mosquito distribution is essential to identify critical larval reproduction and egg laying sites. The use of ovitraps began in 1965. Oviposition traps for *A. aegypti* populations were shown to be effective for larval research and studies on vector frequency (Fay and Eliason, 1966; Regis et al., 2008).

Ovitraps provide useful data on the spatio-temporal distribution of mosquitoes because this monitoring allows one to check the presence and density of the vector at a local scale (Fisher et al., 2017; Nunes et al., 2011). In addition, ovitraps might be employed for vector control because they remove eggs from the environment; they are a sensitive and low-cost tool to aid in epidemiological studies (Depoli et al., 2016; Gomes, 2002).

Data obtained with this tool can monitor the impact of control measures such as the use of insecticides. They can also help monitor the effectiveness of vector reduction programs in urban areas. Although ovitraps are effective, they are a short-term method, replacement is required within approximately 5–7 days so that larval hatching does not occur and traps do not become oviposition sites (Alarcón et al., 2014; Gomes, 1998; Gomes, 2002; Regis et al., 2008).

The association of ovitraps and larval control agents could improve field traps and prevent potential larval hatching. This would ensure the safety of these traps (Depoli et al., 2016; Regis et al., 2013). However, it is necessary to analyze the best control agent associated with ovitraps because the selection of a potential oviposition site involves visual, olfactory, and tactile responses. In this respect, grass infusion is an important attractant for laying eggs (Reiter et al., 1991) as is the presence of a conspecific larvae has been reported (Nunes et al., 2011; Santana et al., 2006).

Manaus is an Amazon city with climatic conditions that favor *A. aegypti* and *A. albopictus* year-round. It has abundant rain and high temperatures as well as a diversity of natural and artificial larval habitat. Therefore, integrating a monitoring model that combines ovitraps with control agents might be an effective alternative for surveillance and control of mosquitoes that are vectors of human pathogens. Thus, the aim of this study was to analyze the effectiveness of different control agents associated with ovitraps for *A. aegypti* and *A. albopictus* under laboratory and field conditions.

Materials and methods

Obtaining Aedes aegypti *and* Aedes albopictus *mosquitoes*

To obtain females, *A. aegypti* and *A. albopictus* populations were kept and stabilized in an insectary of the Laboratory of Biological Control and Biotechnology of Malaria and Dengue (LCBBMD) of the National Institute of Amazonian Research (INPA), Manaus, Amazonas, Brazil. The insectary started obtaining eggs using oviposition traps, also known as ovitraps. Ovitraps are black and round plastic pots measuring 9 x 11 cm with a capacity of 500 mL. Inside the ovitraps, there is a five-mm thick Duratree paddle, Eucatex® brand, 15 cm long by three cm wide. This is placed vertically with the rough surface of the material facing outwards to provide a substrate for oviposition and egg adherence. The main attractant of females in the traps was a grass infusion with total a volume of 300 mL at 1.2% of Colonião grass (*Megathyrsus maximus* Jacq) fermented for seven days at a mean room temperature of 32 °C (range of 38 °C and 20 °C) according to Koeppen's classification (1948).

Twenty ovitraps were mounted and distributed in the INPA Campus. After one week, ovitraps were collected and the Duratree paddles containing the eggs were submerged in distilled water for larval hatching. The larvae were fed with cat food (Whiskas®) minced in fine particles. The water in the containers was changed every two days to prevent feed fermentation—this can damage gas exchange during the immature stages. After emergence, the adults were captured with a Castro grabber, and the species identity was confirmed using external morphological characteristics of adults, especially in the thorax using stereoscopic microscope, where they were identified with the help of a mosquito identification guide (Consoli and Lourenço-de-Oliveira, 1994; Forattini, 2002; WRBU, 2018).

The adults were subsequently maintained in a cage for mating and maintenance of adult individuals. Cotton wrapped in gauze and soaked in sugared water at 12% was added as a source of carbohydrates, and females were fed a blood meal twice a week using an adequately anesthetized hamster (*Mesocricetus auratus*) following the procedure approved by the INPA Ethical Committee for the Use of Animals (CEUA: 02/2014 – "Breeding of vectors under laboratory conditions").

Obtaining larvae of Toxorhynchites haemorrhoidalis *Fabricius, 1787*

Toxorhynchites haemorrhoidalis is a natural predator of culicids and other insects. To obtain immature *T. haemorrhoidalis*, eight cut tires (25 × 15 × 11 cm), containing well water and a small amount of litter were distributed throughout the INPA *Campus* and monitored daily. We also actively searched for immature stages in the natural larval habitat. The larvae were kept in the insectary of the LCBBMD and fed on the third and fourth instar larvae of *Aedes* spp.; 56 third instar larvae of *T. haemorrhoidalis* were used.

Substrates used in the experiment

Table 1 lists the associations used in the oviposition process of *A. aegypti* and *A. albopictus* under laboratory and field conditions.

Control agents were diluted using falcon tubes and dissolved with a Vortex agitator and a BRANSONIC® ultrasonic until the desired concentrations were reached based on the manufacturers' recommendations (Table 1).

