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Insecticide resistance in vector Chagas disease: Evolution, mechanisms and management

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ABSTRACT

Chagas disease is a chronic parasitic infection restricted to America. The disease is caused by the protozoa *Trypanosoma cruzi*, which is transmitted to human through the feces of infected triatomine insects. Because no treatment is available for the chronic forms of the disease, vector chemical control represents the best way to reduce the incidence of the disease. Chemical control has been based principally on spraying dwellings with insecticide formulations and led to the reduction of triatomine distribution and consequent interruption of disease transmission in several areas from endemic region. However, in the last decade it has been repeatedly reported the presence triatomnes, mainly *Triatoma infestans*, after spraying with pyrethroid insecticides, which was associated to evolution to insecticide resistance. In this paper the evolution of insecticide resistance in triatomines is reviewed. The insecticide resistance was detected in 1970s in *Rhodnius prolixus* and 1990s in *R. prolixus and T. infestans*, but not until the 2000s resistance to pyrthroids in *T. infestans* associated to control failures was described in Argentina and Bolivia. The main resistance mechanisms (i.e. enhanced metabolism, altered site of action and reduced penetration) were described in the *T. infestans* resistant to pyrethrods. Different resistant profiles were demonstrated suggesting independent origin of the different resistant foci of Argentina and Bolivia. The deltamethrin resistance in *T. infestans* was showed to be controlled by semi-dominant, autosomally inherited factors. Reproductive and developmental costs were also demonstrated for the resistant *T. infestans*. A discussion about resistance and tolerance concepts and the persistence of *T. infestans* in Gran Chaco region are presented. In addition, theoretical concepts related to toxicological, evolutionary and ecological aspects of insecticide resistance are discussed in order to understand the particular scenario of pyrethroid resistance in triatomines.

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1. Introduction

Chagas disease is a chronic parasitic infection restricted to America where it is currently estimated that 9–11 million people carry the disease, and 50–60 million people are at risk of acquiring the infection (Schofield et al., 2006; 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).

Current therapeutic options are limited mainly to two drugs (benznidazole and nifurtimox), and the phase of the disease is one of the factors that determine the success of treatment. Briefly, the therapeutic response is high when treatment is administered in the acute phase of the disease, especially in children, but the effectiveness diminishes in adult patients with chronic disease. However, recent clinical studies showed good results in chronic patients treated with benznidazole, and new candidate drugs are currently being evaluated with encouraging results but not conclusive (e.g. posaconazole) (Ministerio da Saude Brazilian, 2005; Gascón et al., 2007 Molina et al., 2014). In this context, vector chemical control represents the best way to reduce the incidence of the disease. Chemical control has been based principally on spraying dwellings and peridomiciliary areas with insecticide formulations applied by professional sprayers. This is how the chemical control, historically based on the use of organochlorine, organophosphate, carbamate and pyrethroid insecticides, led to the reduction of *Triatoma infestans* distribution, one of the main vectors of the disease, and consequent interruption of disease transmission in several areas of the Southern Cone (Zerba, 1999a; Dias et al., 2002). For example, intradomiciliary infestation by *T. infestans* was eliminated in some areas of Brazil in 2006, in Chile in 1999 and in Uruguay in 1997 (Moncayo and Silveira, 2009). However, in the last decade it has been repeatedly reported the intradomiciliary presence of triatomines in some localities from endemic countries (principally in Argentina and Bolivia, but also in Paraguay, Chile and Venezuela) after spraying with pyrethroid insecticides and within the residual period accepted for such treatment. Assuming that the dose and formulation of the insecticide was adequate, the unsatisfactory control could be generated by: 1) control actions were not performed properly or the insecticide did not reach all insect colonies, 2) after a successful spraying occurred events of colonization by insects from neighbors areas, and 3) presence of resistant insects to that insecticide (Picollo et al., 2005; Gürtler, 2009; Germano et al., 2012).

For a long time it was considered that chemical control in triatomines, mainly from the use of pyrethroids, would be successful for a long time mainly because the low probability of evolution of insecticide resistance. This assumption was based on two main biological characteristics of triatomines: 1) low genetic variability which implies low probability of individuals genetically less susceptible to insecticides, and 2) long cycle life (so even if there are resistant individuals, the selection process would be very slow) (Guhl and Schofield, 1996; Monteiro et al., 2001). However the insecticide resistance evolved in triatomines, which was well documented by numerous studies which showed a substantial body of evidence. Resistance to organochlorinated insecticides was detected in *Rhodnius prolixus* from Venezuela in the 1970s and resistance to pyrethroid insecticides was described in *T. infestans* from Argentina and Bolivia since the late 1990s, where high resistance levels were associated with field control failures (Gonzalez-Valdivieso et al., 1971; Picollo et al., 2005; Santo-Orihuela et al., 2008; Germano et al., 2012). In addition, recent studies using genetic markers with high levels of polymorphims showed that *T. infestans* from Argentina and Bolivia has a high degree of genetic differentiation between groups of insects from different localities and strong populations structure both macro and microgeographical level (Pérez de Rozas et al., 2007 Marcet et al., 2008; Pizarro et al., 2008), evidencing the evolutionary potential of this species. After a decade of studies, the resistance to insecticides triatomines, particularly in *T. infestans*, is evidenced as a complex problem: the resistance evolved in different areas of the geographic distribution of the species (Picollo et al., 2005; Toloza et al., 2008; Germano et al., 2010a), different resistance profiles were found in different areas (Germano et al., 2012), and different resistance mechanisms were described (Picollo et al., 2005; Santo-Orihuela et al., 2008; Pedrini et al., 2009). Thus, the argument described above, both in one of its premise and its conclusion, was ruled out changing our position regarding the consequences of the actions of chemical control in populations of triatomines.

