17. Practical application of olfactory cues for monitoring and control of *Aedes aegypti* in Brazil: a case study

Alvaro E. Eiras, Martin Geier, Andreas Rose and Owen Jones

Abstract

The increasing importance of managing the mosquito vectors of dengue, mainly *Aedes aegypti*, is recognised world-wide as the disease becomes more prevalent in many countries. The complexity of the urban environment where the vectors breed makes it especially difficult to organise control efforts to reduce their numbers and their impact on disease transmission. Existing control programmes depend on larval surveys for population assessment but these are often unreliable and become available too late for the application of control measures to have a significant impact on disease transmission. The need for better and more timely information about the mosquito vectors of dengue has been recognised in Brazil and several new technologies for more reliable information on vector distribution and population densities have emerged that are making control efforts more effective. Olfactory cues from oviposition sites are now being used to lure gravid *Ae. aegypti* females into sticky traps for population assessments and indices of dengue risk. The development of the so-called MosquiTRAP which incorporates an oviposition attractant is described and its efficiency relative to more traditional monitoring methods is discussed. Volatile chemicals that attract host-seeking female *Ae. aegypti* have also been identified. These have been formulated for controlled release ('BG-Lure') and incorporated into novel trap designs ('BG-Sentinel') with much improved capture of *Ae. aegypti* compared to the standard traps that have been used previously. Both trapping systems are now being used to monitor and survey these important disease vectors. The MosquiTRAP is also an important cornerstone of an on-line GIS-based surveillance system called MI Dengue (Dengue Intelligent Monitoring System) which is being implemented in Brazil. Results to date have demonstrated that the accuracy of its predictions relating to dengue risk is much greater than that obtained using more traditional methods. Real-time decision-making related to vector management is now possible using the new surveillance systems, and this should greatly impact the management of the disease.

Keywords: *Aedes aegypti*, oviposition attractant, host odours, MosquiTRAP, BG-Sentinel, MI-Dengue, disease risk assessment, Brazil

Introduction

Dengue is ranked as the most important mosquito-borne viral disease in the world, where in the last 50 years incidence has increased 30-fold. The World Health Organization (WHO) has estimated that 2.5 billion people live in over 100 countries of Africa, South-East Asia, the eastern Mediterranean, the western Pacific, and the Americas where dengue viruses can be transmitted. Up to 50 million infections occur annually, with 500,000 cases of dengue haemorrhagic fever and 22,000 deaths, mainly among children (WHO 2009).

The virus responsible for dengue fever (DF) and dengue haemorrhagic fever (DHF) belongs to the family *Flaviviridae* (the same family as the yellow fever virus), and four different viral serotypes exist: DEN-1, DEN-2, DEN-3, and DEN-4 (Gubler 2004). Infection with one dengue serotype provides immunity for years, but it does not protect against infection with the other serotypes. Dengue haemorrhagic fever is more common in children and it can lead to shock from blood loss, and even death (Gubler 1998). Dengue virus is transmitted to humans during blood feeding by infected mosquitoes, which have usually acquired the virus by ingesting blood of infected and viremic humans and possibly also by transovarial transmission. The mosquito *Aedes aegypti* (L.) (*Diptera*: *Culicidae*) is considered the main vector, although other *Aedes* species, including *Aedes albopictus* (Skuse), have been implicated in rural epidemics as well as some urban ones (Effler at al. 2005). The mosquito *Ae. aegypti* lives in close association with humans in urban and suburban environments. The mosquito feeds predominantly on human blood and breeds in artificial containers such as drums, buckets, tyres, flowerpots, and vases (Forattini *et al.* 1995). Therefore, the epidemiology of dengue is highly related to the biology of the mosquito vector and human behaviour, as well as to the environment and the virus itself.

As there is no effective vaccine for dengue, vector control is the main approach for control and prevention. Although insecticide spraying has been used extensively, larval source reduction (eliminating or cleaning water-filled containers that can harbour *Ae. aegypti* larvae) is considered the most effective way of reducing and controlling the mosquito populations (Gubler 1998).

Scientific interest in research on dengue has grown in many countries, and opportunities for investigators to obtain funds for that research have considerably improved. Funding agencies, such as governments and international organisations, have increased the amount of money available for research in response to dengue's growing global incidence and impact on public health. In Brazil, special programme funds have been allocated by the Federal and State governments directed towards the achievement of new knowledge, control tools and new technologies for dengue and other neglected diseases (CNPq 2009).

In subtropical countries such as Brazil, mosquito population density, dengue transmission, and the number of DF cases start to increase at the beginning of the rainy season (October), with dengue cases peaking more than three months later (Coutinho *et al.* 2006, Forattini *et al.* 1995). In Brazil, dengue fever re-emerged as a major urban epidemic in 1986 (Marques *et al.* 1993) and currently is considered the most important arthropod-borne viral disease (MS 2007a,b). In 2000, all 26 states had reported DF cases and during 1986-2007 a total of 4,559,818 cases were officially reported (MS 2007a) with 493 deaths (MS 2007b).

Transmission of dengue depends on a variety of variables in the relation between virus, mosquito vector and human host (Focks *et al.* 1995, Kuno 1997). In the assessment of dengue risk, important factors are the level of herd immunity in the human population and the ratio of mosquito to human density, which influences the probability of vector-host contacts. To measure mosquito density, *Ae. aegypti* control programmes usually still mainly rely on the surveillance of immature mosquito stages (eggs, larvae and pupae) to determine the dengue risk. These surveillance methods rely on tedious and repetitive house-to-house surveys. Data from these surveys are used to calculate indices like the Premise Index (PI) or the Breteau Index (BI). The Premise Index was described in the early 1920s for yellow fever outbreaks and is based on the percentage of premises infected with *Aedes* larvae or pupae (Connor and Monroe 1923), whereas the BI indicates the number of containers with larvae and/or pupae of *Ae. aegypti* (Breteau 1954). Both indices are used by WHO as indicators of dengue transmission risk (Focks 2003).

In general, these traditional indices are poorly related to the risk of dengue transmission (Coelho *et al.* 2008, Focks 2003, Méndez *et al.* 2006, Reiter *et al.* 1991, Scott and Morrison 2002). More advanced assessments of immature stages therefore focus only on pupae, as their abundance is more closely linked to adult population size (Focks 2003). A direct surveillance of the adult mosquito population was previously possible only with much time and effort (using aspirators or human landing collections) or were inefficient due to low catching rates of nonspecific adult traps (Schoeler *et al.* 2004). However, studies in Brazil, combining newly-developed specific traps for adult *Ae. aegypti* with geographic information systems, are now showing that an efficient and real-time surveillance of adult vectors is possible, resulting in a quick determination of dengue risk and effective vector control.

Overview of standard collections methods for dengue vector

The collection of host-seeking dengue mosquitoes directly from human volunteers (human-landing collection or catch) can provide a real-time surveillance of the mosquito population that is actually involved in producing a dengue risk. During a dengue outbreak, however, when it is important to assess the efficacy of the control measures, human-landing collections expose the field workers to an intolerable risk of infection. In addition, the human-landing collection is time consuming, labour intensive and yields variable results due to differences in human attractiveness and in the skill of the field workers. Therefore other methods to collect adult mosquitoes are usually preferred for the collection of dengue vectors. These methods focus either on the detection of dengue mosquito eggs in oviposition traps (ovitraps), on finding their larvae or pupae, or on collecting adult mosquitoes (Service 1993, Silver 2008).

Resting adults can be collected using aspirators, which is labour intensive and costly, but collects large numbers of mosquitoes. Conventional adult traps use only visual cues to attract mosquitoes (Fay-Prince trap, Wilton trap). Catch rates are usually too low when set in relation to their price and the fact that these traps need electricity adds to their inconvenience. The addition of carbon dioxide raises the attractiveness of visual traps, but its use on a larger scale will also be too costly and complicated in most urban dengue-risk areas.

Recent research has led to the development of new traps and attractants that are highly specific for dengue mosquitoes. Sticky traps with oviposition attractants capture mosquitoes that have already ingested a quantity of blood sufficient to produce eggs (e.g. MosquiTRAP™). A new ventilator trap (BG-Sentinel™) used with a combination of odorant substances found on human skin focuses on host-seeking dengue mosquitoes. The BG-Sentinel trap has been shown to have much better catching rates than conventional traps (Kröckel *et al.* 2006, Meeraus *et al.* 2008). Both trap types allow for more specific and refined vector control campaigns in situations where the traditional tools are often marginally effective.

Dengue mosquito monitoring through the detection of eggs: ovitraps

Eggs are collected with an oviposition trap (ovitrap), which is commonly used to detect the presence of *Aedes* mosquitoes in low density areas. The ovitrap was first described by Fay and Eliason (1966) and consists of a black container filled partially with water and a wooden paddle placed vertically as an oviposition substrate. This trap requires laboratory infra-structure and human resources for counting the eggs deposited on the oviposition substrate and identifying the larval species of *Aedes*.

Historically, ovitraps have provided useful information in the early detection of new infestations, spatial (presence or absence) and temporal (seasonal) distribution and in monitoring the impact of control measures on *Ae. aegypti*, including those using pesticides (Focks 2003, Reiter and Nathan 2001, Reiter *et al.* 1991). However, many comparative studies between the larval survey and ovitrap use have shown that the ovitrap, besides being more sensitive in the detection of *Ae. aegypti*, also has a lower cost but demonstrates limited operational viability in entomological surveillance (Braga *et al.* 2000, Rawlins *et al.* 1998). When ovitraps are used in urban areas where several species of the *Stegomyia* subgenus occur sympatrically, the eggs are morphologically indistinguishable and, thus there is a need for identification of larvae.

Further studies are necessary in order to use ovitraps as a methodological approach to indicate risk of dengue fever and yellow fever, because of the difficulties involved in calculating the population density of adult vectors (Focks 2003).

Dengue mosquito monitoring through the detection of larvae and/or pupae

Active search for immature stages

The search of premises for containers that harbour larvae or pupae is still a widespread surveillance method for dengue vectors. The results are usually used to calculate surveillance indices such as the House Index (percentage of houses with larvae and/or pupae, also: Premise Index), the Container Index (percentage of water-containers with larvae and/or pupae) or the Breteau Index (number of positive containers per 100 houses) (Silver 2008). However, these indices do not provide for a significant assessment of the number of adults that are actually produced and the associated dengue transmission risk (Coelho *et al.* 2008, Focks 2003, Méndez *et al.* 2006, Reiter *et al.* 1991, Scott and Morrison 2002).

Because the time from pupa to adult is short and pupal mortality is low, the number of pupae found at a given premise provides the best estimation of adult density (Focks 2003). Linking the number of pupae to human density can thus give an improved estimate of an important factor in dengue risk assessment, the relation of mosquito density to human density. Focks (2003) therefore promotes a 'pupal/ demographic survey method'. This method combines interviews to determine the number of people living or sleeping at the examined premises with the examination of the water-holding containers for pupae. Pupae are collected and, in the case of the presence of various container-breeding species, kept in the laboratory until adult emergence for identification. Using standardised classification methods to identify and focus on the most productive containers should both make surveillance and the control through source reduction more efficient (Barrera *et al.* 2006, Chadee *et al.* 2007, Focks and Alexander 2006, Koenraadt *et al.* 2007). However, pupal habitats may vary greatly in different settings, making a comparison and standardisation difficult.

Larval traps

Larval traps of various designs made of automobile tyres (tyre-section traps) have been used for monitoring *Aedes* oviposition activity (WHO 1999). A standard larval trap ('larvitrap') consists of a water-filled section of a tyre that facilitates visual inspection of the water *in situ* and allows for the ready transfer of larvae to another container for examination. However, such a trap has many disadvantages such as low sensitivity and the eggs laid in the trap have to be in contact with water for hatching. In Brazil, the tyre-section larval trap was evaluated in the field and the results showed that ovitraps are much more sensitive than larval traps (Marques *et al.* 1993). In spite of the difference in sensitivity, larval traps are still widely used in *Ae. aegypti* surveillance programmes and studies in Brazil (Dibo *et al.* 2005) and other countries, such as Argentina (Micieli and Campos 2003), Peru (Morrison *et al.* 2004), Nicaragua (Lugo *et al.* 2005), Thailand (Tsuda *et al.*

2006), Columbia (Cuéllar-Jiménez *et al.* 2007, Maestre-Serrano *et al.* 2008) or Cuba (Suárez Ramírez and Colás Bonne 2008).

Floating larval trap

An alternative larval trap, the 'floating larval trap', was developed and evaluated for eliminating biases commonly associated with human collection (larval survey) (Harrison *et al.* 1982). The trap collects diving mosquito larvae and pupae when they float back to the water surface. Both field and laboratory data revealed that the trap was highly efficient and sensitive for detecting immature stages of *Ae. aegypti* and *Culex quinquefasciatus* Say. The trap was claimed to function as a random sampling device, providing an equal chance for the capture of immatures. However, fewer 1st and 2nd instars larvae are collected because they often remain closer to the water surface and do not dive as deeply when disturbed. Specimens captured in the trap remained alive for at least 48 h. Although the size of this instrument (>13 cm diam.) could probably be reduced, its use excludes important *Ae. aegypti* larval habitats such as vases, tyres, small plastic containers and most footbaths.

Detection of adult dengue mosquitoes

Human landing collection

Although laborious, collecting mosquitoes directly from a human host obviously provides the best indication of the number of mosquitoes that are currently involved in the transmission cycle and can therefore give key information in the assessment of the dengue risk. The method has been used to determine daily biting periodicity in different areas of the world (Chadee and Martinez 2000). However, catch rates depend on the time of day and the skill, motivation and attractiveness of the individual field worker. Results are therefore variable and may lack the accuracy needed for a reproducible and meaningful comparison of data, especially in the study of seasonal variations. Because the technique exposes field workers to infected mosquitoes, it is currently undergoing ethical restrictions (Focks 2003, PNCD 2002). The human landing collection is therefore not used as a standard surveillance method for dengue mosquitoes, but remains an important reference method in the assessment and comparison of other collection methods (Jones *et al.* 2003, Kröckel *et al.* 2006, Schoeler *et al.* 2004).

