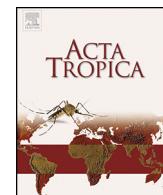




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Microgeographical study of insecticide resistance in *Triatoma infestans* from Argentina

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ABSTRACT

Chagas disease is a chronic parasitic infection restricted to America where it is currently estimated that 90 million people are at risk of acquiring the infection. Chemical control with pyrethroid insecticides has been effective to reduce disease transmission in several areas of the Southern Cone, although insecticide resistance has evolved and diminished the campaigns' results. Considering previous reports on the different levels of resistance between *Triatoma infestans* from different geographical areas, the objective of this work was to determine if *T. infestans* populations are toxicologically structured within localities. Response to the insecticide was measured and compared between houses of two Argentine localities. Different toxicity of deltamethrin was detected between dwellings of Chaco province, accounting for both susceptible and resistant houses within the same locality. However no difference was found among houses of Salta province. The results obtained in this work suggest that geographical structure is present not only at the between localities level, but also at the microgeographical level.

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1. Introduction

Chagas disease is a chronic parasitic infection restricted to America where it is currently estimated that 15 million people carry the disease, and 90 million people are at risk of acquiring the infection (Rodrigues Coura and Dias, 2009). The disease is caused by the protozoa *Trypanosoma cruzi*, which is transmitted to human through the feces of infected blood-sucking insects belonging to the subfamily Triatominae (Heteroptera: Reduviidae). *Triatoma infestans* (Klug, 1834) is the triatomine species responsible for most cases of Chagas disease in the continent (Dias and Schofield, 1999). Because no treatment is available for the chronic forms of the disease, control campaigns rely primarily on vector insect control (WHO, 2006). Chemical control, historically based on the use of organochlorine, organophosphate, carbamate and pyrethroid insecticides, led to the reduction of *T. infestans* distribution and consequent interruption of disease transmission in several areas of the Southern Cone (Dias et al., 2002; WHO, 2006; Zerba, 1999). However the insecticide resistance evolved in *T. infestans* populations. Resistance to deltamethrin was detected in South America since 1990s where low, medium and high resistance levels have been reported (Germano et al., 2010a, 2012; Picollo et al., 2005; Santo

Orihuela et al., 2008; Vassena et al., 2000). Moreover, high resistance levels correlated with field control failures were reported for the Argentine provinces of Salta and Chaco (Carvajal et al., 2012; Picollo et al., 2005). After a decade of studies, the resistance to insecticides in *T. infestans* is evidenced as a complex problem: the resistance evolved in different areas of the geographic distribution of the species, different resistance profiles were found in different areas, and different resistance mechanisms were described (i.e. enhanced metabolism, modified site of action, reduced penetration) (Fabro et al., 2012; Germano et al., 2010a, 2012; Pedrini et al., 2009; Picollo et al., 2005; Santo Orihuela et al., 2008; Toloza et al., 2008). Considering the high levels of structuring of *T. infestans* populations that have been found in the past (Marcat et al., 2008; Pérez de Rosas et al., 2007, 2008), we evaluate the toxicological response to deltamethrin in insects from individual houses of the same locality and we demonstrate that some houses host resistant insects while other houses host susceptible insects.

2. Materials and methods

2.1. Study design and insect rearing

Insects were collected at La Esperanza, Province of Chaco, Argentina and Acambuco, Province of Salta, Argentina, two rural areas where infestation after chemical treatment with deltamethrin (25 mg/m^2) had been previously reported (Germano et al., 2012, 2013). Last chemical treatment in these areas had taken

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Table 1Survivorship to discriminant dose and resistance levels (LDR) to deltamethrin in *T. infestans* from individual dwellings from Acambuco, Salta, Argentina.

