Spatial and temporal country-wide survey of temephos resistance in Brazilian populations of Aedes aegypti

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The organophosphate temephos has been the main insecticide used against larvae of the dengue and yellow fever mosquito (Aedes aegypti) in Brazil since the mid-1980s. Reports of resistance date back to 1995; however, no systematic reports of widespread temephos resistance have occurred to date. As resistance investigation is paramount for strategic decision-making by health officials, our objective here was to investigate the spatial and temporal spread of temephos resistance in Ae. aegypti in Brazil for the last 12 years using discriminating temephos concentrations and the bioassay protocols of the World Health Organization. The mortality results obtained were subjected to spatial analysis for distance interpolation using semi-variance models to generate maps that depict the spread of temephos resistance in Brazil since 1999. The problem has been expanding. Since 2002-2003, approximately half the country has exhibited mosquito populations resistant to temephos. The frequency of temephos resistance and, likely, control failures, which start when the insecticide mortality level drops below 80%, has increased even further since 2004. Few parts of Brazil are able to achieve the target 80% efficacy threshold by 2010/2011, resulting in a significant risk of control failure by temephos in most of the country. The widespread resistance to temephos in Brazilian Ae. aegypti populations greatly compromise effective mosquito control efforts using this insecticide and indicates the urgent need to identify alternative insecticides aided by the preventive elimination of potential mosquito breeding sites.

Key words: insecticide resistance survey - dengue - distance interpolation - distribution maps - mosquito larvae

Vector-borne neglected (tropical) diseases such as dengue are an increasing worldwide issue of concern, particularly given current rates of urbanisation, international travel and trade, and climate change, all of which favor the spread of such diseases and their vectors (Hsieh & Chen 2009, Guzman et al. 2010, Gubler 2012). Mass gatherings and large sporting events are also associated with higher risks of health incidents. The 2014 FIFA World Cup held in Brazil is an example that drew attention and incited debate that focused particularly on dengue due to potential vector outbreaks (Hay 2013). The concern is understandable and justifiable, even if the risks were generally small (Lowe et al. 2014, van Panhuis et al. 2014). As a result, no serious incident came to past. The 2016 Olympic Games to be held in Rio de Janeiro are bound to draw a similar level of international attention.

The lack of effective vaccines or pharmaceutical treatments for dengue, typical of the neglected diseases, places mosquito vector control in the forefront of prevention efforts for this disease (Gubler 2004, Halstead 2012). This scenario prevails throughout the affected tropical and subtropical regions of the world, which roughly encompasses about half of the global population (Guzman et al. 2010). Control of the dengue mosquito vector [Aedes aegypti (L.)], which also transmits chikungunya, zika and yellow fever (YF) (thus the common name “yellow fever mosquito”), relies heavily on insecticide use - but there are few compounds available and their use is usually guided by the countries’ health officials (OPAS 1995, Funasa 2001, 2002, Braga & Valle 2007, Araujo et al. 2013, Macoris et al. 2014, Tomé et al. 2014).

The organophosphate temephos is globally the most commonly used insecticide against mosquito larvae due to its high efficacy, low cost and low vertebrate toxic-
ity (WHO 2009). The result of this overreliance on temephos in controlling YF mosquito larvae is evolution and spread of temephos resistance among populations of this pest species. Such resistance has been detected in various countries since 1995 (Macoris et al. 1995, Mazzari & Georghiou 1995, Rawlins & Wan 1995, Bisset Lazcano et al. 2009, Melo-Santos et al. 2010, Bisset et al. 2013, Giresales et al. 2013). Furthermore, the use of temephos for the control of larvae of Ae. aegypti also apparently led to incidental selection for temephos resistance in co-occurring mosquito species populations (Campos & Andrade 2003, Alves et al. 2011, Phlophiro et al. 2011, Amorim et al. 2013), as has also been reported among other co-occurring arthropod pest species (Guedes et al. 2016).