Sampling resting adults with aspirators

Aspirators are used to capture resting adult mosquitoes, especially indoors (Nasci 1981) in dark places and this method provides an estimate of adult density in the sampled houses (Morrison *et al.* 2004; Schoeler *et al.* 2004). Aspirators are either carried on the back of the technician (e.g. CDC backpack aspirator, John W. Hock Co., Gainesville, FL, USA) or hand held as in the Nasci aspirator. As the field worker needs to enter private homes, this method is invasive and requires high tolerance of the inhabitants. It is also labour intensive and results may be affected by the efficiency of field technicians (Clark *et al.* 1994, Favaro *et al.* 2006). Using backpack aspirators is therefore usually considered more for research than for routine activities of dengue control programmes (Favaro *et al.* 2008).

Traps for gravid Aedes aegypti

Artificial oviposition sites can be used to attract and catch gravid mosquito females. Their attractiveness for dengue mosquitoes can be augmented by the addition of decomposing organic matter in fermenting infusions made from grass, hay, leaves or other plant material (Chadee 1993, Du and Millar 1999, Reiter *et al.* 1991, Sant'Ana *et al.* 2006, Takken and Mboera 2000). The CDC gravid trap (John W. Hock Co., Gainesville, FL, USA) was originally designed by Reiter (1983). A battery-powered fan draws mosquitoes attracted by grass or hay infusions into a catch bag. In contrast, sticky oviposition traps use glue boards to capture landing mosquitoes and do not need electricity. Different models have been developed to capture gravid *Ae. aegypti* (Fávaro *et al.* 2006, Gama *et al.* 2007, Kay *et al.* 2000, Muir and Kay 1998, Ordóñez-Gonzalez *et al.* 2001, Ritchie *et al.* 2003, 2004) and *Ae. albopictus* (Facchinelli *et al.* 2007). Like the CDC gravid trap, they use decomposing organic material in water to attract mosquito females searching for an oviposition site. Although these infusions are relatively easy to produce, they need approximately one to three weeks time to ferment. Their composition also changes with age and time in the traps, potentially altering their attractiveness and making a comparison of trapping results more difficult.

Traps using visual cues

The use of light traps constitutes a method for capturing a large variety of adult insects and is often used to collect nocturnal mosquito species. However, light traps are not effective at capturing the diurnal *Ae. aegypti* (Silver 2008). The Fay-Prince trap (John W. Hock Co., Gainesville, FL, USA) is a daytime trap with black and white contrasts and uses a fan to draw approaching mosquitoes into a catch bag (Fay and Prince 1970). Another trap designed to capture day-active mosquitoes such as *Ae. aegypti* is the Wilton trap (Wilton 1985) (John W. Hock Co., Gainesville, FL, USA). The Wilton trap consists of a glossy black cylinder with a fan and collection cup inside. Both traps catch mosquitoes of both sexes and all physiological stages. The catch rates for visual traps are, however, only a fraction of the human landing collection (Jones *et al.* 2003, Kröckel *et al.* 2006, Schoeler *et al.* 2004).

Traps using carbon dioxide

Carbon dioxide is a general attractant to a large majority of mosquito species. Liberated either from dry ice or pressurised gas bottles, CO_2 enhances the catch rates of the Fay-Prince and the Wilton traps, but results for *Ae. aegypti* are poor compared to human landing collections (Canyon and Hii 1997, Schoeler *et al.* 2004). Some trap types that are commercially available for the end consumer[1] produce CO_2 from the combustion of propane gas. The reaction is usually supported by a catalyst and yields warm, humid CO_2 of a concentration attractive to mosquitoes (Kline 2002). The attractiveness of these traps for dengue mosquitoes has not been investigated. As these traps are relatively expensive, they are not practical for use in routine monitoring programmes. Other traps with carbon dioxide that are routinely used to monitor mosquitoes, such as the EVS-trap or the CDC-light-traps with CO_2, also catch *Ae. aegypti* and *Ae. albopictus* (Russel 2004), but the costs, efforts and logistics that come along with the use of CO_2 from any of these sources currently do not make theirr application feasible in most field situations.

[1] E.g. Woodstream Mosquito Magnet, Blue Rhino Skeeter Vac, Flowtron Mosquito Power Trap, Lentek Mosquito Trap Guardian (list does not claim to be exhaustive).

The need for simple, efficient, and specific collection tools

A suitable tool for collecting adult dengue vectors in routine monitoring operations should be sensitive enough to detect adults also when they occur in low densities. The tool should be robust and workable for routine uses under various conditions and the caught mosquitoes should be easily removable from the collection device to be identified and/or processed for further virus detection. The collection rate should correlate very closely with human landing collections in order to establish meaningful risk indicators.

MosquiTRAP for collection of gravid *Aedes aegypti*

Olfactory cues

The deposition of eggs by mosquitoes is mediated by olfactory cues, which originate from the breeding site as a result of microbial activity (Bentley and Day 1989; Clements 1999). Apparently, Metha (1934) was the first to report the existence of olfactory cues in recognition and selection of oviposition sites by mosquitoes. Water containing decomposing organic matter was found attractive for gravid mosquitoes and has been widely used for mosquito surveillance and possibly, mosquito control (Chadee *et al.* 1993, Eiras 2001, Millar *et al.* 1992, Reiter *et al.* 1991, Takken and Mboera 2000). Hay and grass infusions have been reported since the 1980's to be attractive for ovipositing females of a wide range of mosquito species, including *Ae. albopictus* (Holck *et al.* 1988), *Ae. aegypti* (Chadee *et al.* 1993), *Ae. triseriatus* (Say) (Holck *et al.* 1988), *Cx. nigripalpus* Theobald (Ritchie 1984), *Cx. pipiens* Linnaeus (Reiter 1986), *Cx. quinquefasciatus* (Mboera *et al.* 2000). The infusions are usually made of many different organic materials, such as leaves, grass and sod (Allan and Kline 1995, Chadee *et al.* 1993, Rawlins *et al.* 1998, Reiter *et al.* 1991).

There are differences in the selection of breeding sites between *Ae. albopictus* and *Ae. aegypti*. The former species breeds in natural and manmade containers and is often most abundant in rural and sylvan areas, but is able to rapidly colonise suburban an urban areas with sylvan characteristics (Hornby *et al.* 1994). The second species is a container-inhabiting mosquito that occurs in urban areas and oviposits in many breeding sites such as tyres, plant vases, and other water-holding containers associated with human activities. Many reports have shown that gravid *Ae. aegypti* and *Ae. albopictus* lay more eggs in ovitraps when baited with grass infusions compared to clean water (Chadee *et al.* 1993, Reiter *et al.* 1991, Sant'Ana *et al.* 2006).

Many organic materials have been used for increase the efficacy of ovitraps for sampling *Aedes* eggs in the field, such as oak leaves (*Quercus* spp.) (Allan and Kline 1995), hay infusions of *Axonopus compressus* (Sw.) Beauv. (Rawlins *et al.* 1998), Bermuda hay infusions (Reiter *et al.* 1991), infusions of dried *Sclerica bractaeda* (L.) (Chadee *et al.* 1993), palletised plant-based animal feeds (Ritchie *et al.* 2003) and leaves of *Panicum maximum* Jacq. (Sant'Ana *et al.* 2006). Sant'Ana *et al.* (2006) have shown that the oviposition responses of *Aedes (Stegomyia)* mosquitoes changed with respect to grass species, stage of plant development and type of fermentation, thus suggesting that grass leaves have different chemical profiles and bacteria and other microorganisms present in the infusions and that they may produce several chemical lures for gravid mosquitoes. Neglecting to take into account the age of infusions for monitoring seasonal abundance of mosquitoes by ovitraps baited with grass infusions may constitute a significant source of variability in the results of these studies. The period of fermentation of grass infusions has a significant effect on the response of gravid mosquitoes and infusions of known and standardised fermentation periods should be used only. For instance, studying various ages of fermentations (e.g. 1, 3, 5, 7, 15, 20 and

30 days), Sant'Ana *et al.* (2006) demonstrated that the best fermentation ages for grass infusions of *P. maximum* were 15 and 20 days. Most likely, the period of fermentation may change the chemical profile of volatiles produced by grass infusions and, consequently, the oviposition response of *Ae. aegypti* and *Ae. albopictus* may be affected.

Identification of oviposition attractants for Aedes aegypti *from* Panicum maximum *grass infusion*

The use of specific synthetic oviposition attractant chemicals in monitoring programmes for *Aedes (Stegomyia)* mosquitoes would eliminate the need to create infusions and would allow for the preparation of precise formulations of an oviposition medium. Therefore, synthetic oviposition attractants can eliminate variation in infusion quality and attractiveness.

One important aspect of the use of oviposition attractants in trapping devices is to understand the role of the individual active chemicals. Several studies used dual-choice bioassays at close-range, where the oviposition behaviour comes at the end of a behavioural sequence and thus, they did not discriminate between long-range and short-range responses. As it is likely that oviposition stimulants act mainly at close-range, such studies may underestimate the role of these stimulants at long range.

There are several publications on the evaluation of synthetic oviposition attractants for mosquitoes in the field (Allan and Kline 1995, Beehler *et al.* 1994, Mboera *et al.* 2000). However, only few chemical identifications of oviposition attractants have been conducted for *Culex* (Du and Millar 1999, Hwang *et al.* 1980, Leal *et al.* 2008) and *Aedes* mosquitoes (Bentley *et al.* 1979, Ponnusamy *et al.* 2008, Sant'Ana 2003). Some compounds such as nonanal, p-cresol, indole and 3-methyl-indole have been identified as oviposition attractants for *Cx. quinquefasciatus* and *Cx. tarsalis* mosquitoes (Du and Millar 1999, Leal *et al.* 2008) and *Aedes* mosquitoes (Bentley *et al.* 1979). Some specific bacteria associated with carboxylic acids and methyl esters have been described in binary choice assays as potent oviposition stimulants for gravid *Ae. aegypti,* but the elucidation of these compounds has not yet been completed (Ponnusamy *et al.* 2008)

Oviposition attractants for gravid *Ae. aegypti* were isolated and identified from *P. maximum* grass infusions (Sant'Ana *et al.* 2006) and these are considered an important tool for increasing the catch of gravid *Ae. aegypti* in the sticky trap MosquiTRAP (Eiras and Resende 2009, Eiras and Sant'Ana 2002, Favaro *et al.* 2006, 2008, Lourenço-de-Oliveira *et al.* 2008). The chemical identification of volatiles produced by grass infusion of *P. maximum* that enhanced oviposition responses of gravid female *Ae. aegypti* was possible only by means of a combination of studies using electrophysiology (electroantennography), gas-chromatography coupled with mass-spectrometry (GC-MS), gas-chromatography coupled with electroantennography (GC-EAD) and behaviour using ovitraps (Eiras *et al.* 2001, Eiras and Sant'Ana 2002; Sant'Ana 2003; Eiras *et al.* 2004). Firstly, the grass infusions of different ages (Sant'Ana *et al.* 2006) were extracted individually by solvent and by solid micro phase extraction (SMPE) and submitted individually to GC-MS, GC-EAD and EAG and compared with results from oviposition responses. It was interesting that the responses of gravid female *Ae. aegypti* antennae to EAG extract of grass infusions of different ages followed a similar pattern to the oviposition responses to grass infusion extracts in the field (Figure 1), showing that either the quantity of active compounds increased with ageing of the grass infusion or that new compounds were produced as the grass infusions fermented. Later, the active compounds present in infusion extracts of high EAG and oviposition responses were submitted to GC-MS for identification. Seven compounds were identified as candidates for an oviposition stimulant or attractant of gravid *Ae. aegypti*: nonanal, benzothiazol, decanal, p-cresol, indole, 3-methyl indole and limonene) (Eiras and

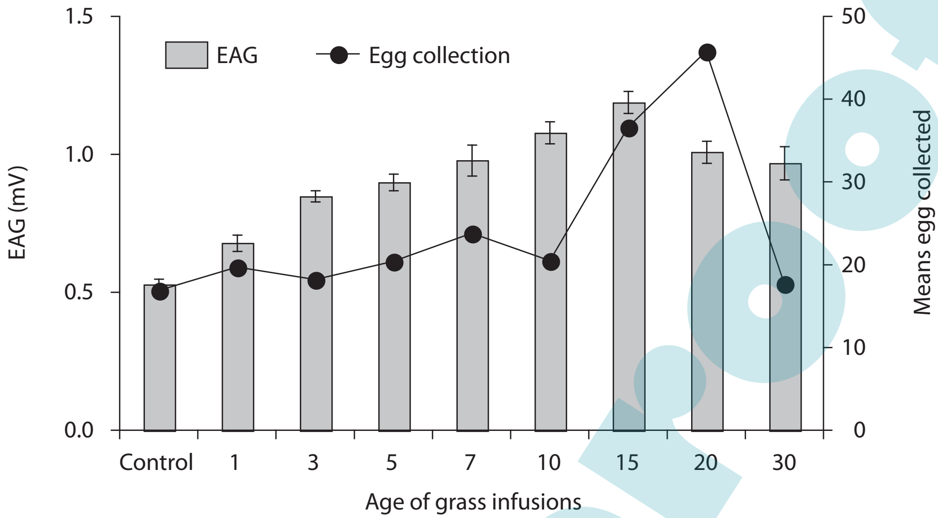

Figure 1. Behavioural (ovitrap) and electrophysiological (EAG) responses of gravid Aedes aegypti *to* Pannicum maximum *grass infusions of different ages (Sant'Ana 2004).*

Sant'Ana 2002 - patent pending). The EAG studies of the seven individually identified compounds showed that the aldehydes (nonanal) elicited the highest antennal responses of gravid female *Ae. aegypti*. The GC analysis showed that the grass infusion produced a large number of volatiles. The compound 3-methylindole has a very unpleasant and strong smell even in small concentrations, and has been shown to modify the response of gravid female *Ae. aegypti* (Allan and Kline 1995, Sant'Ana 2003). However, 3-methylindole was revealed to have a repellent effect on *Ae. albopictus* in laboratory assays and in the field using ovitraps (Trexler *et al.* 2003). By contrast, 3-methylindole is an oviposition stimulant for *Cx quinquefasciatus* at very low concentrations (Mboera *et al.* 2000).

Based on the fact that many substances are produced by grass infusions and that only a few key chemical compounds are responsible for attracting gravid mosquitoes to breeding sites, many of the volatiles present in infusions of organic material are likely to be produced by other sources, such as plants or decaying materials. The responses of gravid *Ae. aegypti* mosquitoes to a single compound could result in attraction to an inadequate place for oviposition, but the complexity of chemical signals may be crucial as an indicator of a potential breeding site.

