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Insecticide resistance of *Triatoma infestans* (Hemiptera, Reduviidae) vector of Chagas disease in Bolivia.

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OBJECTIVE: To define the insecticide resistance status of *Triatoma infestans* to deltamethrin (pyrethroid), malathion (organophosphate) and bendiocarb (carbamate) in Bolivia.

METHODS: Fifty populations of *T. infestans* were sampled in Bolivian human dwellings. Quantal response data were obtained by topical applications of 0.2µl of insecticide-acetone solutions on nymphs N1 of the F1 generations. For most populations, dose-mortality relationships and resistance ratios (RR) were analyzed. Discriminating concentrations were established for each insecticide with a susceptible reference strain and used on the other field populations. A tarsal-contact diagnostic test using insecticide impregnated papers was designed to rapidly identify deltamethrin resistant populations in the field.

RESULTS: Discriminating concentrations for topical applications were 5, 70 and 120 ng active ingredient /insect for deltamethrin, bendiocarb and malathion respectively. The diagnostic concentration for deltamethrin was 0.30% for the one hour exposure by tarsal contact. All populations sampled in human dwellings exhibited significant levels of resistance to deltamethrin, from 6 to 491 and varied among regions. Resistant populations did not recover complete susceptibility to deltamethrin when the synergist piperonyl-butoxite (PBO) was used. None of the sampled populations exhibited significant resistance to bendiocarb (all RR_{50} <1.8) or malathion (all RR_{50} < 2.2).

CONCLUSION: In Bolivia, most "domestic" *T. infestans* populations are resistant to deltamethrin. Because insecticide vector control is the only selection pressure, resistance likely originates from it. Switching from pyrethroids to organophosphates or carbamates could be a short term solution to control this vector, but other alternative integrated control strategies should also be considered in the long term.

Keywords: *Triatoma infestans*, insecticide, resistance, Bolivia

Introduction

With more than 10 million human cases, Chagas disease is one of the most important parasitic diseases in Latin America. It is caused by the protozoan *Trypanosoma cruzi* (Kinetoplastida, Trypanosomatidae) and is the major cause of cardiopathy in the world (Yacoub *et al.* 2008). The parasite is transmitted mainly by blood sucking insects of the Triatominae family (Heteroptera, Reduviidae) which are responsible of more than 80% of human cases (Schofield 1994). In Bolivia, 55% of the territory is considered endemic for Chagas disease and involves around four million people at-risk (≈50% of the total population). As well as in all the countries of the "southern cone" (Southern Peru, Bolivia, Argentina, Paraguay, Brazil), the kissing bug *Triatoma infestans* is the main vector (WHO 2002). It entirely completes its life cycle in human dwellings (in intra- and peri- domicile environments) and present vector control strategies are therefore based on indoor and outdoor sprayings of persistent insecticides (pyrethroids). In Bolivia this spraying is the responsibility of the Ministry of Health which manages a National Program for the Control of Chagas Disease (NPCCD) (Rojas Cortez, 2007).

Despite some good results obtained with insecticide sprayings in the southern cone countries since the 1950's (Zerba, 1999), endemic vector-borne transmission still occurs in large areas of Southern Peru, Bolivia and Argentina. Several factors may explain the maintenance of Chagas transmission in these regions (Gürtler *et al.*, 2007) among which insecticide resistance has recently been pointed out as one of the most significant (Vassena *et al.* 2007). Indeed, since 1997, resistance to pyrethroids has been detected in certain areas of Argentina (Vassena & Picollo 2003, Gonzalez Audino *et al.* 2004) and in 2002, failures in field insecticide treatments have been associated with deltamethrin resistance (Picollo *et al.* 2005). Recently, high levels of deltamethrin resistance have been observed in Bolivia, in the vicinity of Yacuiba, Sucre and Mataral (Santo Orihuela *et al.* 2008, Toloza *et al*. 2008), confirming preliminary data obtained in 2003-2005 (Vassena *et al.* 2007). In Bolivia however, the geographical extend and the magnitude of insecticide resistance is still unknown despite some recent alarming reports of treatment failures by technicians of the NPCCD.

The aim of the present study is therefore to assess the resistance status of various Bolivian field populations of *T. infestans* to deltamethrin, the pyrethroid insecticide used by the Bolivian NPCCD. An organophosphate insecticide (malathion) and a carbamate (bendiocarb) have been proposed as alternatives to deltamethrin in Bolivia (Vassena *et al.* 2007) and therefore are also tested here. A diagnostic concentration for each of the three tested

insecticide is set for topical applications. A tarsal-contact diagnostic test is also proposed to rapidly assess the resistance status of field populations to deltamethrin. This study is the first one carried out at a large geographic scale in Bolivia.

Methods

***T. infestans* populations.** Fifty field populations of *T. infestans* were collected between 2006 and 2009 by means of active search in infested human dwellings (Pinchin *et al.* 1981) by the personnel of the NPCCD before routine insecticide treatments. Localities were chosen without any previous knowledge of the *T. infestans* population resistance status. Three localities were in the Cochabamba Department, 20 in the Chuquisaca Dept., 20 in the Tarija Dept. and 7 in the La Paz Dept., covering most of the geographical distribution of the species in this country (only populations from the Santa-Cruz Dept. are lacking) (Fig. 1).

In Chivisivi (La Paz Dept.) a *T. infestans* population (normal phenotype) was also captured with sticky traps in a sylvatic rocky environment 500 m away from any house or human activities. Sylvatic populations are considered as independent entities without any evident interchanges with "human dwelling" populations (Noireau 2009). Field populations were reared in the insectary following Núñez & Segura (1987) until the F1 generation from which first-instar nymphs (N1) were used for the bio-assays. The laboratory strain CIPEIN (Picollo *et al.* 1976) was used as a susceptible reference strain for bio-assays.

Chemicals. Technical grade insecticides were deltamethrin (100% purity, AgrEvo, Berkham, UK), malathion (98.4% purity, Supelco, Bellefonte PA, USA) and bendiocarb (96% purity, Aventis CropScience (Bayer), Cambridge, UK). Pipernonyl-butoxide (PBO) (Sigma-Aldrich, France) was used as a synergist for deltamethrin.