Laboratory experiments

From a stabilized *A. aegypti* and *A. albopictus* insectary, 25 fertilized females from generation F_1 were previously selected from each species for each experiment. Five days after the blood meal, females were placed in an aluminum-screened cage (55 × 47 × 47 cm). Five 160 mL white plastic cups were placed inside—one for each treatment ontaining 50 mL of each treatment and a paper filter as the oviposition substrate (Table 1). Subsequently, a container with 12% sugar in water was added as a source of carbohydrates for females.

Containers were placed in the four corners of the cage and one in the center. The container positions were changed clockwise daily for four days to discard any external influence on oviposition; three

Table 1
Treatments used in oviposition experiments with *Aedes aegypti* and *Aedes albopictus* under laboratory and field conditions in the period ranging from March to July, 2015, Manaus – Amazonas – Brazil.

Treatments	Control agents	Concentration	Batch/validity
Grass infusion + Vectobac® WG	*Bacillus thuringiensis israelensis* (gI + Bti)*	0.004 g/L	237-445-PG – Jan/2016
Grass infusion + Natular™ DT	*Saccharopolyspora spinosa* (gI + Ss)*	0.007 g/L	1309190010 – Oct/2015
Grass infusion + Sumilar®	Pyriproxyfen (gI + P)*	0.01 g/L	4303F425 – Jan/2016
Distilled water + *T. haemorrhoidalis*	*T. haemorrhoidalis* (dW + Th)*		
Grass infusion	Control (gI)*	1.2%	

Legend: *Entomopathogenic bacterium: *Bacillus thuringiensis israelensis*; *Entomopathogenic bacterium: *Saccharopolyspora spinosa*; *Insect Growth Regulator (IGR): Pyriproxyfen; Predatory invertebrate: *Toxorhynchites haemorrhoidalis*; Control: Grass Infusion.

replicates were performed. The females were captured and sacrificed at the end of each replicate. Experiments were conducted under controlled temperature, humidity and photoperiod conditions (27 ± 2 °C; 80–90%; 12L:12D).

The eggs obtained at the end of each replicate were dried on absorbent paper for 60 minutes at 27 ± 2 °C and humidity of 80–90%. They were then stored under the same temperature and humidity conditions in 750 mL cups for subsequent egg counting using a ZEISS Stemi 2000 50X stereoscopic microscope.

Field experiments

For the field oviposition tests, 50 ovitraps were distributed (10 for each treatment) (Table 1). Traps were installed in the INPA campus. They were placed near buildings at ground level with shelter from the sun and rain. We focused on areas free of constant movement of people and animals; they were located 30 meters apart from each other. The experiment lasted five consecutive weeks, and the Duratree paddles were replaced every seven days. Eggs were counted using a stereoscope microscope (ZEISS Stemi 2000 50X).

After egg counting, paddles were submerged separately in disposable 600 mL flasks containing 400 mL of distilled water and 0.0055 g cat food (Whiskas®) ground into minced in fine particles to feed the larvae and obtain adults. After the adults emerged, the species were identified using external morphological analysis. The specimens that were not identified because of the difficulty in visualizing the patterns of ornamentation of the scales were forwarded live one after the other to a stereoscopic microscope, where they were identified with the help of a mosquito identification guide (Consoli and Lourenço-de-Oliveira, 1994; Forattini, 2002; WRBU, 2018).

Statistical analysis

The following indices were calculated: OPI – ovitraps positivity index, EDI – egg density index, and VDI (mean eggs) – vector density index. The OPI is the ratio between the number of positive traps and the number of traps examined multiplied by one hundred (Gomes, 1998). The EDI is the ratio between the number of eggs and the number of positive traps (Gomes, 2002). The VDI is the mean number of eggs in each type of treatment obtained by the ratio between the number of eggs and the number of traps examined (Avendanha, 2007). In relation to the data of number of eggs obtained in each treatment, an analysis was first made to know if the data had a normal distribution or not, to later verify which statistic would be the most adequate, parametric or non-parametric. After these initial tests, ANOVA was used for the data that presented normal distribution, followed by Tukey's multiple comparison test ($p < 0.05$), while the data that did not present normality were chosen by non-parametric Kruskal tests Wallis, followed by the Dunn test ($p < 0.05$). The statistical software SPSS® 14.0 for Windows® (SPSS Inc. 2005 Headquarters, Chicago, IL, USA) was used for analysis assistance.

Results

Oviposition at the laboratory

The number of *A. aegypti* eggs collected in all treatments was higher than the number of *A. albopictus* eggs (4215 and 2074 eggs, respectively). This had a statistical difference using Tukey's test ($F = 21.836$; $P = 0.0019$). No differences were found between the mean number of *A. aegypti* ($F = 1.046$; $P = 0.4320$) eggs and *A. albopictus* eggs ($F = 0.5312$; $P = 0.7179$) either in the grass infusion (control) or with control agents using Tukey's test at 5% significance level (Table 2).

Both species had the highest mean number of eggs with grass infusion (control); however, *A. aegypti* had the highest mean in ovitraps with distilled water + *T. haemorrhoidalis* and the lowest value with grass infusion + *S. spinosa*. Results for *A. albopictus* were exactly the opposite, but this difference was not statistically significant (Tukey's, $p > 0.05$).

