The efficacy of a combined larvicide–adulticide in ultralow volume and fumigant canister formulations in controlling the dengue vector Aedes aegypti *(Diptera: Culicidae) in Northwest of Argentina*

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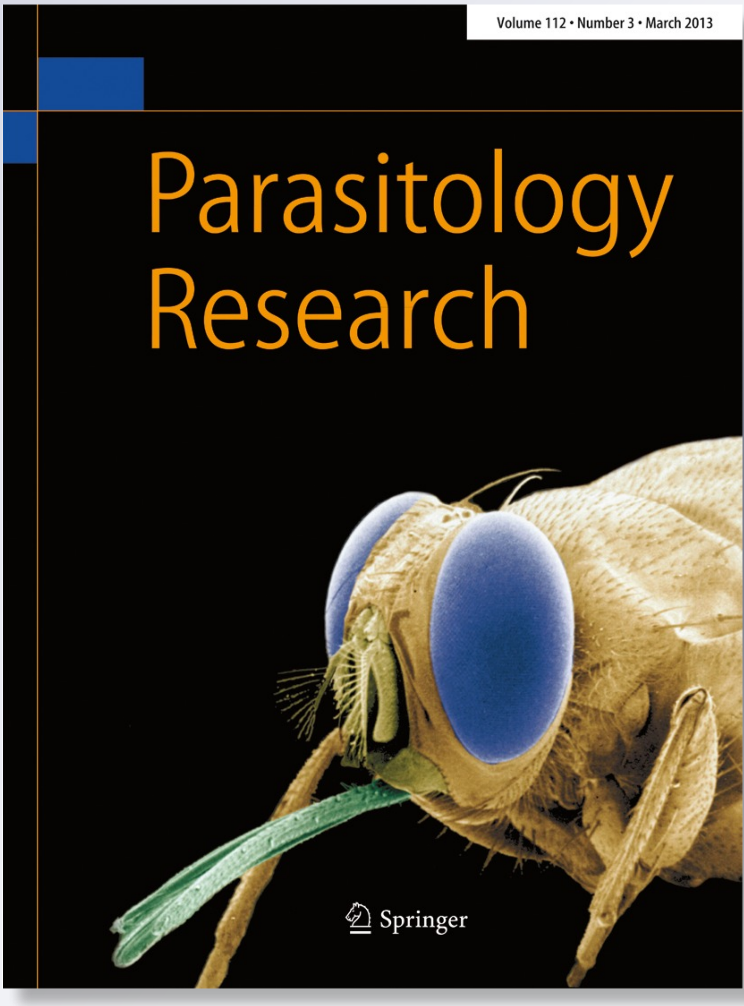

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ORIGINAL PAPER

The efficacy of a combined larvicide–adulticide in ultralow volume and fumigant canister formulations in controlling the dengue vector *Aedes aegypti* (Diptera: Culicidae) in Northwest of Argentina

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Abstract The efficacy of an ultralow volume formulation (ULV) and fumigant canister, containing both permethrin and pyriproxyfen, was compared with that of standard permethrin applications in a field assay conducted in Banda del Río Salí, Tucumán (north-western Argentina). Five treatment areas were established: first area was sprayed with a ULV formulation of 10 % permethrin, a second area was treated using a fumigant canister containing 10 % permethrin and 3 % pyriproxyfen, the third area was sprayed with a ULV formulation of 10 % permethrin and 3 % pyriproxyfen, the fourth area with ULV formulation of 10 % permethrin using a portable aerosol generator and the fifth area was a left untreated area. Immature and adult *Aedes aegypti* individuals placed in containers and sentinel cages were positioned within the treated and control areas. The effects of treatment and time on larval, pupal and adult survival were tested. We also investigated the effects of treatment and time on the numbers of larval and pupal deaths, on the proportion of larvae that metamorphosed into pupae and adults, and on the proportion of dead adults. Larval *A. aegypti* survivorship in 250 mL containers revealed a significant treatment effect and significant treatment × time interaction 2 and 24 h after the application of the ULV treatment with 10 % permethrin using the portable aerosol generator. The number of dead larvae in 20 L containers differed significantly by treatment and by time. ULV treatment with 10 % permethrin and 10 % permethrin plus 3 % pyriproxyfen using the cold fogger truck mount ULV resulted in the greatest numbers of dead larvae; most larvae died 2 weeks after application. Adult *A. aegypti* mortality in all treatments did not differ significantly 2 and 24 h after application. In addition, we found no significant differences in adult mortality between cages exposed at 3 m and those at 6 m from the ULV application line. However, there was a significant difference in adult mortality between the 10 % permethrin treatment applied with cold fogger truck mount ULV and that applied using the portable aerosol generator ($P<0.001$). The larval index known as Breteau index (BI) was higher before treatment than after treatment in different areas. After the treatments, the lowest value of BI was observed in the area treated with the fumigant canister formulation, and a long-lasting effect was observed with the formulation of 10 % permethrin and 3 % pyriproxyfen.

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Introduction

Dengue fever is the most important arbovirus disease in the world, with an estimated 50 million cases annually and 30,000 deaths per year (Kourí et al. 2007; Kroeger and Nathan 2006; Garelli et al. 2011). *Aedes aegypti* (Diptera:

Culicidae) (L.), the main vector of dengue, urban yellow fever and chikungunya virus, is a highly domestic and anthropophilic mosquito found inside or close to human dwellings in urban settings (Kyle and Harris 2008;, Garelli et al. 2011). Around 1960, the vector was eliminated from Argentina; however, since 1987, it has again been detected in the northern region of the country (Salta, Jujuy, Misiones and Formosa provinces) with a prevalence of DEN-1 and DEN-2 antibodies (Aviles et al. 1999; Carbajo et al. 2001; Boffi 2002; Masuh et al. 2008). In 2009, it produced the largest epidemic in the country's history, with 26,612 human cases of DEN-1 reported in 9 provinces (Boletín Epidemiológico 2011). The epidemic included severe clinical cases and, for the first time in Argentina, grave cases with mortality attributable to this cause (Sistema Nacional de Vigilancia de la Salud (SNVS) 2012). In 2010, 1,136 cases of DEN-1, DEN-2 and DEN-4 antibodies were reported, and there were 192 reported cases of DEN-1 and DEN-2 in 2011 with the majority occurring in the Salta, Santa Fe and Misiones provinces. Meanwhile, in Bolivia, Paraguay and Brazil, 10,914 cases of DEN-1, DEN-2 and DEN-3; 22,012 cases of DEN-1 and DEN-2 and 254,734 cases of DEN-1, DEN-2, DEN-3 and DEN-4 were registered in each country, respectively (Sistema Nacional de Vigilancia de la Salud (SNVS) 2012; PAHO 2011).

To date, there is no vaccine for dengue; control depends largely on the control of the vector *A. aegypti*. This species breeds in natural and artificial environments (Goma 1964; Masuh et al. 2008), and due to its capacity to readily adapt to novel artificial environments, it can invade new habitats (Gratz and Knudsen 1996; Lucia et al. 2009).