Formulation of lures for Aedes aegypti *from synthetic oviposition attractants*

The majority of semiochemicals that have been described as oviposition attractants for *Aedes* species would volatilise very quickly if simply added to the water surface of an oviposition trap. Some form of controlled-release technology is required to release the active substances at a rate that is optimal for continued attraction of the target species to the oviposition trap. In the case of *Aedes* species, two types of controlled-release devices have been used. One was a solid matrix polymer, within which the active ingredient nonanal was evenly distributed while the other was released from a reservoir system that took the form of sealed tube. The reservoir system tend to release more chemicals at the beginning of their field life in contrast with that released from solid matrix polymer, which was more towards the end of their field life (Figure 2). There is a high

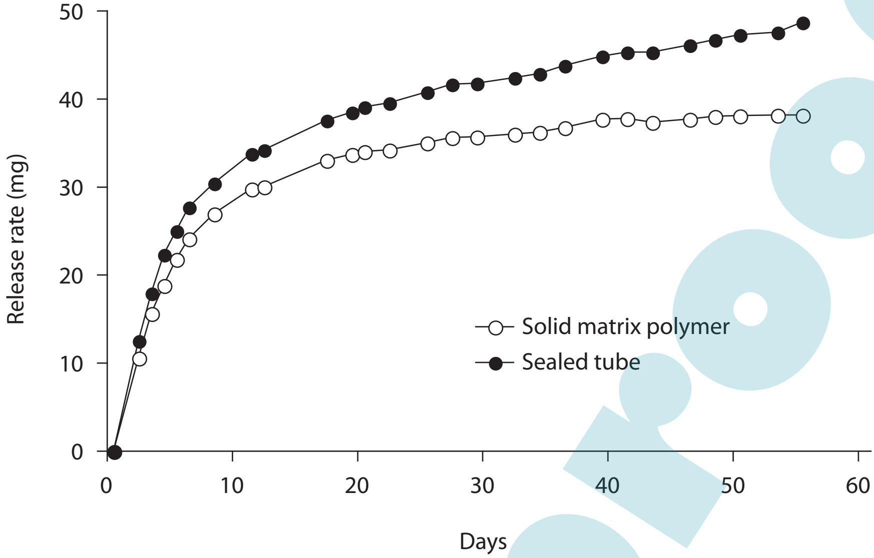

Figure 2. Release rate of nonanal (AtrAedes) at solid matrix tube and sealed tube dispensers at 25.1±1.87 °C (Data not published).

evaporation rate during the first 10 days before both devices get a steady release rate. This is often referred to as a 'first order' release profile, while the preferred profile would be one where the amount of active substance released remains constant during the whole field life of the dispenser and referred to as a 'zero order' release profile. Dispensers with a reservoir system tend to show a zero order release profile and therefore are preferred as dispensers, as the attraction rate remains more consistent during the whole length of the life of the dispenser in the field.

Laboratory bioassays

Behavioural studies of gravid female *Ae. aegypti* exposed to synthetic lures were carried out in a dual-port olfactometer (Geier and Boeckh 1999) in response to log-doses of nonanal. It is interesting that the response of host-seeking *Ae. aegypti* to a human hand in the olfactometer bioassay is usually about 85-100%, whereas the response of gravid females to grass infusions is about 40-50% (Mota 2003). The response of gravid *Ae. aegypti* to oviposition attractants was consistently within the range of 31 to 45% responding whereas the response of host-seeking mosquitoes to human odour was about 80% (Table 1). The synthetic oviposition attractant (nonanal) and the Atr*Aedes* lure elicited similar responses in gravid *Ae. aegypti*.

Semi field test

Semi-field systems have been used for a variety of studies on mosquitoes, especially on the behavioural ecology of malaria vectors (Knols *et al.* 2002, Schmied *et al.* 2008) and for evaluating trapping devices for *Ae. aegypti* (Kline 2002). Although there are some limitations associated with these studies, for instance that longe-range dispersal cannot be studied. Semi-field cages are considered a controlled environment that offers intermediate conditions between the laboratory and the field for behavioural studies, where both laboratory and wild-caught insects can be tested.

Table 1. Behavioural responses of host seeking and gravid female Aedes aegypti *to different stimuli in a dual port olfactometer (Data not published).*

	Stimulus	N	Control	Test
Host-seeking female	Human hand	30	81.9%±10.8	1.1%±2.6*
Gravid female	Grass infusion	33	35.0%±4.0	21.1%±3.8^{ns}
	Nonanal (10 µg)	8	31.8%±13.1	22.6%±6.4^{ns}
	AtrAedes™ Lure	8	43.4%±13.6	25.2%±6.0^{ns}

* Means P<0.05 and ns = means P>0.05.

In Brazil, a semi-field experimental area with eight test cages was developed for studying behavioural responses of gravid *Ae. aegypti* mosquito to evaluate prototype traps, controlled release devices and the development of new formulations of synthetic oviposition attractants (Roque and Eiras 2008). As laboratory-reared mosquitoes are used in these field cages, the age and physiological status of test mosquitoes, which are free of viruses and bacterial contamination, are well established. Field cage results showed that gravid *Ae. aegypti* mosquitoes had similar responses in a field cage as in field tests in urban areas, confirming that the semi-field set up is reliable, safe and gives reproducible results.

Behavioural studies in semi-field cages (Roque and Eiras 2008) showed that the MosquiTRAP baited with a formulation of synthetic oviposition cues caught similar proportions of gravid *Ae. aegypti* to traps baited with infusions of 10-45 days of fermentation (Figure 3). However, the oviposition cues caught a significantly higher proportion of mosquitoes than infusions aged 5 or 60 days (P<0.01) (Roque 2007). Therefore, the synthetic oviposition cue can replace the natural grass infusions. The behavioural response of gravid *Ae. aegypti* is likely to be governed by a complex mixture of

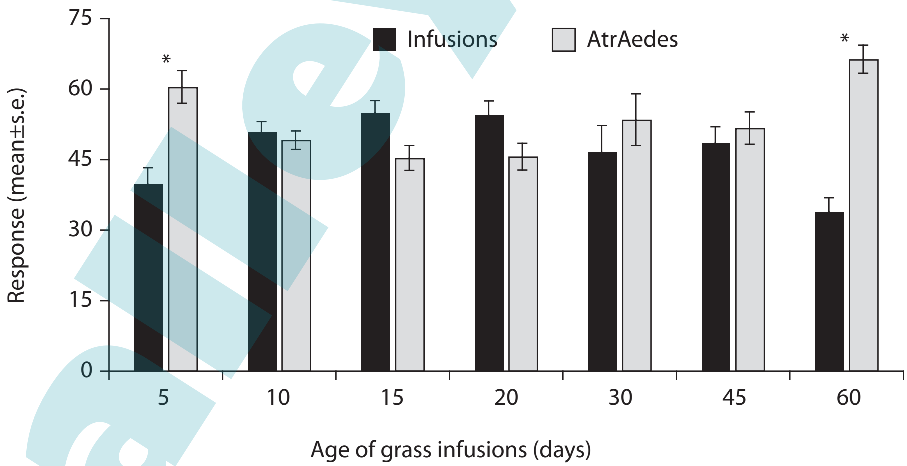

Figure 3. Behavioural responses of gravid females Aedes aegypti *to the sticky trap (MosquiTRAP) within field cages to grass infusion (*Panicum maximum*) and synthetic oviposition attractants (AtrAedes) (Roque 2007).*
** Means* P*<0.05.*

several compounds having specific release rates. A single compound at a very precise release rate can replace the grass infusion. Again, oviposition behaviour is probably mediated by a complex combination of many compounds rather than by one single compound (Eiras 2001). Moreover, the concentration of the compound in the odour plume may play an import role as chemical signal to gravid mosquitoes.

Field evaluation of oviposition lures for Aedes aegypti

Field studies showed that the Atr*Aedes* lures are still attractive after 30 days of field use (Eiras *et al.* 2004) and their performance as bait for the Brazilian sticky trap (MosquiTRAP) has been evaluated with adult collection methods, using an aspirator. In Brazil, the sensitivity of the sticky trap was compared with manual aspirations (Nasci 1981) to detect the presence of *A. aegypti* females during the rainy season for 23 weeks and it was concluded that the sensitivities of both methods were similar (Favaro *et al.* 2008). The authors pointed out that the relative efficiency of use of the MosquiTRAP was 30 traps per person per day whereas that of manual aspirators was five collections per person per day. Collection with aspirators requires the collector to enter indoor sites, thus causing potential problems with the residents. In a dengue endemic area in Rio de Janeiro city during the rainy season, the sticky trap baited with Atr*Aedes* lures was also compared with CDC backpack aspirators in a mark-release-recapture experiment. From a total of 1,240 gravid dust-marked *Ae. aegypti* released in the field, 127 (10.2%) were recaptured by baited MosquiTRAPs whereas only 47 (3.8%) were recaptured with the backpack aspirator at 10, respectively 8, days after release(Maciel de Freitas *et al.* 2008).

***Traps for collection of gravid adult* Aedes aegypti**

Only a few traps are specifically designed for catching previously blood fed, i.e. gravid female *Ae. aegypti*. The CDC gravid trap (John W. Hock Co., Gainesville, FL, USA) was originally designed by Reiter (Reiter 1993) to attract and catch gravid mosquitoes. It has a battery-powered fan mounted inside that draws mosquitoes attracted by grass or hay infusions into a catch bag. Later, the CDC-gravid trap was modified by Dennet *et al.* (2007) and visual aspects were incorporated to increase the catch of gravid *Ae. albopictus.* The modified gravid trap did not turn out to be effective at catching gravid *Ae. albopictus,* probably because the standard oviposition attractant used had been developed for *Culex* spp. and may not have been attractive to *Ae. albopictus*.

Several sticky trap models have been developed to capture gravid *Ae. aegypti* (Favaro *et al.* 2006, Gama *et al.* 2007, Kay *et al.* 2000, Muir and Kay 1998; Ordonez *et al.* 1997, Ordonez-Gonzalez *et al.* 2001, Ritchie *et al.* 2003, 2004) and *Ae. albopictus* (Facchinelli *et al.* 2007). The efficacy of a sticky ovitrap lined with an adhesive paper strip was tested in a dengue area in Guadelupe, Mexico using mark-release-recapture (MRR) methods (Ordonez-Gonzalez *et al.* 2001). A similar type of sticky ovitrap with natural oviposition attractants was developed and evaluated in an *Ae. aegypti* infested area in Cairns, Australia, where an association between the number of adult females captured and the risk of dengue transmission was found (Ritchie *et al.* 2004). The Australian sticky ovitraps have also been used for surveillance and behavioural investigations of *Ae. aegypti* and *Ae. polynesiensis* (Marks) in Moorea, French Polynesia (Russell and Ritchie 2004) and in the Torres Straits, Australia (Ritchie *et al.* 2006). In Italy, Facchichelli *et al.* (2007) also developed a new sticky trap design, which was shown to be specific for *Ae. albopictus* in field trials in Rome. The MosquiTRAP has been used for studies of mark-release-recapture in a suburban residential area of Rio de Janeiro (Brazil) (Maciel de Freitas and Lourenço de Oliveira 2009, Maciel de Freitas *et al.* 2008) and in Cairns, Australia (Russell *et al.* 2005).

In Brazil the MosquitoTRAP is used for monitoring populations of *Ae. aegypti* in urban areas (Eiras and Resende 2009). Such sticky traps are a low-cost device without the need for electricity, laboratory facilities or human resources, providing faster results than the ovitrap, larval survey and other adult traps for dengue control. Mosquito behaviour in sticky traps appears to be similar in different areas (Favaro *et al.* 2006, Gama *et al.* 2007, Ritchie *et al.* 2004); once stuck, the mosquito remains in resting position. Those that escape, usually loose one or more legs that remain adhered to the sticky card. Identification of collected mosquitoes is still possible with their thoraces, which usually remain somewhat visible (Gama *et al.* 2007, Ritchie *et al.* 2003).

The MosquiTRAP Version 1.0 (patent pending) was developed in 2001 at the Federal University of Minas Gerais and consisted of a 1 litre black plastic cylinder filled with 300 ml of grass infusion (as a natural oviposition attractant) and a removable sticky card on which the mosquitoes are captured (Eiras 2002). During the dry season of 2003, field studies showed that the MosquiTRAP Version 1.0 was more sensitive to detect the presence of *Ae. aegypti* in urban areas than the traditional larval survey used in Brazil (Gama *et al.* 2007), especially during the dry season when the larval method cannot detect this mosquito. Therefore, the Brazilian National Programme of Dengue Control (NPDC) stimulated and provided funds for further studies. In 2004-2005, the Brazilian NPDC sponsored field evaluations of the MosquiTRAP version 2.0 and the MI-Dengue technology in 10 Brazilian cities, aiming to supplement new tools and develop new entomological indices for dengue vector control (Eiras *et al.* 2005, 2007). For such studies, it was necessary to develop a new sticky trap prototype that could be produced on a large scale. The MosquiTRAP Version 2.0 was developed at the University of Minas Gerais and prototyped by professional designers, looking for easy and rapid functionality for field workers. This trap version was funded by several Brazilian public grants that encouraged the creation of a 'spin-off' company, namely Ecovec Ltda., in order to commercialise it.

The MosquiTRAP Version 2.0 was evaluated and compared with larval surveys and ovitraps in 10 Brazilian cities. The results demonstrated that field workers were able to identify 95 to 100% of *Ae. aegypti* captured in MosquiTRAPs and that the larval survey was less sensitive than the sticky trap, whereas the ovitrap was more sensitive than both methods (Eiras *et al.* 2005).

The position in which the sticky trap is placed in houses is very important in order to avoid rejection by the residents. Studies in Brazil demonstrated that the MosquiTRAPs placed outdoors captured five times more females than traps placed indoors (Favaro *et al.* 2006). This is probably because *Ae. aegypti* feeds on human blood indoors and lays eggs outdoors, where more breeding places are available. Outdoor traps have also a great advantage of being a non-invasive method of mosquito sampling because trap inspection does not require house entry for mosquito inspections, which is obviously inconvenient for homeowners as well as for vector control workers. Beside the sticky trap being easy to manipulate, it was readily accepted by the residents and local community. A study in Brazil has shown that the MosquiTRAP had a high level of acceptance by the local community (95.6 to 99.3%), whereas the larval survey had a low acceptance (60-90%). The time spent for sticky trap inspection is about 3-5 min whereas the larval survey is about 12-25 min (Eiras *et al.* 2005). The selectivity of the MosquiTRAP in catching gravid *Ae. aegypti* was shown by Favaro *et al.* (2006): of 488 dissected females, 426 were gravid (87.3%).