Routine applications of temephos against mosquito larvae in Brazil began in the 1980s (Funasa 1994, 2001, Sucen 1997). The initial suppression of Ae. aegypti in Brazil by 1955 was followed by its subsequent return in the 1970s (Schatzmayr 2000, Lourenço-de-Oliveira et al. 2004). Dengue became endemic in the country and has become an increasingly serious problem since 1986 despite established vector control programs in the country that still continue today (Lourenço-de-Oliveira et al. 2004, Maciel-de-Freitas et al. 2014). By the 1990s, concern emerged in Brazil regarding likely control failures and detection of temephos-resistant mosquito populations, which led to systematic surveys of insecticide resistance in the country and a series of reports on the phenomenon (Macoris et al. 1995, 2003, 2007, 2014, Campos & Andrade 2003, Lima et al. 2003, 2006, Melo-Santos et al. 2010, Gambarra et al. 2013, Diniz et al. 2014).

A few studies on the underlying mechanisms of temephos resistance followed the initial detection of this phenomenon in Brazil. Despite of an initial report of altered (acetylcholinesterase) target site sensitivity detected in a Brazilian population of Ae. aegypti resistant to temephos from Uberlândia (MG), current evidence suggests the prevalence of enhanced detoxification by metabolising enzymes in an apparently mixed pattern (Braga & Valle 2007, Melo-Santos et al. 2010, Lima et al. 2011, Gambarra et al. 2013). Congruent findings have been reported from other countries as well (Bisset Lazcano et al. 2009, Bisset et al. 2013, Giresales et al. 2013). Furthermore, recent transcriptome (i.e., the set of all mRNA molecules from a cell) evidence indicates upregulation of detoxification enzymes in insecticide-resistant mosquitoes (Reyes-Solis et al. 2014, Saavedra-Rodriguez et al. 2014). These findings reinforce the perception that multiple metabolic genes are involved in temephos resistance in Ae. aegypti, but with the prevalence of esterase rather than glutathione-S-transferase gene expression (Reyes-Solis et al. 2014, Saavedra-Rodriguez et al. 2014).

Temephos resistance monitoring in populations of the YF mosquito were underway in Brazil by the late 1990s in response to the increasing incidence of dengue in the country (Braga & Valle 2007). Scientific reports of the incidence of temephos resistance have increased since then (Campos & Andrade 2003, Lima et al. 2003, 2006, Macoris et al. 2003, 2007, Melo-Santos et al. 2010, Gambarra et al. 2013, Diniz et al. 2014), but no comprehensive dataset is currently available and no area-wide description of the phenomenon of temephos resistance and its spread has been attempted despite the strategic importance of such information in guiding control policies, protocols and decision-making by Brazilian health officials. The current effort took advantage of the dataset gathered by the National Network of Insecticide Resistance Monitoring (MoReNAA) in Ae. aegypti under the tutelage of the National Program of Dengue Control from the Office of Health Surveillance of the Brazilian Ministry of Health (Brasília, DF, Brazil). The objective of our study was to recognise the spatial and temporal spread of temephos resistance in Brazil for the past 12 years, which we hypothesized, has been acute and has likely encompassed the entire country since 2010.

Our spatial and temporal survey of temephos resistance was performed using standardised procedures for insect sampling and temephos bioassays from the WHO (1981) that were countersigned by the laboratories involved (from MoReNAA) with the support of the Centers for Disease Control and Prevention (CCD, USA), Pan-American Health Organization and the World Health Organization (Braga et al. 2004, Macoris et al. 2005, Braga & Valle 2007). The data obtained was subjected to kriging to select suitable semivariogram models for distance interpolation with the goal of generating geospatial maps of the frequency of temephos resistance in Brazilian populations of Ae. aegypti.

