New Findings of Insecticide Resistance in *Triatoma infestans* (Heteroptera: Reduviidae) From the Gran Chaco

M. D. GERMANO,[1] G. ROCA ACEVEDO, G. A. MOUGABURE CUETO, A. C. TOLOZA, C. V. VASSENA, AND M. I. PICOLLO

Centro de Investigaciones de Plagas e Insecticidas (CIPEIN, CITEFA-CONICET); Juan Bautista de la Salle 4397 (B1603ALO), Villa Martelli, Provincia de Buenos Aires, Argentina

J. Med. Entomol. 47(6): 1077–1081 (2010); DOI: 10.1603/ME10069

ABSTRACT Chagas disease is a major health concern in Latin America, and *Triatoma infestans* (Klug, 1834) is responsible for the majority of cases of Chagas disease in the continent. After the discovery of deltamethrin highly resistant populations in the neighboring areas to Yacuiba (Bolivia) and Salvador Mazza (Argentina), we studied *T. infestans* populations north and southward, with the aim of describing the range of the resistant area. In addition, tests were conducted to describe the susceptibility to fipronil in deltamethrin-resistant populations. Tarija populations were highly resistant to deltamethrin, showing that the resistant area is greater than previously reported. Argentinean and Paraguayan populations were susceptible or presented moderate to low levels of deltamethrin resistance. Resistance to fipronil was found in Bolivian populations, although this insecticide was effective against Argentinean populations. This study shows that the Argentinean and Paraguayan populations are currently under successful control with deltamethrin. However, continuous resistance monitoring is necessary. We found that fipronil is a viable option for Argentina, whereas the resistance found in Bolivia implies that new formulations are needed to control *T. infestans* in Bolivia. Further research is required to find new alternatives of control in those areas that are currently suffering from high infestation rates.

KEY WORDS *Triatoma infestans*, resistance, deltamethrin, fipronil, Gran Chaco

Chagas disease is a major health concern in Latin America, and *Triatoma infestans* (Klug, 1834) is the main vector of this disease in the continent. After >20 yr of pyrethroid spraying campaigns (Zerba 1999), the emergence of deltamethrin resistance brings a new challenge for insect control.

Toxicological tests conducted during the late 1990s showed small levels of deltamethrin resistance associated to control campaigns in Argentina and Brazil (Vassena et al. 2000, Vassena and Picollo 2003). During 2002, though, the first field control failures against *T. infestans* from Argentina and Bolivia were reported. The toxicological tests conducted on field insects showed high deltamethrin resistance levels, which would explain the poor performance of house spraying (Picollo et al. 2005). In addition, recent studies confirmed an increasing deltamethrin resistance in northern Argentina, and the first detection of deltamethrin resistance in central Bolivia (Germano et al. 2010, Toloza et al. 2008).

After the discovery of deltamethrin highly resistant populations in the neighboring areas to Yacuiba (Tarija, Bolivia) and Salvador Mazza (Salta, Argentina) (Picollo et al. 2005), we studied *T. infestans* populations north and southward, with the aim of describing the range of the resistant area. Moreover, after the discovery of fipronil tolerance in the west-central area of Bolivia (Toloza et al. 2008), and considering this insecticide could be an alternative for control, we studied the response to fipronil in deltamethrin-resistant populations. In this study, we present a survey of several areas of Argentina, Bolivia, and Paraguay concerning their response to both insecticides, and discuss their implication in this vector's control.

Materials and Methods

Insect Sampling and Rearing. Considering the first findings of deltamethrin-resistant populations at Salvador Mazza (Salta, Argentina) and Yacuiba (Tarija, Bolivia), collection sites were selected with the aim of determining the range of the deltamethrin-resistant area. Insects were collected from different areas where domiciliary infestation after insecticide treatment had been reported, and were selected in a wide range that would provide a general knowledge of the Gran Chaco situation. In Bolivia, surveyed areas were

[1] Corresponding author: Centro de Investigaciones de Plagas e Insecticidas (CIPEIN, CITEFA-CONICET), Juan Bautista de la Salle 4397 (B1603ALO), Villa Martelli, Provincia de Buenos Aires, Argentina (e-mail: mgermano@citefa.gov.ar).