Vector control efforts typically target the immature stages, attempting to reduce larval populations in domestic habitats (Lucia et al. 2009). However, such efforts require considerable time and effort, with the result that sometimes not all containers are treated, e.g. those within dwellings (Harburguer et al. 2009).

The most widely used larvicide during the past 40 years has been organophosphorus temephos, which is not toxic to humans at the recommended application doses (Brooks et al. 1966; Bang and Pant 1972; Chadee 1984; Donalisio et al. 2002; Tawatsin et al. 2007; Garelli et al. 2011). However, *A. aegypti* now exhibit an acquired resistance to this larvicide in Brazil, Argentina and Bolivia (Macoris et al. 2003; Braga Ima et al. 2004; Seccacini et al. 2008; Harburguer et al. 2009).

The World Health Organization (WHO) recommends the use of the pyrethroid permethrin, the biolarvicide *Bacillus thuringiensis* var *israeliensis* (*Bti*) and the insect growth regulators (IGRs) methoprene and pyriproxyfen for treating drinking water (Lee et al. 1996; Chavasse and Yap 1997; WHO (World Health Organization) 1999, 2007a, 2007b). Pyriproxyfen affects the hormonal balance organisms and it can suppress the embryogenesis, metamorphosis and development into adults (Itaya 1987; Koehler and Patterson 1991; Harburguer et al. 2009).

The most common type of intervention used during prevention efforts as well as during epidemic outbreaks is the ground application of spatial sprays. These sprays deliver a minimal volume of insecticide formulation per unit area, known as the ultralow volume (ULV; PAHO 1994; Lucia et al. 2009). However, ULV applications have been reported as ineffective in controlling not only larvae inside and outside dwellings (Yap et al. 1997) but also adult *A. aegypti* individuals (Perich et al. 1990) resting under beds, behind furniture and in closed rooms, where it is difficult for aerosol droplets to reach (Perich et al. 2000; Harburguer et al. 2009).

Another method frequently used for vector control is the indoor, thermal active system known as the fumigant canister. This canister is a smoke-generating device that typically releases pyrethroid formulations. It was developed for the indoor control of Chagas disease vectors (Zerba 1995; Zerba et al. 1996; Gonzalez Audino et al. 1999) and has been shown to provide excellent control of adult *A. aegypti* inside dwellings (Masuh et al. 2003; Harburguer et al. 2009)

Until today in Argentina, control efforts against *A. aegypti* were focused on domiciliary control, primarily at the community level. This approach implies changes in relation to the re-organisation of the environment, which is not always successful. Several components inhibit the success of these efforts: (a) the shortage of control efforts with sufficient coverage, i.e. coverage of at least 90 % of each locality; (b) the prevalence of different types of containers that favour vector development in the urban habitat; (c) insufficient resources to eliminate containers and (d) insufficient human resources capable of successfully carrying out preventative measures. In addition to these social factors, technical shortcomings hinder control. For example, the inefficacy of ULV spatial sprays and the persistence of adult mosquitoes inside dwellings can act to limit the exposure of adults to the insecticide (e.g. if interior spaces are not prepared prior to spraying (e.g. with open windows or doors) or if adults reside inside closed cabinets, closets or rooms, the resulting limited circulation of insecticide will have little impact on those adults). Another limitation is that ULV spatial sprays inside dwellings have limited performance, particularly during epidemics when demand is high. Foregoing the use of ULV spatial sprays raises questions about the need of alternative approaches to remedy or improve the effectiveness of interventions in such situations.

Therefore, we present the results of a multi-faceted approach conducted in Tucumán province, which evaluated (a) the efficacy of a new combination larvicidal–adulticidal product (permethrin plus pyriproxyfen) in ULV spatial sprays (cold fogger truck mount ULV), (b) its efficacy in controlling both adult and immature mosquitoes relative to the conventional permethrin application via spatial ultralow volume formulation sprays (using cold foggers truck mount ULV and portable aerosol generator ULV) and (c) the efficacy of this product applied via fumigant canister in the same locality. It is hoped that the results obtained in the present study will be useful to be implemented during vector control programs and then, to limit dengue transmission.

Materials and methods

Study site

Banda del Río Salí (26° 51′ S; 65° 10′ W, 425 msnm) is in the Cruz Alta Department in eastern Tucumán Province. It is known as the "National Capital of Sugar" due to the presence of sugarcane crops favoured by the subtropical climate. The city has 64,591 inhabitants. It is one of the most populated cities of Tucumán Province and lies 3 km east of San Miguel de Tucumán, the capital of the Province.

Five areas (A, B, C, D and E) of approximately nine blocks each and separated from one another by green areas (e.g. primary vegetation, secondary vegetation, sugarcane crops) were subject to either (A) ULV treatment with 10 % permethrin using cold fogger truck mount ULV, (B) fumigant canister treatment with 10 % permethrin plus 3 % pyriproxyfen, (C) an untreated area as a control, (D) ULV treatment with 10 % permethrin plus 3 % pyriproxyfen using cold fogger truck mount ULV and (E) ULV treatment with 10 % permethrin using portable aerosol generator ULV. At the all the areas, infestation rates were measured before, during and after treatment. The areas were selected based on similar socio-economical characteristics (e.g. housing materials, running water, animals living in or close to dwellings and the presence of natural barriers (such as the Salí River) or green areas (i.e. natural vegetation or crops) that were considered buffer zones.

Chemical products

Formulation A: composition: 10 % permethrin (3-phenoxyphenyl) methyl 3-(2,2-dichloroethenyl)-2,2-(dimethyl cyclopropane carboxylate), cis: trans relationship 45:55, provided as an emulsifiable concentrate (EC) by Chemotecnica S.A. (Argentina). Dose: 10 mL per ha.

Formulation B: composition: emulsifiable concentrate (EC); 3 % pyriproxyfen (2-(1-methyl-2-(4-phenoxyphenoxy) ethoxy) pyridine) plus 10 % permethrin as an emulsifiable concentrate (EC), formulated by Chemotecnica S.A. (Argentina).

Polyethyleneglycol 1000 could be used as an antievaporant for ULV treatments.

Dose: 100 mL per ha.

Formulation C: fumigant canister with permethrin plus pyriproxyfen. Composition: 1 % permethrin (1-RS)-cis-trans-3-(2,2-dichlorovinyl)-2,2-(dimethyl cyclopropane carboxylate of phenoxybenzyl) plus 0.2 % pyriproxifen (4-phenoxyphenyl (RS)2-(2pyridyloxy) propyl ether). Fumigant components and inert c.s.p. 50 g.

Dose: One tablet each 25–50 m^3/10–20 m^2

The fumigant canister was provided as tablets by Chemotecnica S.A. (Argentina).

The cold fogger truck mount ULV covered a 3-ha area. Adult cages were placed at dwellings located in the central zone. The portable aerosol generator ULV was applied to a randomly chosen set of 20 dwellings in the central zone, comprising an area of nine blocks (three blocks per side), fully treated.