**MATERIALS AND METHODS**

**Insects and insecticide** - Mosquito populations were sampled through the MoReNAA (Table I, Fig. 1) as described by Macoris et al. (2003). Briefly, between 100-200 oviposition traps (i.e., ovitraps) were used for this purpose in each city. The ovitraps were placed outdoors in a grid pattern for four weeks, always in the second semester of each year (Fay & Eliason 1966, Jakob & Bevier 1969, Funasa 1999). Egg clutches thus collected were used to establish laboratory colonies of over 3,000 individuals from each city (i.e., sampling site). First-generation larvae raised in the laboratory were used in the bioassays (Lima et al. 2003, Macoris et al. 2003). Technical grade temephos (>90% pure) was obtained from the Brazilian Ministry of Health and diluted with acetone at the desired concentration for subsequent use in the diagnostic bioassays.

**Diagnostic bioassays of temephos resistance** - The diagnostic bioassays were performed following the standardised procedures of the WHO (1981, 1992). The concentration of temephos required to identify resistant insects (i.e., the diagnostic concentration) was initially established as 14.0 µg a.i./L. but was subjected to yearly calibration and validation with the standard susceptible Rockefeller strain, as described by Braga et al. (2004) and Macoris et al. (2005). The diagnostic concentration was applied as a 1 mL solution to each of the experimental containers, reaching a final 250 mL volume of contaminated water solution (except for the controls, for which only 1 mL acetone was used). Deionised and distilled water were used to prepare the bioassay solutions. Twenty-five individuals (3rd-4th instar mosquito larvae) were placed in 250 mL transparent glass contain-
TABLE I  
Sample site identification and geographical coordinates of collection sites for populations of the yellow fever mosquito *Aedes aegypti* used in the spatio-temporal survey of temephos resistance in Brazil