Fig. 1. Geographical location of sampled areas.

Villamontes, Tierras Nuevas, El Palmar, and Villa El Carmen (Tarija Department). In Argentina, surveyed areas were San Carlos, Corralito and Banda Sur (Salta Province), Capital, La Noria (Santiago del Estero Province), Taco Pozo (Chaco Province), Palo Blanco (Catamarca Province), and El Nochero (Santa Fé Province). In Paraguay, the surveyed area was Jerico (Boquerón Department). Surveyed areas' geographical location is presented in Fig. 1.

Individuals were captured from infested house dwellings with the use of manual forceps and without the use of dislodging agent. Captured insects and their offspring were raised at the laboratory under controlled temperature (28 ± 1°C), humidity (50–70%), and photoperiod (12:12 L:D). A pigeon was weekly provided as a blood meal source (WHO 1994).

Chemicals. Technical grade deltamethrin (99.0%) and fipronil (95.5%) used for bioassay were obtained from Ehrestorfer, Augsburg, Germany. The analytical grade acetone used for dilutions was purchased from J. T. Baker (Edo. de Mexico, Mexico).

Insecticide Susceptibility Determination. Every field sample was analyzed to account for its response to deltamethrin. Those samples that resulted in resistance to deltamethrin were also tested for fipronil effectiveness, as a mean to determine this compound viability as a control alternative. A laboratory strain (CIPEIN), which has been reared at the laboratory since 1975 and never exposed to insecticide treatment, was used as a reference for insecticide susceptibility (Picollo et al. 1976).

Lethal doses were determined according to the World Health Organization protocol (WHO 1994). Bioassays were conducted on the F_1 of the field-collected insects, and consisted of the topical application of 0.2 μl of insecticide in acetone solutions. The insecticide application was conducted by means of a 10 μl Hamilton syringe with automatic dispenser, on the dorsal abdomen of first instar nymphs. These were 3–5 d old and had been starved since eclosion. Three replicates of at least four doses in a range that produced between 10 and 90% mortality were conducted, and control groups received only pure acetone. Insects were held at the same laboratory conditions for 24 h, when mortality was evaluated. To this aim, treated insects were placed at the center of a circular filter paper (11 cm diameter), and their behavior was observed. Only those nymphs that were able to walk to the paper border, with or without mechanical stimulation, were considered alive.

Data Analysis. Lethal doses 50% (LD_{50}) and resistance ratios (RR) were calculated according to Robertson and Preisler (1992), using POLO Plus software (Robertson et al. 2007). Studied populations were considered resistant if the RR confidence limits did not include the number 1. LD_{50} values are expressed in nanograms per insect units.

Results

Susceptibility values (LD_{50}), slopes of regression lines, and RR to deltamethrin in the studied populations are shown in Table 1. All Bolivian RRs were significantly >1, indicating that the studied populations were resistant to deltamethrin. Also, five Argentinean populations were resistant to deltamethrin, with RR ranging from 3.7 to 8.4. Clearly, Bolivian populations showed very high resistance levels, which resulted in significantly higher levels than those determined for the Argentinean-resistant populations. The sample from Paraguay resulted in slight resistance to deltamethrin.

Susceptibility values, slopes of regression lines, and RR to fipronil in the studied populations are shown in Table 2. Villa El Carmen and El Palmar populations showed RR values that differed significantly from 1 (RR = 92.7 and 55.5, respectively), indicating that those populations were resistant to fipronil. Three Argentinean populations presented low levels of resistance to this insecticide (Banda Sur, San Carlos, and Capital). On the contrary, Tierras Nuevas and Corralito populations resulted in susceptibility to fipronil.

