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Fenitrothion: an alternative insecticide for the control of deltamethrin-resistant populations of *Triatoma infestans* in northern Argentina

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Abstract. Deltamethrin-based campaigns to control *Triatoma infestans* (Klug) (Hemiptera: Reduviidae) have decreased in success as a result of the development of insecticide resistance. We compared the *in vitro* effects of the pyrethroid deltamethrin and two doses of the organophosphate fenitrothion, presented on different materials, on *T. infestans* from La Esperanza, Argentina. Laboratory tests demonstrated a decrease in susceptibility to deltamethrin in the field population [LD_{50}: 30.32 nanograms per insect (ng/i)] compared with the reference population (LD_{50}: 0.13 ng/i), giving a high resistance ratio of 233.42. By contrast, similar susceptibility to fenitrothion was assessed in both the field and reference populations (LD_{50}: 21.65 ng/i and 21.38 ng/i, respectively). The effectiveness of the formulated insecticides varied according to the surfaces to which they were applied. The application of fenitrothion formulations to glass or brick resulted in mortality of 90–100%. The application of fenitrothion formulations to wood or mud caused mortality in the range of 6.7–56.7%. Resistant insects presented low mortality when exposed to the deltamethrin formulation and high mortality when exposed to fenitrothion formulations. Moreover, the insecticides demonstrated residual activity only when applied to glass. The present work demonstrates that fenitrothion is an alternative to pyrethroids for the management of deltamethrin-resistant insects in La Esperanza. However, this effectiveness is not sustained over time.

Key words. *Triatoma infestans*, deltamethrin-resistant, alternative control, fenitrothion.

Introduction

Triatoma infestans (Klug) is currently considered to be the main vector of Chagas' disease in South America. This insect inhabits domestic and peridomestic environments in structures that facilitate the transmission of *Trypanosoma cruzi* (Kinetoplastida: Trypanosomatidae). This triatomine has historically been controlled with the use of insecticides; pyrethroids have been the preferred option since the 1980s (Zerba, 1999).

Despite vector control efforts, deltamethrin-based control campaigns decreased in success during the early 2000s. The field control failures were soon found to be related to insecticide resistance (Picollo *et al.*, 2005; Toloza *et al.*, 2008; Germano *et al.*, 2010). Studies conducted during the last decade showed that insecticide resistance in *T. infestans* evolved in several areas of the geographic distribution of the species (Picollo *et al.*, 2005; Toloza *et al.*, 2008; Germano *et al.*, 2010), and showed different profiles of resistance (Germanto *et al.*,

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2012) based on different resistance mechanisms (Picollo *et al.*, 2005; Santo Orihuela *et al.*, 2008; Pedrini *et al.*, 2009; Fabro *et al.*, 2012).

Recently, a locality in rural northern Argentina (La Esperanza, Chaco Province) was reported to be infested after repeated spraying with pyrethroids. The current study examines the toxicological effects of, respectively, the pyrethroid deltamethrin and two doses of the organophosphate fenitrothion, presented on different materials, on *T. infestans* populations from La Esperanza, Chaco, Argentina. We discuss the possible role of non-pyrethroids as an alternative for the control of pyrethroid-resistant populations.

Materials and methods

The study was carried out in La Esperanza, Province of Chaco, Argentina (26°03′ S, 60°27′ W). La Esperanza is located on a semi-arid plain and most of its houses are constructed with adobe walls. Houses were selected because their owners and the authorities of vector control campaigns reported domiciliary infestation after insecticide treatment.

Individuals were captured from infested dwellings using manual forceps following treatment with 0.2% tetramethrin (Icona SA, Buenos Aires, Argentina) as a dislodging agent. Captured insects and their offspring were raised in the laboratory under controlled temperature (26 ± 1 °C) and relative humidity (50–70%) conditions and a photoperiod of LD 12 : 12 h. A pigeon was provided weekly as a bloodmeal source [World Health Organization (WHO), 1994]. A reference *T. infestans* colony was obtained from descendents of insects provided by the Coordinación Nacional de Control de Vectores (Punilla, Córdoba, Argentina). These were reared under the same controlled laboratory conditions as the field populations.

Technical grade deltamethrin (99.5%) and fenitrothion (98.0%) used for bioassay were obtained from Dr Ehrenstorfer GmbH, Augsburg, Germany. The analytical grade acetone used for dilutions was purchased from Merck Argentina SA, Buenos Aires, Argentina.

Formulated deltamethrin [suspension concentrate (SC) 2.5%; Bayer SA, Buenos Aires, Argentina] and fenitrothion [wettable power (WP) 40%; Chemotécnica SA, Buenos Aires, Argentina] were provided by the Ministry of Health of Argentina.