Several studies have been conducted to compare the sensitivity of the sticky trap with that of the ovitrap (Facchinelli *et al.* 2007, Fávaro *et al.* 2006, 2008, Gama *et al.* 2007, Honorio *et al.* 2009, Lourenço-de-Oliveira *et al.* 2008, Ritchie *et al.* 2003, 2004) and the aspirator (Favaro *et al.* 2008, Maciel de Freitas *et al.* 2008). Generally, gravid *Ae. aegypti* lay eggs in both natural and artificial containers, indoors or outdoors and exhibit an oviposition pattern known as 'skip-oviposition', which means that a single gravid female lays her eggs in several containers (Cobert and Chadee 1993). Consequently, one recipient, such as an ovitrap, may contain eggs from several *Ae. aegypti* females (Reiter *et al.* 1991), which precludes the possibility of knowing the number of females that laid eggs in the ovitrap. The sensitivity of the ovitrap (presence of at least one egg) and the sticky trap (presence of at least one adult female *Ae. aegypti*) was measured in field trials. Gama *et al.* (2007) reported that during the dry season, when the population of *Ae. aegypti* is low, the ovitrap is more sensitive (16-67%) than the sticky trap (0-32%) whereas Favaro *et al.* (2006) showed a similar sensitivity of the MosquiTRAP (82.1%) and the ovitrap (89.7%) during the rainy season. In Australia, Ritchie *et al*. (2003) reported that the sensitivity of sticky ovitraps (67.5%) was slightly higher than that of oviposition traps (64%). The sensitivity of a trap to detect the presence of disease vectors and to measure population fluctuations with time is a key aspect in effective monitoring of dengue risk and dengue control programmes.

A new MosquiTRAP design (Version 3.0) (Figure 4) consists of a polyethylene mat black container (33 cm high by 15 cm wide), divided into two parts: a base, filled with approximately 300 ml of tap water, and an upper part with a funnel-shaped opening, facilitating the mosquito's entry and hindering its exit (Eiras and Resende 2009). The sticky card is attached from the water line in the base to the upper part of the trap and the synthetic oviposition attractant (Atr*Aedes*) is attached to the sticky card. This new MosquiTRAP model #3.0 was compared with the previous model #2.0 in semi-field and urban areas of Brazil. In the semi-field, the new version caught a significantly higher number of gravid *Ae. aegypti* (total of 244; mean 5.5±0.46) than the previous version (total of 91; mean 2.1±0.29) (Eiras *et al.* 2005). In urban areas (Boa Vista, Roraima State, Brazil) 40 sticky traps of each version were set in 40 blocks and the evaluation ran for 12 weeks. The previous MosquiTRAP version 2.0 caught 47 gravid female *Ae. aegypti* whereas the new version 3.0 caught 127.

This new MosquiTRAP version has been evaluated for dispersion of *Ae. aegypti* (Maciel de Freitas and Lourenço-de-Oliveira 2009, Maciel de Freitas *et al.* 2007, 2008) and temporal patterns of *Ae. aegypti* population dynamics (Honorio *et al.* 2009) and in a novel field data acquisition of adult female *Ae. aegypti* and processing-generating GIS real-time web-site surveillance system (Eiras and Resende 2009).

Figure 4. The Brazilian sticky trap MosquiTRAP™: (A) Sticky trap assembled and (B) its inner parts: (1) upper opening; (2) funnel shape entering; (3) sticky card set on rings and (4) lower pot with water (Eiras et al. *2007).*

Traps for collection of host-seeking adult *Aedes aegypti*

Host odours attractive for Aedes aegypti

Composition of host odours attractive for Aedes aegypti

In the collection of mosquitoes in general, the most effective attractant is carbon dioxide (Chapter 5, this volume). For the highly anthropophilic *Ae. aegypti,* carbon dioxide is, however, not very effective if used as the sole attractant for gravid females. As the gas is either liberated from dry ice, from pressurised bottles or from the combustion of propane gas, its use is costly, complicated and not feasible in typical dengue endemic urban environments. The search for *Ae. aegypti* attractants other than carbon dioxide first focused on lactic acid. This substance, present on human skin as well as in breath, was repeatedly shown to attract *Ae. aegypti*, but until the beginning of the 1990's only in combination with carbon dioxide (Acree *et al.* 1968, Eiras and Jepson 1991, 1994, Smith *et al.* 1970).

Attraction to lactic acid without the addition of carbon dioxide was first shown in an Y-tube olfactometer that allowed for an especially fine distinction of the effect of olfactory attractants (Geier and Boeckh 1999). The apparatus also demonstrated the attraction of female *Ae. aegypti* to a human skin extract. This extract had been produced by rubbing the skin of a human test subject with ethanol-soaked cotton pads and a subsequent extraction of these pads. Although lactic acid was also a component of the skin extract, the extract was more attractive than lactic acid alone and was the same as that of a human finger introduced into the olfactometer.

Ammonia has also been found to be an attractive component. It had already been shown to be attractive for other haematopageous insects (Hribar *et al.* 1992, Taneja and Guerin 1997), but earlier authors could not demonstrate the attraction of *Ae. aegypti* to ammonia (Brown *et al.* 1951, Müller 1968, Rössler 1961;). It had never before been tested in combination with lactic acid. Reinvestigation of this combination showed a synergistic effect of ammonia with lactic acid (Geier *et al.* 1999a). However, the combination of lactic acid and ammonia was again not as attractive as the complete skin extract, indicating that yet other components play an additional role.

Because fatty acids are widely present on human skin, their possible role as attractants for mosquitoes had already been proposed by earlier authors (Carlson *et al.* 1973, Knols *et al.* 1997, Rössler 1961). This was confirmed for *Ae. aegypti* by Bosch *et al.* (2000), whose experiments suggested two groups of carboxylic acids with different chain lengths: C1 to C3 and C5 to C8 (C9 and C11 carboxylic acids reduced the attractiveness of lactic acid in the olfactometer).

Later investigations showed that hexanoic acid (caproic acid) was one substance with which the attractiveness of the lactic acid and ammonia combination for *Ae. aegypti* could be augmented further, but only in a closely defined proportion to each other. A dispenser liberating the different substances in the right quantities for a prolonged period of time would have to account for the different physical and chemical properties of the individual components. Due to their relatively large volume and vapour pressure, reservoir devices have been used mainly for lactic acid controlled release. Tube structures such as those described earlier for oviposition attractants have been used with the factor determining the release rate being the material from which the tube is made. For ammonia and hexanoic acid, solid polymer release matrices have been used. In this way, each substance in the three-component lure has its own release mechanism so that the ratios between the three components remain the same during the whole of the field life of the lure. The same principle of separated release devices for each compound in one dispenser unit is also used in an advanced version of a commercially-available dispenser, the Sweetscent™ dispenser, in which thin polymer coatings determine the release rate of each compound out of its reservoir pouch. Figure 5 shows the BG-Lure™ and the Sweetscent™, which are made up of the three controlled release formulations of the active substances, all contained in a mesh bag for ease of manipulation and placement in the BG Sentinel trap.

A

B

Figure 5. (A) Bg-Lure™ and (B) the Sweetscent™ dispensers containing lactic acid, ammonia and hexanoic acid (Source: Biogents AG).

Plume structure of host odours attractive for Aedes aegypti

Not only the composition, but also the structure of attractant odour plumes carries important information for host-seeking *Ae. aegypti*. This was indicated for the first time by a closer analysis of its flight behaviour in a Y-tube olfactometer (Geier *et al.* 1999b). The attractiveness of carbon dioxide, of human skin odours rubbed on a glass support and of L-(+)-lactic acid were tested in homogeneous, turbulent and filamentous odour plumes. A marked difference between carbon dioxide on the one side and the skin odours on the other side was observed. A turbulent and filamentous carbon dioxide plume with large fluctuations in the concentration lured more mosquitoes upwind than a homogeneous CO_2 plume. The opposite was found with plumes of human skin odour: the highest number of mosquitoes flew upwind in the homogeneous plume, whereas in filamentous plumes, their numbers were significantly lower. 3-D video analyses of the flight behaviour of individual *Ae. aegypti* females in a larger wind tunnel confirmed these findings (Dekker *et al.* 2005). Heterogeneous odour plumes, both from skin odour and from carbon dioxide, elicited a meandering flight pattern with many turns and transversal parts. Homogeneous skin odour resulted in a quick and direct upwind flight, while mosquitoes presented with a homogeneous carbon dioxide plume flew mostly sidewards, much as in the control experiments (see also Chapter 6, this volume).

The Biogents-Sentinel trap design

The patented design of the BG-Sentinel incorporates attractive host-odour plumes for *Ae. aegyti* (Figure 6) (Geier *et al.* 2004 – patent pending). A faint upward air stream, charged with artificial skin odours, is produced through a gauze making up the top of the trap. The individual components are lactic acid, ammonia and caproic acid, all liberated in a fixed ratio from a specially designed dispenser, the BG-Lure, which is placed inside the trap. The artificial host-odour plume mimics the upward convection currents produced by a human body. Approaching mosquitoes are furthermore attracted over short range by the black and white contrast between the top of the trap and the centre tube, where the mosquitoes are drawn into a catch bag. One fan produces the attractive plume and sucks the mosquitoes into a catch bag.

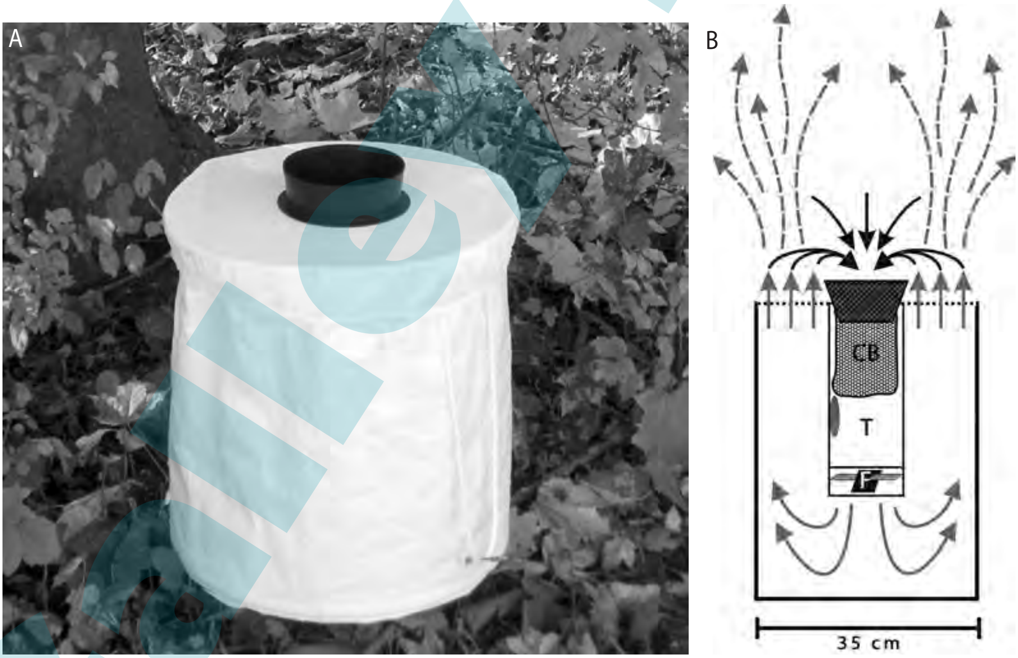

Figure 6. (A) BG-Sentinel trap™ assembled and (B) its functional diagram: longitudinal section of the trap. CB: catch bag; T: black tube; F: fan; arrows indicate the direction of the airflow.

A number of field studies have demonstrated that the BG-Sentinel trap is an excellent tool for capturing *Ae. aegypti* (Feltner and Ferrao 2008, Krueger and Hagen 2007, Maciel de Freitas *et al.* 2006, 2007, Meeraus *et al.* 2007, 2008, Morrison *et al.* 2008, Williams *et al.* 2007, Solberg *et al.* 2007). Kröckel *et al*. (2006) compared the BG-Sentinel to the Fay-Prince trap and a trap that produces carbon dioxide through the combustion of propane gas (the Mosquito Magnet Liberty™), and human landing collections in Belo Horizonte, Brazil. They reported significantly better catching rates for female *Ae. aegypti* with the BG-Sentinel (between 3 and 15 times better catching rates than the MML trap and between 8 and 15 times better rates than the Fay-Prince traps (Figure 7). Compared to human landing collections, the BG-Sentinel trap (without the addition of carbon dioxide) catches about 40% fewer female *Ae. aegypti* than a human collector. The trap also captures male *Ae. aegypti*.

In comparison to a backpack aspirator (used in a standard protocol of 20 minutes per premise) in Rio de Janeiro, Brazil, the BG-Sentinel (placed at each premise for 24 hours) captured significantly more female and male *Ae. aegypti* (Maciel de Freitas *et al.* 2006). A similar field study in Cairns, Australia, confirmed significantly higher catching rates of the BG-Sentinel than a back pack aspirator and EVS-trap baited with carbon dioxide (Williams *et al.* 2006). Additional experiments demonstrated the BG-Sentinel's potential as an *Ae. aegypti*-population sampling tool both in dengue risk assessment as well as the assessment of the success of mosquito control programmes (Williams *et al.* 2007). As with a conventional CDC light trap, the BG-Sentinel requires electrical power to operate the ventilator. Although the ventilator requires only about three Watts, it has some disadvantages in routine monitoring operations. Passive trapping systems that use sticky surfaces to catch mosquitoes do not require electricity and are low cost tools, but the catching rates of these systems are much lower (between 10 to 50 times, Kröckel *et al.* 2006). For a sensitive and high resolution spatial analysis of dengue vectors both trapping systems are useful, the

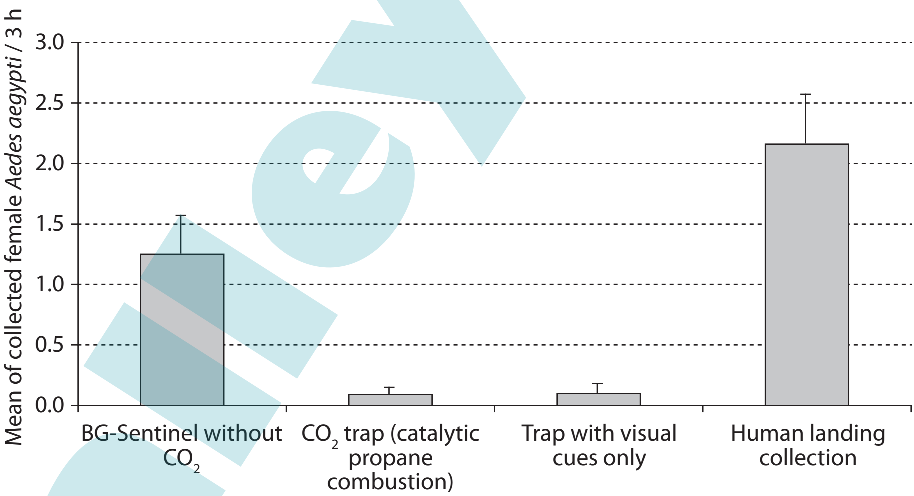

Figure 7. Mean values (± standard error) for caught Aedes aegypti *(syn.* Stegomyia aegypti*) from three-hour catching periods over an eight week period. BG-Sentinel trap with lure dispenser BG Lure (n=32); CO_2 trap: Mosquito Magnet Liberty generates moist, warm CO_2 (n=32); Trap with visual cues only: Fay Prince trap with black and white contrast (n=30); Human landing collection: Voluntary test person as a mosquito catcher (n=32) (Kröckel* et al. *2006).*

sticky traps to provide a high spatial resolution and the BG-Sentinels to provide a fast and highly sensitive measure of the density of adult vectors.