The following fieldwork areas were studied:

1. Zone A: This zone contained nine blocks. The central block contained all of the dwellings, adult cages, 250 mL and 20 L water containers; the latter were sampled to measure the infestation rate in and around dwellings, e.g. front yards, backgrounds, galleries, patios, terraces and storage units. Ten percent permethrin was applied via cold fogger truck mount ULV.
2. Zone B: Similar to Zone A, except that fumigant canister were employed at all dwellings.
3. Zone C (Control): A nine block area without treatment, where the infestation rates before, during and treatment in other zones were measured.
4. Zone D: Similar to Zone A, except the combination formulation of 10 % permethrin plus 3 % pyriproxyfen was applied via cold fogger truck mount ULV.
5. Zone E: Similar to Zone A, except 10 % permethrin was applied via portable aerosol generator ULV.

Biological material

We used to the trials susceptible strains of *A. aegypti* (Rockefeller strain) according to CIPEIN (Centro de Investigaciones de Plagas e Insecticidas CITEFA-CONICET) and the established conditions required by INAL (Instituto Nacional de Alimentos) and ANMAT (Administración Nacional de Medicamentos, Alimentos y Tecnologías Médicas). *A. aegypti* specimens were reared at the laboratory of the Vector Control National Program (National Ministry of Health) in San Miguel de Tucumán (Tucumán, Argentina).

ULV treatments

We used a cold insecticide, vehicle-mounted generator called the Curtis Dyna-Fog 2PTM ULV Maxi-ProTM (Westfield, IN, USA) for ULV insecticide applications. Flow was regulated at 1 L of mixture every 2 min 40 s, which is the amount of time considered necessary to spray 1 ha (Lucia et al. 2009). Ten grams of permethrin/ha (formulation A) was applied in the zone A and 10 g of permethrin plus 2 g pyriproxyfen/ha (formulation B) was sprayed in the zone D.

We also used a cold insecticide, portable aerosol generator ULV called the Motan Fontan® Portstar N (Isny, Germany) for ULV insecticide application. The deposit volume of insecticide was 2.5 L. The flow was regulated to 1 L of mixture per hour. Polyethylene glycol antievaporant (6 % of concentration in volume) was used. The application time per environment was approximately 3 s. Ten grams of permethrin/ha (formulation A) was applied in the zone E.

The applications were performed during the peak of mosquito flight activity (i.e. before dawn or after sunset). The sprays were performed on February 10, 2011.

Monitoring *A. aegypti* populations

Population levels of *A. aegypti* larvae were determined by visual observation using larval sampling following PAHO (1994). The Breteau index (BI; defined as the number of containers with larvae per 100 inspected houses) was calculated as estimates of vector abundance. Entomological studies were conducted in the city for 10 weeks from January 4 to March 10, 2011.

Each selected area contained a central zone (with approximately 20 dwellings) where entomological studies were conducted. BI was calculated weekly from 3 weeks prior to insecticides applications until the population levels returned to pre-treatment values.

The treatment and control areas were similar between of them considering the kind with respect to dwelling type, socio-economic and urban characteristics, vegetation and entomological indexes, among others factors, relevant to *A. aegypti* population dynamics and the application of insecticide(s). To avoid the effect of mosquitoes migrating into the areas of close neighbours and thereby escaping treatment, we expanded the treatment area to include a buffer zone of approximately 1,000 m beyond the central area; alternatively, we also noted the presence of natural barriers, such as rivers, open spaces and dense areas of vegetation or forest.

Adult bioassays

The field bioassays were performed following the World Health Organization's protocols (Reiter and Nathan 2003; WHO (World Health Organization) 2005) with minor modifications as described below.

We placed cylindrical (15 cm tall × 3 cm diameter) screened sentinel cages built with 18-mesh nylon in each central zone following Lucia et al. (2009). Ten to 12 h before ULV application, ten adults (five female, five male) aged 24–36 h and fed on raisins were transferred to each cage. Four groups of ten cages were randomly assembled at each area: groups 1, 2, 3 and 4 were hung outside of the dwellings 3 and 6 m from the pulverisation line, at 1.5 m from the floor. Five additional cages were placed in the control area (without treatment). Screened cages were returned to the laboratory 30 min after ULV application and kept at room temperature. Hydration was provided, and adult mortality in the treated cages was assessed at 2 and 24 h.

Larval bioassay

We followed the methodology of Reiter and Nathan (2003) and later modified by Masuh et al. (2003) for bioassays. In each central zone, five plastic 250 mL containers (7.5 cm diameter) were placed alongside white plastic 20 L containers (29 cm diameter) containing tap water; each pair was placed outside each selected house 5 m from the pulverisation line. On the day before ULV treatment, 15 late-third or early-fourth instar *A. aegypti* larvae were placed in each type of container and provided food.

Screened plastic 250 mL containers were returned to the laboratory 30 min following ULV application and they were inspected daily for 3 weeks to investigate the inhibition of adult emergence.

The 20-L containers were covered with nylon gauze to prevent either the escape of adults or the entrance of other insects; containers were then held under field conditions under a roof. Once a week, emerged adults and the surviving larvae and pupae were removed from the treatment and control containers and placed with 100 mL water into new 250 mL containers. These containers were then evaluated in the laboratory to assess adult emergence. Fifteen fresh late-third or early-fourth instar *A. aegypti* larvae were introduced into the containers. The water level in the containers was held constant for water loss/evaporation. The entire process was repeated weekly until no inhibition effect on adult emergence was observed.

The experiment was terminated once no statistically significant larvicidal effect was observed (i.e. no adult emergence inhibition effect) between the treatment groups and the untreated control.

Adult emergence inhibition (EI%) was adjusted for larval or pupal mortalities in the corresponding controls according to Mulla et al. (1974) and calculated as follows:

$$EI\% = 100 - 100\ (T/C)$$

where T is the percent emergence in treated containers and C is the percent emergence in control containers.

Statistical analysis

A one-way analysis of variance (ANOVA) was used to analyse the effects of the different treatments and time on larval, pupal and adult survival. The Kruskal–Wallis test and the Kruskal–Wallis multiple comparisons test were used to test for differences among treatments. A multilevel Poisson regression was conducted to test for the effects of treatment and time on the number of dead larvae and pupae on the proportion of larvae metamorphosing into pupae and adults and on the proportion of dead adults. Analyses were performed implemented with the program HLM 6 Hierarchical Linear and Nonlinear Modeling (Scientific Software International; Raudenbush et al. 2000). A P value of <0.05 was considered statistically significant.

Results and discussion

Most control measures implemented during dengue emergences were focused on the larvicidal activities in the oviposition sites of *A. aegypti* and on the permethrin application by ultralow volume spatial sprays (by cold fogger truck mount ULV and portable aerosol generator ULV; Chadee 1985; Perich et al. 2000; Lucia et al. 2009). Other efforts have used temephos (an organophosphate insecticide) as a larvicide to control the vector population (Brooks et al. 1966; Bang and Pant 1972; Chadee 1984; Donalisio et al. 2002; Tawatsin et al. 2007). Recent studies have identified residual effects of this insecticide (depending on formulation, application procedure and experimental conditions) ranging between 1 and 6 months (Thavara et al. 2005; Pontes et al. 2005; Palomino et al. 2006; Garelli et al. 2011).