House code	Dwelling structure ^a	Latitude/longitude	Survivorship to DD (%) (n) ^b	LD ₅₀ (ng/i) (95% CL) (n) ^b	Slope ± SE ^c	LDR (95% CL) ^c	Toxicological status ^d
Susceptible reference ^e	–	–	0 (30)	0.13 (0.11–0.15) (125)	2.15 ± 0.70	–	–
Locality reference ^f	–	–	–	6.4 (4.58–8.92) (169)	1.7 ± 0.21	32.5 (31.8–44.6)	R
A1	P	22°09'19"S 63°54'49"W	70 (34)	4.87 (1.99–11.27) (140)	1.85 ± 0.30	37.51 (25.89–54.35)	R
A2	P	22°20'11"S 63°50'35"W	62 (39)	2.59 (1.94–3.43) (135)	2.14 ± 0.32	19.90 (14.57–27.18)	R
A3	P	22°20'11"S 63°50'35"W	45 (40)	2.16 (1.22–3.26) (139)	1.28 ± 0.25	16.71 (10.35–26.94)	R

^a Site of insect collection within houses, namely domestic (D) or peridomestic (P) structures.^b Number of insects used for bioassays (n).^c Slope and lethal dose ratios (LDR) with respective standard error (SE) and 95% confidence limits (CL).^d Toxicological status expressed as resistant (R) vs susceptible (S) to deltamethrin.^e Data from Picollo et al. (2005).^f Data from Germano et al. (2013).

place between 1 and 5 years before insect collection, which took place between November 2009 and March 2010 at Acambuco and from August to December 2011 at La Esperanza. A susceptible strain raised at the laboratory since 1975 was used as a reference, as its response to deltamethrin had been repeatedly verified with susceptible field strains (Germano et al., 2010b; Picollo et al., 1976; Tolosa et al., 2008).

Every house was numbered and georeferenced. Both the house code and the structure from which the individuals were collected were recorded for insect rearing and further analysis. All of the captured insects from Acambuco were found at the peridomestic structures, namely chicken coops, pig and goat corrals. In the case of La Esperanza, infestation was generally higher and in addition to the houses that presented peridomestic infestation, some presented domiciliary infestation.

The search of insects was conducted in all of the houses from both localities. Insects were collected using timed manual collections, such that two trained workers did the search during 30 min, one in the domestic area, and the other in the peridomestic areas. Approximately 30% of the houses presented *T. infestans* infestation, although only those houses in which 10 or more adults were found were included in this assay, since a sufficient number of insects for bioassays were required. Considering that bioassays were conducted on the F1 (see below), a second requirement for house inclusion was that at least three adult females were available for reproduction.

Individuals were captured from infested dwellings using manual forceps and with the use of 0.2% tetramethrin as a dislodging agent (Icona SA, Buenos Aires, Argentina). Captured insects and their offspring were reared at the laboratory maintaining the house population independence, under controlled temperature ($26 \pm 1^\circ\text{C}$), humidity (50–70%) and photoperiod (12:12 L:D). A pigeon was weekly provided as a blood meal source (WHO, 1994).

2.2. Bioassays

Tests to determine insecticide susceptibility were done on the first generation obtained from the field collected insects, according to the World Health Organization protocol (WHO, 1994). The tests were conducted by topical application on *T. infestans* first

instars (5–7-d-old, mean weight 1.3 ± 0.2 mg) starved since eclosion. Each insect was treated with 0.2 µl of deltamethrin diluted in acetone, applied on the dorsal abdomen using a 10 µl Hamilton syringe provided with a repeating dispenser. Survivorship to a discriminant dose was used as a reference for insecticide response. This dose was previously established as twice the minimum dose which causes total mortality on the reference strain, and its value is 2 nanograms per insect (ng/i). For dose–mortality assays at least four doses in a range that produced between 10 and 90% mortality were tested, and each dose was replicated at least three times. The concentrations ranged from 0.02 to 500 ng/i of deltamethrin, and 10 insects were used for each replicate. Topical application with acetone was conducted for controls. After treatment, insects were held at the rearing laboratory conditions for 24 h, when mortality was recorded. The criterion for mortality was the inability to walk from the center to the border of an 11 cm diameter filter paper disk, meaning that only those nymphs which were able to reach the paper border, with or without mechanical stimulation, were considered alive.

2.3. Chemicals

Technical grade deltamethrin (99.0%) used for bioassay was obtained from Ehrestorfer, Germany. The analytical grade acetone used for dilutions was purchased from J.T. Baker, Mexico.

2.4. Data analysis

Mortality data were corrected using Abbott's formula (Abbott, 1925). Dose–mortality data were subjected to probit regression analysis (Litchfield and Wilcoxon, 1949) to estimate the lethal dose to kill 50% of the population (LD₅₀) by using POLO PC (LeOra, 1987). Lethal dose ratio (LDR) and 95% confidence limits (CL) were calculated as described by Robertson et al. (2007). Studied populations were considered resistant if the LDR 95% confidence limits did not include the number one. Fisher's exact test was used to evaluate the association between the toxicological profile (resistant vs susceptible) and the original location of the insect within dwellings (domestic or peridomestic structures).