The high collection rates of the BG-Sentinel allow an accurate determination of vector population densities and provide sufficient sample material to establish virus infection rates by pooling mosquitoes. In the transmission of vector-borne diseases, two factors play a role: first, the density of vectors and, second, the incidence of disease agents in their population (infection rate). With efficient sampling tools for the vectors involved and with quick tests for the presence of viruses in these vectors, it is thus possible to assess transmission risk by calculating the Vector Index. This gives the most meaningful risk index which can be used in the efficient planning and rapid implementation of the control of vector-borne diseases (J Arias, personal communication).

Although originally developed for catching host-finding females, it has been demonstrated that the BG-Sentinel trap also catches gravid females, parous and nulliparous females, males, and even blood-fed females in larger numbers than expected (Maciel de Freitas *et al.* 2006, Williams *et al.* 2006). Catching a broad spectrum of mosquitoes in different physiological stages allows a more precise analysis of the mosquito population in a certain area.

A semi-field study showed that an established population of *Ae. aegyti* in a greenhouse could be completely eradicated by the placement of one BG-Sentinel trap. Already two days after the trap's placement, the human biting rate in the greenhouse was reduced from 15 bites per minute to less than one bite per minute (Obermayr 2006). Within six weeks of continuous operation of the trap, the mosquito population was completely extinguished with no eggs, larvae or adult mosquitoes being found. A large scale field study with about 600 households is currently ongoing in Manaus, Brazil to demonstrate that the traps can be also used for control of local mosquito populations and for lowering the risk of dengue transmission in private homes.

Traps for sampling host-seeking Aedes albopictus

The BG-Sentinel with the BG-Lure has also proven to be a useful trap for another species of the subgenus *Stegomyia*, *Aedes albopictus*. This was reported for the first time in 2005, when *Ae. albopictus* were detected in two locations in Italy where ovitraps failed to collect this species (Bitzhenner *et al.* 2005). In the same year, *Ae. albopictus* was detected in BG-Sentinel traps placed on islands in the Torres strait near Australia (Ritchie *et al.* 2006). In 2006, *Ae. albopictus* was found in BG-Sentinels placed in Libreville, Gabon (Krüger and Hagen 2007). A study comparing the CDC Light trap baited with carbon dioxide to the BG-Sentinel and another new trap, the CMT-20TM was published by Meeraus *et al.* (2007). The BG-Sentinel baited only with the BG-Lure captured six times more *Ae. albopictus* that the CDC trap with carbon dioxide. Adding CO_2 to the BG-Sentinel and the BG-Lure improved the catching rate to 33 times that of the carbon dioxide-baited CDC trap. The CMT-20 (with SkinLure^{TM}, an attractant containing lactic acid) collected significantly more *Ae. albopictus* when used with CO_2 than without CO_2, but did not collect significantly more *Ae. albopictus* than the CO_2-baited CDC light trap.

The high catching rates of the BG-Sentinel traps for *Ae. albopictus* have led to the idea of using the traps for mosquito control. A long-term field study in Cesena, Italy, was conducted from May to October 2008 in order to investigate the effect of the traps on local mosquito populations. Human biting rates as well as egg collections with ovitraps were significantly reduced in areas with trap placement compared to areas without traps (Englbrecht *et al.* 2009).

Practical application of population data of *Aedes aegypti*

The role of GIS in public health surveillance

Geographic Information Systems (GIS) have been widely used by many countries for health surveillance. In Canada, the Public Health Agency of Canada developed a *Disease Surveillance On-Line* web site that provides mapping and other services for cancer, cardiovascular diseases, major chronic diseases, notifiable diseases, and injury surveillance (PHAC 2009). Mosquito surveillance systems are also currently using GIS as an important tool to monitor the distribution and spread of West Nile virus (BCCDC 2009), dengue (Sithprasasna *et al.* 1997) and malaria (WHO 2009).

Geographic information systems consist of an automated computer-based system that has the ability to capture, retrieve, manage, display and analyse large quantities of spatial and temporal data in a geographical context. The system comprises hardware (computer and printer), software (GIS software), digitised base maps, information and a whole set of procedures such as data collection, management and updating (WHO 2009). Vector-borne diseases such as that carried by *Ae. aegypti* can be mapped temporally and spatially. Such information, when mapped together creates a powerful tool for monitoring and controlling the dengue vector. Operations for dengue control based on GIS were established in Singapore in 1998. Streets, residential buildings and other relevant databases were obtained and mapped to form the base map layer, such as *Aedes* breeding sites, dengue case incidences, complainants' addresses, sensitive areas, weather data (rainfall, temperature and relative humidity) were mapped into the GIS (Ai-leen and Song 2000).

In Recife, northeastern Brazil, a new approach to dengue vector surveillance based on the use of spatial analysis techniques, such as the Kernel density estimator, for the identification of hotspots of vector populations using data from oviptraps, can be very useful for guiding vector control operations. The long-term use of modified ovitraps provided with *Bacillus thuringiensis* var. *israelensis* (*Bti*) as a larvicide was evaluated in various intra-urban landscapes. Results from the first year of egg collections indicated that this could be a promising strategy for detecting *Ae. aegypti* population outbreaks (Regis *et al.* 2008). It is also possible to integrate remote sensing satellite imagery, trap placement, meteorological data and census data. Similarly, environmental data such as temperature, relative humidity, and rainfall can be recorded daily at meteorological point-stations and also integrated into the system. The main disadvantage of the spatial analysis technique is that it still relies on the use of oviposition traps, requiring much personnel and laboratory facilities. Although their use is recommended for the detection of low populations of *Ae. aegypti*, their value in the assessment of vector abundance, also between blocks or neighborhoods is questionable (Focks 2003).

Intelligent Dengue Monitoring System

Because of the difficulties of field data collection of larval surveys by the Brazilian National Programme for Dengue Control, a new field data acquisition of adult female *Ae. aegypti* and processing-generating GIS real-time web-site surveillance system was developed and evaluated in Brazil. The technology is known as 'Intelligent Dengue Monitoring system' (MI-Dengue) and was developed by a 'spin-off' company of the Federal University of Minas Gerais, Belo Horizonte, Brazil. The MI-Dengue consists of (a) a MosquiTRAP baited with synthetic oviposition attractant that captures adult female *Ae. aegypti*, which allows for the identification of the adult vectors during trap inspection; (b) recording and sending entomological data on electronic spreadsheets or by mobile phone during trap inspection and (c) an internet site that is an integrated real-time adult mosquito surveillance system providing entomological indices and GIS technology (Eiras and Resende 2009). The MI-Dengue permits researchers to send and make data available on the internet for municipalities' health managers to access information on the density of gravid female *Ae. aegypti* on georeferenced maps and in analytical tables of the sites monitored with MosquiTRAP.

The MI-Dengue web-data system consists of three software programmes developed to simplify information: (a) 'geo-dengue collection' installed in portable devices (e.g. palmtops or cell phones) to record *Ae. aegypti* field capture data; (b) 'geo-dengue monitoring' to process the field data and produce tables with entomological indices and graphs with trends for analysis; and (c) 'geo-dengue', which produces georeferenced maps of mosquitoes captured with MosquiTRAP and makes them available to users on the Internet on a weekly basis.

The MI-Dengue technology provides a wide range of GIS tools for public health surveillance and can be adapted for surveillance of other diseases, such as AIDS, tuberculosis, malaria, leishmaniosis, etc.

Data acquisition

Field data collected by ovitrap, larval surveys, adult traps and aspirator are traditionally recorded on printed spreadsheets, attached to a clipboard by field workers. Later, the spreadsheets are sent to be typed and consolidated on a personal computer. Not only is this method prone to typing errors, it is also time consuming and labour intensive if a large amount of field data is collected.

An alternative and faster method of acquisition and transport of entomological data is by means of an electronic spreadsheet installed in palmtop computers or mobile phone (Eiras and Resende 2009). These data are transferred automatically to the municipality's database, and the internet site automatically generates the GIS maps and entomological tables for the city council's dengue control service. The electronic spreadsheet allows for recording household information (e.g. resident's name, address, postal code, and emplacement of the trap within the residence) as well as number of adult mosquitoes caught in each trap/premise.

There are several advantages of using electronic spreadsheet or mobile phone over conventional field data acquisition systems. The field data can be accessed immediately (premises visited and scheduled for visits, trap locations, residents' names, and so on.) and the entomological indices can be produced automatically. The operation cost is low because these applications and systems are maintained and operated automatically and there is no delay between data reported to the database and data available for web mapping and public health access.

GIS for identifying infestations of female adult Aedes aegypti

Infestations of gravid female *Ae. aegypti* are measured by the MI-Dengue system in five different ways for focused vector control: (1) weekly monitoring of *Ae. aegypti* infestation in the urban blocks; (2) monitoring re-infestation of blocks; (3) hot spot areas; (4) entomological index and (5) monitoring infection of adult mosquitoes with dengue virus (Eiras and Resende 2009).

All blocks of urban areas are weekly monitored for *Ae. aegypti* infestation and the number of female *Ae. aegypti* captured with the sticky trap is used to establish colour categories for classifying blocks as green (zero captures), yellow (one female *Ae. aegypti*/MosquiTRAP/week = low infestation), orange (two females/trap/week = medium infestation), and red (> three females/trap/week = high infestation) (Eiras and Resende 2009). These parameters were based on studies by Ritchie *et al.* (2004) showing that in Australia, more than two gravid females *A. aegypti*/trap/week was associated with a risk of dengue cases.

These weekly data on infested sites and vector infestation levels provided important information to the municipal dengue control workers who assisted municipal health managers in targeting and optimising their *Ae. aegypti* control activities. The georeferenced maps produced by MI-Dengue technology and provided weekly on the internet, allowed municipal health managers to identify urban blocks by colour according to the number of female *Ae. aegypti* specimens captured. Thus, dengue control activities could be focused on infested blocks within a 200-meter radius. This focused vector control strategy, supported by a weekly infestation monitoring system, allowed spatial localisation of vector infestation and the evaluation of control measures within the radius represented by the trap.

Entomological index

Two indices are used to calculate the EI: (1) the Mean Female *Aedes* Index (MFAI), which is the mean number of female *Ae. aegypti* specimens caught by traps installed per week and is used for monitoring vector infestations in neighborhoods and municipalities (Figure 8) (Eiras and Resende 2009); (2) the Temporal Mean Female *Aedes* Index (MFAIt), which is the average of MFAI values for the three previous, consecutive weeks and is calculated on a monthly basis for *Ae. aegypti*. The MFAIt in neighbourhoods are colour-coded green (MFAIt<0.2), yellow (0.2<MFAIt<0.4), and red (MFAIt>0.4). The MFAIt was developed based on correlations between the number of dengue cases and the MFAI entomological index during an outbreak of dengue in Vitoria city, Espírito Santo State, Brazil, where clusters of dengue cases and mosquitoes were observed. The entomological index t is classified as 'risk of dengue' if MFAIt≥0.4, while 'dengue alerts' are issued when MFAIt ranges between 0.20 and 0.39. The situation is considered 'risk-free' in terms of dengue when MFAIt≤0.2. Using this trategy, the municipality of Presidente Epitacio, Sao Paulo, Brazil, experienced a shift from 'dengue alert' to 'risk free' was observed). A reduction in entomological indices after several weeks of monitoring indicated a strong change in the *Ae. aegypti* population density that was influenced either by environmental conditions or by vector control in the targeted and priority areas, or both (Table 2) (Eiras and Resende 2009).

Monitoring re-infestation of blocks

Three variables are used as follows: (a) recurrence of positive traps (at least one female *Ae. aegypti* caught); (b) time (chronological order of the week in which the capture occurred), and (c) number of female *Ae. aegypti* in the traps during the previous four weeks in the monitored blocks (Eiras and Resende 2009).