| Region       | State                  | City          | Longitude     | Latitude     |
|--------------|------------------------|---------------|---------------|--------------|
| North        | Rondônia (RO)          | Cacoal        | -61,447222    | -11,438611   |
| North        | Rondônia (RO)          | Guajará-Mirim | -65,339444    | -10,782778   |
| North        | Rondônia (RO)          | Porto Velho   | -63,903889    | -8,761944    |
| North        | Rondônia (RO)          | Jaru          | -62,466389    | -10,438889   |
| North        | Rondônia (RO)          | Vilhena       | -60,145833    | -12,740556   |
| North        | Acre (AC)              | Rio Branco    | -67,810000    | -9,974722    |
| North        | Amazonas (AM)          | Manaus        | -60,025000    | -3,101944    |
| North        | Roraima (RR)           | Boa Vista     | -60,673333    | 2,819722     |
| North        | Pará (PA)              | Ananindeua    | -48,372222    | -1,365556    |
| North        | Pará (PA)              | Belém         | -48,504444    | -1,455833    |
| North        | Pará (PA)              | Benevides     | -48,244722    | -1,361389    |
| North        | Pará (PA)              | Dom Elizeu    | -47,505000    | -4,285000    |
| North        | Pará (PA)              | Marabá        | -49,117778    | -5,386611    |
| North        | Pará (PA)              | Marituba      | -48,341944    | -1,355278    |
| North        | Pará (PA)              | Rondon do Pará| -48,067222    | -4,776111    |
| North        | Pará (PA)              | Sta. Bárbara do Pará | -48,294444 | -1,223611 |
| North        | Pará (PA)              | Santarém      | -54,708333    | -2,443056    |
| North        | Pará (PA)              | Tucuruí       | -49,672500    | -3,766111    |
| North        | Amapá (AP)             | Macapá        | -51,066389    | 0,038889     |
| North        | Tocantins (TO)         | Araguaina     | -48,207222    | -7,911111    |
| North        | Tocantins (TO)         | Palmas        | -48,360278    | -10,127778   |
| Northeast    | Maranhão (MA)          | Bacabal       | -44,791667    | -4,291667    |
| Northeast    | Maranhão (MA)          | São Luís      | -44,302778    | -5,259722    |
| Northeast    | Piauí (PI)             | Parnaíba      | -41,776667    | -2,904722    |
| Northeast    | Piauí (PI)             | Teresina      | -42,801944    | -5,089167    |
| Northeast    | Ceará (CE)             | Caucaia       | -38,653056    | -3,736111    |
| Northeast    | Ceará (CE)             | Fortaleza     | -38,543056    | -3,717222    |
| Northeast    | Ceará (CE)             | Juazeiro do Norte | -39,315278 | -7,213056 |
| Northeast    | Rio Grande do Norte (RN)| Caicó      | -37,097778    | -6,458333    |
| Northeast    | Rio Grande do Norte (RN)| Jardim do Seridó | -36,774444 | -6,584444   |
| Northeast    | Rio Grande do Norte (RN)| Parnamirim   | -35,262778    | -5,915556    |
| Northeast    | Rio Grande do Norte (RN)| Mossoró     | -37,344167    | -5,187500    |
| Northeast    | Rio Grande do Norte (RN)| Natal       | -35,209444    | -5,795000    |
| Northeast    | Rio Grande do Norte (RN)| Pau dos Ferros | -38,204444 | -6,109167  |
| Northeast    | Paraíba (PB)           | Alagoa Grande | -35,630000 | -7,158333   |
| Northeast    | Paraíba (PB)           | Bayeux        | -34,932222    | -7,125000    |
| Northeast    | Paraíba (PB)           | João Pessoa   | -34,863056    | -7,115000    |
| Northeast    | Paraíba (PB)           | Santa Rita    | -34,978056    | -7,113889    |
| Northeast    | Paraíba (PB)           | Souza         | -38,228056    | -6,759167    |
| Northeast    | Pernambuco (PE)        | Araripina     | -40,498333    | -7,576111    |
| Northeast    | Pernambuco (PE)        | Cabo de Sto Agostinho | -35,035000 | -8,286667   |
| Northeast    | Pernambuco (PE)        | Jaboatão dos Guararapes | -35,014722 | -8,112778  |
| Northeast    | Pernambuco (PE)        | Moreno        | -35,092222    | -8,118611    |
| Northeast    | Pernambuco (PE)        | Olinda        | -34,855278    | -8,080889    |
| Northeast    | Pernambuco (PE)        | Petrolina     | -40,500833    | -9,398611    |
| Northeast    | Pernambuco (PE)        | Recife        | -34,881111    | -8,053889    |
| Northeast    | Pernambuco (PE)        | Tamandaré     | -35,104722    | -8,759722    |
| Northeast    | Alagoas (AL)           | Arapiraca     | -36,661111    | -9,752500    |
| Northeast    | Alagoas (AL)           | Maceió        | -35,735278    | -9,665833    |
| Northeast    | Sergipe (SE)           | Aracaju       | -37,071667    | -10,911111   |
| Region      | State            | City            | Longitude | Latitude   |