Discussion

Bolivian populations presented high levels of resistance to deltamethrin, which demonstrates that the known resistant area reported by Picollo et al. (2005) is greater than previously expected. Chemical control with deltamethrin is strongly jeopardized in Tarija department, given that doses required to eliminate insects from house dwellings are increased by a 300× factor and more. Previous findings of resistance in this department (Santo Orihuela et al. 2008) showed similar ratios of resistance to the Salvador Mazza area, but our present results demonstrate a greater level of resistance to deltamethrin. However, and even though the resistant area is extended to the north with increasingly greater resistance, this phenomenon does not affect the whole Gran Chaco area studied.

The nearest population to the Salvador Mazza-resistant area, Banda Sur, presented the highest resistance level found in the survey of Argentinean areas.

Table 1. Bioassay statistics and resistance ratios to deltamethrin in *T. infestans* from Argentina, Bolivia, and Paraguay

Country	Province/ Department	Population	n^a	Slope ± SE	LD_{50} (ng/i)[b] (95% CL)	RR[b] (95% CL)
Reference		CIPEIN[c]	125	2.1 ± 0.66	0.1 (0.12–0.15)	–
Argentina	Salta	S. Mazza[c]	90	1.4 ± 0.10	31.1 (12.9–84.7)	133.1 (105.6–167.7)
		Banda Sur	121	1.8 ± 0.31	5.1 (3.07–8.70)	39.0 (26.37–57.85)
		Corralito	138	3.1 ± 0.25	0.02 (0.01–0.03)	0.1 (0.07–0.18)
		San Carlos	148	1.7 ± 0.27	1.1 (0.51–2.54)	8.4 (5.86–12.13)
	Catamarca	Palo Blanco	220	2.7 ± 0.09	0.6 (0.07–0.69)	4.5 (2.47–8.13)
	Santiago del Estero	Capital	151	2.0 ± 0.31	0.5 (0.27–0.80)	3.8 (2.82–5.22)
		La Noria	145	1.8 ± 0.35	0.04 (0.02–0.05)	0.3 (0.15–0.45)
	Chaco	Taco Pozo	138	2.0 ± 0.31	0.5 (0.22–0.80)	3.7 (2.68–5.10)
	Santa Fé	El Nochero	151	3.1 ± 0.25	0.1 (0.11–0.14)	0.9 (0.50–1.61)
Bolivia	Tarija	T. Nuevas	155	0.9 ± 0.17	70.1 (26.63–1,240.21)	541.6 (260.23–1,127.32)
		V. El Carmen	184	0.6 ± 0.14	56.7 (24.47–313.15)	438.0 (147.47–1300.60)
		El Palmar	166	0.7 ± 0.11	33.2 (23.40–53.80)	299.8 (163.80–548.80)
		Villamontes	115	0.8 ± 0.07	32.0 (16.45–128.06)	247.4 (173.08–353.63)
Paraguay	Boquerón	Jerico	90	1.6 ± 0.10	0.5 (0.01–1.59)	3.7 (2.99–4.52)

ng/i, Nanograms per insect units.
[a] Number of insects used for bioassays.
[b] LD_{50}, RR, and 95% confidence limits (CL) calculated following Robertson et al. (2007).
[c] Data from Picollo et al. (2005).

However, this is still significantly smaller than the Salvador Mazza resistance ratio. This indicates that the resistance to the studied pyrethroid is not so high in areas that are not bordering to the resistant Yacuiba-Salvador Mazza area. Interestingly, we found an incipient resistance to deltamethrin in San Carlos, at the south of Salta province. However, its nearby population Corralito resulted in more susceptibility to deltamethrin than the reference population CIPEIN.

The insects from Catamarca, Chaco, Santa Fé, and Santiago del Estero provinces were susceptible or slightly resistant to deltamethrin. The Paraguay survey showed a small RR. These results suggest that deltamethrin is currently a considerable and valuable tool for chemical control of *T. infestans* in these areas.