Monitoring block infestations with adult female Aedes aegypti *using GIS*

The city council's mosquito control unit can access GIS-maps on the website for the weekly infestations of the blocks by female *Ae. aegypti*, and can thus obtain a spatial and temporal understanding of the infestations (Eiras and Resende 2009). The black dots on the maps represent the exact trap location by GPS. The users (city council) can have access to the georeferenced information (latitude and longitude), household address, block and neighbourhood numbers, and number of mosquitoes captured during each epidemiological week by clicking on these

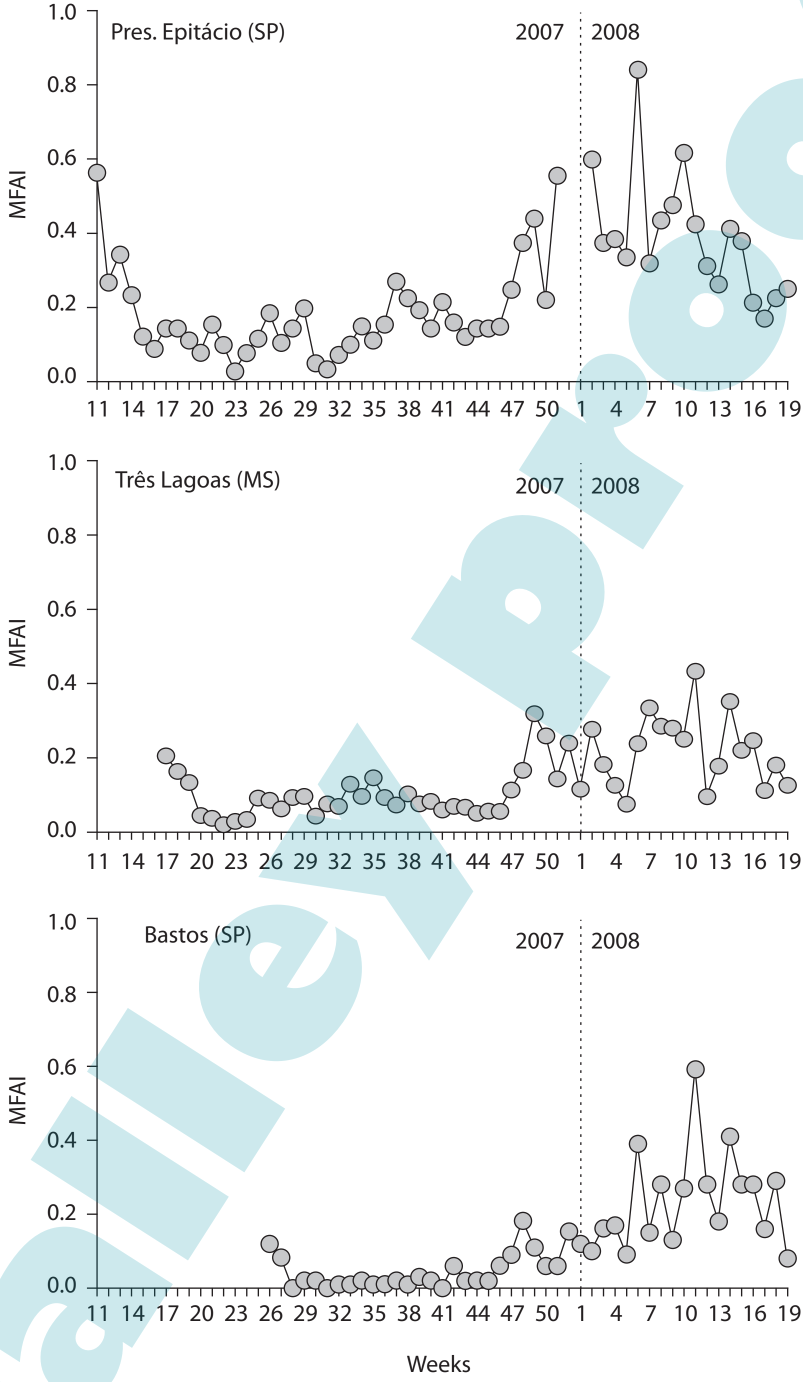

Figure 8. Weekly monitoring of Mean Female Aedes *Index (MFAI) furnished by MI-Dengue in three municipalities in 2007 and 2008 (Eiras and Resende 2009).*

Table 2. Example of evolution in entomological indices in neighbourhoods in the Municipality of Presidente Epitacio (São Paulo State, Brazil), classified according to temporal Mean Female Aedes *Index (MFAIt) in the previous four epidemiological weeks (Eiras and Resende 2009).*

Entomological weeks	% neighbourhood classified		
	Non-critical (MFAIt<0.2)	Dengue alert (0.2<MFAIt<0.4)	Critical (MFAIt>0.4)
7-10	0.0	44.4	55.6
8-11	22.2	33.3	44.4
9-12	22.2	44.4	33.3
10-13	22.2	66.7	11.1
11-14	33.3	55.6	11.1
12-15	66.7	33.3	0.0
13-16	88.9	0.0	11.1
14-17	88.9	11.1	0.0

dots (traps). The weekly infestation levels are provided by colour-coded information on the blocks, based on the number of female *Ae. aegypti* captured in each block. The dengue vector control measures can be based on the weekly observations of infestation levels, where effort is concentrated in given areas or neighbourhoods of red blocks, thus facilitating implementation of targeted control measures.

Monthly monitoring of Ae. aegypti *reinfestation in the neighbourhoods*

Eiras and Resende (2009) showed in preliminary studies that using the MI-Dengue technology in three cities in Brazil reduced entomological indices after seven weeks of monitoring adult female *Ae. aegypti* followed by directional control measures. The MI-Dengue technology was used in three districts (30,000-75,000 inhabitants) during one year and vector control was based on the website information of 'dengue alert' or 'hot spots' of adult female *Ae. aegypti*. Results showed that there was a trend of shifting from categories 'dengue alert' and 'critical' to 'risk free', indicating a strong seasonal variation in the *Ae. aegypti* population density that was probably influenced by the climatic conditions and/or targeted control measures. The incidence of dengue cases was also significantly reduced when compared with the neighbourhood districts (Figure 9). Although, this was a pilot study, further data are currently being collected in 45 Brazilian cities during 2009 with the populations of the cities ranging from 30,000 to 700,000 inhabitants.

Hot spot areas

Cluster analysis of the capture of female mosquitoes by MosquiTRAP is based on the weekly mean number of female *Aedes* index per block. Space-time Permutation Scan Statistical Model (STPSSM) (Kulldorff *et al.* 2005) and Poisson Scan Statistic Model (PSSM) (Kulldorff and Nagarwalla 1995, Kulldorff *et al.* 1998) were used to identify whether *Ae. aegypti* clusters in space are randomly distributed or not (Figure 10).

Figure 9. Total autochthonous dengue cases and incidence in municipalities that (A) did not adopt MI-Dengue, (B) partially MI-Dengue and (C) fully adopted MI-Dengue in early 2008. Numbers in parenthesis means percentage of (-) reduction or (+) increasing in incidence of autochthonous dengue in 2008 (adapted from Eiras and Resende 2009).

A

B

Figure 10. (A) Presence of hot spot areas of gravid female Aedes aegypti *in Vitoria (Espirito Santo State, Brazil) detected by MI-Dengue weekly monitoring at week 12 (2009). The window shown on the screen allowed to follow weekly maps. (B) GIS of infected mosquitoes with dengue virus (small circles are dengue cases and bigger circles are infected* Aedes aegypti *mosquitoes with dengue virus).*

Measurement of dengue- infected Aedes aegypti *mosquitoes using GIS*

In order to control and prevent dengue transmission, it is also important to rapidly detect and type the virus in adult mosquitoes. For instance, in Brazil, the control of adult *Ae. aegypti* is trigged only when a person gets dengue fever (PNCD 2002). Thus, information indicating the number of *Ae. aegypti* infected by dengue virus may replace the current indicator for adult *Ae. aegypti* control.

Recently in Brazil, a rapid and stable method for detection and typing of dengue virus (DEN) in *Ae. aegypti* was established and associated with the MI-Dengue technology. Vitoria city, Espirito Santo State, Brazil, has been using the MI-Dengue technology since January 2007 and approximately 1,450 sticky traps were placed all over the urban area. The entomological index and GIS maps are produced on a weekly basis. The adult female *Ae. aegypti* mosquitoes that are trapped in the MosquiTRAP, are pooled in groups of about 20 mosquitoes and submitted for virus detection and type identification by reverse transcriptase RT-PCR. Data on infected mosquitoes is plotted in GIS web-online for the city council. At the moment of writing, it takes 10 days to analyse mosquitoes for virus infection because samples need to be transported and the analysis is done manually. In future, it is expected that the detection of virus in mosquitoes can be automated. It was found that *Ae. aegypti* was infected with virus DENV-1, DENV-2 and DENV-3 in urban areas during many months (Figure 10B). The hot spot areas with infected mosquitoes were considered priorities for vector control. In a preliminary study of an outbreak of dengue in Vitoria city, it was observed that 85% of dengue cases occurred within a 200 m radius of sticky traps having caught infected female *Ae. aegypti* (AE Eiras, unpublished data) This method is very helpful and it has been used as baseline to develop a new early surveillance system to provide early warning for dengue fever epidemics, in furnishing information for epidemiologic studies, and for effective vector control measures.

Monitoring gravid female *Ae. aegypti* with MosquiTRAP and MI-Dengue allows the Municipal Health Secretariats to conduct weekly follow-up of infestation trends in neighbourhoods and municipalities. The practicality of MosquiTRAP and MI-Dengue to monitor female adult *Ae. aegypti* in real time is substantial when compared with other georeferenced studies that use sampling of immature forms of the mosquito (eggs and larvae) (Ai-leen and Song 2000; Chansang and Kittayapong 2007), which require time and infrastructure for identification, quantification, and data processing. Our experience has shown that these innovative technological tools are not difficult to learn nor are they difficult to integrate into existing dengue control programmes.

Potential use of traps for dengue suppression

Efficient tools for the monitoring of adult *Ae. aegypti*, such as the MosquiTRAP and the BG-Sentinel trap, have a wide range of applications in both dengue control operations and research. Both trap types allow the detection of virus-positive female *Ae. aegypti* during standard weekly monitoring (even if the incidence of these females is very low) and at the peak of acute epidemics.

A novel strategy has been proposed to effectively suppress dengue transmission in urbanised areas by the use of innovative mosquito traps. This has not been tried before, probably because of the low performance, high costs, and complexity of existing trapping technologies. The BG-Sentinel and MosquiTRAP have the potential to be produced economically at a large scale due to their simple technology. Although the efficacy of the trap has been demonstrated already in scientific studies and monitoring programmes, the trap has not been used before as a control tool to suppress dengue transmission. In 2009, two pilot projects were designed to prove that a lure and kill control strategy encompassing environmentally-friendly traps for adult vectors can suppress dengue transmission. Both projects are currently being implemented in Manaus city, Amazon State, Brazil, and the Salvador, Bahia State, Brazil). Using an integrated community-based approach for proper education and training of the trap users, the traps are installed in the household or premises, public buildings, and working places. They can be placed indoors as well as outdoors, depending on the situation and prevalence of the vectors. The traps should run continuously throughout the whole season or year. The theoretical benefits of both traps are increased vector mortality, reduction of human biting rates, early indication of breeding sites through monitoring, enhancement of individual responsibility in community based programmes, environmentally friendly (no toxic substances are used) and sustainable. These are important preconditions for a successful implementation of the traps and the acceptance of the users. Education and training of the trap user is very important to achieve all the benefits of the traps. Therefore the implementation of the proposed trapping strategy has to be integrated in already existing or newly implemented community-based dengue control programs. The use of the traps is expected to have a positive effect on the continued motivation of individuals to take action against the vectors, by source reduction, using additional traps, or applying insecticide treatments.

Acknowledgments

Alvaro Eiras thanks to CNPq, FAPEMIG, SEBRAE, FINEP and the Programa Nacional de Controle da Dengue (PNCD), the Brazilian Health Ministry and the 'spinoff' Ecovec for supporting the development and evaluation of the MI-Dengue technology.

References

Acree F Jr., Turner, RB, Gouck HK, Beroza M and Smith N (1968) L-Lactic acid: a mosquito attractant isolated from humans. Science 161: 1346-1347.

Ai-leen GT and Song RJ (2000) The use of GIS in ovitrap monitoringfor dengue control in Singapore Dengue Bull 24: 110-116.

Allan SA and Kline DL (1995) Evaluation of organic infusions and synthetic compounds mediating oviposition in *Aedes albopictus* and *Aedes aegypti* (*Diptera*: *Culicidae*). J Chem Ecol 21: 1847-1860.

Barrera R, Amador M and Clark GG (2006) Use of the pupal survey technique for measuring *Aedes aegypti* (*Diptera*: *Culicidae*) productivity in Puerto Rico. Am J Trop Med Hyg 74: 290-302.

Beehler JW, Millar JG and Mulla MS (1994) Field evaluation of synthetic compounds mediating oviposition in *Culex* mosquitoes (*Diptera*: *Culicidae*). J Chem Ecol 20: 281-291.

Bentley MD and Day JF (1989) Chemical ecology and behavior aspects of mosquito oviposition. Annu Rev Entomol 34: 401-421.

Bentley MD, McDaniel IN, Yatagai M, Lee H-P and Maynard R (1979) p-Cresol: an oviposition attractant of *Aedes triseriatus*. Environ Entomol 8: 206-209.

Bitzhenner M, Guaraglia Ch, Geier M, Rose A and Talbalaghi A (2005) Evaluation of the BG-Sentinel, a new monitoring trap for mosquitoes, in northern Italy. Poster presentation at the 4th International Congress of Vector Ecology, Reno, Nevada, USA, 2-7 October 2005.

Bosch JO, Geier M and Boeckh J (2000) Contribution of fatty acids to olfactory host finding of female *Aedes aegypti*. Chem Senses 25: 323-330.

Braga IA, Gomes AC, Nelson M, Mello RCG, Bergamaschi DP and Souza JMP (2000) Comparação entre pesquisa larvária e armadilha de oviposição, para detecção de *Aedes aegypti*. Rev Bras Med Trop 33: 347-353.

Breteau H (1954) La fiève jaune en Afrique occidentale française. Un aspect de la médecine préventive massive. Bulletin WHO 11: 453-481.