Topical application bioassays. Serial dilutions of the insecticides in acetone were prepared and 0.2µl of solution was topically applied on the dorsal surface of the abdomen of each N1 (WHO 1994, CIPEIN-CITEFA 2001), with a 10µl Hamilton Microliter 701 micro-syringe (Hamilton Co, Reno NE, USA) mounted on a repeating dispenser (Hamilton PB600). The mean weight of N1 was 1.69 mg (± 0.31). At least 15 (and up to 100) insects per dose per replicate were used. At least three replicates were carried out for each insecticide/population experiment. For each experiment, at least 4 doses flanking the LD_{50} (insecticide dose that killed 50% of the population) and causing >0 or <100% mortality were used. For each

experiment, a control group received only acetone. Treated and control insects were maintained in a climatic chamber (Sanyo MLR 351-H, Japan) under controlled conditions of temperature (27°C ± 1°C), relative humidity (60% RH ± 5%) and photoperiod 12:12h (Light:Dark). Mortality was recorded at 24h. The criterion for mortality was the inability of the nymphs to walk out of a filter paper disk of 7 cm diameter (Vassena *et al*. 2000; Picollo *et al*. 2005). Probit analysis (Finney 1971) was performed on mortality data using PROBIT ver.2 software (Raymond *et al*. 1993). When results exhibited a large χ^2 for the Log-probit lines (which was the case for only the El Chaco population for deltamethrin and San Francisco del Inti population for bendiocarb) and because there was no sign of systematic deviation from linear regression, the heterogeneity factor *H* (Finney, 1971) was computed to continue the computrations. Resistance ratios (RR) were computed relative to the susceptible reference strain CIPEIN as $RR = LD_{field.population} / LD_{CIPEIN}$. When lines where parallel, RRs were computed at LD_{50} (*i.e.*, RR_{50}) (Finney, 1971) and in addition, at LD_{90} (*i.e.*, RR_{90}) when probit lines were not parallel (Robertson *et al*. 2007).

Bio-assays with piperonyl-butoxide (PBO). A dose of 1000 ng active ingredient (a.i.) / insect PBO (Vassena *et al* 2000) in 0.2 µl acetone solution was first topically applied on the dorsal surface of the abdomen of N1. Insects were then left one hour before being processed for topical application of insecticide as explained above. Then, resistance ratios were computed relative to the reference strain CIPEIN and the percentage of effective reduction in RR_{50} (with and without PBO) was computed as:

$$P_{reduc}\% = \frac{RR_{50withoutPBO} - RR_{50withPBO}}{RR_{50withoutPBO} - 1} x100$$

If the only mechanism of resistance is based on detoxifying enzymes such as oxydases (cytochrome P_{450}), P_{reduc} *%* would be expected to be 100% (*i.e.*, a complete recovery of susceptibility to deltamethrin) as $RR_{50\ with\ PBO}$ would be 1.

If P_{reduc} *%* is significantly different from 0% (*i.e.*, it exists some synergistic effect with PBO) but also significantly different from 100% (*i.e.*, the population does not recover total susceptibility to insecticide with PBO), then in addition to detoxifying oxydases, others types of resistance mechanisms are likely to be involved (Scott 1990). The significance in reduction of resistance ratios was estimated by computing the resistance ratio of the population "with" and "without" application of PBO ($RR_{PBO} = DL50_{without\ PBO} / DL50_{with\ PBO}$). Therefore, P_{reduc} *%*

was considered as not significantly different from 0 if the value one was inside the 95% confidence interval of RR_{PBO}.

Determination of diagnostic doses (DD) for topical application assays. For a given insecticide, the diagnostic concentration is twice the minimum concentration that causes 100% mortality of individuals of the susceptible strain (WHO 2006). At a diagnostic dose, the usual OMS rough threshold for detecting resistance is 80% mortality (Brown & Pal 1973) and therefore, with a single dose assay, it is possible to rapidly detect the presence of resistant individuals in a field population. Diagnostic concentrations for each of the tested insecticides were determined with the CIPEIN strain. A log-probit base line was first computed and then, a trial-and-error process was used with concentrations lower and higher than the computed LD_{99}. For each concentration, at least 25 (and up to 50) N1 were used. This process was repeated several times to determine the observed LD_{100} and then, the diagnostic concentration was set to 2 x LD_{100}.

Design of a tarsal-contact diagnostic test for field use. Graduated series of filter papers (Whatman n°1) 12x15 cm were impregnated with 2ml of insecticide solution (insecticide diluted in acetone with silicone oil [Dow Corning 556 cosmetic grade 3.6 mg/cm2]) following WHO (1996). Depending on their developmental stage, groups of 5 to 10 *T. infestans* of the CIPEIN strain were allowed to walk on an impregnated filter paper during one hour, below OMS plastic cones which maintained the insects on the paper. Then, as for topical application bioassays, insects were returned to plastic glass with folded paper in climatic chambers (27°C ± 1°C, 60% RH ± 5%), for the 24h dosage/mortality relationship. For each concentration, at least 20 insects were used. At least three replicates were carried out for each bio-assay. For each developmental stage, Log-probit lines were estimated and lethal concentrations 99 (LC_{99}) were computed. Then, "trial and error" assays were carried out with impregnated papers flanking the LC_{99} to determine the observed LC_{100}. The concentration for diagnostic paper was set to 2 x LC_{100}.

Results

Resistance to deltamethrin. (Tab. 1). The minimum dose of deltamethrin that caused 100% mortality in the CIPEIN strain of *T. infestans* when topically applied was 2.5 ng a.i./insect in agreement with the computed LD_{99} of 1.72 ng a.i./insect (95% confidence

interval : 1.29 – 2.53). Therefore, the discriminating dose was set to 5 ng a.i./insect. At this dose, few populations exhibited 100% mortality and for the majority of them, mortality was <80% indicating that most of the tested populations were resistant. In resistant populations, mortality rates were generally far below 80% and often close or equal to zero, indicating high levels of resistance, as also evidenced by large resistance ratios (RR_{50}) which ranged from 6 to 491. However, regional differences existed: Most of the susceptible populations were from La Paz Department, a few ones from Chuquisaca and none from the Tarija Department. Resistance ratios were higher in the Tarija Department (mean RR = 183, SD=138, n=10) than in the Chuquisaca Department (mean RR = 68, SD=114, n=14) and La Paz Department (mean RR=6.6, SD=4, n=2). Similarly, the mean mortality rate *M* at the diagnostic dose was lower in the Tarija Department (*M*=18.3%, SD=16.2, n=12) than in the Chuquisaca Department (*M*=51%, SD=33.1, n=21) and the La Paz Department (*M*=75.2%, SD=35.8, n=7). The sylvatic population was susceptible (100% mortality at the diagnostic dose). Generally, Log-probit lines were parallel to that of the reference strain CIPEIN, except for some populations for which slopes values were significantly lower (Yuchan, San Francisco del Inti, Estación Caiza, Saladito, San Antonio, Tentami, Villa El Carmen, Sausal, Barrial, Barrio Itaty).

Evaluation of PBO synergist effect. (Tab. 2) The percentage of effective reduction in RR_{50} for deltamethrin with PBO was variable and ranged 0 – 74%. PBO decreased significantly the RR_{50} values in some of the tested populations: Estación Caiza, San Antonio, Yuchan and Tiguipa (RR_{PBO} ranged [1.1 -1.6], [1.7 – 3.0], [1.6 – 2.2] and [2.6 - 5.4)] respectively) indicating that oxidative mechanisms are likely to play a significant but variable role in deltamethrin resistance. However other mechanisms may be involved because the reduction was never 100% (although significant).