Fipronil insecticide was tested on deltamethrin-resistant samples with the aim to propose this insecticide as a control alternative. This compound was effective against Argentinean and Tierras Nuevas populations, but the other populations from Tarija presented a moderated level of resistance to fipronil. Resistance to fipronil was also recently detected in central Bolivia by Toloza et al. (2008), who proved a high resistance to this insecticide in populations with moderate resistance levels to deltamethrin. This insecticide has not been used in the field for chemical control of *T. infestans*, which implies that the resistance found is not a consequence of selection after fipronil treatment. Also, the fact that deltamethrin's action site is sodium channels, and that of fipronil is γ-aminobutyric acid-gated chlorine channels implies that the possibility of a cross-resistance phenomenon as a result of altered action site between these compounds is highly improbable. However, as fipronil presents a mode of action that is similar to that of lindane and dieldrin, it is possible that the early use of these compounds in Bolivia (Dias 1997, Schofield 1994) would cause a cross-resistance phenomenon to fipronil. Cross-resistance between dieldrin and fipronil has been described fundamentally in cockroaches, even when dieldrin had not been used for control for several generations before the implementation of fipronil (Holbrook et al. 2003, Kristensen et al. 2005), and lindane cross-resistance to fipronil has recently been demonstrated for *Musca domestica* (Kristensen et al. 2004).

Table 2. Bioassay statistics and resistance ratios to fipronil in *T. infestans* from Argentina and Bolivia

Country	Province/ Department	Population	n^a	Slope ± SE	LD_{50}[b] (95% CL)	RR[b] (95% CL)
Reference		CIPEIN[c]	150	1.4 ± 0.11	2.1 (1.28–3.46)	–
Argentina	Salta	S. Mazza[d]	180	0.9 ± 0.11	4.9 (3.25–5.61)	1.9 (1.00–3.70)
		Banda Sur	90	1.0 ± 0.09	6.2 (4.36–8.98)	3.0 (2.58–3.57)
		San Carlos	128	1.2 ± 0.24	4.9 (2.34–12.99)	2.2 (1.11–4.41)
		Corralito	90	0.7 ± 0.23	2.3 (0.36–7.52)	1.09 (0.16–7.04)
	Santiago del Estero	Capital	158	1.1 ± 0.18	4.5 (2.31–9.90)	2.0 (1.04–3.91)
Bolivia	Tarija	T. Nuevas	145	0.8 ± 0.08	0.4 (0.20–0.60)	0.19 (0.11–0.35)
		V. El Carmen	121	2.4 ± 0.56	205.9 (148.0–267.1)	92.7 (53.52–160.66)
		El Palmar	176	0.9 ± 0.21	123.1 (50.7–215.3)	55.5 (26.95–114.31)

[a] Number of insects used for bioassays.
[b] LD_{50}, RR, and 95% confidence limits (CL) calculated following Robertson et al. (2007).
[c] Data from Santo Orihuela et al. (2008).
[d] Data from Toloza et al. (2008).

Given that both deltamethrin and fipronil are metabolized by monooxygenases, it may be possible that a cross-resistance phenomenon is present because of this mechanism. In research conducted by Scott and Wen (1997), monooxygenase metabolism was related to pyrethroid-fipronil cross-resistance in *M. domestica.* Also, small resistance ratios to fipronil were found in highly deltamethrin-resistant strains of *Spodoptera litura* that presented an increased monooxygenase metabolism (Huang and Han 2007). Whether this could be the case in *T. infestans* remains unknown.

Further interesting differences among the Yacuiba and Salvador Mazza populations were previously reported (Toloza et al. 2008). These authors demonstrated that deltamethrin resistance is expressed only in nymphs from Bolivia, whereas both the eggs and nymphs from Salvador Mazza express resistance to this insecticide. Additional toxicological evidence for these populations was provided by Santo Orihuela et al. (2008), who proved that the esterase activity was increased only in the Salta population Salvador Mazza. These toxicological differences, in addition to the results presented in this work, leave room for thought of natural differences among the different populations of *T. infestans.*

Several studies have demonstrated that populations of *T. infestans* are highly structured (Marcet et al. 2008; Pérez de Rosas et al. 2007, 2008), and recent research confirmed that Bolivian and Argentinean populations are part of different haplotype clusters (Monteiro et al. 1999, Piccinali et al. 2009). However, hypothesis on *T. infestans* origin and geographic spread suggests that this insect would have its origin in the Bolivian Andes, and then migrated to the Gran Chaco area. A founder effect in the Argentinean Austral Chaco could have led to a genetic material loss, which would play an important role in the genetic structure of *T. infestans* populations (Panzera et al. 2004). The question remains open about the possibility of different genetic backgrounds, in addition to different selection pressure and ecological variables, allowing the different outcome of pyrethroid treatment found in both countries. Further research on nontreated populations would allow clarifying this possibility and its implications in vector control.