Oviposition in the field

The highest mean number of eggs for both species was obtained with grass infusion + *S. spinosa*, and the lowest value was obtained with grass infusion + Pyriproxyfen. This resulted in a mean number of eggs that was twice as low as grass infusion + *S. spinosa*; this corroborates the significant difference between treatments (Tukey, $P = 0.0124$) (Table 3). Except for the treatment with grass infusion + Pyriproxyfen, all treatments had a higher mean number of eggs than the grass infusion (control) (Table 3); however, there was no statistical difference between treatments (Tukey, $p > 0.05$).

Nearly all treatments had an OPI of 100% except distilled water + *T. haemorrhoidalis*, which obtained 97% of positivity (Fig. 1).

The EDI values of both species showed that biotic agents received a higher amount of eggs than the chemical agent Pyriproxyfen (Fig. 1). Grass infusion + *S. spinosa* and grass infusion + *B. thuringiensis israelensis* showed higher EDIs (150 and 130, respectively; Fig. 1). On the other hand, treatment with grass infusion + Pyriproxyfen had the lowest density (70 eggs; Fig. 1). The same result was observed for the VDI, which was highest with grass infusion + *S. spinosa* and the lowest with grass infusion + Pyriproxyfen.

The mosquito emergence rate obtained from eggs captured in the field was higher for *A. albopictus*: 4734 specimens comprising of 2270 males and 2464 females (1:1 ratio). In contrast, 494 specimens were obtained for *A. aegypti*: 272 males and 222 females (1:1 ratio). Therefore, *A. albopictus* predominated throughout the study area.

The rate of mosquito emergence was analyzed in the treatments. The highest number of adults occurred with grass infusion + *S. spinosa* and grass infusion (control). In these treatments, the number of adult *A. albopictus* was approximately ten times as high as that obtained for *A. aegypti* (Fig. 2). The control agents had little interference in the egg phase of these insects.

The lowest values for adult emergence were seen with grass infusion + *B. thuringiensis israelensis* (Fig. 2) despite this treatment

Table 2
Mean number of *Aedes aegypti* eggs and *Aedes albopictus* eggs obtained with ovitraps containing grass infusion and different control agents under laboratory conditions, in the period ranging from March to April, 2015, Manaus – Amazonas – Brazil.

Treatments	*Aedes aegypti*			*Aedes albopictus*		
	Mean	Maximum	Minimum	Mean	Maximum	Minimum
* Grass infusion + *B. thuringiensis israelensis*	270.[a*]	345	171	124 [a]	254	56
* Grass infusion + *S. spinosa*	222.[a]	421	92	164.7 [a]	208	135
* Grass infusion + Pyriproxyfen	249.3 [a]	279	193	117.3 [a]	177	50
* Distilled water + *T. haemorrhoidalis*	289.7 [a]	306	269	98 [a]	220	32
* Grass infusion (Control)	373.7 [a]	440	287	187.3 [a]	278	94

Legend: *Same letters in the column do not differ according to the Tukey's test at 5% significance level.

Table 3
Total mean of eggs (*Aedes aegypti* and *Aedes albopictus*) obtained by the association of grass infusion and different control agents using ovitraps under field conditions in the period ranging from March to April, 2015, Manaus – Amazonas – Brazil.

Treatments	Mean	Maximum	Minimum
* Grass infusion + *B. thuringiensis israelensis*	1322.8 [ab*]	2025	831
* Grass infusion + *S. spinosa*	1528.2 [a]	1932	1121
* Grass infusion + Pyriproxyfen	712.6 [b]	903	380
* Distilled water + *T. haemorrhoidalis*	1158.[ab]	1525	765
* Grass infusion (Control)	988.2 [ab]	1299	533

Legend: *Same letters in the column do not differ according to the Tukey's test at 5% significance level.

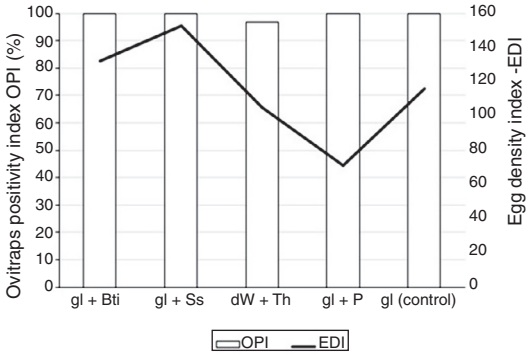

Fig. 1. Ovitraps Positivity Index (OPI) and Egg Density Index (EDI) with association of grass infusion with different control agents using ovitraps in the period ranging from June to July 2015, Manaus – Amazonas – Brazil. *Legend*: *(gl + Bti) – grass infusion + *Bacillus thuringiensis israelensis*; *(gl + Ss) – grass infusion + *Saccharopolyspora spinosa*; *(dW + Th) – distilled water + *Toxorhynchites haemorrhoidalis*; *(gl + P) – grass infusion + Pyriproxyfen; *(gl) – grass infusion (control).