Susceptibility to bendiocarb. (Tab. 3). The minimum dose of bendiocarb that caused 100% mortality in the CIPEIN strain of *T. infestans* when topically applied was 35 ng a.i./insect in agreement with the computed LD_{99} of 32.63 ng a.i./insect (95% confidence interval: 26.26 – 41.01). Therefore, the discriminating dose was set to 70 ng a.i./insect. At this discriminating dose, the percentage of mortality observed in field "domestic" populations were all usually >95-97% % indicating the absence of resistance to bendiocarb. Some kind of tolerance could be evoked because most RR_{50} were significantly >1 (their 95% confidence intervals did not flanked this value), but only slightly above (range 1.4 – 1.8). On the contrary, for some populations such as Tambo Ackachilla and Tambo Atajo, mortality at the

diagnostic dose was close to the 80% threshold and therefore, resistance might be in the process of appearance. These populations should be monitored.

Susceptibility to malathion. (Tab. 4).The minimum dose of malathion that caused 100% mortality in the CIPEIN strain of *T. infestans* when topically applied was 60 ng a.i./insect, and therefore, the discriminating dose was set to 120 ng a.i./insect. At this discriminating dose, the percentage of mortality observed in field "domestic" populations were all >91% indicating the absence of resistance to malathion. For the three populations for which Log-probit lines were computed, RR_{50} were significantly >1, but only slightly above this value (range: 1.5 – 2.2) indicating that no marked resistance existed. Slopes of the Log-probit lines were generally high, as a consequence of homogeneity of response of the *T. infestans* populations to this insecticide.

Contact-tarsal test with deltamethrin. Table 5 summarizes the results for the Log-probit lines and the determination of the observed LC_{100} obtained for each larval developmental stage. For all nymphal stages, the LC_{100} were close to 0.15% except for N1 for which it was 0.09%. Because the diagnostic test should be used in field conditions (*i.e.*, where all *T. infestans* developmental stages are captured) during routine surveillance, a single consensual diagnostic concentration that could be used for all developmental stages would be easier to handle. Results indicate that except for N1, a consensual LC_{100} would be 0.15% and therefore that the diagnostic concentration would be set to 0.30%. So, the protocol for the diagnostic test would be: (1) expose field captured triatomines (all developmental stages, including N1) to 0.30% deltamethrin impregnated filter papers for one hour in groups of 5 to 10 individuals; (2) transfer the insects in resting cups with folded paper at room temperature (if possible at ≈27°C); (3) compute the percentage of mortality after 24 hours and use the OMS criterion (Brown & Pal 1973) to conclude. However, special attention should be paid in the interpretation of N1 mortality (see discussion).

Discussion

Insecticide pressure on Bolivian "domestic" populations of *T. infestans* comes from vector control measures of the NPCCD. Present results indicate that resistance to deltamethrin is widespread and high. In some places in the centre and south of Bolivia where the NPCCD

reported insecticide field treatments failures, resistance ratios for deltamethrin where >50, which is the rough threshold computed by Picollo *et al*. (2005) above which field treatment failures were also reported in Argentina. Few populations are still susceptible, of which the sylvatic one. The field diagnostic test for deltamethrin designed in this study is of simple use and could adequately be carried out by the technicians of the NPCCD to complete the geographical distribution of deltamethrin resistance in Bolivia. The diagnostic concentration of 0.30% deltamethrin on impregnated papers would probably overestimate the diagnostic concentration for N1. If only N1 are tested and 100% mortality is found, the test would conclude that the population is susceptible even though it is resistant. However, in field conditions, the capture of N1 only is very unlikely and generally, a mixture of all development stages is sampled which would make the test effective. Testing N1 could still be informative if they survive the discriminate dosis because their survival would indicate that the resistance level in the population is high.

Villages in the Tarija Department (South Bolivia) exhibited the higher levels of resistance (generally RR>150) and correlates to the lower percentages of mortality at the diagnostic dose (generally <10%). These are in agreement with results from *T. infestans* population from Southern Bolivia (Vassena *et al.* 2007, Santo Orihuela *et al.* 2008) and Brazil (Vieira Sonoda *et al*. 2009). The significant decrease in RR with PBO in some populations, such as Yuchan, San Antonio, Tiguipa and Estación Caiza, indicated that oxidases played a significant role in deltamethrin resistance (Ffrench-Constant & Rough, 1990). However, because the populations did not recover complete susceptibility with PBO (and even, for some populations, there was no decrease in RR) other mechanisms would be involved such as esterares or *kdr* (Santo Orihuela *et al*. 2008). In *T. infestans*, glutathione S-transferases are known to participate in pyrethroid and organophosphate resistance (Sivori *et al.* 1997) and therefore may also participate in the observed deltamethrin resistance. The heterogeneity in the resistance levels to deltamethrin and in the mechanisms involved (variations in PBO response) are likely to originate from non homogenous insecticide pressure (frequencies of interventions vary among villages and insecticide doses may vary among "domestic" and "peridomestic" environments, the later being more difficult to treat (Gürtler et al. 2004)). In addition *T. infestans* populations are generally highly structured genetically (although not isolated) (Pérez de Rosa *et al*. 2007, 2008, Abrahan *et al.* 2008, Pizarro *et al*. 2008, Segura *et al*. 2009) and this also can partly account for the observed differences in resistance patterns.

Because there is no marked resistance to bendiocarb and malathion (as measured by diagnostic dose assays), alteration of insecticide biding-sites for acetylcholin esterase is

unlikely to occur in *T. infestans*. (Mc Cord & Yu 1987). Therefore, because these enzymes are strongly suspected for deltamethrin resistant *T. infestans* populations in Bolivia, they might also be involved for bendiocarb, giving various RR_{50} >1 (although only slightly above one). However, to better characterize the metabolic resistance, biochemical assays would be needed (Hemingway 1998).

In addition to metabolic resistance, the knockdown resistance (*kdr*) mechanism is common in many insect species resistant to pyrethroids. Its mode of action consists of pointed mutations in the sodium channel genes leading to nerve insensitivity and cross-resistance with DDT (Soderlund & Knipple 2003). Because the tested populations of *T. infestans* did not recover total susceptibility to deltamethrin with PBO (and even in some cases, PBO did not have any effect), and because RR are high, the most probable complementary mechanism is the *kdr* mechanism which has already been suggested by Santo Orihuela *et al.* (2008) for *T. infestans* populations of Argentina.

In combination with insecticide resistance reports in Argentina, our results also raise the question of the apparition of insecticide resistance in the Andean populations of *T. infestans*. On the other hand, non Andean populations of *T. infestans* of Paraguay and from some regions of Argentina seem to be susceptible to pyrethroids despite the same insecticide pressure. The classification of *T. infestans* populations into two distinct groups Andean and non-Andean has long been underlined (Panzera *et al*. 2007), and differences in ecological responses of the two groups mentioned (Catalá *et al.* 2006). Andean populations of *T.infestans* from Bolivia seem to have the potential to develop insecticide resistance faster than non-Andean populations. Correlating genetic and insecticide resistance data will bring new insights into resistance management programs.