One option under consideration is the use of *B. thuringiensis* var. *israliensis* (*Bti*) with the target *A. aegypti* to reduce the vector population once established. Efforts often fail because several conditions must be met to obtain consistent results; these include continuity based on the residual product, an application cycle of once every 2 or 3 months, a high level (>90 %) of coverage, simultaneous removal of breeding containers and temephos application in containers of high productivity (e.g. car tires).

The advantages of combining adulticidal and larvicidal active ingredients in spatial treatments were described by Tidwell et al. (1994); in another study, Yap et al. (1997) used a combination of Pesguard PS102 and *B. thuringiensis* var. *israliensis* in an aqueous base to increase the larvicidal effect of pyrethroid ULV formulations.

Here, we report on the efficacy of a combination larvicidal–adulticidal product (permethrin and pyriproxyfen) by ULV spatial sprays and fumigant canister formulations. Our results may be useful for developing future vector control programs and stopping the transmission of the dengue virus.

In 2009, during the largest epidemic recorded in recent decades in the country, cases of DEN-1 were reported in 9 provinces; among them, Tucumán Province was one of the most affected provinces (Dantur Juri 2009). Banda del Río Salí is a city experiencing continuous growth. It is located in the Cruz Alta Department and registered 103 cases during the outbreak; the vector appears well established. The National Coordination of Vector Control of Argentina has documented larval and adult *A. aegypti* in many houses, residing primarily inside dwellings: in walls, under beds and behind furniture. In some houses, some mosquitoes of the *Culex* genus were identified, which have no effect on the development of *A. aegypti* larvae.

The effects of A, B and C formulations (based on 10 % permethrin, 3 % pyriproxyfen plus 10 % permethrin and 1 % permethrin plus 0.2 % pyriproxyfen) were compared among four zones and among containers type (250 mL and 20 L containers and adult cages).

Survival of larval *A. aegypti* in 250 mL containers among treatments is illustrated in Fig. 1. There was a significant treatment effect and a significant treatment × time interaction ($P<0.001$) on larvae survival at 2 and 24 h after application of the ULV formulation with 10 % permethrin using the portable aerosol generator. No significant treatment effect on pupal mortality was detected ($P<0.432$); a significant effect of time was observed between 2 and 24 h following insecticide application ($P<0.001$; Fig. 2).

There was no effect of either treatment or time on the adult survival ($P<0.786$).

Among the 20-L containers, we observed a significant effect of treatment and time on the number of dead larvae ($P<0.037$). ULV treatment with 10 % permethrin using the cold fogger truck mount ULV and ULV treatment with 15 % permethrin plus 3 % pyriproxyfen using the cold fogger truck

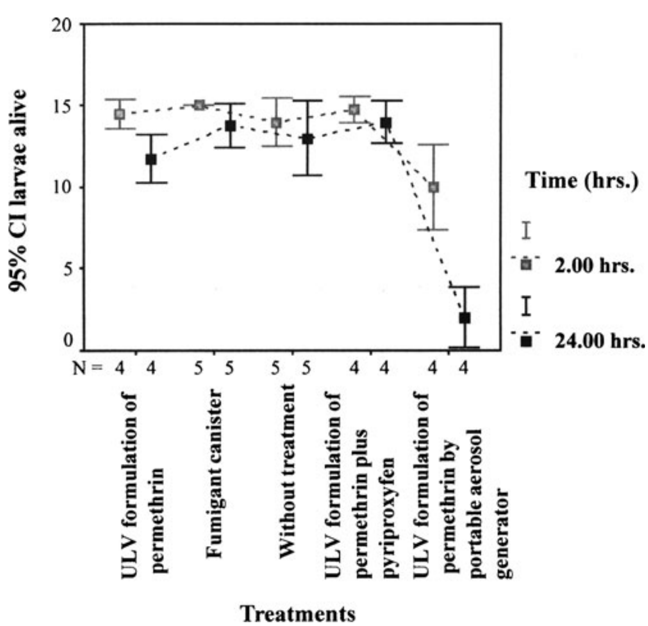

Fig. 1 Larval *A. aegypti* survival (95 % CI) in 250 mL containers for the areas treated considering treatment × time interaction in the field essay carried out in Banda del Río Salí between January and March 2011. *CI* confidence interval

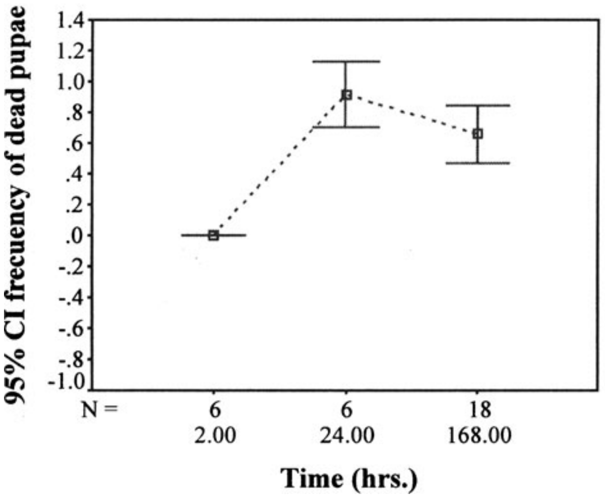

Fig. 2 Pupal *A. aegypti* survival (95 % CI) in 250 mL containers for the areas treated considering the time in the field essay carried out in Banda del Río Salí between January and March 2011. *CI* confidence interval

mount ULV produced the most larval lethality (Fig. 3); most larvae died after 2 weeks (Fig. 4). Accounting for the proportion of dead pupae with respect to both treatment and time, we observed no significant effects ($P<0.862$).

The proportion of larvae metamorphosing to pupae did not differ significantly between treatments using the formulations A, B and C ($P<0.660$). However, the proportion of larvae metamorphosing to pupae varied significantly with time ($P<0.001$); the majority of the larvae became pupae (i.e. metamorphosed to the next stage) after 2 weeks (Fig. 5).

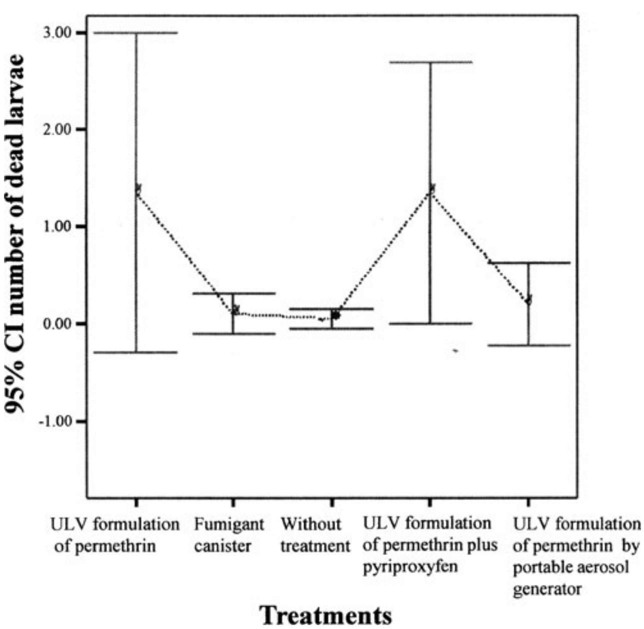

Fig. 3 Number of dead larvae (95 % CI) in 20 L containers considering the treatment in the field trial carried out in Banda del Río Salí between January and March 2011. *CI* confidence interval