Table 2Survivorship to discriminant dose and resistance levels (LDR) to deltamethrin in *T. infestans* from individual dwellings from La Esperanza, Chaco, Argentina.

House code	Dwelling structure ^a	Latitude/longitude	Survivorship to DD (%) (n) ^b	LD ₅₀ (ng/i) (95% CL) (n) ^b	Slope ± SE ^c	LDR (95% CL) ^c	Toxicological status ^d
Susceptible reference ^e	–	–	0 (30)	0.13 (0.11–0.15) (125)	2.15 ± 0.70	–	–
Locality reference ^f	–	–	–	30.32 (10.21–79.71) (224)	0.64 ± 0.12	233.42 (116.78–466.57)	R
E29	D	26°03'28"S 60°25'10"W	100 (30)	122.67 (76.61–219.15) (192)	1.02 ± 0.18	946.11 (557.53–1605.51)	R
E50	D	26°02'49"S 60°24'18"W	100 (30)	–	–	–	R
E168	D	26°06'28"S 60°23'54"W	24 (37)	0.15 (0.04–0.32) (197)	0.88 ± 0.17	1.20 (0.64–2.21)	S
E35	P	26°04'30"S 60°25'1"W	100 (30)	181.18 (104.27–542.95) (115)	1.21 ± 0.32	1419.61 (635.49–3171.23)	R
E36	P	26°04'00"S 60°24'60"W	100 (33)	68.78 (–) (60)	1.34 ± 0.36	529.09 (235.79–1187.20)	R
E58	P	26°02'03"S 60°25'56"W	100 (31)	–	–	–	R
E83	P	26°00'03"S 60°25'32"W	97 (30)	191.89 (–) (161)	0.72 ± 0.19	1417.70 (444.60–4521.11)	R
E131	P	26°05'01"S 60°25'45"W	40 (30)	1.11 (0.50–4.28) (108)	0.75 ± 0.18	8.62 (3.28–22.67)	R
E179	P	26°07'29"S 60°21'00"W	0 (30)	0.35 (–) (56)	2.69 ± 0.71	2.74 (1.59–4.72)	R
E3	P	26°02'56"S 60°28'44"W	9 (33)	0.14 (0.03–0.44) (175)	0.68 ± 0.15	1.09 (0.54–2.21)	S
E76	P	26°02'43"S 60°28'36"W	30 (30)	0.13 (0.01–0.48) (51)	0.88 ± 0.23	0.97 (0.31–3.06)	S
E84	P	26°01'26"S 60°25'31"W	19 (32)	0.16 (0.03–0.39) (200)	0.74 ± 0.16	1.23 (0.63–2.41)	S
E96	P	26°01'26"S 60°28'57"W	0 (30)	–	–	–	S
E109	P	25°59'46"S 60°29'29"W	7 (30)	0.14 (0.07–0.24) (164)	0.89 ± 0.15	1.07 (0.58–1.98)	S
E156	P	26°06'27"S 60°24'11"W	3 (30)	0.12 (0.01–0.30) (165)	0.83 ± 0.18	0.90 (0.40–2.03)	S

^a Site of insect collection within houses, namely domestic (D) or peridomestic (P) structures.^b Number of insects used for bioassays (n).^c Slope and lethal dose ratios (LDR) with respective standar error (SE) and 95% confidence limits (CL).^d Toxicological status expressed as resistant (R) vs susceptible (S) to deltamethrin.^e Data from Picollo et al. (2005).^f Data from Germano et al. (2013).

3. Results

Bioassay results for the sampled houses at Acambuco are presented in Table 1. Lethal doses and lethal dose ratios did not significantly differ between houses. The latter ranged from 18 to 37, showing that deltamethrin effectiveness in this locality is low.

Per house measures did not differ from the overall values previously reported for this locality.

Results for La Esperanza locality are presented in Table 2. Of the 16 samples tested, 9 houses presented deltamethrin resistant insects and 7 presented susceptible individuals. The LDRs were significantly different between dwellings, ranging from

complete susceptibility ($LDR \approx 1$) to high levels of resistance ($LDR > 1400$).