Resistance profiles of *T. infestans* might be more complex and diversified than expected, reflecting the various insecticide pressures and genetic structures of the populations. A better management of chemicals can be a short term solution to insecticide resistance (Tabashnick 1990). Because our results indicated that there was no resistance to bendiocarb, this molecule could be suggested as a short term alternative where resistance to deltamethrin is high. In that case, a careful monitoring should be carried out in areas where *Anopheles pseudopunctipennis*, a malaria vector, also occurs in sympatry (Lardeux *et al.* 2008) and is simultaneously controlled by the Bolivian Ministry of Health with insecticide indoor sprayings.

New control strategies including integrated approaches are needed to definitively overcome the resistance problem of *T. infestans*. Housing improvement and ecosystem

management using an Eco-Bio-Social approach (WHO 2002, Gürtler *et al.* 2007) are strongly encouraged. The use of insecticidal paints using organophosphates (Amelotti *et al*. 2009) is promising but poses different challenges at the operational level. Insecticide rotations or mixtures of organophosphates or carbamates with pyrethroids and the search for insecticide synergy would also be worth considered. Restoration of pyrethroid susceptibility using low rates of other insecticides (Bielza *et al.* 2007) could also be studied. Finally, high-tech strategies using genetics (such as paratransgenesis) are promising (Durvasula *et al.* 2008) but more time is needed to be fully operational.

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References

Abrahan L, Hernández L, Gorla D & Catalá S (2008) Phenotypic Diversity of *Triatoma infestans* at the microgeographic level in the Gran Chaco of Argentina and the Andean valleys of Bolivia. *Journal of Medical Entomology* **45**, 660-666.

Amelotti I, Catalá S & Gorla D (2009) Experimental evaluation of insecticidal paints against *Triatoma infestans* (Hemiptera: Reduviidae), under climatic conditions. *Parasites and Vectors* **2**: 30

Bielza P, Espinoza PJ, Quinto V, Abellan J & Contreras J (2007) Synergism studies with binary mixtures of pyrethroid, carbamate and organophosphate insecticides on *Frankliniella occidentalis* (Pergande). *Pest Management Science* **63**, 84-89.

Brown AWA & Pal R (1973) *Résistance des Arthropodes aux Insecticides*. 2nde édition, Série de Monographies n°38. Organisation Mondiale de la Santé, Genève, 541 p.

Catalá S, Abrahan L & Hernández ML (2006) Variación fenotípica en poblaciones de *Triatoma infestans*. Reunión final de discusión, Proyecto SSA-European Community, CRILAR, Argentina, 2-5 octubre 2006.

CIPEIN CITEFA (2001) Protocolo de evaluación de efecto insecticida en *Triatoma infestans*. In *Monitoreo de la Resistencia a Insecticidas en Triatominos en America Latina* (ed. RELCOT). Fundación Mundo Sano, Buenos Aires, Argentina, 53-59.

Durvasula RV, Sundaram RK, Kirsch P, Hurwitz I, Crawford CV, Dotson E & Beard CB (2008) Genetic transformation of a Corynebacterial symbiont from the Chagas disease vector *Triatoma infestans. Experimental Parasitology* **119**, 94-98.

Finney DJ (1971) *Probit Analysis.* Third edition. Cambridge University Press, Cambridge, 333 p.

Ffrench-Constant R & Roush RT (1990) Resistance detection and documentation: the relative roles of pesticidal and biochemical assays. In *Pesticide Resistance in Arthropods* (ed. R Roush & B Tabashnick). Chapman & Hall, New York, USA, 4-38.

Gonzalez Audino P, Vassena C, Barrios S, Zerba E & Picollo MI (2004) Role of enhanced detoxication in a deltamethrin-resistant population of *Triatoma infestans* (Hemiptera, Reduviidae) from Argentina. *Memorias do Instituto Oswaldo Cruz* **99**, 335-339.

Gürtler RE, Canale DM, Spillmann C, Stariolo R, Salomón OD, Blanco S & Segura EL (2004) Effectiveness of residual spraying of peridomestic ecotopes with deltamethrin and permethrin on *Triatoma infestans* in rural western Argentina: a district-wide randomized trial. *Bulletin of the World Health Organization* **82**, 196-205.

Gürtler RE, Kitron U, Cecere MC, Segura EL & Cohen JE (2007) Sustainable vector control and management of Chagas disease in the Gran Chaco, Argentina. *PNAS* **104**, 16194-16199.

Hemingway J (1998) Techniques to detect insecticide resistance mechanisms (Field and laboratory manual). *WHO/CDS/CPC/MAL/98.6* 35p.

Lardeux F., Tejerina R, Aliaga C, Ursic-Bedoya R, Lowenberger C & Chavez T (2008) Optimization of a semi-nested multiplex PCR to identify *Plasmodium* parasites in wild-caught *Anopheles* in Bolivia, and its application to field epidemiological studies. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **102**, 485—492

McCord Jr E & Yu SJ (1987) The mechanisms of carbaryl resistance in the fall armyworm, *Spodoptera frugiperda* (J.E. Smith). *Pesticide and Biochemistry Physiology*. **27**, 114–122.

Noireau F (2009) Wild *Triatoma infestans*, a potential threat that needs to be monitored. *Memorias do Instituto Oswaldo Cruz* **104**, 60-64.

Núñez JA & Segura EL (1987) Rearing of Triatominae. In *Chagas Disease Vectors Vol. II* (ed. R. Brenner & AM Stoka). CRC Press, Boca Raton, FL, USA, 29-40.

Panzera F, Pérez R, Lucero C, Ferréandis I, Ferreiro MJ, Calleros L & Romero V (2007) Cambios genómicos en la subfamilia Triatominae, con énfasis en *Triatoma infestans*. In *Triatominos de Bolivia y la enfermedad de Chagas* (ed. M. Rojas Cortez). Ministerio de Salud y Deportes de Bolivia – Programa Nacional de Chagas, La Paz, Bolivia, 149-172.

Pérez de Rosas AR, Segura EL & García BA (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. *Molecular Ecology*, **16**, 1401 – 1412.

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

Pinchin R, Fanara DM, Castleton CW, Oliveira Filho AM (1981) Comparison of technique for detection of domestic infestation with *Triatoma infestans* in Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **75**, 691-694.

Picollo MI, Vassena CV, Santo Orihuela PL, Barrios S, Zaidemberg M & Zerba E (2005) High resistance to pyrethroid insecticides associated with ineffective treatments in *Triatoma infestans* (Hempiptera : Reduviidae) from Northern Argentina. *Journal of Medical Entomology* **42**, 637-642.