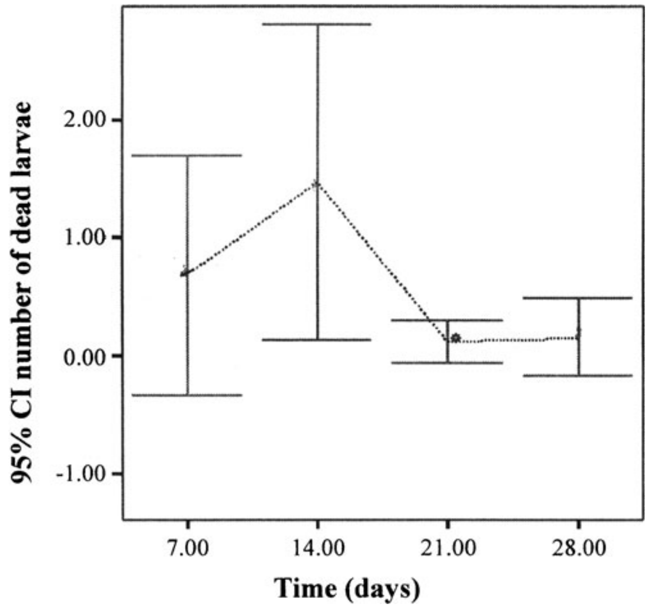

Fig. 4 Number of dead larvae (95 % CI) in 20 L containers considering the time in the field trial carried out in Banda del Río Salí between January and March 2011. *CI* confidence interval

The proportion of larvae metamorphosing into adulthood did not vary by treatment ($P<0.320$); however, the proportion did vary significantly with time ($P<0.001$), with adults appearing after 2 weeks (Fig. 6). The proportion of larvae that metamorphosed into subsequently dying adults varied with time, with the majority dying within the first 5 days of adulthood ($P<0.001$; Fig. 7); however, this proportion did not vary with treatment ($P<0.720$).

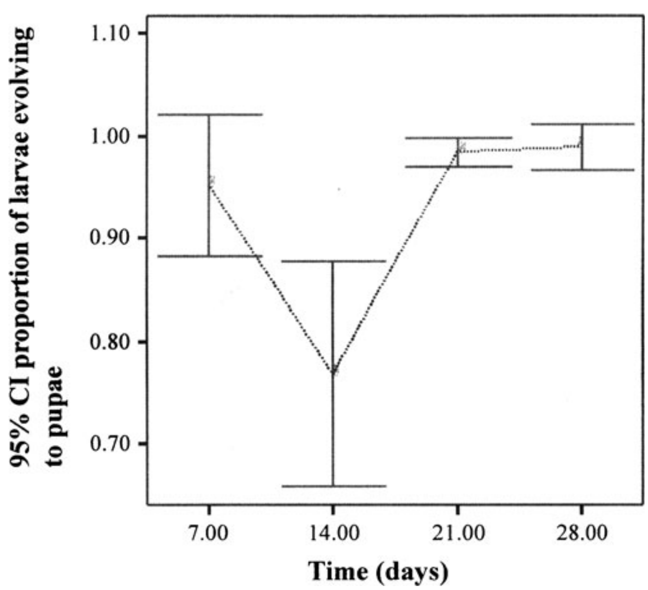

Fig. 5 Proportion of larvae metamorphosing to pupae (95 % CI) in 20 L containers considering the time in the field trial carried out in Banda del Río Salí between January and March 2011. *CI* confidence interval

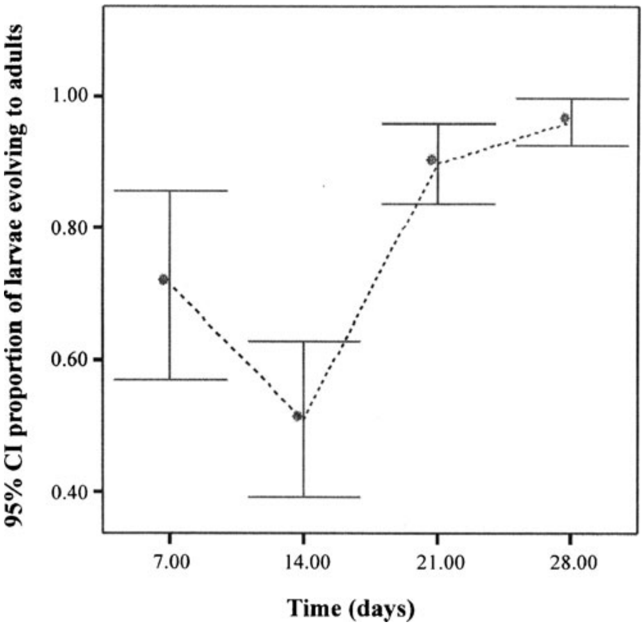

Fig. 6 Proportion of larvae metamorphosing into adulthood (95 % CI) in 20 L containers considering the time in the field trial carried out in Banda del Río Salí between January and March 2011. *CI* confidence interval

In all treatments, adult *A. aegypti* did not differ between 2 and 24 h after the applications; moreover, there were no significant differences in adult mortality between cages exposed at 3 m (exterior of dwellings) and those exposed at 6 m (inside dwellings) from the ULV spraying application line. However, we observed a significant effect of treatment on adult mortality: ULV treatment with 10 % permethrin using portable aerosol generator significantly increased adult mortality ($P<0.001$; Fig. 8).

We found that the control group do not allowed finding significant differences between the effects of the different treatment on the adult survival into the analysis. We therefore removed it from analysis; as result, there was more than one significant effect on the adult survival: in addition to ULV treatment with 10 % permethrin using portable aerosol generator, there was also a significant effect of ULV treatment with 15 % permethrin plus 3 % pyriproxyfen using cold fogger truck mount ULV ($P<0.001$; Fig. 9).

Among treatments, there was a significant difference between the ULV treatment with 10 % permethrin using cold fogger truck mount ULV (G1) and the ULV treatment with 15 % permethrin plus 3 % pyriproxyfen using cold fogger truck mount ULV (G4) on adult mortality ($P<0.006$). Similarly, effects on adult mortality differed between the ULV treatment with 10 % permethrin using cold fogger truck mount ULV (G1) and the ULV treatment with 10 % permethrin using portable aerosol generator (G5; $P<0.001$), between the combination larvicidal-adulticidal (1 % permethrin plus 0.2 % pyriproxyfen) product applied using the fumigant canister (G2) and the ULV treatment with 10 % permethrin using the portable aerosol generator (G5; $P<0.001$) and between the ULV treatment with 15 % permethrin plus 3 % pyriproxyfen using the cold fogger truck mount ULV (G4) and the ULV treatment with 10 % permethrin using the portable aerosol generator (G5; $P<0.001$).