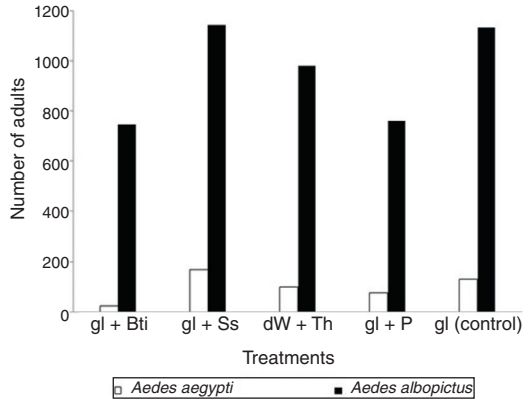

Fig. 2. Total *Aedes aegypti* and *Aedes albopictus* adults obtained through egg collection in ovitraps containing grass infusion (control) and associated with different control agents. *Legend*: *(gl + Bti) – grass infusion + *Bacillus thuringiensis israelensis*; *(gl + Ss) – grass infusion + *Saccharopolyspora spinosa*; *(dW + Th) – distilled water + *Toxorhynchites haemorrhoidalis*; *(gl + P) – grass infusion + Pyriproxyfen; *(gl) – grass infusion (control).

having the second highest mean number of eggs in the field (Table 3). No differences were seen in larval hatching rate between grass infusion (control) and control agents either for *A. aegypti* ($P = 0.1481$; $H = 6.78$) nor for *A. albopictus* ($P = 0.093$; $H = 7.96$) using the Kurskall Wallis test with a significance level of 5% (Fig. 2).

Discussion

Biological characteristics are determining factors for oviposition and might directly influence the higher number of *A. aegypti* eggs observed in the laboratory (Phasomkusolsil et al., 2014) especially because mosquitos are an intradomiciliar and anthropophilic insect. Females of this species complete their gonotrophic cycle with two blood meals. The mean number of eggs at each oviposition is 120; *A. albopictus* needs multiple meals to generate an average of 60 to 65 eggs (Clements, 1999; Forattini, 2002).

The high number of eggs seen in both species in ovitraps with grass infusion (control) might be explained by the proven effectiveness of this substrate as an attractant to female *Aedes* spp. (Nunes et al., 2011; Reiter et al., 1991; Santana et al., 2006).

The difference found in the treatment with distilled water + *T. haemorrhoidalis* with higher average number of *A. aegypti* eggs can be explained by the behavior of this species. It oviposits in pre-existing immature-containing breeding sites, since in most urban environments, specimens do not establish strong ecological relations of competition with other insects, such as coexistence with predatory invertebrates, and thus become completely generalist in relation to oviposition sites (Forattini, 2002). On the other hand, *A. albopictus* coexists with *T. haemorrhoidalis* in the suburban environment of Manaus. *T. haemorrhoidalis* is a natural predator of culicids in vegetated areas—sites where *A. albopictus* occurs. It consequently colonizes different types of natural breeding sites establishing ecological relationships with other insects, e.g. habitat competition (Bailey et al., 1983; Forattini, 2002; WHO, 1984). This might explain the low number of *A. albopictus* eggs in ovitraps containing larvae of this predatory species.

The choice of potential oviposition sites is related to several environmental factors including water spray intensity, size of the water surface relative to light, presence of immature culicid stages, and certain chemical components that might act as repellents or attractants depending on the concentration (Allan and Kline, 1995; Chadee et al., 1990).

The laboratory experiments had controlled conditions. It isolated the different factors related to oviposition and maximized the treatment differences. This allowed us to relate the factors found to be effective in ovitraps for each control agent. These agents can attract more fertilized females leading to better study and control of these species.

Under field conditions, more eggs were collected in ovitraps with grass infusion + *S. spinosa.* One study carried out in Mexico also showed the effectiveness of this bacterium as an attractant for mosquito females in the field where the oviposition rate of *A. aegypti* was higher than in treatments with temephos or distilled water (Solís-Valdez et al., 2015). However, in this study, grass infusion + *S. spinosa* only differed statistically from the chemical Pyriproxyfen (Tukey, $P = 0.0124$; Table 3). Ovitraps with grass infusion + *B. thuringiensis israelensis*, were preferred for oviposition corroborating prior field results (Carrieri et al., 2009; Depoli et al., 2016; Jahan and Sajjad Sarwar, 2013; Stoops, 2005).

Spinosad® is a new generation larvicide derived from the bacterium *S. spinosa*. It is toxic to several insect species of the orders Lepidoptera, Thysanoptera, and Diptera including mosquitoes (Bond et al., 2004; Dias et al., 2017; Huseyin et al., 2005). The effectiveness of *S. spinosa* was verified in the field for *A. aegypti* over 13 weeks during the dry season and 10 weeks during the rainy season (Marina et al., 2011). This is important information from an ecological standpoint because it shows feasibility at a large scale. Persistence in the field—combined with the effectiveness of the bacterium as an attractant to females and control of immature stages—makes this tool an important alternative. This information is important considering the climatic conditions in Manaus. It has a high solar incidence and abundant rains every year, but these do not limit the use of ovitraps associated with *S. spinosa*.

However, the major concern in using this bacterium for biological pest or vector control is the selection of resistant insects. This has already been observed in agricultural pests (Moulton et al., 2000; Rehan and Freed, 2014; Rinkevich and Scott, 2009) and in *Culex quinquefasciatus* Say, 1823 (Su and Cheng, 2012, 2014a, 2014b) and *A. albopictus* (Khan et al., 2011).