Picollo MI, Wood E., Zerba E., Licastro SA & Ruveda MA (1976) Métodos de laboratorio para medir la actividad de insecticidas en *Triatoma infestans*. *Acta Bioquímica Clínica Latinoamericana* **10**, 67-71.

Pizarro JC, Gilligan LM & Stevens L (2008) Microsatellites reveal a high population structure in *Triatoma infestans* from Chuquisaca, Bolivia. *PLoS Neglected Tropical Diseases* **2(3)**, e202, doi:10.1371/journal.pntd.0000202

Raymond M, Prato G & Ratsira D (1993) PROBIT, CNRS-UMII, Licence L93019. Avenix, 34680 St. Georges d'Orques, France.

Robertson JL, Russell RM, Preisler HK & Savin E (2007) *Bioassays with Arthropods.* 2nd edition, CRC Press, Boca Raton, FL, USA 199 p.

Rojas Cortez M (Ed.) (2007) *Triatominos de Bolivia y la enfermedad de Chagas*. Ministerio de Salud y Deportes, Programa Nacional de Chagas, La Paz, Bolivia 352 p.

Santo Orihuela PL, Vassena C., Zerba EN & Picollo MI (2008) Relative contribution of monooxygenase and esterase to pyrethroid resistance in *Triatoma infestans* (Hemiptera: Reduviidae) from Argentina and Bolivia. *Journal of Medical Entomology* **45**, 298-306.

Schofield CJ (1994) *Triatominae : Biology and Control*. Eurocommunica Publications, West Sussex UK, 80 p.

Scott JG (1990) Investigating mechanisms of insecticide resistance: methods, strategies and pitfalls. In *Pesticide Resistance in Arthropods* (ed. R Roush & B Tabashnick). Chapman & Hall, New York, USA, 39-57.

Segura EL, Torres AG, Fusco O & Garcia A (2009) Mitochondrial 16S DNA variation in populations of *Triatoma infestans* from Argentina. *Medical and Veterinary Entomology* (2009) **23**, 34–40

Sivori JL, Casabé N, Zerba EN & Wood EJ (1997) Induction of glutathione S-transferase activity in *Triatoma infestans*. *Memorias del Instituto Oswaldo Cruz* **92**, 797-802.

Soderlund DM & Knipple DC (2003) The molecular biology of knockdown resistance to pyrethroid insecticides. *Insect Biochemistry and Molecular Biology* **33**, 563–577.

Tabashnick BE (1990) Modeling and evaluation of resistance management tactics. In *Pesticide Resistance in Arthropods* (ed. R Roush & B Tabashnick). Chapman & Hall, New York, USA, 153-182.

Toloza AC, Germano M, Cueto GM, Vassena C, Zerba E & Picollo MI (2008) Differential patterns of insecticide resistance in eggs and first instars of *Triatoma infestans* (Hemiptera: Reduviidae) from Argentina and Bolivia. *Journal of medical entomology* **45**, 421-426.

Vassena CV & Picollo MI (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://sertox.com.ar/retel/n03-004.pdf)

Vassena CV, Picollo MI, Santo Orihuela P & Zerba EN (2007) Desarrollo y manejo de la resistencia a insecticidas piretroides en *Triatoma infestans*: situación en Bolivia. In *Triatominos de Bolivia y la enfermedad de Chagas* (ed. M. Rojas Cortez). Ministerio de Salud y Deportes de Bolivia – Programa Nacional de Chagas, La Paz, Bolivia, 229-255.

Vassena CV, Picollo MI & Zerba EN (2000) Insecticide resistance in Brazilian *Triatoma infestans* and Venezuelan *Rhodnius prolixus*. *Medical and Veterinary Entomology* **14**, 51-55.

Vieira Sonoda I, Caldas D'Avila Pessoa G, Cortez Rojas M, Dias Pinto JC, Romanha AJ & Diotaiuti L (2009) Susceptibility of *Triatoma infestans* to deltamethrin in Rio Grande do Sul, Brazil. *Memorias do Instituto Oswaldo Cruz* **104**, 668-670

WHO (World Health Organization). (1994) Protocolo de evaluación de efecto insecticida sobre Triatominos. *Acta Toxicologica Argentina* **2**,: 29-32.

WHO (World Health Organization). (1996) Report of the WHO informal consultation on the evaluation and testing of insecticides. *CTD/WHOPES/IC/96.1*. 69 p.

WHO (World Health Organization) (2002) Control of Chagas disease. *WHO Technical Report Series*, 905. 109 p.

WHO (World Health Organization). (2006) Guidelines for testing mosquito adulticides for indoor residual spraying and treatment of mosquito nets. *WHO/CDS/NTD/WHOPES/GCDPP/2006.3*. 60 p.

Yacoub S, Mocumbi AO & Yacoub MH (2008) Neglected tropical cardiomyopathies: I. Chagas disease. *Heart* **94**, 244-248.

Zerba EN. (1999) Susceptibility and resistance to insecticides of Chagas disease vectors. *Medicina (Buenos Aires)* **59** (Supl. II) 41-46.

Table 1. Deltamethrin. Log-probit lines, lethal doses (LD_{50} and LD_{90}), resistance ratios at LD_{50} and LD_{90} for various field populations of *T. infestans* (CH for Chuquisaca, CO for Cochabamba, LP for La Paz and TJ for Tarija Departments respectively). LD_{50} are in ng a.i./insect. RR_{50} and RR_{90} are computed relative to the susceptible reference strain CIPEIN. For LD_{50}, RR_{50} and RR_{90} the 95% confidence interval is in parenthesis. For the percentage of mortality at the diagnostic dose (5 ng a.i./insect for deltametrhin), the number of N1 tested is in parenthesis. (*) population from peridomestic environments only.