Bioassays

Tests to determine insecticide susceptibility were performed using topical application, according to the WHO protocol (WHO, 1994). The test was conducted on *T. infestans* first-instar nymphs (5–7 days old, mean weight 1.3 ± 0.2 mg) that had been starved since eclosion. Each insect was treated with 0.2 μL of increasing doses of the insecticide diluted in acetone and applied to the dorsal abdomen using a 10-μL Hamilton syringe. Each dose was replicated at least three times. Mortality was recorded 24 h later and was defined by the inability of the nymph to walk to the edge of a filter paper (11 cm in diameter), with or without mechanical stimulation (WHO, 1994).

Deltamethrin was selected for toxicity studies because it represents the main insecticide used since the 1980s in fumigation campaigns for vector control of Chagas' disease. The organophosphate fenitrothion was selected because it has a different mode of action and data on its previous use in the field were available (Cichero *et al.*, 1983).

The effectiveness of formulated insecticides was tested on four different surfaces: glass; wood; adobe (mud and straw), and brick. Insecticides were applied to a square area (36 cm^2) on each material, using a 1-mL pipette and a constant flow to achieve uniform impregnation. Treated surfaces were dried for 1 h at room temperature, with the exception of glass, which was dried for 24 h. Each replicate consisted of a control group (water), SC 2.5% deltamethrin in water at 25 mg (AI)/m^2, WP 40% fenitrothion in water at 1 g (AI)/m^2, and WP 40% fenitrothion in water at 2 g (AI)/m^2. Six replicates were conducted for each insecticide and each formulation.

Groups of five nymphs (fifth-instar nymphs aged 10–20 days, starved since the last moult) were confined in glass rings (5.5 cm in diameter) and exposed for 1 h to the treated surfaces. After exposure, the insects were placed in clean flasks with filter papers and were maintained under the laboratory conditions described earlier for 72 h, prior to recording mortality.

The impregnated surfaces were kept under both indoor (domiciliary conditions) and outdoor (peridomiciliary) conditions in order to evaluate the effects of environmental factors on the residual efficacy of insecticide formulations on each substrate.

At 15 days after the initial treatment, tests were repeated in order to evaluate the residual activity of the formulated insecticides. In the case of glass, assays were repeated systematically until no mortality was detected.

Statistical analysis

Mortality data were corrected using the formula described by Abbott (1925). Dose–mortality values were subjected to probit regression analysis (Litchfield & Wilcoxon, 1949) using POLO-PC software (LeOra Software, 1987). Mean lethal dose values (LD_{50}) and confidence intervals (CIs) are expressed as nanograms per insect (ng/i). Resistance ratios (RRs) and 95% CIs were calculated as described by Robertson *et al.* (2007). Study populations were considered resistant when RRs were significantly different from 1 (i.e. when the 95% CI of the RR did not include the number 1).

Results

The toxicological responses of first-instar nymphs to deltamethrin and fenitrothion are shown in Table 1. Deltamethrin was more effective than fenitrothion in the reference susceptible colony (LD_{50} deltamethrin: 0.13 ng/i; LD_{50} fenitrothion: 21.5 ng/i). However, a significant decrease in susceptibility to deltamethrin was assessed in the field population (LD_{50}: 30.32 ng/i), which showed a high RR of

Table 1. Toxicological effects of deltamethrin and fenitrothion on first-instar nymphs of *Triatoma infestans*.

Insecticide	Population	*n*	DL_{50}, ng/i(95% CI)	Slope ± SE	RR(95% CI)
Deltamethrin	Reference	125	0.13* (0.12–0.15)	2.15 ± 0.70	–
	La Esperanza	224	30.32 (10.21–79.71)	0.64 ± 0.12	233.42 (116.78–466.57)
Fenitrothion	Reference	150	21.65 (5.35–67.91)	1.42 ± 0.11	–
	La Esperanza	161	21.38 (17.40–28.80)	4.47 ± 0.87	1.00 (0.76–1.33)

*Data from Picollo *et al.* (2005).
n, number of insects used in the bioassay; ng/i, nanograms per insect; 95% CI, 95% confidence interval; SE, standard error; RR, resistance ratio.

Table 2. Activity against *Triatoma infestans* of formulated insecticides applied on different surfaces at 1 h and 15 days after impregnation.