The effectiveness of *B. thuringiensis israelensis* is proven and selective for the control of different dipteral groups such as Culicidae, Simuliidae, and Chironomidae (Bravo et al., 2011). It has been widely employed successfully in different regions of the world. There was a limited selection of resistant insects due to the specific mechanism of action and safety (these effects are not accumulative; Bravo et al., 2011; Gill, 1995). By integrating *B. thuringiensis israelensis*, our results point toward an important alternative as a surveillance tool. This tool could aid in *A. aegypti* and *A. albopictus* control in urban Manaus.

The lowest mean oviposition was observed in ovitraps with grass infusion + Pyriproxyfen. This corroborates other studies finding a preference for oviposition in substrates containing biological products (Quiroz-Martínez et al., 2012; Solís-Valdez et al., 2015). According to Forattini (2002), the microbial activity attracts female mosquitoes to lay their eggs in containers with this substrate.

The low number of eggs obtained in ovitraps containing distilled water + *T. haemorrhoidalis*, is in accordance with the results obtained for *A. albopictus* under laboratory conditions. It is important to emphasize that Manaus is a city with strong anthropogenic influence, with urban occupation among native and secondary vegetation mosaics, a characteristic that is present in the study area, with natural occurrence of *T. haemorrhoidalis*, which coexists with the mosquito species tested. This coexistence might have influenced the low effectiveness of these traps in the field, due to the well-known predatory potential of this species, mainly of *A. albopictus*.

The number of eggs obtained here using ovitraps and their respective OPI, EDI, and VDI indices highlight the effectiveness of this tool in monitoring these vectors. The removal of eggs from the environment is analogous to the control of immature stages with a control agent. This makes this method more effective and safer for mosquito-control programs. There were a high number of eggs found in the different control agents with no repellents, and this strategy could monitor vectors and assist in vector control.

Studies using ovitraps to estimate density of *A. aegypti* and *A. albopictus* show that organic matter infusions, mostly those made of grass, have been used to maximize the effect of the trap, serving as an attractant to females (Reiter et al., 1991; Santana et al., 2006; Villaseca et al., 2001). According to Nunes et al. (2011), grass infusion associated to ovitrap is more effective than ovitraps with water. This was also observed in our study with ovitraps containing only grass infusion and grass infusion associated with different control agents.

Studies demonstrate the feasibility of ovitraps for monitoring and control of *A. aegypti* in the long-term (Chism and Apperson, 2003; Perich et al., 2003; Rapley et al., 2009). These traps, associated to enthomopathogens, cause reduction in the density of mosquitoes of medical importance, preventing larvae from developing, thus ensuring more time in the environment and preventing traps from becoming a potential breeding site (Depoli et al., 2016; Regis et al., 2008).

Although some mosquitoes' populations resistant to Natular™ DT – *S. spinosa* have been reported, this product should not be excluded from control programs, as it has been shown to be effective when associated to ovitraps. However, this larvicide is more indicated in specific situations, i.e., where it has not been previously used and when surveillance is required because of its excess use.

Ovitraps associated to Vectobac® WG – *B. thuringiensis israelensis* also play an important role in the oviposition of *A. aegypti* and *A. albopictus*. The fact that it is highly selective for the target organisms, that no selection of resistant mosquitoes has been detected, and that it does not repel females in the oviposition process makes the integration of this biolarvicide with ovitraps the best surveillance/control tool for *Aedes*.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors thank the team of the Laboratory of Biological Control and Biotechnology of Malaria and Dengue of the National Institute of Research of the Amazon, Foundation for Research Support of the State of Amazonas, and National Council for the Scientific and Technological Development for their financial support.

References

Alahmed, A.M., 2012. Mosquito fauna (Diptera: Culicidae) of the eastern region of Saudi Arabia and their seasonal abundance. J. King Saud Univ. 24, 55–62.

Alarcón, E.P., Segura, A.M., Rúa-Uribe, G., Parra-Henao, G., 2014. Evoluación de ovitrampas para vigilância y control de *Aedes aegypti* em dos centros urbanos Del Urabá antioqueño. Biomedica 34, 409–424.

Allan, A.S., 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, 1847–1860.

Avendanha, J.S., 2007. Monitoramento vetorial e do vírus Dengue, Belo Horizonte Minas Gerais. Rev. Inst. Adolfo Lutz (Impr.) 66, 207.

Bailey, D.L., Jones, R.G., Simmonds, P.R., 1983. Effects of indigenous *Toxorhynchites rutilus rutilus* on *Aedes aegypti* breeding in tire dumps. Mosq. News 43, 33–37.

Beech, C.J., Nagaraju, J., Vasan, S.S., Rose, R.I., Othman, R.Y., Pillai, V., Saraswathy, T.S., 2009. Risk analysis of a hypothetical open field release of a self-limiting transgenic *Aedes aegypti* mosquito strain to combat dengue. J. Mol. Microbiol. Biotechnol. 17, 99–111.

Bond, J.G., Marina, C.F., Williams, T., 2004. The naturally-derived insecticide spinosad is highly toxic to *Aedes* and *Anopheles* larvae. Med. Vet. Entomol. 18, 50–56.