T. infestans population	Probit line			LD_{50} (95% CI)	RR_{50} (95% CI)	RR_{90} (95% CI)	% mortality with diagnostic assay (n tested insects)
	n tested insects	slope (standard error)	χ^2 (df) *P*				
CIPEIN (sensible reference strain)	739	2.73 (0.21)	1.29 (4) *P*=0.86	0.24 (0.22 - 0.26)	-	-	100%
Barrio Itaty (CH)	202	1.44 (0.20)	1.11 (4) *P*=0.89	67.33 (49.02 – 96.83)	277 (234 -328)	735 (505 -1070)	6% (50)
Hicupe (CH)	-	-	-	-	-	-	44% (45)
Huacareta (CH)	-	-	-	-	-	-	66% (101)
Huacaya * (CH)	-	-	-	-	-	-	67% (95)
Icla (CH)	174	2.79 (0.46)	1.85 (3) *P*=0.60	1.69 (1.35 – 2.00)	6.9 (5.5 – 8.9)	-	88% (49)
Jatun Cka Cka * (CH)	309	2.06 (0.37)	7.68 (5) *P*=0.17	1.29 (0.98 -1.55)	5.2 (4.2 – 6.5)	-	98% (45)
La Abra (CH)	-	-	-	-	-	-	40% (40)
La Mendoza (CH)	-	-	-	-	-	-	40% (63)*
Corso (CH)	322	3.81 (0.47)	3.84 (3) *P*=0.28	1.44 (1.30 – 1.57)	5.9 (4.9 – 7.1)	-	100% (40)
El Chaco (CH)	298	2.34 (0.59)	43.3(4) *P*=0	2.04 (1.73 – 2.42)	8.5 (5.2 – 10.9)	-	72% (229)
Machareti (CH)	77	2.13 (0.15)	1.38 (3) *P*=0.71	8.07 (4.27 – 11.17)	33 (24 – 47)	-	17% (77)
Rodeo Porvenir * (CH)	319	2.59 (0.33)	0.56 (3) *P*=0.90	2.65 (2.31 – 3.03)	11 (9 – 13)	-	82% (83)
Pajcha (CH)	108	3.40 (0.79)	0.52 (2) *P*=0.77	5.08 (4.25 – 6.37)	21 (16 – 28)	-	48% (56)
Sotomayor * (CH)	74	3.47 (1.15)	0.14 (3) *P*=0.98	3.32 (2.09 – 4.05)	14 (9 – 19)	-	58% (40)*
Sucre (CH)	-	-	-	-	-	-	48% (180)
Tambo Ackachilla (CH)	254	2.97 (0.45)	0.62 (3) *P*=0.89	1.26 (1.02 – 1.47)	5 (4 – 6)	-	100% (57)
Tambo Atajo (CH)	242	2.64 (0.42)	1.56 (4) *P*=0.81	1.30 (1.01 – 1.54)	5 (4 – 7)	-	91% (58)
Tentami (CH)	322	1.38 (0.23)	2.19 (4) *P*=0.70	78.44 (61.76 – 103.83)	323 (283 – 367)	936 (630 – 1390)	0% (35) 5% (57)*
Tiguipa Estación (CH) Intradomicile	162	2.47 (0.76)	0.94 (2) *P*=0.62	55.98 (34.78 – 90.43)	230 (150 – 353)	-	0% (52)
Tiguipa Estación (CH) Peridomicile	1007	1.59 (0.18)	3.55 (3) *P*=0.31	34.47 (27.13 -43.43)	142 (121 – 166)	307 (225 – 419)	3% (62)
Tihuacana * (CH)	58	3.72 (1.26)	0.68 (3) *P*=0.87	3.27 (2.14 – 4.07)	13 (9 – 19)	-	77% (40)
Anamo (CO)	-	-	-	-	-	-	73% (22)
Mataral (CO)	324	3.11 (0.35)	5.94 (3) *P*=0.11	3.35 (2.88 – 3.78)	14 (11 - 17)	-	67% (148)
Tunal Kassa (CO)	-	-	-	-	-	-	92% (53)
Candial (LP)	-	-	-	-	-	-	100% (17)
Chivsivi (LP)	67	7.09 (2.73)	$5\ 10^{-6}$(2) *P*= 0.99	1.41 (1.00 – 1.86)*	5.8 (3.3 – 10.1)	-	100% (11) 100% (39) sylvatic
Colopampa (LP)	70	5.04 (1.02)	1.83 (3) *P*=0.61	2.31 (1.84 – 2.73)	9.5 (6.5 – 13.8)	-	95% (22)
El Palomar * (LP)	-	-	-	-	-	-	100% (25)

Los Sotos (LP)	-	-	-	-	-	-	0% (54)
Malavi * (LP)	-	-	-	-	-	-	76% (45)
Parani (LP)	271	3.09 (0.38)	9.90 (5) *P*=0.08	0.91 (0.80 – 1.04)	3.7 (3.0 – 4.6)	-	92% (36)
Sapini (LP)	-	-	-	-	-	-	63% (8)
Barrial (TJ)	440	1.46 (0.14)	5.92 (3) *P*=0.12	40.11 (32.21 – 49.23)	165 (146 - 187)	424 (328 -548)	31% (70)
Estación Caiza (TJ)	697	1.14 (0.14)	2.28 (3) *P*=0.51	49.46 (39.50 – 60.06)	204 (184 – 227)	924 (692 – 1233)	1% (96)
La Grampa (TJ)	-	-	-	-	-	-	6% (65)
Quinchao (TJ)	153	1.36 (0.21)	2.69 (3) *P*=0.44	31.70 (21.19 – 54.45)	131 (106 – 161)	530 (312 – 900)	15% (92)
Rio Negro (TJ)	-	-	-	-	-	-	8% (84) 33% (33)*
Saladito (TJ)	212	1.39 (0.18)	4.04 (3) *P*=0.25	23.34 (16.72 – 35.51)	96 (80 – 115)	274 (187 – 401)	17% (139)
San Antonio (TJ)	666	1.24 (0.12)	8.16 (5) *P*=0.15	76.37 (62.45 – 96.95)	315 (281 - 353)	1157 (866 – 1546)	9% (128)
San Francisco del Inti (TJ)	273	1.01 (0.16)	4.71 (5) *P*=0.45	7.41 (4.61 – 10.69)	30 (26 – 35)	189 (137 – 260)	43% (92)
Sausal (TJ)	171	1.51 (0.34)	1.29 (2) *P*=0.52	119.12 (87.00 – 189.04)	491 (409 – 589)	1170 684 – 2002)	5% (79)
Tierras Nuevas (TJ)	473	1.76 (0.18)	0.671 (4) *P*=0.95	35.82 (30.34 – 42.09)	148 (125 – 174)	-	38% (288)
Villa El Carmen (TJ)	438	1.36 (0.20)	1.33 (3) *P*=0.72	52.00 (41.35 – 64.00)	214 (190 - 241)	-	3% (94)
Yuchan (TJ)	2428	0.82 (0.04)	8.74 (5) *P*=0.12	8.10 (6.73 – 9.57)	33 (30 - 36)	405 (333 – 493)	44% (468)

Table 2. Deltamethrin. Probit lines with prior PBO application for some field populations of *T. infestans*, resistance ratios at LD_{50} and percentage of effective reduction (P_{reduc}) in RR_{50} with PBO (see formula in text). A 100% reduction would indicate a complete recovery of susceptibility to deltamethrin with PBO. * indicates that P_{reduc} % is significant (*i.e.*, RR_{PBO} >1). In parenthesis, the 95% confidence intervals of the computed parameters LD_{50} and RR_{50}.