As reported by Lucia et al. (2009) to Wanda (Misiones), the adulticide effect did not differ significantly between 2 and 24 h: the result was immediate and did not change after 24 h. Adults exposed in the treatment cages did not exhibit

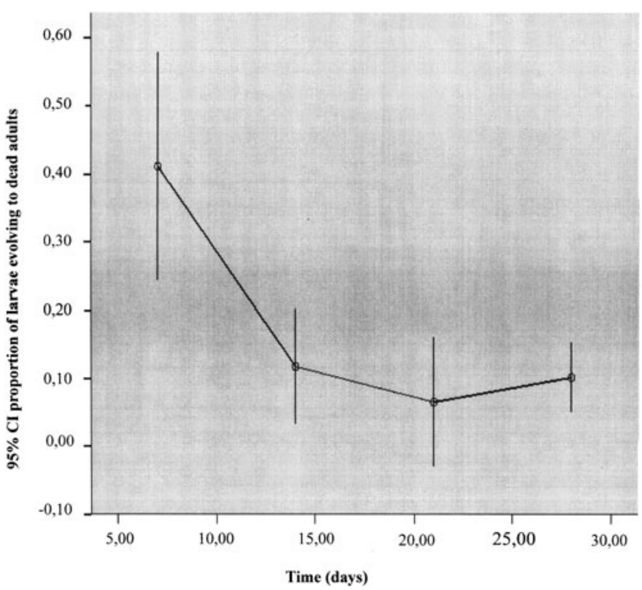

Fig. 7 Proportion of larvae that metamorphosed into dying adults (95 % CI) in 20 L containers according to the time in the field trial carried out in Banda del Río Salí between January and March 2011. CI: Confidence Interval

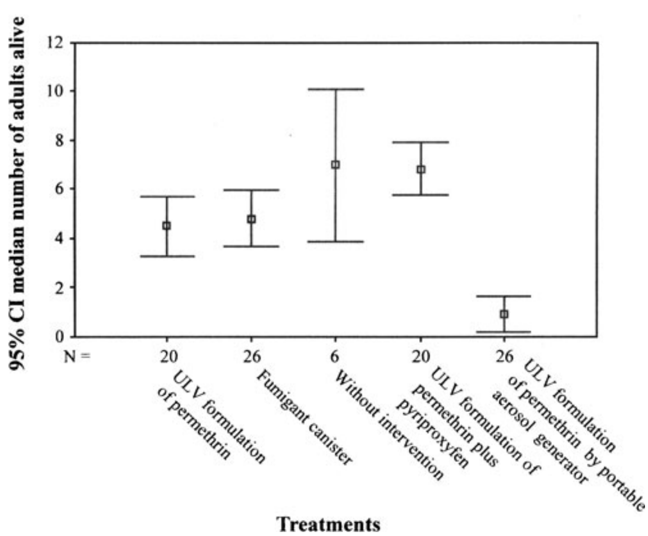

Fig. 8 Median number of adult *A. aegypti* mortality (95 % CI) in 20 L containers considering the treatment in the field trial carried out in Banda del Río Salí between January and March 2011. *CI* confidence interval

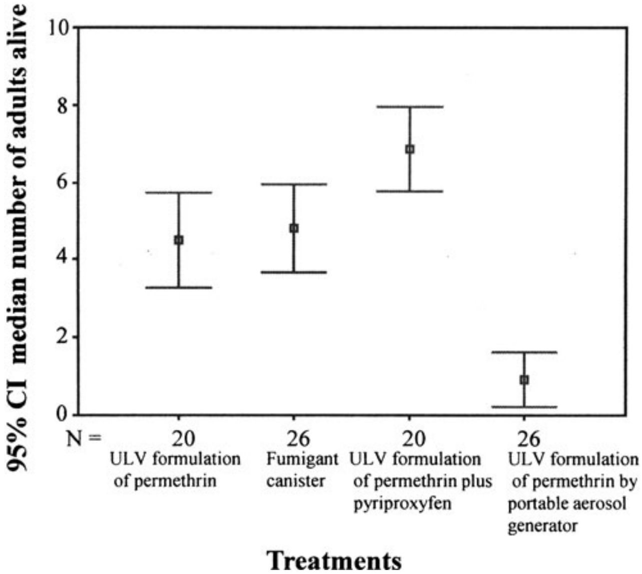

Fig. 9 Median number of adult *A. aegypti* mortality (95 % CI) in 20 L containers, without control group, considering the treatment in the field trial carried out in Banda del Río Salí between January and March 2011. *CI* confidence interval

any posterior mortality. This finding conflicts with the recommendations of the WHO (Reiter and Nathan 2003), which have suggested transferring adults from treatment to maintenance cages in order to place additional, mortal stress on mosquito adults.

With respect to the percent of adult emergence inhibition (EI%) in 250 mL containers, we observed both treatment and time effects. EI% values in containers from areas treated with formulation A applied with the cold fogger truck mount ULV and the portable aerosol generator were relatively low (3 % and 33 %, respectively) at 2 h but increased by 24 h, reaching 40 % and 60 %, respectively. The area treated with 10 % permethrin plus 3 % pyriproxyfen using the cold fogger truck mount ULV showed 100 % inhibition at 2 h, later decreasing to 56 % at 24 h. In contrast, there was almost no effect of the fumigant canister with 1 % permethrin and 0.2 % pyriproxyfen at 2 h; EI% increased over time, reaching 35 % at 24 h.

Within 20 L containers, adult emergence inhibition (EI%) differed among areas. In areas treated with formulation A using the cold fogger truck mount ULV and the portable aerosol generator, EI% values were 38 % and 18 %, respectively. The values decreased between day 14 and day 21, approaching zero by day 28. The effect of 10 % permethrin plus 3 % pyriproxyfen (formulation B) using cold fogger truck mount ULV was found to be lower than 10 % of inhibition, increasing at day 14 to 20 % but then approaching zero by day 21. The effect of the fumigant canister with 1 % permethrin plus 0.2 % pyriproxyfen (formulation C) was very low (4 %) from the outset and decreased over the following weeks to near zero.

In the present study, the larvicidal effect (measured as the inhibition of adult emergence) appeared to vary with the size of the container. In the 250-mL containers, inhibition values were relatively low (3 % and 33 %) within the area treated with formulation A at 2 h, but they increased over time, reaching 40 % and 60 % at 24 h. In the area treated with formulation B, inhibition was near 100 %, decreasing later to 56 % by 24 h indicating a promissory effect of IGR. The effect of formulation C was near zero by 2 h but increased to 35 % by 24 h. Within the 20 L containers, EI% using formulation A never exceeded 38 % and by day 28 was near zero. Formulation B produced lower than 10 % of inhibition, increasing after 14 days to 20 % but decreasing to almost zero by day 21. The effect of formulation C was initially very low (4 %) and decreased over the following weeks to near zero.

In summary, comparing 250 mL and 20 L containers, we found that the container size influenced the insecticide dilution, which possibly affected EI%. Therefore, spatial ULV treatments with permethrin as well as with permethrin plus pyriproxyfen, and fumigant canister with permethrin plus pyriproxyfen, might perform well in 250 mL containers.

In contrast, Lucia et al. (2009) found that the 250-mL and 20-L containers used for larvicidal treatments had no diluting effect on the insecticide, and it could have affected EI%. To these authors, spatial ULV treatments with permethrin plus pyriproxyfen might perform well over a wide range of containers.