Bravo, A., Likitvivatanavong, S., Gill, S.S., Soberón, M., 2011. *Bacillus thuringiensis*: a story of a successful bioinsecticide. Insect Biochem. Mol. Biol. 41, 423–431.

Calvo, E.P., Coronel-Ruiz, C., Velazco, S., Velandia-Romero, M., Castellanos, J.E., 2016. Diagnóstico diferencial de dengue y chikungunya en pacientes pediátricos. Biomédica 36, 35–43.

Carrieri, M., Masetti, A., Albieri, A., Maccagnani, B., Bellini, R., 2009. Larvicidal activity and influence of *Bacillus thuringiensis* var. *israelensis* on *Aedes albopictus* oviposition in ovitraps during a two-week check interval protocol. J. Am. Mosq. Control. Assoc. 25, 149–155.

Carvalho, D.O., McKemey, A.R., Garziera, L., Lacroix, R., Donnelly, C.A., Alphey, L., Capurro, M.L., 2015. Suppression of a field population of *Aedes aegypti* in Brazil by sustained release of transgenic male mosquitoes. PLoS Negl. Trop. Dis. 9, e0003864.

Carvalho, F.D., Moreira, L.A., 2017. Why is *Aedes aegypti* Linnaeus so successful as a species? Neotrop. Entomol. 46, 243–255.

Chadee, D.D., Corbet, P.S., Greenwood, J.J.D., 1990. Egg-laying yellow fever mosquitoes avoid sites containing eggs laid by themselves or by conspecifics. Entomol. Exp. Appl. 57, 295–298.

Chism, B.D., Apperson, C.S., 2003. Horizontal transfer of the insect growth regulator pyriproxifen to larval microcosms by gravid *Aedes albopictus* and *Ochlerotatus triseriatus* mosquitoes in the laboratory. Med. Vet. Entomol. 17, 211–220.

Clements, A.N., 1999. The Biology of Mosquitoes: Development, Nutrition and Reproduction, 2nd ed. Chapman and Hall, London.

Consoli, R.A.G.B., Lourenco-de-Oliveira, R., 1994. Principais mosquitos de importância sanitária no Brasil, 1st ed. Fiocruz, Rio de Janeiro, RJ.

Darbro, J.M., Graham, R.I., Kay, B.H., Ryan, P.A., Thomas, M.B., 2011. Evaluation of entomopathogenic fungi as potential biological control agents of the dengue mosquito *Aedes aegypti* (Diptera: Culicidae). Biocontrol Sci. Technol. 21, 1027–1047.

Depoli, P.A.C., Zequi, J.A.C., Nascimento, K.L.C., Lopes, J., 2016. Eficácia de Ovitrampas com Diferentes Atrativos na Vigilância e Controle de Aedes. EntomoBrasilis 9, 51–55.

Dias, L.S., Macoris, M.L.G., Andrighetti, M.T.M., Otrera, V.C.G., Dias, A.S., Bauzer, L.G.S.R., Rodovalho, C.M., Martins, A.J., Lima, J.B.P., 2017. Toxicity of spinosad to temephos-resistant *Aedes aegypti* populations in Brazil. PLOS ONE 12, e0173689.

Dutra, H.L.C., Rocha, M.N., Dias, F.B.S., Mansur, S.B., Caragata, E.P., Moreira, L.A., 2016. Wolbachia blocks currently circulating Zika virus isolates in Brazilian *Aedes aegypti* mosquitoes. Cell Host Microbe 19, 771–774.

Fay, R.W., Eliason, D.A., 1966. A preferred oviposition site as a surveillance method for *Aedes aegypti*. Mosq. News 26, 531–534.

Ferreira, F.A.D.S., Arcos, A.N., Sampaio, R.T.D.M., Rodrigues, I.B., Tadei, W.P., 2015. Effect of *Bacillus sphaericus* Neide on *Anopheles* (Diptera: Culicidae) and associated insect fauna in fish ponds in the Amazon. Rev. Bras. Entomol. 59, 234–239.

Fisher, S., De Majo, M.S., Quiroga, L., Paez, M., Schweigmann, N., 2017. Long-term spatio-temporal dynamics of the mosquito *Aedes aegypti* in temperate Argentina. Bull. Entomol. Res. 107, 225–233.

Forattini, O.P., 2002. Culicidologia médica: identificação, biologia e epidemiologia, 2nd ed. Edusp, São Paulo.

Gill, S.S., 1995. Mechanism of action of *Bacillus thuringiensis* toxins. Mem. Inst. Oswaldo Cruz. 90, 69–74.

Gomes, A.C., 1998. Medidas dos níveis de infestação urbana para *Aedes* (*Stegomyia*) *aegypti* e *Aedes* (*Stegomyia*) *albopictus* em programas de Vigilância Entomológica. Inf. Epidemiol. SUS 7, 49–57.

Gomes, A.C., 2002. Vigilância entomológica. Inf. Epidemiol. SUS 1, 79–90.

Gubler, D., 2005. The emergence of epidemic dengue fever and dengue hemorrhagic fever in the Americas: a case of failed public health policy. Rev. Panam. Salud Pública 17, 221–224.