|-------------|------------------|-----------------|-----------|------------|
| Northeast   | Sergipe (SE)     | Barra dos Coqueiros | -37.038611 | -10.908889 |
| Northeast   | Sergipe (SE)     | Iubaiana        | -37.425278 | -10.685000 |
| Northeast   | Bahia (BA)       | Barreiras       | -44.990000 | -12.152778 |
| Northeast   | Bahia (BA)       | Eunápolis       | -39.580278 | -16.377500 |
| Northeast   | Bahia (BA)       | Feira de Santana | -38.966667 | -12.266667 |
| Northeast   | Bahia (BA)       | Ilhéus          | -39.049444 | -14.788889 |
| Northeast   | Bahia (BA)       | Itabuna         | -39.280278 | -14.785556 |
| Northeast   | Bahia (BA)       | Jacobina        | -40.518333 | -11.180556 |
| Northeast   | Bahia (BA)       | Jequié          | -40.083611 | -13.857500 |
| Northeast   | Bahia (BA)       | Potiguará       | -39.876667 | -15.597222 |
| Northeast   | Bahia (BA)       | Salvador        | -38.510833 | -12.971111 |
| Northeast   | Bahia (BA)       | Teixeira de Freitas | -39.741944 | -17.535000 |
| Northeast   | Bahia (BA)       | Vitória da Conquista | -40.839444 | -14.866111 |
| Midwest     | Mato Grosso do Sul (MS) | Campo Grande     | -54.646389 | -20.442778 |
| Midwest     | Mato Grosso do Sul (MS) | Corumbá          | -57.653333 | -19.009167 |
| Midwest     | Mato Grosso do Sul (MS) | Coxim           | -54.760000 | -18.506667 |
| Midwest     | Mato Grosso do Sul (MS) | Três Lagoas     | -51.678333 | -20.751111 |
| Midwest     | Mato Grosso do Sul (MS) | Ponta Porã      | -55.725556 | -22.536111 |
| Midwest     | Mato Grosso do Sul (MS) | Dourados        | -54.805556 | -22.211111 |
| Midwest     | Mato Grosso (MT)  | Cuiabá          | -56.096667 | -15.596111 |
| Midwest     | Mato Grosso (MT)  | Várzea Grande   | -56.132500 | -15.646667 |
| Midwest     | Goiás (GO)       | Aparecida de Goiânia | -49.243889 | -16.823333 |
| Midwest     | Goiás (GO)       | Goiânia         | -49.253889 | -16.678611 |
| Midwest     | Goiás (GO)       | Itumbiara       | -49.215278 | -18.419167 |
| Midwest     | Goiás (GO)       | Luziânia        | -47.950278 | -16.252500 |
| Midwest     | Goiás (GO)       | Novo Gama       | -48.039444 | -16.059167 |
| Midwest     | Goiás (GO)       | Rio Verde       | -50.928056 | -17.798056 |
| Midwest     | Goiás (GO)       | Uruaçu          | -49.140833 | -14.524722 |
| Midwest     | Distrito Federal (DF) | Brasília        | -47.929722 | -15.779722 |
| Southeast   | Minas Gerais (MG) | Belo Horizonte  | -43.937778 | -19.920833 |
| Southeast   | Minas Gerais (MG) | Formiga         | -45.426389 | -20.464444 |
| Southeast   | Minas Gerais (MG) | Januária        | -44.361667 | -15.488056 |
| Southeast   | Minas Gerais (MG) | Montes Claros   | -43.861667 | -16.735000 |
| Southeast   | Minas Gerais (MG) | Teófilo Otoni   | -41.505278 | -17.857500 |
| Southeast   | Minas Gerais (MG) | Ubá             | -42.942778 | -21.120000 |
| Southeast   | Minas Gerais (MG) | Uberaba         | -47.939444 | -19.748333 |
| Southeast   | Minas Gerais (MG) | Uberlândia      | -48.277222 | -18.918611 |
| Southeast   | Espírito Santo (ES) | Cach. de Itapemirim | -41.112778 | -20.848889 |
| Southeast   | Espírito Santo (ES) | Cariacica      | -40.420000 | -20.263889 |
| Southeast   | Espírito Santo (ES) | Colatina       | -40.630556 | -19.539444 |
| Southeast   | Espírito Santo (ES) | Serra          | -40.307778 | -20.128611 |
| Southeast   | Espírito Santo (ES) | Viana          | -40.496111 | -20.390278 |
| Southeast   | Espírito Santo (ES) | Vila Velha     | -40.292500 | -20.329722 |
| Southeast   | Espírito Santo (ES) | Vitória        | -40.337778 | -20.319444 |
| Southeast   | Rio de Janeiro (RJ) | Cabo Frio      | -42.018611 | -22.879444 |
| Southeast   | Rio de Janeiro (RJ) | C. dos Goytacazes | -41.324444 | -21.754167 |
| Southeast   | Rio de Janeiro (RJ) | Duque de Caxias | -43.311667 | -22.785556 |
| Southeast   | Rio de Janeiro (RJ) | Iaperuna       | -41.887778 | -21.205000 |
| Southeast   | Rio de Janeiro (RJ) | Niterói        | -43.103611 | -22.883333 |
| Southeast   | Rio de Janeiro (RJ) | Nova Iguacu    | -43.451111 | -22.759167 |
| Southeast   | Rio de Janeiro (RJ) | Rio de Janeiro  | -43.207500 | -22.902778 |
| Southeast   | Rio de Janeiro (RJ) | São Gonçalo    | -43.053889 | -22.826944 |
| Southeast   | Rio de Janeiro (RJ) | São João de Meriti | -43.372222 | -22.803889 |
ers containing temephos-contaminated water (except in the control treatments) and four replicates were used for each locally collected population. Mortality assessment of the mosquito larvae was performed after 24 h exposure. The larvae were considered dead if they were unable to rise to the surface when dorsally prodded.