The presence and abundance of immature of *A. aegypti* individuals at treated and control areas was calculated by larval index, Breteau index (BI). Before insecticide application, BI values exceeded 44 in all areas, ranging from 170 to 44. As also reported by Lucia et al. (2009), post-treatment values from the control area were higher than pre-treatment values, approaching 150.

To evaluate insecticide impact, BI was measured until pre-treatment values were re-established. In areas treated with formulation A, 2 weeks elapsed before pre-treatment levels were reached; the lowest BI value (50) was stable for 1 week after application. For the area treated with formulation B, approximately 3 weeks elapsed before restoration of pre-treatment levels; the lowest BI value (72) was observed for 1 week following application. For formulation C, the lowest BI value (11) remained constant for 1 week after application; by week 2, the BI value reached a high of 118.

In summary, the lowest BI value was observed in the area treated with formulation C, followed by the areas treated with formulations A and B. Lastly, after the treatment with formulations A and C, 2 weeks elapsed before the original index values were restored. In contrast, for formulation B, 3 weeks elapsed before the pre-treatment value was restored, suggesting relatively higher performance; a similar result was reported by Lucia et al. (2009) in the north-eastern region of Argentina.

Conclusion

Dengue is an important public health concern affecting millions of people living in dengue-endemic countries. To effectively control the disease, early detection by national agencies is necessary. Early detection can not only prevent outbreaks but also help manage *A. aegypti* population levels. Vector control is an essential tool of dengue national control programmes. During an epidemic, ULV spraying with adulticides is used to prevent transmission. This treatment is not enough for controlling the adult mosquito population living indoor dwellings as well as the larval population living inside and/or outside dwellings (Yap et al. 1997; Perich et al. 2001; Mani et al. 2005). An alternative approach is to use larvicides in containers, but this method has generally encouraged increases in the diversity and multiplicity of larval habitats. Encouraging community participation in the domiciliary control of containers housing *A. aegypti* larvae is challenging because it requires behavioural modification and the re-organisation of human environments, which is often resisted. To succeed, disease control efforts require the participation of national institutions with capable personnel that can conduct clean-up campaigns and chemical treatments. In addition, the collaboration of educational institutions, with meetings, talks and interactive symposia, is needed. Our results show that larval *A. aegypti* survivorship in 250 mL containers were influenced by the treatment x time interaction after the application of 10 % permethrin using the portable aerosol generator ULV. There were effects of the treatment and time on larval survivorship in 20 L containers. The 10 % permethrin and 10 % permethrin plus 3 % pyriproxyfen formulations using the cold fogger truck mount ULV resulted in the greatest numbers of dead larvae. Adult *A. aegypti* mortality differ significantly considering the treatments with 10 % permethrin applied with cold fogger truck mount ULV and by using the portable aerosol generator. The Breteau index (BI) of areas treated with permethrin required approximately 2 weeks to return to pre-treatment levels. In the area treated with formulation B, approximately 3 weeks elapsed before the return to pre-treatment levels; for formulation C, the lowest BI value remained stable for 1 week after application, increasing to the high value by week 2. In summary, the lowest BI value obtained 1 week post-treatment was observed in the area treated with the permethrin plus pyriproxyfen formulation delivered as fumigant canister product, the next lowest values were observed in the areas treated with permethrin and permethrin plus pyriproxyfen. After treatment, the BI values of areas treated with permethrin and the fumigant canister formulations took 2 weeks to return to pre-treatment levels. In contrast, the BI values of the area treated with permethrin plus pyriproxyfen took 3 weeks to recover, indicating better performance of this formulation. These results indicate that this latter product provides better control of *A. aegypti* and suggest that this new formulation could be a new successful alternative for controlling dengue vector populations.

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References

Aviles G, Rangeon G, Vorndam V, Briones A, Baroni P, Enria D, Sabattini M (1999) Dengue reemergence in Argentina. Emerg Infect Dis 5:1–4

Bang YH, Pant CP (1972) A field trial of Abate larvicide for the control of *Aedes aegypti* in Bangkok, Thailand. Bull World Health Organ 46:416–425

Boletín Epidemiológico (2011) Dirección Nacional de Epidemiología. Departamento de Vigilancia Epidemiológica. Ministerio de Salud de la Nación (Argentina). Dengue. Pp. 25–26

Boffi R (2002) Erradicación y reinfestación por el *Aedes aegypti* en la Republica Argentina Master dissertation. Universidad Nacional General San Martin, Argentina

Braga Ima A, Lima JBP, Soares SS, Valle D (2004) *Aedes aegypti* resistance to temephos during 2001 in several municipalities in the states of Rio de Janeiro, Sergipe and Alagoass, Brazil. Mem Inst Oswaldo Cruz 99:199–203

Brooks GD, Shoof HF, Smith EA (1966) Evaluation of five formulations of Abate against *Aedes aegypti*, Savannah, Georgia, 1965. Mosq News 26:580–582

Carbajo AE, Schweigmann N, Curto SI, Garin A, Bejaran R (2001) Dengue transmission risk maps of Argentina. Trop Med Int Health 6:170–183

Chadee DD (1984) An evaluation of temephos in water drums in Trinidad, WI. Mosq News 44:51–53

Chadee DD (1985) An evaluation of malathion ULV spraying against caged and natural populations of *Aedes aegypti* in Trinidad, West Indies. Cahiers ORSTOM Series Entomol and Med Parasitol 23:71–74

Chavasse DC, Yap HH (1997) Chemical methods for the control of vectors and pests of public health importance Document WHO/CTD/WHOPES/97.2. World Health Organization, Geneva

Dantur Juri MJ (2009) Brote de dengue en el NOA: situación epidemiológica en la provincia de Tucumán. Bol Soc Entomol Arg 20 (1–2):6–7

Donalisio MRC, Leite OF, Mayo RC, Alves M, de Souza A, Rangel O, Andrade VR, Oliveira SS, Matias VLM (2002) Use of temephos for control of field population of *Aedes aegypti* in Americana São Paulo, Brazil. Dengue Bull 26:173–177

Garelli FM, Espinosa MO, Weinberg D, Trinelli MA, Gürtler RE (2011) Water Use Practices Limit the Effectiveness of a Temephos-Based *Aedes aegypti* Larval Control Program in Northern Argentina. PLoS Negl Trop Dis 5(3):e991

Goma LK (1964) Laboratory observations on the oviposition habits of *Aedes aegypti* (Linnaeus). Ann Trop Med Parasitol 58:347–349

Gonzalez Audino P, Licastro S, Zerba E (1999) Thermal behaviour and biological activity of pyrethroids in smoke-generating formulations. Pestic Sci 55:1187–1193

Gratz N, Knudsen B (1996) The rise and spread of dengue, dengue hemorrhagic fever and its vectors. A Historical Review (up to 1995). CTD/FIL (DEN) 96.7, WHO

Harburguer LV, Seccacini E, Masuh H, González Audino P, Zerba E, Licastro S (2009) Thermal behaviour and biological activity against *Aedes aegypti* (Diptera: Culicidae) of permethrin and pyriproxyfen in a smoke-generating formulation. PestManag Sci 65:1208–1214