On the other hand, there was not statistical association between the toxicological status (resistant vs susceptible) and the original location of the insects within house dwellings in La Esperanza ($p > 0.05$).

4. Discussion

This is the first report of the susceptibility to deltamethrin in *T. infestans* at the microgeographical level, i.e. the toxicological response to insecticide in insects from different dwellings of the same locality. This study demonstrated that *T. infestans* from different dwellings of La Esperanza present different toxicological phenotype to deltamethrin. Particularly, we demonstrated that some houses host resistant insects and other houses host susceptible insects. This pattern was not identified in Acambuco locality, possibly associated to the fewer amount of highly infested houses in this area. However, it is to note that the resistance level in Acambuco houses was similar to the overall lethal dose ratio found in the past at this locality ($RR \approx 30$) (Germano et al., 2012), which may indicate that differences among houses of this locality are not apparent. Conversely, the overall resistance ratio in La Esperanza has been established at approximately $230 \times$ (Germano et al., 2013), while per-house values ranged from complete susceptibility to over $1400 \times$ resistance. The wide range of lethal dose ratios found for these locality's dwellings suggests that the conventional calculation for lethal doses approaches its average toxicological phenotype and evidences the likely outcome of spraying campaigns, although it does not necessarily account for the individual house response. This finding would indeed provide an explanation for the fact that residual foci are usually found on some houses, while others remain under an effective control.

On the other hand, it has been shown that deltamethrin residual activity is higher at the domestic than at the peridomestic structures, which are highly exposed to environmental conditions such as extreme temperatures, rain or sun exposition (Germano et al., 2013; Rojas de Arias et al., 2004). However, the toxicological condition of susceptible or resistant was not associated with the location of insects within houses (domestic vs peridomestic). This finding suggests that differences in persistence of the insecticide at both structures would not be a determinant factor in the selection for deltamethrin resistance in *T. infestans* from La Esperanza, reinforcing the notion that chemical control success in rural houses depends fundamentally on the initial impact of the insecticide, and on species dependant conditions such as biological cycle and/or recolonization times (Germano et al., 2013).

The results of the present study show that the geographical complexity of the phenomenon of resistance in *T. infestans* is greater than the known at present, demonstrating important differences in resistance status at the microgeographical level. Those differences highlight the complex spatial distribution of toxicological phenotypes of insects that persist after chemical control actions, and suggest a high level of population structure in *T. infestans* on which the insecticide exerts its selective pressure. Regarding the later, Pérez de Rosas et al. (2007, 2008) demonstrated through the analysis of microsatellite loci that *T. infestans* populations are highly structured. In particular, a significant genetic difference between insects from different houses in the same locality was reported, and genetic structure within the same house dwellings (e.g. domestic vs peridomestic area) was suggested (Marcket et al., 2008; Pérez de Rosas et al., 2008). Microgeographic structuring in *T. infestans* was also demonstrated by morphometry studies. Hernández et al. (2011) showed significant differences between habitats (chicken coops and goat corrals) in a zone of the arid Chaco region of

Argentina, using morphometric combined analysis of the head and wing. Considering that in this study toxicological differences were identified at the between houses level, it is likely that the similarities in the toxicological profile at the within house level imply that even when morphological differences may appear related to the habitat, the exchange of insects between domestic and peridomestic structures is large enough to sustain similar toxicological phenotypes. On the contrary, the differences that were found at the between house level may be consequence of a low gene flow between these insects which allows genetic divergence affecting toxicological characters. Thus, the results we present would confirm the low exchange of individuals between areas and possibly reflect *T. infestans* dispersal capacity, which generally covers less than 1.5 km (Cecere et al., 2006). In particular, these results may well agree with previous reports of spatial structuring in populations located at distances ranging from 1.4 to 13.5 km (Gaspe et al., 2012), which are in the range of distances between the houses studied in this research (0.5–20.19 km).