British Columbia Centre for Disease Control (BCCDC) (2009) Interactive GIS mapping for West Nile virus. Available at http: //maps.bccdc.org.
Brown AWA, Sarkaria DS and Thompson RP (1951) Studies on the responses of the female *Aedes* mosquito. Part I. - The search for attractant vapours. Bull Ent Res 42: 105-114.
Canyon DV and Hii JLK (1997) Efficacy of carbon dioxide, 1-octen-3-ol, and lactic acid in modified Fay-Prince traps as compared to man-landing catch of *Aedes aegypti*. J Am Mosq Control Assoc 13: 66-70.
Carlson DA, Smith N, Gouck HK and Goodwin DR (1973) Yellow fever mosquitoes: compounds related to lactic acid that attract females. J Econ Entomol 66: 329-331.
Chadee DD (1993) Oviposition response of *Aedes aegypti* (L.) to the presence of conspecific eggs in the field in Trinidad, WI. J Florida Mosq Contr Ass 64: 63-66.
Chadee DD, Lakkan A, Ramdath WR and Persad RC (1993) Oviposition response of *Aedes aegypti* mosquitoes to different concentrations of hay infusion in Trinidad, West Indies. J Am Mosq Control Assoc 9: 346-348.
Chadee DD and Martinez R (2000) Landing periodicity of *Aedes aegypti* with implications for dengue transmission in Trinidad, West Indies. J Vector Ecol 25(2): 158-163.
Chadee DD, Doon R and Severson DW (2007) Surveillance of dengue fever cases using a novel *Aedes aegypti* population sampling method in Trinidad, West Indies: the cardinal points approach. Acta Trop 104: 1-7
Chansang C and Kittayapong P (2007) Application of mosquito sampling count and geospatial methods to improve dengue vector surveillance. Am J. Trop Med Hyg 77: 897-902.
Clark GG, Seda H and Gubler DJ (1994) Use of the 'CDC backpack aspirator' for surveillance of *Aedes aegypti* in San Juan, Puerto Rico. J Am Mosq Control Assoc 10: 119-124.
Clements AN (1999) The biology mosquitoes. Volume 2: sensory reception and behaviour. CABI, Wallingford, UK.
Cobert PS and Chadee DD (1993) An improved method for detecting substrate preferences shown by mosquitoes that exhibit skip oviposition. Physiol Entomol 18: 114-118.
Coelho GE, Burattini MN, Teixeira MG, Coutinho FAB and Massad E (2008) Dynamics of the 2006/2007 dengue outbreak in Brazil. Mem Inst Oswaldo Cruz 103: 535-539.
Connor ME and Monroe WM (1923) *Stegomyia* índices and their value in the yellow fever control. Am J Trop Med 3: 9-19.
Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (2009) Edital doenças neglegenciadas - MCT/CNPq/CT-Saúde/MS/SCTIE/DECIT No. 034/2008. Available at: http: //www.cnpq.br/editais/ct/encerrados.htm.
Coutinho FAB, Burattini MN, Lopez LF and Massad E (2006) Threshold conditions for a non-autonomous epidemic system describing the population dynamics of dengue. Bull Math Biol 68: 2263-2282.
Cuéllar-Jiménez ME, Velásquez-Escobar OL, González-Obando R and Morales-Reichmann CA (2007) Detección de *Aedes albopictus* (Skuse) (*Diptera*: *Culicidae*) en la ciudad de Cali, Valle del Cauca, Colombia. Biomédica 27: 273-279.
Dekker T, Geier M and Cardé RT (2005) Carbon dioxide instantly sensitizes female yellow fever mosquitoes to human skin odours. J Exp Biol 208: 2963-2972
Dennett JA, Wuithiranyagool T, Reyna-Nava M, Bala A, Tesh RB, Parsons RE and Bueno RJr (2007) Description and use of the Harris County Gravid trap for West Nile virus surveillance 2003–06. J Am Mosq Control Assoc. 23: 359-362.
Dibo MR, Chiaravalloti-Neto F, Battigaglia M, Mondini A, Favaro EA, Barbosa AC and Glasser CM (2005) Identification of the best ovitrap installation sites for gravid *Aedes* (*Stegomyia*) *aegypti* in residences in Mirassol, state of São Paulo, Brazil. Mem Inst Oswaldo Cruz,100: 339-343
Du YJ and Millar JG (1999) Electroantennogram and oviposition bioassay responses of *Culex quinquefasciatus* and *Culex tarsalis* (*Diptera*: *Culicidae*) to chemicals in odors from bermuda grass infusions. J Med Entomol 36: 158-166.
Effler PV, Pang L, Kitsutani PI, Vorndam V, Nakata M, Ayers T, Elm J, Tom T, Reiter P, Rigau-Perez JG, Hayes JM, Mills K, Napier M, Clark GG and Gubler (2005) Dengue fever, Hawaii, 2001–2002. Emerg Infect Dis 11: 742-749.
Eiras AE (2001) Mediadores químicos entre hospedeiros e insetos vetores de doenças médico-veterinárias. In: Vilela EF and Lúcia MTD (eds) Feromônios de insetos: biologia, química e emprego no manejo de pragas. Editora Holos, Ribeirão Preto, SP, Brasil.
Eiras AE (2002) Armadilha para captura de mosquitos. Brazilian Patent PI0203907, 9 Sept 2002.
Eiras AE and Jepson PC (1991) Host location by *Aedes aegypti* (*Diptera*: *Culicidae*): A wind tunnel study of chemical cues. Bull Entomol Res 81: 151-160.

Eiras AE and Jepson PC (1994) Responses of female *Aedes aegypti* (*Diptera*: *Culicidae*) to host odours and convection currents using an olfactometer bioassay. Bull Entomol Res 84: 207-211.

Eiras AE and Resende MC (2009) Preliminary evaluation of the 'Dengue-MI' technology for *Aedes aegypti* monitoring and control. Cad Saude Publica 25 Suppl 1: S45-58.

Eiras AE and Sant'Ana AL (2002) Atraentes de oviposição para mosquitos. Brazilian Patent PI 0106701-0, Dec 2001.

Eiras AE, Resende MC and Silva IM (2007) Proposta de validação da MosquiTRAP e do sistema de monitoramento (MI-Dengue): uma nova tecnologia para a geração de novos índices de vigilância entomológica para o Programa Nacional de Controle da Dengue (PNCD). Research Report, Fundação Nacional de Saúde (FUNASA), Brasilia, Brasil.

Eiras AE, Sant'Ana AL and Stein K (2001) Identification of volatiles from grass infusions that attract gravid *Aedes aegypti* mosquito. Presentation at the 3rd International Congress of Vetor Ecology, Barcelona, Spain, 16-21 September 2001.

Eiras AE, Silva IM and Resende MC (2005) Proposta de novo método de monitoramento e de novos índices de vigilância entomológica usando MosquiTRAP, uma nova armadilha para a captura de adultos do mosquito *Aedes aegypti*. Research Report, Fundação Nacional de Saúde (FUNASA), Brasilia, Brasil.

Eiras AE, Silva IM, Roque RA, Matosinhos IM and Geier M (2004) Behavioural responses of gravid *Aedes aegypti* (*Diptera*: *Culicidae*) to synthetic oviposition attractants identified from grass infusions volatiles. Presentation at the XXII International Congress of Entomology, Brisbane, Queensland, Australia, 15-21 August 2004.

Englbrecht Ch, Venturelli C, Rose A and Geier M (2009) Continuous trapping of adult Asian tiger mosquitoes (*Aedes albopictus*) with BG-Sentinel traps reduced the human landing rate and density indices in an urban environment in Cesena, Italy. Oral presentation at the 5th European Mosquito Control Association Workshop, Turin, Italy, 9-13 March 2009.

Facchinelli L, Valerio L, Pombi M, Reiter P, Constantini C and Della Torre A (2007) Development of a novel sticky trap for container-breeding mosquitoes and evaluation of its sampling properties to monitor urban populations of *Aedes albopictus*. Med Vet Entomol 21: 183-195.

Favaro AE, Dibo MR, Mondini A, Ferreira AC, Barbosa AAC, Eiras AE, Barata EAMF and Chiaravalloti-Neto F (2006) Physiological state of *Aedes (Stegomyia) aegypti* mosquitoes captured with MosquiTRAPs in Mirassol, São Paulo, Brazil. J Vector Ecol 31: 285-291.

Favaro EA, Mondini A, Dibo MR, Barbosa AAC, Eiras AE, Barata EAMF and Chiaravalloti-Neto F (2008) Assessment of entomological indicators of *Aedes aegypti* (L.) from adult and egg collections in São Paulo, Brazil. J Vector Ecol 33: 8-16.

Fay RW and Eliason DA (1966) A preferred oviposition site as a surveillance method for *Aedes aegypti*. Mosq News 26: 531-535.

Fay RW and Prince WH (1970) A modified visual trap for *Aedes aegypti*. Mosq News 30: 20-23.

Feltner H and Ferrao P (2008) Evaluating efficacy of the BG-Lure attractant using three mosquito trap designs in the city of Alexandria, Virginia. Presenation at the 33rd Annual Conference of the Mid-Atlantic Mosquito Control Association, Baltimore, Maryland, USA, 27-29 Feb 2008. Available at: http://www.bg-sentinel.com/bilder/Feltner_Ferrao-2008-Evaluating_efficacy_of_BG-Lure.pdf.

Focks DA (2003) A review of entomological sampling methods and indicators for dengue vectors. TDR/ IDE/Den/03.1 WHO, Geneva, Switzerland.

Focks DA and Alexander N (2006) Multicountry study of *Aedes aegypti* pupal productivity survey methodology: findings and recommendations. TDR/IRM/Den /06.1 WHO, Geneva.

Focks DA, Daniels E, Haile DG and Keesling JE (1995) A simulation model of the epidemiology of urban dengue fever: literature analysis, model development, preliminary validation, and samples of simulation results. Am J Trop Med Hyg 53: 489-506.

Forattini OP, Kakitani I, Massad E and Marucci D (1995) Studies mosquitoes (*Diptera*: *Culicidae*) and anthropic environment. 9. Synanthropic and epidemiological vector role of *Aedes scapularis* in South-Eastern Brazil. Rev Saúde Pública 29: 199-207.

Gama RA, Silva EM, Silva IM, Resende MC and Eiras AE (2007) Evaluation of the sticky MosquiTRAP™ for detecting *Aedes* (*Stegomyia*) *aegypti* (L.) (*Diptera*: *Culicidae*) during the dry season in Belo Horizonte, Minas Gerais, Brazil. Neotrop Entomol 36: 294-302.

Geier M and Boeckh JA (1999) A new Y-tube olfactometer for mosquitoes to measure the attractiveness of host odours. Entomol Exp Appl 92: 9-19.

Geier M, Bosch JO and Boeckh J (1999) Ammonia as an attractive component of host odour for the yellow fever mosquito, *Aedes aegypti*. Chem Senses 24: 647-653.

Geier M, Bosch JO and Boeckh J (1999) Influence of odour plume structure on upwind flight of mosquitoes towards hosts. J Exp Biology 202: 1639-1648.

Geier M, Rose A and Eiras AE (2004) Insect trap. International Patent WO2004/054358A2, 1 Jul 2004

Gubler DJ (1998) Dengue and dengue hemorrhagic fever. Clin Microbiol Rev 11: 480-496.

Gubler DJ (2004) The changing epidemiology of yellow fever and dengue, 1900 to 2003: full circle? Comp Immun Microbiol Infect Dis 27: 319-330.

Harrison BA, Callahan MC, Watts DM and Panthusiri L (1982) An efficient floating larval trap for sampling *Aedes aegypti* populations (*Diptera*: *Culicidae*). J Med Entomol 19: 722-727.

Holck AR, Meek CL and Holck JC (1988) Attractant enhanced ovitraps for the surveillance of container breeding mosquitoes. J Am Mosq Control Assoc 4: 97-98.

Honorio NA, Codeço CT, Alves FC, Magalhães MAFM and Lourenço-de-Oliveira R (2009) Temporal distribution of *Aedes aegypti* in different districts of Rio de Janeiro, Brazil, measured by two types of traps. J Med Entomol 46: 1001-1014

Hornby JA, Moore DE and Miller TW (1994) *Aedes albopictus*, distribution, abundance, and colonization in Lee County, Florida, and its effect on *Aedes aegypti*. J Am Mosq Control Assoc 10: 397-402.

Hribar LJ, Leprince DJ and Foil LD (1992). Ammonia as an attractant for adult *Hybomitra lasiophthalma* (*Diptera*: Tabanidae). J Med Entomol 29: 346-348.

Hwang YS, Kramer WL and Mulla M (1980) Isolation and identification of oviposition repellents for *Culex* mosquitoes. J Chem Ecol 6: 71-80.

Jones JW, Sithiprasasna R, Schleich S and Coleman RE (2003) Evaluation of selected traps as tools for conducting surveillance for adult *Aedes aegypti* in Thailand. J Am Mosq Control Assoc 19: 148-150.

Kay BH, Sutton KA and Russell BM (2000) A sticky entry-exit trap for sampling mosquitoes in subterranean habitats. J Am Mosq Contr Assoc 16: 262-265.

Kline DL (2002) Evaluation of various models of propane-powered mosquito traps. J Vector Ecol 27: 1-7.

Knols BGJ, Van Loon JJA, Cork A, Robinson RD, Adam W, Meijerink J, De Jong R, Takken W (1997) Behavioural and electrophysiological responses of the female malaria mosquito *Anopheles gambiae s.s.* Giles (*Diptera*: *Culicidae*) towards Limburger cheese volatiles. *Bull Entomol Res.* 87: 151-159.

Knols BGJ, Njiru BNN, Mathenge EM, Mukabana WR, Beier JC and Killeen GF (2002) MalariaSphere: A greenhouse-enclosed simulation of a natural *Anopheles gambiae* (*Diptera*: *Culicidae*) ecosystem in western Kenya. Malaria J 1: 19.

Koenraadt CJM, Jones JW, Sithiprasasna R and Scott TW (2007) Standardizing container classification for immature *Aedes aegypti* surveillance in Kamphaeng Phet, Thailand. J Med Entomol 44: 938-944.

Kröckel U, Rose A, Eiras AE and Geier M (2006) New tools for surveillance of adult yellow fever mosquitoes: Comparison of trap catches with human landing rates in an urban environment. J Am Mosq Control Assoc 22: 229-238.

Krueger A and Hagen RM (2007) Short communication: first record of *Aedes albopictus* in Gabon, Central Africa. Trop Med Int Health 12: 1105-1107.

Kulldorff M, Athas WF, Feurer EJ, Miller BA and Key CR (1998) Evaluating cluster alarms: a space-time scan statistic and brain cancer in Los Alamos, New Mexico. Am J Pub Health 88: 1377-1380.

Kulldorff M, Heffernan R, Hartman J, Assunção R and Mostashari F (2005) A space-time permutation scan statistic for disease outbreak detection. PLoS Med 2: e59.

Kuno G (1997) Factors influencing the transmission of dengue viruses. In: Gubler DJ and Kuno G (eds) Dengue and dengue hemorrhagic fever. CABI, Wallingford, UK, pp 61-88.

Leal WS, Barbosa RMR, Xu W, Ishida Y and Syed Z (2008) Reverse and conventional chemical ecology approaches for the development of oviposition attractants for *Culex* mosquitoes Plos One 3: e3045.

Lourenço-de-Oliveira R, Lima JB, Peres R, Alves FC, Eiras AE and Codeço CT (2008) Comparison of different uses of adult traps and ovitraps for assessing dengue vector infestation in endemic areas. J Am Mosq Control Assoc 24: 387-392.

Lugo E del C, Moreno G, Zachariah MA, López MM, López JD, Delgado MA, Valle SI, Espinoza PM, Salgado MJ, Pérez R, Hammond SN and Harris E (2005) Identification of Aedes albopictus in urban Nicaragua. J Am Mosq Control Assoc 21: 325-327.