Gubler, D.J., Reiter, P., Ebi, K.L., Yap, W., Nasci, R., Patz, J.A., 2001. Climate variability and change in the United States: potential impacts on vector- and rodent-borne diseases Environ. Health Perspect. 109, 223–233.

Huseyin, C., Yanikoglu, A., Cilek, J.E., 2005. Evaluation of the naturally-derived insecticide spinosad against *Culex pipiens* L. (Diptera: Culicidae) larvae in septic tank water in Antalya, Turkey. J. Vector Ecol. 30, 151–154.

Jahan, N., Sajjad Sarwar, M., 2013. Field evaluation of lethal ovitraps for the control of dengue vectors in Lahore Pakistan. Pak. J. Zool. 45, 305–315.

Khan, H.A.A., Akram, W., Shehzad, K., Shaalan, E.A., 2011. First report of field evolved resistance to agrochemicals in dengue mosquito *Aedes albopictus* (Diptera: Culicidae), from Pakistan. Parasit. Vectors 4, 146.

Koeppen, N.W., 1948. Climatologia, Com um estudio de los climas de la tierra. Fondo Cultural Econômico, México.

Lopes, J., 1999. Ecologia de mosquitos (Diptera, Culicidae) em criadouros naturais e artificiais de área rural do norte do Paraná, Brasil. VIII. Influência das larvas predadoras (*Toxorhynchites* sp., *Limatus durhamii* e *Culex bigoti*) sobre a população de larvas de *Culex quinquefasciatus* e *Culex eduardoi*. Rev. Bras. Zool. 16, 821–826.

Marina, C.F., Bond, J.G., Casas, M., Muñoz, J., Orozco, A., Valle, J., Williams, T., 2011. Spinosad as an effective larvicide for control of *Aedes albopictus* and *Aedes aegypti*, vectors of dengue in southern Mexico. Pest Manag. Sci. 67, 114–121.

Martins, V.P., Silveira, D.A., Ramalho, I.L., 2013. *Aedes albopictus* no Brasil: aspectos ecológicos e riscos de transmissão da dengue. Entomotropica 28, 75–86.

Medeiros, E.D.S., Rodrigues, I.B., Litaiff-Abreu, E., da S Pinto, A.C., Tadei, W.P., 2013. Larvicidal activity of clove (*Eugenia caryophyllata*) extracts and eugenol against *Aedes aegypti* and *Anopheles darlingi*. Afr. J. Biotechnol. 12, 836–840.

Moore, C.G., Mitchell, C.J., 1997. *Aedes albopictus* in the United States: ten-year presence and public health implications. Emerg. Infect. Dis. 3, 329–334.

Moulton, J.K., Pepper, D.A., Dennehy, T.J., 2000. Beet armyworm (*Spodoptera exígua*) resistance to spinosad. Pest Manag. Sci. 56, 842–848.

Nunes, L.S., Trindade, R.B.R., Souto, R.N.P., 2011. Avaliação da atratividade de ovitrampas a *Aedes* (*Stegomyia*) *aegypti* Linnaeus (Diptera: Culicidae) no bairro Hospitalidade, Santana, Amapá. Biota Amazônia 1, 26–31.

PAHO (Pan American Health Organization), 2018. Available at: http://www.paho.org/hq/index.php?option=com_content&view=article&id=11585&Itemid=41688&lang=en (accessed 02.01.18).

Perich, M.J., Kardec, A., Braga, I.A., Portal, I.F., Burge, R., Zeichner, B.C., Wirtz, R.A., 2003. Field evaluation of a lethal ovitrap against dengue vectors in Brazil. Med. Vet. Entomol. 17, 205–210.

Phasomkusolsil, S., Pantuwatana, K., Tawong, J., Khongtak, W., Monkanna, N., Kertmanee, Y., Schuster, A.L., 2014. Factors influencing the feeding response of laboratory-reared *Aedes aegypti*. Southeast Asian J. Trop. Med. Public Health 45, 40.

Pinheiro, V.C.S., Tadei, W.P., 2002. Frequency, diversity, and productivity study on the *Aedes aegypti* most preferred containers in the city of Manaus, Amazonas, Brazil. Rev. Inst. Med. Trop. São Paulo 44, 245–250.

Quiroz-Martínez, H., Garza-Rodríguez, M.I., Trujillo-González, M.I., Zepeda-Cavazos, I.G., Siller-Aguillon, I., Martínez-Perales, J.F., Rodríguez-Castro, V.A., 2012. Selection of two oviposition sites by female *Aedes aegypti* exposed to two larvicides. J. Am. Mosq. Control Assoc. 28, 47–49.

Rapley, L.P., Johnson, P.H., Williams, C.R., Silcock, R.M., Larkman, M., Long, S.A., Ritchie, S.A., 2009. A lethal ovitrap-based mass trapping scheme for dengue control in Australia: II Impact on populations of the mosquito *Aedes aegypti*. Med. Vet. Entomol. 23, 303–316.

Regis, L., Monteiro, A.M., Melo-Santos, M.A.V.D., Silveira Jr., J.C., Furtado, A.F., Acioli, R.V., Souza, W.V.D., 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.