Furthermore, the different toxicological profiles of *T. infestans* from different locations have been regarded as independently originated (Germano et al., 2012). The presented findings reinforce that hypothesis and extend it to the microgeographical level, as insects from relatively close dwellings have been shown to develop different levels of resistance to deltamethrin after the exposure to similar levels of treatment. In conclusion, the resistance to deltamethrin in *T. infestans* is a complex phenomenon both at organismic (i.e. resistance mechanisms) and population (i.e. resistance levels and geographical distribution) levels. The diversity of insecticide toxicity between different dwellings of the same locality presents a challenge for vector control but also provides an explanation for the finding of residual insect foci after chemical treatment. Nonetheless locality-based estimations of insecticide susceptibility remain as an important tool for vector control as they provide an efficient overview of the locality's situation and have proven to be effective for control failure detection in the past (Carvajal et al., 2012; Picollo et al., 2005). This knowledge has a significant practical application, as it provides rational basis for the choice of alternative insecticides and allows addressing alternative control actions in a more accurate manner.

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References

- Abbott, W.S., 1925. A method for computing the effectiveness of an insecticide. *J. Med. Entomol.* 18, 265–267.
- Carvajal, G., Mouagabre-Cueto, G., Toloza, A.C., 2012. Toxicity of non-pyrethroid insecticides against *Triatoma infestans* (Hemiptera: Reduviidae). *Mem. Inst. Oswaldo Cruz* 107, 675–679.
- Cecere, M.C., Vazquez-Prokopek, G.M., Gürtler, R.E., Kitron, U., 2006. Reinfestation sources for Chagas disease vector, *Triatoma infestans*, in Argentina. *Emerg. Infect. Dis.* 12, 1096–1102.
- Dias, J.C.P., Schofield, C.J., 1999. The evolution of Chagas disease (American Trypanosomiasis) control after 90 years since Carlos Chagas discovery. *Mem. Inst. Oswaldo Cruz* 94, 103–121.
- Dias, J.C.P., Silveira, A.C., Schofield, C.J., 2002. The impact of Chagas disease control in Latin America – a review. *Mem. Inst. Oswaldo Cruz* 97, 603–612.
- Fabro, J., Sterkel, M., Capriotti, N., Mouagabre-Cueto, G., Germano, M., Rivera-Pomar, R., Ons, S., 2012. Identification of a point mutation associated with pyrethroid resistance in the para-type sodium channel of *Triatoma infestans*, a vector of Chagas disease. *Infect. Genet. Evol.* 12, 487–491.