Geostatistical analyses - These analyses were based on the geographical coordinates of each mosquito sampling site from which the mosquito populations were obtained and used to calculate the distance between sampling sites. The distances from the sampling sites and the mortality data obtained from the diagnostic bioassays were subjected to alternative kriging methods (stable, circular, spherical, exponential and Gaussian) to select suitable semivariogram functions for distance interpolation (Isaacs & Srivastava 1989). The semivariogram functions obtained using each group of models allowed the estimation of three parameters to determine their respective shapes: range ($h_r$), partial sill ($C$), and nugget ($C_0$). The range ($h_r$) and partial sill ($C$) refer to the point in the semivariogram function in which a plateau
RESULTS

General temephos mortality findings - The diagnostic bioassays assessing mosquito larval mortality by temephos were performed to estimate the frequency of temephos-resistant individuals in the sampled insect populations. This frequency of resistant individuals is indicated as an average mortality score ranging from 80.31% between 1999-2000 and dropping to less than 50% between 2010-2011 (Table II). The number of insect samples tested per year ranged from 25 (from 2010-2011) to 74 (between 2000-2001) and had a broad range of mortality response within each year, resulting in a high standard deviation of larval mortality per year (Table III).

Semivariogram model selection - Suitable semivariogram models were obtained for each biannual dataset of temephos mortality using the diagnostic insecticide resistance bioassays. The selected semivariogram models are exhibited in Table III, along with their respective parameters for model selection. The plots from each model and the respective observed data are exhibited in Fig. 2.

| Year         | Sampling sites (n) | Minimum | Maximum | Mean  | SD    | Skewness (g) | Kurtosis (g) |
|--------------|--------------------|---------|---------|-------|-------|--------------|--------------|
| 1999-2000    | 64                 | 13.15   | 100.00  | 80.31 | 24.62 | -1.22        | 3.40         |
| 2000-2001    | 74                 | 10.80   | 100.00  | 71.53 | 26.34 | -0.68        | 2.38         |
| 2002-2003    | 58                 | 2.00    | 99.80   | 62.48 | 30.16 | -0.51        | 2.08         |
| 2004-2005    | 59                 | 1.50    | 98.45   | 53.41 | 33.69 | -0.18        | 1.39         |
| 2006-2007    | 39                 | 6.40    | 97.60   | 52.33 | 24.48 | -0.16        | 1.97         |
| 2008-2009    | 46                 | 6.00    | 96.70   | 50.60 | 24.99 | 0.05         | 1.82         |
| 2010-2011    | 25                 | 7.50    | 88.20   | 49.99 | 28.16 | -0.12        | 1.55         |