Regis, L.N., Acioli, R.V., Silveira Jr., J.C., Melo-Santos, M.A.V., Souza, W.V., Ribeiro, C.M.N., Braga, C., 2013. Sustained reduction of the dengue vector population resulting from an integrated control strategy applied in two Brazilian cities. PLOS ONE 8, 1–12.

Rehan, A., Freed, S., 2014. Selection, mechanism, cross resistance and stability of spinosad resistance in *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). Crop Prot. 56, 10–15.

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

Rinkevich, F.D., Scott, J.G., 2009. Transcriptional diversity and allelic variation in nicotinic acetylcholine receptor subunits of the red flour beetle *Tribolium castaneum*. Insect Mol. Biol. 18, 233–242.

Rossati, A., Bargiacchi, O., Kroumova, V., Garavelli, P.L., 2015. The mosquito-borne viruses in Europe. Recenti Prog. Med. 106, 125–130.

Santana, A.L., Roque, R.A., Eiras, A.E., 2006. Characteristics of grass infusions as oviposition attractants to *Aedes* (*Stegomyia*) *aegypti* (Diptera: Culicidae). J. Med. Entomol. 43, 214–220.

Santos, R.L.C.D., 2003. Updating of the distribution of *Aedes albopictus* in Brazil (1997–2002). Rev. Saude Publica 37, 671–673.

Silva, V.C.D., Scherer, P.O., Falcão, S.S., Alencar, J., Cunha, S.P., Rodrigues, I.M., Pinheiro, N.L., 2006. Diversidade de criadouros e tipos de imóveis frequentados por *Aedes albopictus* e *Aedes aegypti*. Rev. Saude Publica 40, 1106–1111.

Soares-da-Silva, J., Ibiapina, S.S., Bezerra, J.M.T., Tadei, W.P., Pinheiro, V.C.S., 2012. Variation in *Aedes aegypti* (Linnaeus) (Diptera, Culicidae) infestation in artificial containers in Caxias, state of Maranhão, Brazil. Rev. Soc. Bras. Med. Trop. 45, 174–179.

Soares-da-Silva, J., Pinheiro, V.C.S., Litaiff-Abreu, E., Polanczyk, R.A., Tadei, W.P., 2015. Isolation of *Bacillus thuringiensis* from the state of Amazonas, in Brazil, and screening against *Aedes aegypti* (Diptera, Culicidae). Rev. Bras. Entomol. 59, 1–6.

Soares-da-Silva, J., Queirós, S.G., Aguiar, J.S., Viana, J.L., Neta, M., Silva, M.C., Tadei, W.P., 2017. Molecular characterization of the gene profile of *Bacillus thuringiensis* Berliner isolated from Brazilian ecosystems and showing pathogenic activity against mosquito larvae of medical importance. Acta Trop. 176, 197–205.

Solís-Valdez, C.B., Barrientos-Contreras, J., Escobar-González, B., Zepeda-Cavazos, I.G., Rodríguez-Castro, V.A., Quiroz-Martínez, H., 2015. Oviposición de *Aedes aegypti* L. (Diptera: Culicidae) en Ovitrampas con dos Larvicidas en El Fuerte, Sinaloa México. Southwest. Entomol. 40, 575–580.

Stoops, C.A., 2005. Influence of *Bacillus thuringiensis* var. *israelensis* on oviposition of *Aedes albopictus* (Skuse). J. Vector Ecol. 30, 41–44.

Su, T., Cheng, M.L., 2012. Resistance development in *Culex quinquefasciatus* to spinosad: a preliminary report. J. Am. Mosq. Control Assoc. 28, 263–267.

Su, T., Cheng, M.L., 2014a. Cross resistances in spinosad-resistant *Culex quinquefasciatus* (Diptera: Culicidae). J. Med. Entomol. 51, 428–435.

Su, T., Cheng, M.L., 2014b. Laboratory selection of resistance to spinosad in *Culex quinquefasciatus* (Diptera: Culicidae). J. Med. Entomol. 51, 421–427.
Vega-Rúa, A., Zouache, K., Girod, R., Failloux, A.B., Lourenço-de-Oliveira, R., 2014. High level of vector competence of *Aedes aegypti* and *Aedes albopictus* from ten American countries as a crucial factor in the spread of Chikungunya virus. J. Virol. 88, 6294–6306.
Villaseca, P., León, W., Palomino, M., Mostorino, R., Lecca, L., 2001. Validación de sustratos atractivos a oviposición para ladetección de *Aedes aegypti*. Rev. Peru. Med. Exp. Salud Publica 18, 77–81.
WHO (World Health Organization), 1984. Report of the seventh meeting of the scientific working group on biological control of vectors. Mimeographed document TDR/BCV/SWG – 7/84. 3, 33.
WRBU (Walter Reed Biosystematics Unit), 2018. Mosquito identification resources. Available at: http://www.wrbu.org/VecID_MQ.html (accessed 21.01.18).
Zequi, J.A.C., Lopes, J., Santos, F.P., Vilas-Bôas, G.T., 2015. Efficacy and persistence of two *Bacillus thuringiensis israelensis* formulations for the control of *Aedes aegypti* (Linnaeus, 1762) under simulated field conditions. Int. J. Mosq. Res. 2, 05–09.