Maciel de Freitas R, Codeco CT and Lourenço de Oliveira R (2007) Daily survival rates and dispersal of *Aedes aegypti* females in Rio de Janeiro, Brazil. Am J Trop Med Hyg 76: 659-665.

Maciel de Freitas R, Eiras AE and Lourenço de Oliveira R (2006) Field evaluation of effectiveness of the BG-Sentinel, a new trap for capturing adult *Aedes aegypti* (*Diptera*: *Culicidae*). Mem Inst Oswaldo Cruz 101: 321-325.

Maciel de Freitas R, Eiras AE and Lourenco de Oliveira R (2008) 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 and Lourenco de Oliveira R (2009) Presumed unconstrained dispersal of *Aedes aegypti* in the city of Rio de Janeiro, Brazil. Rev Saúde Pública 43: 8-12.

Maestre-Serrano R, Vergara-Sanchez C, Berrueco-Rodriguez G, Bello-Novoa B and Brochero H (2008) Presencia de *Haemagogus equinus* Theobald, 1903 (*Diptera*: *Culicidae*) en los municipios de Soledad y Malambo en el departamento del Atlántico, Colombia, 1998-2005. Biomédica 28: 99-107.

Marques CCA, Marques GRAM, Brito M, Santos Neto LG, Ishibashi VC and Gomes FA (1993) Estudo comparativo de eficácia de larvitrampas e ovitrampas para vigilância de vetores de dengue e febre amarela. Rev Saúde Pública 27: 237-241.

Mboera LEG, Takken W, Mdira KY and Pickett JA (2000) Sampling gravid *Culex quinquefasciatus* (*Diptera*: *Culicidae*) in Tanzania with traps baited with synthetic oviposition pheromone and grass infusions. J Med Entomol 37: 172-176.

Meeraus W, Johnson J and Arias JR (2007) Field comparison of novel and industrial standard traps for collecting *Aedes albopictus* in northern Virginia. Poster presentation at the 73rd Annual Meeting of American Mosquito Control Association, Orlando, Florida, USA, 1-5 April 2007.

Meeraus WH, Armistead JS and Arias JR (2008) Field comparison of novel and gold standard traps for collecting *Aedes albopictus* in northern Virginia. J Am Mosq Control Assoc 24: 244-248.

Méndez F, Barreto M, Arias JF, Rengifo G, Muñoz J, Burbano ME and Parra B. (2006) Human and mosquito infections by dengue viruses during and after epidemics in a dengue-endemic region of Colombia. Am J Trop Med Hyg. 74(4): 678-83.

Metha DR (1934) Effect of saline and free ammonia on the oviposition of *Anopheles culicifacies* and *Anopheles subpiscus* (Rossi). Rev Mal Surv India 4: 411-420.

Micieli MV and Campos RE (2003) Oviposition activity and seasonal pattern of a population of *Aedes (Stegomyia) aegypti* (L.) (*Diptera*: *Culicidae*) in subtropical Argentina. Mem Inst Oswaldo Cruz 98: 659-663.

Millar JG, Chaney JD and Mulla MS (1992) Identification of oviposition attractants for *Culex quinquefasciatus* from fermented Bermuda grass infusions. J Am Mosq Control Assoc 8: 11-17.

Morrison AC, Gray K, Getis A, Astete A, Sihuincha M, Focks D, Watts D, Stancil JD, Olson JG, Blair P and Scott TW (2004) Temporal and geographic patterns of *Aedes aegypti* (*Diptera*: *Culicidae*) production in Iquitos, Peru. J Med Entomol 41: 1123-1142.

Morrison AC, Zielinski-Gutierrez E, Scott TW and Rosenberg R (2008) Defining challenges and proposing solutions for control of the virus vector *Aedes aegypti*. PLoS Med 5: 0362-0366.

Mota RN (2003) Construção de um olfatômetro de dupla escolha para estudos de orientação de fêmeas grávidas de *Aedes aegypti* (Linnaeus, 1792) (*Diptera*: *Culicidae*) à atraentes de oviposição. MSc Dissertation, Universidade Federal de Minas Gerais, Brazil.

Ministério da Saúde (MS) (2007a) Graphic showing the historical series of reported dengue cases between 1980 and 2005. Available at: http://portal.saude.gov.br/portal/arquivos/ pdf/Graficos_DNC_dengue_verde.pdf.

Ministério da Saúde (MS) (2007b) Graphic showing the historical series of mortality due to dengue cases between 1980 and 2005. Available at: http: //portal.saude.gov.br/portal/arquivos/pdf/Graficos_DNC_dengue_vermelho.pdf.

Muir LE and Kay BH (1998) *Aedes aegypti* survival and dispersal estimated by mark-release-recapture in Northern Australia. Am J Trop Med Hyg 58: 277-282.

Müller W.(1968). Die Distanz- und Kontaktorientierung der Stechmücken *Aedes aegypti* (Wirtsfindung, Stechverhalten und Blutmahlzeit). Z vergl Physiol 58: 241-303.

Nasci RS (1981) A light weight battery-powered aspirator for collecting mosquitoes in the field. Mosq News 41: 808-811.

Obermayr R (2006) Are new trapping technologies useful for mosquito control interventions? Vector Ecol Newsl 37 (3): 11-12.

Ordonez JG, Fernandez Salas I and Flores-Leal A (1997) Monitoring dispersal of marked *Aedes aegypti* females under field conditions using sticky ovitraps in Monterrey, northeastern Mexico. J Am Mosq Assoc 13: 121.

Ordóñez-Gonzalez JG, Mercado-Hernandez R, Flores-Suarez AE and Fernández-Salas I (2001) The use of sticky ovitraps to estimate dispersal of *Aedes aegypti* in Northeastern Mexico. J Am Mosq Contr Assoc 17: 93-97.

PNCD (2002) Programa Nacional de Controle da Dengue. Ministério da Saúde Fundação Nacional de Saúde, Brasília, Brasil. Available at: http://bvsms.saude.gov.br/bvs/publicacoes/pncd_2002.pdf.

Public Health Agency of Canada (PHAC) (2009) Notifiable diseases on-line. Available at: http://dsol-smed.phac-aspc.gc.ca/dsol-smed/ndis/index-eng.php.

Ponnusamy L, Xu N, Nojima S, Wesson DM, Schal C and Apperson CA (2008) Identification of bacteria and bacteria-associated chemical cues that mediate oviposition site preferences by *Aedes aegypti*. PNAS 105: 9262-9267.

Rawlins SC, Martinez R, Wiltshire S and Legall G (1998) A comparison of surveillance systems for the dengue vector *Aedes aegypti* in port of Spain, Trinidad. J Am Mosq Control Assoc 14: 131-136.

Regis L, Monteiro AM, Varjal de Melo-Santo MA, Silveira Jr JC, Freire Furtado A, Veiga Aciolis R, Santo GM, Nkazawa M, Sa Carvalho M, Ribeiro Jr PJ, Viera de Souza W (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.

Reiter P (1983) A portable, battery-powered trap for collecting gravid *Culex* mosquitoes. Mosq News 4: 496-498.

Reiter P (1986) A standardized procedure for the quantitative surveillance of certain *Culex* mosquitoes by egg raft collection. J Am Mosq Control Assoc 3: 494-501.

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

Reiter P, Nathan MB (2001) Guidelines for assessing the efficacy of insecticide space sprays for the control of the Dengue vector *Aedes aegypti*. WHO/CDS/CPE/PVC/2001.1, WHO, Geneva, Switzerland.

Ritchie SA, Long S, Hart A, Webb CE and Russell RC (2003) An adulticidal sticky ovitrap for sampling container-breeding mosquitoes. J Am Mosq Control Assoc 19: 235-242.

Ritchie SA (1984) Hay infusion and isopropyl alcohol-baited CDC light trap; a simple effective trap for gravid *Culex* mosquitoes. Mosq News 44: 404-407.

Ritchie SA, Moore P, Carruthers M (2006) Discovery of a widespread infestation of *Aedes albopictus* in the Torres Strait, Australia. J Am Mosq Control Assoc 22: 358-365.

Ritchie SA, Long S, Smith G, Pike A and Knox TB (2004) Entomological investigations in a focus of dengue transmission in Cairns, Queesland, Australia, by using the sticky ovitraps. J Med Entomol 41: 1-4.

Roque RA (2007) Formulação e avaliação de atraentes sintéticos de oviposição, identificados em infusões do capim colonião (*Panicum maximum*) para fêmeas grávidas do mosquito *Aedes* (*Stegomyia*) *aegypti* (Linnaeus, 1762) (*Diptera*: *Culicidae*) em condições de campo e semi-campo. Dissertation, Universidade Federal de Minas Gerais, Brazil.

Roque RA and Eiras AE (2008) Calibration and evaluation of field cage for oviposition study with *Aedes (Stegomyia) aegypti* female (L.) (*Diptera*: *Culicidae*). Neotrop Entomol 37: 478-485.

Rössler HP (1961) Versuche zur geruchlichen Anlockung weiblicher Stechmücken (*Aedes aegypti* L. (*Culicidae*). Z Vgl Physiol 44: 184-231.

Russell RC (2004) The relative attractiveness of carbon dioxide and octenol in CDC- and EVS-type light traps for sampling the mosquitoes *Aedes aegypti* (L.), *Aedes polynesiensis* Marks, and *Culex quinquefasciatus* Say in Moorea, French Polynesia. J Vector Ecol 29: 309-314.

Russel RC, Webb CE, Williams CR and Ritchie SA (2005) Mark-release-recapture study to measure dispersal of the mosquito *Aedes aegypti* in Cairns, Queensland, Australia. Med Vet Entomol 19: 1-7.

Russell RC and Ritchie SA (2004) Surveillance and behavioral investigations of *Aedes aegypti* and *Aedes polynesisensis* in Moorea, French Polynesia, using a sticky ovitrap. J Am Mosq Control Assoc 20: 370-375.

Sant'Ana AL (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. Dissertation, Universidade Federal de Minas Gerais, Minas Gerais, Brazil.
Sant'Ana AL, Roque RA and Eiras AE (2006) Characteristics of grass infusions as oviposition attractants to *Aedes* (*Stegomyia*) (*Diptera*: *Culicidae*). J Med Entomol 43: 214-20.
Service MW (1993) Mosquito ecology: field sampling methods. Second edition. Chapman & Hall, London, UK.
Schoeler GB, Schleich SS, Manweiler SA and Sifuentes VA (2004) Evaluation of surveillance devices for monitoring *Aedes aegypti* in an urban area of northeastern Peru. J Am Mosq Control Assoc 20: 6-11.
Schmied WH, Takken W, Killen GF, Knols BGJ and Smallegange (2008) Evaluation of two counterflow traps for testing behaviour-mediating compounds for the malaria vector *Anopheles gambiae s.s.* under semi-field conditions in Tanzania. Malaria J 7: 230.
Scott TW and Morrison AC (2002) *Aedes aegypti* density and the risk of dengue-virus transmission. In: Takken W and Scott TW (eds) Ecological aspects for application of genetically modified mosquitoes. Proceedings of the Frontis workshop on ecological challenges concerning the use of genetically modified mosquitoes for disease control. Wageningen, the Netherlands 26-29 June 2002. Wageningen UR Frontis Series, pp 187-206.
Silver JB 2008. Mosquito ecology: field sampling methods. 3rd edition. Springer Co., New York, USA.
Sithprasasna R, Linthicum KJ, Lerdthusnee K and Brewer TG (1997) Use of Geographical information system to study the epidemiology of dengue haemorrhagic fever in Thailand. Dengue Bull 21: 68-72.
Smith CN, Smith N, Gouck HK, Weidhaas DE, Gilbert IH, Mayer MS, Smittle BJ and Hofbauer A (1970) L-Lactic acid as a factor in the attraction of *Aedes aegypti* (*Diptera*: *Culicidae*) to human hosts. Ann Entomol Soc Am 7: 99-117.
Solberg VB, Sithiprasasna R and Fansiri T (2007) Efficacy testing of eight unique or standard mosquito surveillance traps/ methods in Thailand mouses. Presentation at the ESA Annual Meeting, San Diego, California, USA, 9-12 Dec 2007.
Suárez Ramírez N and Colás Bonne M (2008) Afectación de la sensibilidad del sistema de vigilancia entomológica mediante larvitrampas en un área de salud. Medisan 12(4). available at: http://bvs.sld.cu/revistas/san/vol12_4_08/san11408.htm.
Takken W and Mboera LEG (2000) Effects of chemical stimuli on oviposition of *Culex quinquefasciatus* (*Diptera*: *Culicidae*) in Tanzania. Proc Exp Appl Entomol 11: 182-187.
Taneja J. and Guerin PM (1997) Ammonia attracts the haematophagus bug *Triatoma infestans*: behavioural and neurophysiological data on nymphs. J Comp Physiol 181: 21-34.
Trexler JD, Apperson CS, Gemeno C, Perich MJ, Carlson D and Schal C (2003) Field and laboratory evaluations of potential oviposition attractants for *Aedes albopictus* (*Diptera*: *Culicidae*). J Am Mosq Control Assoc 19: 228-34.
Tsuda Y, Suwonkerd W, Chawprom S, Prajakwong S and Takagi M. (2006) Different spatial distribution of *Aedes aegypti* and Aedes albopictus along an urban-rural gradient and the relating environmental factors examined in three villages in northern Thailand. J Am Mosq Control Assoc 22: 222-228.
WHO (1999) Strengthening implementation of the global strategy for dengue fever/ dengue haemorrhagic fever prevention and control. Report of the Informal Consultation. WHO, Geneva, Switzerland.
WHO (2009) Dengue and dengue hemorrhagic fever. Available at: http://www.who.int/csr/disease/dengue/impact/en/.
Williams CR, Long SA, Russel RC and Ritchie SA (2006) Field efficacy of the BG-Sentinel compared with the CDC Backpack Aspirator and CO2-baited EVS trap for collection of adult *Aedes aegypti* in Cairns, Queensland, Australia. J Am Mosq Control Assoc 22: 296- 300.
Williams CR, Long SA, Webb CE, Bitzhenner M, Geier M, Russel RC and Ritchie SA (2007) *Aedes aegypti* population sampling using BG-Sentinel traps in north Queensland Australia: statistical considerations for trap deployment and sampling strategy. J Med Entomol 44: 345-350.
Wilton DP (1985) Preliminary evaluation of a black cylinder suction trap for *Aedes aegypti* and *Culex quinquefasciatus* (*Diptera*: *Culicidae*). J Med Entomol 22: 113-114.