Finally, it is important to note that field control failures have been up to date correlated with RR that are >50 (Picollo et al. 2005), and that small RR imply that the population is currently under successful control. In accordance with these results, the Bolivian Ministry of Health and Sports has reported vectorial infestation rates higher than 20% in the Tarija department, which could be related to the resistance found in these assays. Still, small resistance ratios indicate that field control failures must be prevented by continuous insecticide resistance monitoring and the implementation of optimal control strategies in each area (Rojas Cortez et al. 2007).

Currently, the control of *T. infestans* presents new challenges in the Southern Cone area of the Gran Chaco. The ineffectiveness of field treatments constitutes a hard to overcome obstacle in vector control, and hence, surveillance becomes a necessity for any Chagas control initiative in this area. Further investigation on available insecticide types is required to find new alternatives of control in those areas that are currently suffering from high infestation rates. Furthermore, community participation and rural house improvement will necessarily play an important role in the future of vector control initiatives that aim to attain a sustainable approach to this neglected disease management.

Acknowledgments

We thank Mario Zaidemberg, Héctor Janútolo, and technicians from the Coordinación Nacional de Control de Vectores del Ministerio de Salud de Argentina for the coordination of field sampling in Argentina. We thank Mirko Rojas Cortéz and technicians of the Ministerio de Salud y Deportes de Bolivia for the coordination of field sampling in Bolivia. This work was supported by the UNICEF/United Nations Development Program/World Bank/World Health Organization Special Programme for Research and Training in Tropical Diseases and the Consejo Nacional de Investigaciones Científicas y Técnicas of Argentina.

References Cited

Dias, J.C.P. 1997. Controle da doenca de Chagas, pp. 453–468. *In* Clínica e Terapéutica da Doenca de Chagas: Um Manual Práctica Para o Clínico Peral. Fio Cruz, Río de Janeiro, Brazil.

Germano, M. D., C. V. Vassena, and M. I. Picollo. 2010. Autosomal inheritance of deltamethrin resistance in field populations of *Triatoma infestans* (Heteroptera: Reduviidae) from Argentina. Pest Manag. Sci. 66: 705–708.

Holbrook, G. L., J. Roebuck, C. B. Moore, M. G. Waldvogel, and C. Schal. 2003. Origin and extent of resistance to fipronil in the German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae). J. Econ. Entomol. 96: 1548–1558.

Huang, S., and Z. Han. 2007. Mechanisms for multiple resistances in field populations of common cutworm, *Spodoptera litura* (Fabricius) in China. Pestic. Biochem. Physiol. 87: 14–22.

Kristensen, M., J. B. Jespersen, and M. Knorr. 2004. Cross-resistance potential of fipronil in *Musca domestica.* Pest Manag. Sci. 60: 894–900.

Kristensen, M., K. K. Hansen, and K. M. Jensen. 2005. Cross-resistance between dieldrin and fipronil in German cockroach (Dictyoptera: Blattellidae). J. Econ. Entomol. 98: 1305–1310.

Marcet, P. L., M. S. Mora, A. P. Cutrera, L. Jones, R. E. Gürtler, U. Kitron, and E. M. Dotson. 2008. Genetic structure of *Triatoma infestans* populations in rural communities of Santiago del Estero, northern Argentina. Infect. Genet. Evol. 8: 835–846.

Monteiro, F. A., R. Pérez, F. Panzera, J. P. Dujardin, C. Galvão, D. Rocha, F. Noireau, C. Schofield, and C. B. Beard. 1999. Mitochondrial DNA variation of *Triatoma infestans* populations and its implication on the specific status of *T. melanosoma.* Mem. Inst. Oswaldo Cruz 94: 229–238.