- Gaspe, M.S., Schachter-broide, J., Gurevitz, J.M., Kitron, U., Görtler, R., Dujardin, J.P., 2012. Microgeographic spatial structuring of *Triatoma infestans* (Hemiptera: Reduviidae) populations using wing geometric morphometry in the Argentine Chaco. *J. Med. Entomol.* **49**, 504–514.
- Germano, M.D., Acevedo, G.R., Cueto, G.A.M., Toloza, A.C., Vassena, C.V., Picollo, M.I., 2010a. New findings of insecticide resistance in *Triatoma infestans* (Heteroptera: Reduviidae) from the Gran Chaco. *J. Med. Entomol.* **47**, 1077–1081.
- Germano, M.D., Picollo, M.I., Spillmann, C., Mougabeure-Cueto, G., 2013. Fenitrothion: an alternative insecticide for the control of deltamethrin-resistant populations of *Triatoma infestans* in Northern Argentina. *Med. Vet. Entomol.* <http://dx.doi.org/10.1111/mve.12014>.
- Germano, M.D., Santo Orihuela, P., Roca Acevedo, G., Toloza, A.C., Vassena, C., Picollo, M.I., Mougabeure Cueto, G., 2012. Scientific evidence of three different insecticide-resistant profiles in *Triatoma infestans* (Hemiptera: Reduviidae) populations from Argentina and Bolivia. *J. Med. Entomol.* **49**, 1355–1360.
- Germano, M.D., Vassena, C.V., Picollo, M.I., 2010b. Autosomal inheritance of deltamethrin resistance in field populations of *Triatoma infestans* (Heteroptera: Reduviidae) from Argentina. *Pest Manag. Sci.* **66**, 705–708.
- Hernández, M.S., Abrahan, L.B., Dujardin, J.P., Gorla, D.E., Catalá, S., 2011. Phenotypic variability and population structure of peridomestic *Triatoma infestans* in rural areas of the Arid Chaco (Western Argentine): spatial influence of macro- and microhabitats. *Vector-Borne Zoonotic Dis.* **11**, 503–513.
- LeOra, S., 1987. POLO-PC: A User's Guide to Probit or Logit Analysis, CA, Berkeley.
- Litchfield, J., Wilcoxon, F., 1949. A simplified method of evaluating dose-effect experiments. *J. Pharmacol. Exp. Ther.* **96**, 99–113.
- Marcket, P.L., Mora, M.S., Cutrera, A.P., Jones, L., Görtler, R.E., Kitron, U., Dotson, E.M., 2008. Genetic structure of *Triatoma infestans* populations in rural communities of Santiago del Estero, northern Argentina. *Infect. Genet. Evol.* **8**, 835–846.
- Pedrini, N., Mijailovsky, S.J., Girotti, J.R., Stariolo, R., Cardozo, R.M., Gentile, A., Juárez, M.P., 2009. Control of pyrethroid-resistant Chagas disease vectors with entomopathogenic fungi. *PLoS Negl. Trop. Dis.* **3**, e434.
- Pérez de Rosas, A., Segura, E., Fichera, L., García, B., 2008. Macrogeographic and microgeographic genetic structure of the Chagas disease vector *Triatoma infestans* (Hemiptera: Reduviidae) from Catamarca, Argentina. *Genetica* **133**, 247–260.
- Pérez de Rosas, A., Segura, E., García, B., 2007. Microsatellite analysis of genetic structure in natural *Triatoma infestans* (Hemiptera: Reduviidae) populations from Argentina: its implication in assessing the effectiveness of Chagas disease vector control programmes. *Mol. Ecol.* **16**, 1401–1412.
- Picollo, M.I., Wood, E., Zerba, E.N., Licastro, S.A., Rúveda, M.A., 1976. Laboratory test for measuring toxicity of insecticides in *Triatoma infestans*. *Klug. Acta Bioquím. Latinoam.* **X**, 67–70.
- Picollo, M.I., Vassena, C., Orihuela, P.S., Barrios, S., Zaidemberg, M., Zerba, E., 2005. High resistance to pyrethroid insecticides associated with ineffective field treatments in *Triatoma infestans* (Hemiptera: Reduviidae) from Northern Argentina. *J. Med. Entomol.* **42**, 637–642.
- Robertson, J.L., Preisler, H.K., Russell, R.M., 2007. Polo Plus, Probit and Logit Analysis: User's Guide, 2.0 ed. LeOra Software.
- Rodrigues Coura, J., Dias, J.C.P., 2009. Epidemiology, control and surveillance of Chagas disease: 100 years after its discovery. *Mem. Inst. Oswaldo Cruz* **104**, 31–40.
- Rojas de Arias, A., Lehane, M.J., Schofield, C.J., Maldonado, M., 2004. Pyrethroid insecticide evaluation on different house structures in a Chagas disease endemic area of the Paraguayan Chaco. *Mem. Inst. Oswaldo Cruz* **99**, 657–662.
- Santo Orihuela, P.L., Vassena, C.V., Zerba, E.N., Picollo, M.I., 2008. Relative contribution of monooxygenase and esterase to pyrethroid resistance in *Triatoma infestans* (Hemiptera: Reduviidae) from Argentina and Bolivia. *J. Med. Entomol.* **45**, 298–306.
- Toloza, A.C., Germano, M., Cueto, G.M., Vassena, C., Zerba, E., Picollo, M.I., 2008. Differential patterns of insecticide resistance in eggs and first instars of *Triatoma infestans* (Hemiptera: Reduviidae) from Argentina and Bolivia. *J. Med. Entomol.* **45**, 421–426.
- Vassena, C., Picollo, M.I., Zerba, E., 2000. Insecticide resistance in Brazilian *Triatoma infestans* and Venezuelan *Rhodnius prolixus*. *Med. Vet. Entomol.* **14**, 51–55.
- World Health Organization (WHO), 1994. Protocolo de evaluación de efecto insecticida sobre triatomíos. *Acta Toxicol. Argentina* **2**, 29–32.
- World Health Organization (WHO), 2006. Pesticides and Their Application for the Control of Vectors and Pests of Public Health Importance. World Health Organization, Geneva, pp. 64–66.
- Zerba, E., 1999. Susceptibility and resistance to insecticides of Chagas disease vectors. *Medicina* **59**, 41–46.