SD: standard deviation

| Year         | Kriging | Model      | Nugget (C₀) | Partial sill (C) | Sill (C₀+C) | Proportion (C/C+C₀) | Range (hr, m) | Randomness (C₀/C) | Mean errors |
|--------------|---------|------------|--------------|------------------|-------------|---------------------|---------------|-------------------|-------------|
| 1999-2000    | Ordinary| Gaussian   | 132.963      | 639.079          | 772.042     | 0.827778            | 593820.368    | 0.208054         | -0.027      |
| 2000-2001    | Simple  | Gaussian   | 231.740      | 640.182          | 871.922     | 0.734219            | 632424.376    | 0.361991         | -0.059      |
| 2002-2003    | Simple  | Exponential| 391.601      | 972.709          | 1364.31     | 0.712968            | 3658678.194   | 0.402588         | -0.203      |
| 2004-2005    | Ordinary| Gaussian   | 224.524      | 176.033          | 400.557     | 0.439471            | 695175.201    | 1.275465         | 0.101       |
| 2006-2007    | Ordinary| Exponential| 162.384      | 669.389          | 831.773     | 0.804774            | 1175553.465   | 0.242585         | -0.096      |
| 2008-2009    | Ordinary| Circular   | 57.218       | 723.989          | 781.207     | 0.926757            | 947927.124    | 0.079032         | 0.266       |
| 2010-2011    | Ordinary| Circular   | 367.832      | 262.731          | 630.563     | 0.416661            | 507101.080    | 0.714269         | 1.576       |
Fig. 2: semivariogram models [mortality semivariance (y) as a function of distance (x)] exhibited in Table II and obtained from the diagnostic bioassays of temephos resistance on larvae of the yellow fever mosquito *Aedes aegypti*. Observed points are represented as red symbols, and averages are represented as blue crosses.

**Temporal spread of temephos resistance** - Spatial interpolation using kriging allowed mapping the country-wide spread of temephos resistance in larvae of YF mosquitoes from 1999-2000 until 2010-2011, which is the last year the survey data were available. Initially the efficacy of temephos was high, causing larval mortality of > 80% throughout Brazil, except in the coastal area, which spans from Pará in the north to Piauí in the northeast and encompasses the state of Rio de Janeiro and neighboring parts of São Paulo and Minas Gerais (Fig. 3). However, the frequency of temephos resistant individuals in the insect populations increased steadily during each biannual survey, reflecting a significant reduction in temephos efficacy. This trend reached high levels (< 50% mortality) in about half the country as early as 2004-2005 (Fig. 3). Although the frequency of temephos resistance seems to have been attenuated in the main problem areas observed between 2004-2005, temephos resistance continued to spread within Brazil. By 2010-2011 only Rondônia (in the North), São Paulo (Southeast), Paraná and Santa Catarina (South) exhibited satisfactory temephos efficacy against YF mosquito larvae. New focal areas of temephos resistance were detected in the 2010-2011 survey radiating from near Rio Branco (southern Acre in North Brazil, near Bolivia) and Brasilia (Central Brazil), leading to a country-wide resistance phenomenon.

**DISCUSSION**

The temephos mortality dataset obtained from the diagnostic bioassays performed by the MoReNAa, although not carried out with the objective of spatial in-
Fig. 3: contour maps of temephos resistance in Brazilian populations of the yellow fever mosquito (*Aedes aegypti*) generated using spatial interpolation. The colour legend indicates the represented range of mortality (%) of mosquito larvae obtained in the temephos resistance diagnostic bioassays. Colours tending toward red indicate lower larval mortality and, consequently, a higher frequency of temephos resistance.
interpolation to generate temephos resistance maps for Brazilian populations of *Ae. aegypti*, allowed such interpolations and the inferences necessary to generate the maps. The effort provided a means to clearly illustrate the temporal spread and spatial reach of temephos resistance in *Ae. aegypti* - which greatly increased during the 12-year period of assessment - within the Brazilian territory. Nonetheless, a more fine-tuned survey focusing on diagnostic bioassays of insecticide resistance using larger and better-distributed sampling sites would allow even more comprehensive assessments for eventual decision-making regarding policies and procedures to be adopted. Temephos resistance among Brazilian populations of the YF mosquito is far from novel. All the Brazilian states have adopted the routine use of temephos (1% sand granule formulations) to manage *Ae. aegypti* by controlling its larvae since the early 1990’s (Funasa 1994, 2001, Sucen 1997). The result of this continuous and consistent use of temephos throughout the country led to reports of temephos resistance as early as 1995 (Macoris et al. 1995). The increased incidence of dengue during the 1990s in Brazil attributed to the spread of *Ae. aegypti* enhanced concern regarding insecticide use against the mosquito and the susceptibility of mosquito populations (da Silva Jr et al. 2002, Braga & Valle 2007). The end result was the establishment of an insecticide-resistance monitoring program in the country that focused on populations of the YF mosquito (Braga & Valle 2007). Consistent detection of temephos resistance in different parts of the country soon followed (Campos & Andrade 2003, Lima et al. 2003, 2006, Macoris et al. 2003, 2007, Melo-Santos et al. 2010, Gambarra et al. 2013, Diniz et al. 2014).