T. infestans population	Probit line			LD_{50} (95% CI)	RR_{50} with PBO (95% CI)	P_{reduc} % in RR_{50}
	n tested insects	slope (standard error)	χ^2 (df) *P*			
El Chaco	501	3.16 (0.32)	1.52 (3) *P*=0.67	3.01 (2.73 – 3.39)	12.4 (10.4 – 14.8)	0%
Estación Caiza	344	1.60 (0.22)	1.04 (3) *P*=0.79	37.52 (28.36 – 46.38)	153 (125 – 186)	25% *
San Antonio	125	1.37 (0.32)	1.35 (3) *P*=0.71	33.79 (22.72 – 64.61)	144 (106 – 195)	54% *
Tiguipa peridomicile	413	1.46 (0.18)	0.94 (3) *P*=0.81	9.07 (5.72 – 12.42)	38 (31 - 45)	74% *
Tierras Nuevas	119	1.72 (0.42)	0.21 (2) *P*=0.90	33.82 (24.47 – 52.39)	139 (106 - 184)	6%
Villa El Carmen	41	1.12 (0.78)	0.44 (2) *P*=0.80	39.17	147 (91 – 238)	31%
Yuchan	470	1.09 (0.12)	11.22 (6) *P*=0.08	4.32 (2.97 – 5.81)	18 (15 - 20)	47% *

Table 3. Bendiocarb. Log-probit lines, lethal doses (LD_{50} and LD_{90}), resistance ratios at LD_{50} and LD_{90} for various field populations of *T. infestans* (CH for Chuquisaca, CO for Cochabamba and TJ for Tarija Departments respectively). LD_{50} are in ng a.i./insect. RR_{50} and RR_{90} are computed relative to the susceptible reference strain CIPEIN. For LD_{50}, RR_{50} and RR_{90} the 95% confidence interval is in parenthesis. For the percentage of mortality at the diagnostic dose (70 ng a.i./insect for bendiocarb), the number of N1 tested is in parenthesis.

T. infestans population	Probit line			LD_{50} (95% CI)	RR_{50} (95% CI)	RR_{90} (95% CI)	% mortality diagnostic assay (n tested insects)
	n tested insects	slope (standard error)	χ^2 (df) *P*				
CIPEIN (sensible reference strain)	881	4.99 (0.5556)	10.9 (6) *P*=0.09	11.15 (10.41 -11.93)	-	-	100%
Barrio Itaty (CH)	-	-	-	-	-		100% (15)
Huacaya (CH) *	-	-	-	-	-	-	100% (23)
Icla (CH)	-	-	-	-	-	-	96% (28)
Sucre (CH)						-	100% (16)
Tambo Ackachilla (CH)	-	-	-	-	-	-	88% (25)
Tambo Atajo (CH)	-	-	-	-	-	-	81% (11)
Tentami (CH)	-	-	-	-	-	-	100% (30)
Tiguipa Estación intra domicile (CH)	-	-	-	-	-	-	100% (14)
Mataral (CO)	-	-	-	-	-	-	93% (120)
Tunal Kassa (CO)	-	-	-	-	-	-	100% (24)
Los Sotos (LP)	369	3.45 (0.56)	3.9 (4) *P*=0.42	17.67 (15.86 - 19.70)	1.6 (1.4 - 1.8)	-	100% (88)
Barrial (TJ)	1124	4.36 (0.28)	10.5 (5) *P*=0.06	19.56 (18.56 – 20.66)	1.8 (1.4 – 2.1)	-	98% (102)
Berrety Chaco (TJ)	121	6.09 (1.85)	0.78 (2) *P*= 0.70	15.18 (12.56 -17.00)	1.4 (1.1 - 1.6)	-	100% (22)
Estación Caiza (TJ)	1415	3.32 (0.31)	9.90 (5) *P*=0.08	17.73 (16.54 - 18.83)	1.6 (1.4 - 1.8)	-	95% (79)
La Grampa (TJ)	-	-	-	-	-	-	91% (99)
Laime (TJ)	-	-	-	-	-	-	100% (21)
Lajitas (TJ)	-	-	-	-	-	-	95% (58))
Quinchao (TJ)	-	-	-	-	-	-	100% (65)
Saladito (TJ)	-	-	-	-	-	-	97% (100)
San Antonio (TJ)	-	-	-	-	-	-	94% (93)
San Francisco del Inti (TJ)	328	2.48 (2.22)	23.8 (1) *P*=0	18.80 (8.54 – 41.32)	1.7 (0.8 – 3.5)	3.1 (0.5 – 19)	100% (74)
Sausal (TJ)	-	-	-	-	-	-	100% (108)
Supitin (TJ)	-	-	-	-	-	-	100% (29)
Tierras Nuevas (TJ)	1211	3.33 (0.27)	15.0 (8) *P*=0.06	20.82 (18.48 - 23.46)	1.7 (1.5 – 2.2)	2.5 (1.8 – 3.5)	94% (266)
Villa El Carmen (TJ)	-	-	-	-	-	-	98% (115)
Villa Primavera (TJ)	375	5.28 (0.59)	1.90 (2) *P*=0.38	20.70 (19.28 – 22.08)	1.8 (1.4 – 2.4)	-	100% (51)
Yuchan (TJ)	518	3.16 (0.61)	11.8 (4) *P*=0.02	16.52 (14.30 - 19.09)	1.5 (1.2 - 1.8)	2.1 (1.3 – 3.3)	100% (40)