Itaya N (1987) Insect juvenile hormone analogue as an insect growth regulator Sumitomo Pyrethroid World 8:2–4

Koehler PG, Patterson RJ (1991) Incorporation of pyriproxyfen in a German cockroach management program. J Econ Entomol 84:917–921

Kourí GI, Pelegrino JL, Munster BM, Guzmán MG (2007) Sociedad, economía, inequidades y dengue. Rev Cubana Med Trop 59

Kroeger A, Nathan MB (2006) Dengue: setting the global research agenda. Lancet 368:2193–2195

Kyle JL, Harris E (2008) Global spread and persistence of dengue. Ann Rev Microbiol 62:71–92

Lee YW, Gregorio ER, Khadri MS, Seleena F (1996) Ultra low volume application of *Bacillus thuringiensis* ssp. israeliensis for the control of mosquitoes. J Am Mosq Cont Assoc 12:651–655

Lucia A, Harburguer L, Licastro S, Zerba E, Masuh H (2009) Efficacy of a new combined larvicidal–adulticidal ultralow volume formulation against *Aedes aegypti* (Diptera: Culicidae), vector of dengue. Parasitol Res 104:1101–1107

Macoris MLG, Andrighetti MTM, Takaku L, Glasser CM, Garbeloto VC, Bracco JC (2003) Resistance of *Aedes aegypti* from the state of S. Paulo, Brazil, to organophosphates insecticides. Mem Inst Oswaldo Cruz 98:703–708

Mani TR, Arunachalam N, Rajendran R, Satyanarayana K, Dash AP (2005) Efficacy of thermal fog application of deltacide, a synergized mixture of pyrethroids, against *Aedes aegypti*, the vector of dengue. Trop Med Internat Health 10:1298–1304

Masuh H, Arnstein de Licastro S, Lopez PA, Vega C, Zerba E (2003) Field evaluation of a smoke generating formulation containing beta- cypermethrin against the dengue vector in Argentina. J Am Mosq Cont Assoc 19:53–57

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

Mulla MS, Darwazeh HA, Norland RL (1974) Insect growth regulators: evaluation procedures and activity against mosquitoes. J Econ Entomol 67:329–332

PAHO (1994) Dengue and dengue hemorrhagic fever in the Americas: Guidelines for prevention and control. Scientific Publication 548:28–29

PAHO (2011) Alerta epidemiológica: Actualización sobre la situación de Dengue en las Américas. Organización Panamericana de la Salud. 1–5 pp

Palomino M, Solari L, León W, Vega R, Vergaray M et al (2006) Evaluación del efecto residual del temephos en larvas de *Aedes aegypti* en Lima, Perú. Rev Peru Med Exp Salud Publica 23:158–162

Perich MJ, Tidwell MA, Williams DC, Sardelis MR, Pena CJ, Mandeville D et al (1990) Comparison of ground and aerial ultra-low volume applications of malathion against *Aedes aegypti* in Santo Domingo, Dominican Republic. J Am Mosq Cont Assoc 6:1–6

Perich MJ, Davila G, Turner A, Garcia A, Nelson M (2000) Behavior of resting Aedes aegypti (Culicidae: Diptera) and its relation to ultra-low volume adulticide efficacy in Panama City, Panama. J Med Entomol 37:541–546

Perich MJ, Sherman C, Burge R, Gill E, Quintana M, Wirtz RA (2001) Evaluation of the efficacy of lambda-cyhalothrin applied as ultra-low volume and thermal fog for emergency control of *Aedes aegypti* in Honduras. J Am Mosq Control Assoc 17:221–224

Pontes RJS, Regazzi ACF, Lima JWO, Kerr-Pontes LRS (2005) Residual effect of commercial applications of larvicides temefos and Bacillus thuringiensis israelensis on *Aedes aegypti* larvae in recipients with water renewal. Rev Soc Bras Med Trop 38:316–321

Raudenbush S, Bryk T, Congdon R (2000) HLM 6 Hierarchical Linear and Nonlinear Modeling. Scientific Software International, Inc. (c). http://www.ssicentral.com. Accessed 17 October 2011

Reiter P, Nathan MB (2003) World Health Organization. Report of the WHO Informal Consultation on the evaluation and testing of insecticidas—Guía para la evaluación de la eficacia del rociado espacial de insecticidas para el control del vector del dengue. (WHO/CTD/WHOPES/IC/96.1). Geneva, Switzerland

Seccacini E, Lucia A, Zerba E, Licastro MH (2008) *Aedes aegypti* (L.) resistance to temephos in Argentina. J Am Mosq Control Assoc 24:608–609

Sistema Nacional de Vigilancia de la Salud (SNVS) (2012) Dengue. Ministerio de Salud de la Nación. http://www.msal.gov.ar/dengue/index.php. Accessed 12 March 2012

Tawatsin A, Thavara U, Chompoosri J, Bhakdeenuan P, Asavadachanukorn P (2007) Larvicidal efficacy of new formulations of temephos in non-woven sachets against larvae of *Aedes aegypti* (L.) (Diptera: Culicidae) in water-storage containers. Southeast Asian J Trop Med Public Health 38:641–645

Thavara U, Tawatsin A, Srithommarat R, Zaim M, Mulla MS (2005) Sequential release and residual activity of temephos applied as sand granules to water storage jars for the control of *Aedes aegypti* larvae (Diptera: Culicidae). J Vector Ecol 30:62–72

Tidwell MA, Williams DC, Gwinn GA, Pena CJ, Tedders SH, Gonzalvez GE, Mekuria Y (1994) Emergency control of *Aedes aegypti* in the Dominican Republic using the Scorpion ™ ULV forced-air generator. J Am Mosq Control Assoc 10:403–406

WHO (World Health Organization) (1999) Bacillus thuringiensis. Environmental Health Criteria 217, Geneva

WHO (World Health Organization) (2005) Report of the Eighth WHOPES Working Group Meeting. Geneva. Guidelines for laboratory and field testing of mosquito larvicides (WHO/CDS/WHOPES/GCDPP/2005.13). Geneva, Switzerland

WHO (World Health Organization) (2007a) Pyriproxyfen in drinking water: use for vector control in drinking water sources and containers. Background document for development of WHO guidelines for drinking-water quality. Document WHO/SDE/WSH/07.01.9. Geneva, Switzerland, 13 pp

WHO (World Health Organization) (2007b) Pyriproxyfen in drinking water. Background document for development of WHO guidelines for drinking-water quality. Document WHO/SDE/WSH/07.01.10. Geneva, Switzerland, 13 pp

Yap HH, Chong ASC, Adanan R, Chong NL, Rohaizat B, Abdul Malik Y, Lim SY (1997) Performance of ULV formulations (Pestguard® 102/Vectobac® 12AS) against three mosquito species. J Am Mosq Cont Assoc 13:384–388

Zerba E (1995) Fumigant canisters and other novel insecticide formulations. Public Health Magazine (Bayer) 12:63–67

Zerba E, Melgar Suarez F, Licastro S, Wood EJ, Picollo MI, Malkenson N (1996) Un dispositivo fumigeno insecticida. Argentine Patent 248872