		Mortality, %, mean ± SE			
		1 h		15 days	
Surface	Insecticide	Reference	La Esperanza	Reference	La Esperanza
Glass	Control	0	0	0	0
	SC-deltamethrin	96.8 ± 3.3	16.7 ± 7.5	80.0 ± 11.6	13.3 ± 4.7
	WP-fenitrothion 1	100	100	100	100
	WP-fenitrothion 2	100	100	100	100
Adobe	Control	0	0	0	0
	SC-deltamethrin	63.3 ± 13.1	13.3 ± 6.7	0	0
	WP-fenitrothion 1	16.7 ± 6.1	10.0 ± 6.8	0	6.7 ± 6.7
	WP-fenitrothion 2	40.0 ± 17.3	36.7 ± 8.0	0	0
Wood	Control	0	0	0	0
	SC-deltamethrin	3.3 ± 3.3	13.3 ± 6.7	0	0
	WP-fenitrothion 1	26.7 ± 11.2	6.7 ± 4.2	6.7 ± 6.7	0
	WP-fenitrothion 2	56.7 ± 8.0	26.7 ± 11.2	0	0
Brick	Control	0	0	0	0
	SC-deltamethrin	100	13.3 ± 6.7	0	13.3 ± 4.7
	WP-fenitrothion 1	96.8 ± 3.3	100	0	0
	WP-fenitrothion 2	100	100	6.7 ± 6.7	0

SE, standard error; SC, suspension concentrate; WP, wettable power; SC-deltamethrin, SC 2.5% deltamethrin 25 mg/m^2; WP-fenitrothion 1, WP 40% fenitrothion 1 g/m^2; WP fenitrothion 2, WP 40% fenitrothion 2 g/m^2.

233.42. By contrast, similar susceptibility to fenitrothion was assessed in deltamethrin-susceptible and -resistant populations.

Mortality rates in susceptible and resistant nymphs exposed to impregnated materials are shown in Table 2. The effectiveness of the formulated insecticides varied according to the surface to which they were applied. Maximum mortality was detected in insects exposed to impregnated glass surfaces. As expected, resistant insects presented very low mortality when exposed to the deltamethrin formulation and high mortality when exposed to the fenitrothion formulations. A similar pattern was found when testing impregnated bricks, although lower mortality was registered on wood and adobe blocks. Applications on glass and brick resulted in mortality in the range of 90–100%. Applications on wood and mud produced mortality rates that were lower than expected at 6.7–56.7%. Moreover, fenitrothion-impregnated woods produced slightly higher mortality in deltamethrin-susceptible insects.

The residual effects of formulated insecticides under domestic and peridomestic conditions were measured as the mortality produced at different times subsequent to the first application (Fig. 1). The highest residual activity was registered for fenitrothion-impregnated glass kept under domiciliary conditions (100% at 12 weeks). Under peridomestic conditions, the residual activity of fenitrothion was found to decrease at 8 weeks (for 1 g/m^2) and 12 weeks (for 2 g/m^2).

When mortality was < 10% at 15 days, no residual effects were found under domestic or peridomestic conditions for either insecticide on wood, brick and adobe.

Discussion

The present study demonstrates high resistance to deltamethrin in field populations of *T. infestans* from La Esperanza in the Argentinean Chaco. This is the first report of high resistance levels (RR > 200) in an area of Argentina far from the focus of high resistance detected in 2002 in Salvador Mazza, Salta (RR: 130) (Picollo *et al.*, 2005). However, lower resistance levels (RR < 50) in other Argentine areas have been reported previously (Toloza *et al.*, 2008; Germano *et al.*, 2010, 2012). In particular, Gurevitz *et al.* (2012) concluded that reduced susceptibility to pyrethroids accounted for the failure to suppress *T. infestans* local populations in Pampa del Indio, Chaco, which is located 40 km from La Esperanza. Despite their geographical proximity, levels of resistance to deltamethrin were significantly lower in Pampa del Indio (RR: 7.17) than in La

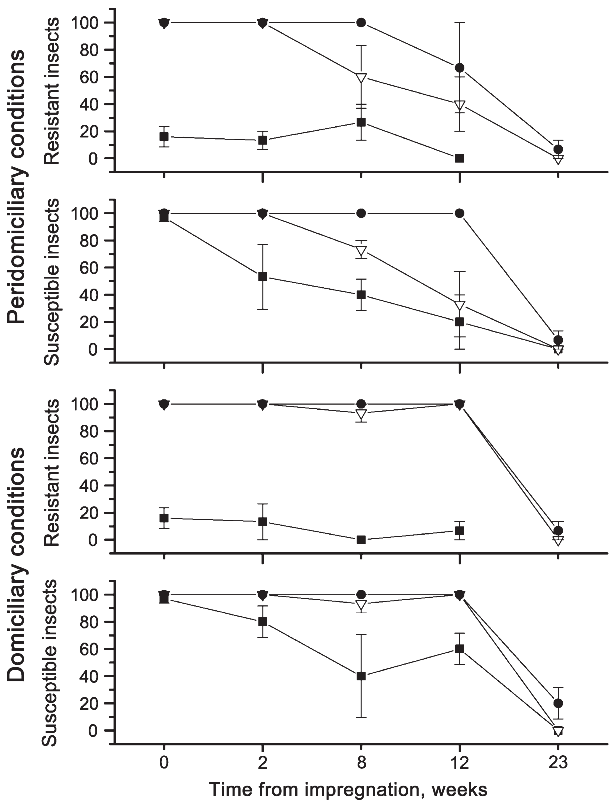

Fig. 1. Residual effects of formulated insecticides on susceptible and resistant *Triatoma infestans*. Assays were conducted on fifth-instar nymphs. Outcomes represent mortality (percentage) at successive timepoints subsequent to initial surface impregnation. ●, deltamethrin 25 mg/m^2; ∇, fenitrothion 1 g/m^2; ■, fenitrothion 2 g/m^2.