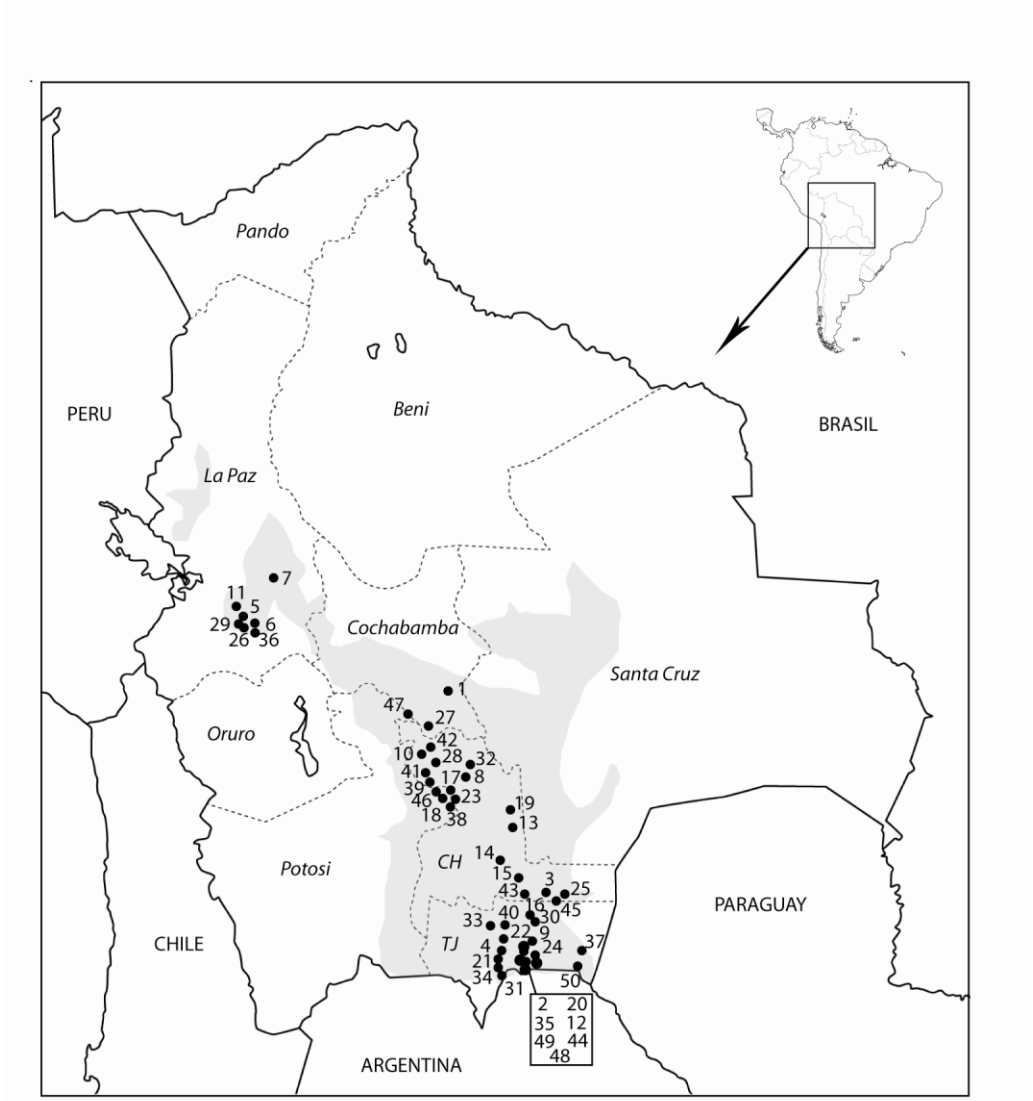
Table 4. Malathion. Log-probit lines, lethal doses (LD_{50} and LD_{90}), resistance ratios at LD_{50} and LD_{90} for various field populations of *T. infestans* (CO for Cochabamba, LP for La Paz and TJ for Tarija Departments respectively). LD_{50} are in ng a.i./insect. RR_{50} and RR_{90} and are computed relative to the susceptible reference strain CIPEIN. For LD_{50}, RR_{50} and RR_{90} the 95% confidence interval is in parenthesis. For the percentage of mortality at the diagnostic dose (120 ng a.i./insect for malathion), the number of N1 tested is in parenthesis.

T. infestans population	Probit line			LD_{50} (95% CI)	RR_{50} (95% CI)	RR_{90} (95% CI)	% mortality with diagnostic assay (n tested insects)
	n tested insects	slope (standard error)	χ^2 (df) *P*				
CIPEIN (sensible reference strain)	722	9.63 (0.74)	6.53 (4) *P*=0.16	17.73 (17.27 – 18.16)	-	-	100%
Mataral (CO)	-	-	-	-	-	-	91% (77)
Los Sotos (LP)	-	-	-	-	-	-	100% (63)
Barrial (TJ)	-	-	-	-	-	-	99% (74)
Cortaderal (TJ)	-	-	-	-	-	-	100% (33)
Estación Caiza (TJ)	-	-	-	-	-	-	100% (80)
Ibopeiti (TJ)	-	-	-	-	-	-	100% (41)
La Grampa (TJ)	170	3.89 (0.12)	2.25 (3) *P*=0.52	38.81 (33.62 – 45.85)	2.2 (1.8 – 2.7)	3.4 (2.4 – 5.0)	96% (50)
Laime (TJ)	141	6.42 (1.04)	7.06 (3) *P*=0.07	31.14 (28.47 – 35.13)	1.8 (1.3 – 2.4)	-	100% (78)
Lajitas (TJ)	-	-	-	-	-	-	95% (58)
Quinchao (TJ)	-	-	-	-	-	-	100% (60)
Saladito (TJ)	-	-	-	-	-	-	99% (71)
San Antonio (TJ)	-	-	-	-	-	-	100% (60)
San Francisco del Inti (TJ)	-	-	-	-	-	-	100% (46)
Sausal (TJ)	-	-	-	-	-	-	100% (85)
Tierras Nuevas (TJ)	-	-	-	-	-	-	100% (64)
Villa El Carmen (TJ)	-	-	-	-	-	-	100 (72)
Yuchan (TJ)	357	14.49 (2.02)	4.62 (3) *P*=0.20	26.67 (26.09 – 27.29)	1.5 (1.2 – 1.8)	-	100 (59)

Table 5. Log-probit analysis, computation of the lethal concentrations 50 and 99 (LC_{50} and LC_{99}) and determination of observed LC_{100} in tarsal-contact bioassays with deltamethrin impregnated papers for each nymphal stage of *T. infestans* from the CIPEIN strain. LC_{50}, LC_{99} and LC_{100} are in % according to the WHO (1996) definition.

T. infestans developmental stage	Probit line			LC_{50} (95% CI)	LC_{99} (95% CI)	observed LC_{100}
	n tested insects	Slope (standard error)	χ^2 (df) *P*			
N1	260	5.15 (0.85)	0.88 (3) *P*=0.83	0.037 (0.032 - 0.041)	0.10 (0.083 - 0.162)	0.09
N2	570	3.81 (0.35)	6.32 (7) *P*=0.50	0.045 (0.042 - 0.049)	0.18 (0.151 - 0.248)	0.12
N3	636	4.69 (0.38)	5.50 (7) *P*=0.60	0.052 (0.048 - 0.056)	0.162 (0.141 - 0.196)	0.15
N4	457	8.64 (1.03)	6.23 (4) *P*=0.18	0.074 (0.069 - 0.078)	0.138 (0.125 - 0.161)	0.15
N5	200	4.57 (1.86)	0.80 (3) *P*=0.85	0.050 (0.004 - 0.067)	0.161 (0.124 - 1.413)	0.15

Figure 1. Distribution of *T. infestans* in Bolivia (shaded area) and sample locations for insecticide resistance studies. Names of Departments are in italics.



1 Anamo	11 El Palomar	21 Laime	32 Rodeo Porvenir	42 Tambo Atajo
2 Barrial	12 Estacion caiza	22 Lajitas	33 Saladito	43 Tentami
3 Barrio Itaty	13 Hicupe	24 Los Sotos	34 San Antonio	44 Tierras Nuevas
4 Berety Chaco	14 Huacareta	25 Machareti	35 San Francisco del Inti	45 Tiguipa
5 Candial	15 Huacaya	26 Malavi	36 Sapini	46 Tihuacana
6 Chivisivi	16 Ibopeiti	27 Mataral	37 Sausal	47 Tunal Kassa
7 Colopampa	17 Icla	28 Pajcha	38 Sotomayor	48 Villa El Carmen
8 Corso	18 Jatun Cka Cka	29 Parani	39 Sucre	49 Villa Primavera
9 Cortaderal	19 La Abra	30 Quinchao	40 Supitin	50 Yuchan
10 El Chaco	20 La Grampa	31 Rio Negro	41 Tambo Ackachilla	