Panzera, F., J. P. Dujardin, P. Nicolini, M. N. Caraccio, V. Rose, T. Tellez, H. Bermúdez, M. D. Bargues, Mas-Coma Santiago, J. E. O'Connor, and R. Pérez. 2004. Genomic changes of Chagas disease vector, South America. Emerg. Infect. Dis. 10: 438–446.

Pérez de Rosas, A., E. Segura, and B. García. 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.

Pérez de Rosas, A., E. Segura, L. Fichera, and B. García. 2008. Macrogeographic and microgeographic genetic structure of the Chagas disease vector *Triatoma infestans* (Hemiptera: Reduviidae) from Catamarca, Argentina. Genetica 133: 247–260.

Piccinali, R. V., P. L. Marcet, F. Noireau, U. Kitron, R. E. Gürtler, and E. M. Dotson. 2009. Molecular population genetics and phylogeography of the Chagas disease vector *Triatoma infestans* in South America. J. Med. Entomol. 46: 796–809.

Picollo, M. I., E. Wood, E. N. Zerba, S. A. Licastro, and M. A. Rúbeda. 1976. Laboratory test for measuring toxicity of insecticides in *Triatoma infestans*, Klug. Acta Bioquímica Latinoamericana X: 67–70.

Picollo, M. I., C. Vassena, P. S. Orihuela, S. Barrios, M. Zaidemberg, and E. Zerba. 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., and H. K. Preisler. 1992. Pesticide Bioassays with Arthropods. CRC, Boca Ratón, FL.

Robertson, J. L., H. K. Preisler, and R. M. Russell. 2007. Polo Plus, Probit and Logit Analysis: user's guide, version 2.0. LeOra Software, FL.

Rojas Cortez, M., R. Barrientos, E. G. Fernández Aráoz, L. Ortiz, A. Sánchez Bustamante, A. Tamayo, E. Durán, J. Chungara, M. Avalos, F. Torrico, C. Billot, C. Alonso-Vega, A. De Muynck, Y. Ordóñez, F. Fernández, B. Chang, F. Durán, A. Nogales, F. Cruz, and E. Villena. 2007. El Programa Nacional de Chagas en Bolivia, pp. 315–327. *In* M. Rojas Cortez (ed.), Triatominos de Bolivia y la Enfermedad de Chagas. Ministerio de Salud y Deportes–Programa Nacional de Chagas, La Paz, Bolivia.

Santo Orihuela, P. L., C. V. Vassena, E. N. Zerba, and M. I. Picollo. 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.

Schofield, C. J. 1994. Triatominae: Biología y Control. Eurocommunica Publications, West Sussex, United Kingdom.

Scott, J. G., and Z. Wen. 1997. Toxicity of fipronil to susceptible and resistant strains of German cockroaches (Dictyoptera: Blattellidae) and house flies (Diptera: Muscidae). J. Econ. Entomol. 90: 1152–1156.

Toloza, A. C., M. Germano, G. M. Cueto, C. Vassena, E. Zerba, and M. I. Picollo. 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. V., and M. I. Picollo. 2003. Monitoreo de resistencia a insecticidas en poblaciones de campo de *Triatoma infestans* y *Rhodnius prolixus*, insectos vectores de la Enfermedad de Chagas: Revista de Toxicología en Línea 3. (http://www.sertox.com.ar/retel/n03/004.pdf).

Vassena, C., M. I. Picollo, and E. Zerba. 2000. Insecticide resistance in Brazilian *Triatoma infestans* and Venezuelan *Rhodnius prolixus.* Med. Vet. Entomol. 14: 51–55.

[WHO] World Health Organization. 1994. Protocolo de evaluación de efecto insecticida sobre triatominos. Acta Toxicológica Argentina 2: 29–32.

Zerba, E. 1999. Susceptibility and resistance to insecticides of Chagas disease vectors. Medicina 59: 41–46.

Received 17 March 2010; accepted 9 July 2010.