Esperanza. Differences in the toxicological characteristics of resistant *T. infestans* in different areas (even in neighbouring areas) suggest that deltamethrin resistance in La Esperanza has evolved independently and is not the result of the dispersion of insects from Salvador Mazza or Pampa del Indio.

Our laboratory bioassays demonstrated similar susceptibilities to fenitrothion in the susceptible reference colony and deltamethrin-resistant populations from La Esperanza. The results are consistent with those reported from other areas in which reduced susceptibilities to pyrethroids have been assessed (Picollo *et al.*, 2005; Toloza *et al.*, 2008; Germano *et al.*, 2010; Roca-Acevedo *et al.*, 2011). Recently, Germano *et al.* (2012) analysed three pyrethroid-resistant *T. infestans* populations and defined at least three resistance profiles according to the toxicological and biochemical characteristics of the studied resistant populations. Despite significant differences in levels of resistance to deltamethrin and fipronil, the expression of resistance in eggs and the role of pyrethroid-esterases, all of the deltamethrin-resistant populations were susceptible to fenitrothion.

Our bioassays with the formulated insecticides showed results similar to those obtained with technical grade insecticides. As expected, the deltamethrin formulation produced higher mortality in susceptible than resistant insects, and the fenitrothion formulation caused high mortality in both susceptible and resistant insects.

The effectiveness of these formulations against susceptible and resistant insects varied according to the surfaces to which they were applied. The two organophosphate formulations were highly effective on glass and brick, but less effective on adobe and wood. Furthermore, the formulated insecticides demonstrated residual activity only when they were applied to glass, into which active ingredient particles cannot penetrate and thus remain available for insect contact. These results clearly indicate the negative influence of surface porosity on the activity of the formulated insecticides.

The relation between residual effect and the nature of the treated substrate was also demonstrated in houses infested with triatomines in a community fumigated with lambdacyhalothrin (WP) in Paraguay (Ferro *et al.*, 1995). These authors found that wood board appeared to sustain higher residual effects than surfaces that contained mud because mud is more permeable than wood board. Similar results were reported by Rojas de Arias *et al.* (2004), who found that a highly porous surface (mud) showed lower initial insecticide activity and residual effect than a surface with lower porosity (lime-coated mud). The latter authors discussed the potential use of lime applied to porous surfaces to improve the performance of insecticide formulations.

In agreement with the present study, Guillén *et al.* (1997) and Gürlter *et al.* (2004) found low residual activity on wood and mud substrates and discussed the importance of time of spraying, climate conditions and level of infestation as fundamental factors affecting insecticide residual activity. Other authors have reported high residual activity of up to 30 days for wood and adobe impregnated with 0.5 g/m^2 and 1.0 g/m^2 of fenitrothion (WP) (Cichero *et al.*, 1983), and for wood impregnated with 25 mg/m^2 deltamethrin (SC and WP), 25 mg/m^2 cyfluthrin (SC) and 25 mg/m^2 lambdacyhalothrin (WP) (Rojas de Arias *et al.*, 2003). The differences in insecticide effect may reflect the higher exposure times used in these studies (8 h and 120 h, respectively). An exposure period of 1 h is a more realistic scenario in sprayed houses because *T. infestans* is active only during the night and engages in maximum activity just after dusk and at dawn, when insects are actively searching for a host and a refuge, respectively (Lazzari, 1992). Individuals of *T. infestans* spend much of their time in a refuge and, once inside a refuge, fall into an inactive state or akinesis (Lazzari & Lorenzo, 2009). It is unlikely that insects will receive a lethal dose of insecticide within a refuge and thus it is unlikely that insects will spend more than 1 h in contact with the insecticide. Insects are likely to make contact with the insecticide only when they are walking on the surfaces of walls and roofs in search of food or refuge.

In summary, the present work demonstrates that fenitrothion is an alternative to pyrethroids for the management of resistant insects in La Esperanza. However, this effectiveness may not be sustained over time when the insecticide is applied to the building materials typically used in endemic zones.

As demonstrated, the insecticide formulations currently used for triatominae control do not show a residual effect when they are evaluated in realistic exposure conditions, even in domestic contexts. Thus, the residual effectiveness of house spraying will depend not on the stability of the formulation, but on its initial impact (i.e. initial mortality) and on the recolonization dynamic of the species.

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