Some of the studies of temephos resistance among Brazilian populations of the YF mosquito explored the mechanisms involved and the existence of fitness costs associated with this resistance. Fitness costs were indeed detected (Diniz et al. 2014). Unfortunately, the studies on the underlying mechanisms of temephos resistance were more confused and patchy, but they were suggestive of the prevailing involvement of enhanced insecticide detoxification as the main mechanism, with esterases likely playing a major role, although not an exclusive one (Braga & Valle 2007, Melo-Santos et al. 2010, Gambarra et al. 2013, Macoris et al. 2014). These findings seem consistent with mechanistic studies of temephos resistance performed with other Latin American populations of the same species (Bisset Lazcano et al. 2009, Bisset et al. 2013, Grisales et al. 2013, Reyes-Solis et al. 2014, Saavedra-Rodriguez et al. 2014).

The twelve-year effort of the MoReNAa achieved a great deal, but no summary of the country-wide survey effort had ever been performed; therefore, creating such a summary was the objective of the current work. The geostatistical tools used here allowed the recognition of both the temporal pattern of the spread of temephos resistance in the country and its gravity by 2011. Despite the early detection of temephos resistance in the mid-1990s, country-wide use of temephos continued; consequently, temephos resistance spread throughout the country during the following years, reaching serious levels by 2002-2003. At this point, nearly half of the country was already having problems because of temephos resistance in *Ae. aegypti*, particularly when considering “resistant” to mean mosquito populations that exhibit mortality levels below the 80% threshold-a threshold that incurs in a high likelihood of control failure (Davidson & Zahar 1973). The scenario has simply gotten worse in subsequent years. Now, nearly all the country (except a part in the South) exhibits temephos resistance.

The use of temephos as a mosquito larvicide in Brazil has been suppressed since late in 2010, which may reverse the spread of resistance and allow for future use of the compound. However, the high frequency of resistant individuals already established in the country potentially limits the extent of such (future) use, even if the fitness cost associated with temephos resistance prevails in the country. Effective, safe and cheap insecticides such as temephos, which are the underlying reasons for its global use as a mosquito larvicide, are hard to come by (Tomé et al. 2014). A few alternatives have emerged and are currently being explored, including a few pyrethroids, but these already exhibit insecticide resistance problems in wide areas in Brazil (e.g., Brito et al. 2013). More recently, insect growth regulators and the bioinsecticide *Bacillus thuringiensis* serovar *israelensis* (Bti) have been explored (Braga & Valle 2007, Fontoura et al. 2012, Araújo et al. 2013).

In conclusion, temephos resistance in Brazilian populations of the YF mosquito spread during the 12-year survey period, showing that resistance is now widespread and there is little hope of achieving effective mosquito control with this insecticide. Alternative insecticides aided by the preventive elimination of potential mosquito breeding sites are necessary. However, the use of these alternative insecticides will also lead to the eventual emergence of resistant mosquito populations - and may have already occurred in the country, considering their present rate of use. Therefore, continuing country-wide surveys are necessary to guide management decisions by the national health officers. In such a context, planned yearly systematic sampling and insecticide resistance diagnostic bioassays are necessary. Moreover, geostatistical analyses to map the levels and spread of the phenomenon are also necessary.

ACKNOWLEDGEMENTS

The authors would like to thank OPAS that allowed the National Insecticide Susceptibility Monitoring Program to be conducted and for making that data available to the present study.

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