In this paper the evolution of insecticide resistance in triatomines is reviewed, including a chronology of the detection of resistance foci, the resistance mechanism involved, the geographical distribution, the genetic basis, and the alternatives to control the resistant foci. In addition, theoretical concepts related to toxicological, evolutionary and ecological aspects of insecticide resistance are discussed in order to understand the particular scenario of pyrethroid resistance in triatomines. Likewise, some new hypotheses about evolution of decreased susceptibility in *T. infestans* are also formulated.

2. Triatomines

All vectors of *T. cruzi* belong to the subfamily Triatominae, family Reduviidae (Hemiptera: Heteroptera), defined by their blood-sucking habit and morphological adaptations associated with host-finding and blood feeding (Schofield and Galvao, 2009). The triatomine group contains an estimated 140 species classified into 5 tribes (Triatomini, Rhodniini, Alberprosenini, Bolboderini and Cavernicolini) and 15–17 genera depending of arrangements proposed (Lent and Wygodzinsky, 1979; Schofield and Galvao, 2009). The triatomines are mainly found in the Americas, between 46°N and 46°S. However, there are some species inhabited areas outside America: *Triatoma rubrofasciata* was reported from port areas of New and Old World tropics, the rubrofasciata complex in areas of East Asia (6 species) and the genus Linshcosteus in Indian subcontinent (6 species) (Schofield and Galvao, 2009). All species are probably capable of transmitting *T. cuzi* although relatively few are of epidemiological significance as vectors. The relative importance of the triatomine species as vectors depends on behavioural and biological features, especially the vector preferred ecotope with adaptation to domestic and peridomestic structures (cor-

rals, chicken coops, wood piles, etc.), degree of anthropophily and host preference, feeding time, dispersal capabilities and time span between blood meal and defecation (Lent and Wygodzinsky, 1979; Schofield 1994). The more important vectors are included mainly in two tribes, Rhodniini and Triatomini, and the species with most epidemiological relevance are *Triatoma infestans,* occurring in a wide area of southern of South America, *Rhodnius prolixus* in northern of South America and Central America (except Panama) and *T. dimidiata* in Ecuador and Peru, as well as in several Central American countries and in the south of Mexico (Lent and Wygodzinsky, 1979)

3. History of chemical control in triatomines

The chemical control for Chagas vectors started in the mid-twentieth century stimulated by the successful experience in controlling Malaria vectors using synthetic insecticides. As in Malaria, the organchlorinated DDT was the first used insecticide. Unexpectedly DDT showed a low efficacy against triatomines and was rapidly discarded. Metabolism mediated by DDT-dehydrochlorinase and DDT-hydroxylase were described as mechanisms associated to tolerance to DDT in *T. infestans*. Delayed penetration in starved nymphs of *T. infestans* was shown to be a complementary cause to that toxicological tolerance (Agosin, 1985; Fontán and Zerba, 1992; Zerba, 1999a). After of failure of DDT, others organochlorinated insecticides, as lindane and dieldrin, were considered as alternative candidates for the Chagas vector control. Only the GAMA isomer of hexachlorocyclohexane (lindane) showed insecticide activity against *T. infestans* and was the first synthetic insecticide introduced for triatomines field control. The lindane was used in field control campaigns for several years mainly in Argentina and Brazil, and dieldrin was introduced in Venezuela for the control of *R. prolixus*. Lindane and/or dieldrin remained the most widely used compounds against domestic Triatominae until the late 1970s when it was progressively replaced by modern synthetic insecticides (Schofield, 1994; Zerba, 1999a; Dias et al., 2002).

The results of the campaigns in those countries were successful; however, the high chemical stability and the potential toxicological and ecotoxicological risks of chlorinated insecticides determined their substitution by compounds with more favorable properties (Zerba, 1999a). The emergence of the less persistent organophosphorus and carbamate insecticides were the alternatives to chlorinated insecticides for the control of insect pests. Propoxur was the first carbamate used for control of triatomines, and field assays with that insecticide were performed in Chile at the beginning of the 70s. The phosphorothionates malathion and fenitrothion were introduced in 1975 into Chagas vector control programs (Zerba, 1999a). These insecticides showed ovicide action and higher initial impact than lindane, but have the disadvantage of unpleasant smell persisting after the application generating the resistance of the resident to indoor spray (Picollo de Villar et al., 1980; Schofield, 1994).

The scenario in controlling insects dramatically changed after the development of pyrethroid insecticides mainly due their high insecticidal activity, their low application rates and toxicological safety, and their rapid environmental degradation (Zerba, 1999a,b). The change in Chagas control happened in the early 80s when the introduction of synthetic pyrethroids was a major advance in campaigns to control of triatomines. The first generation pyrethroids showed excellent triatomicidal activity but were discarded for use in field due to their lack of residual activity as consequence of their photolability. The photostability achieved by pyretrhoids of second and third generation allowed their extensive use in the field control campaigns. Deltamethirn and cypermethrin have been successfully used in the field for triatomine control in Argentina and Brazil since 80s. Since 1990s, the pyrethroids used were restricted to alpha-cyano-substituted pyrethroids as deltamethrin, cypermethrin, beta-cypermethrin, lambda-cyhalothrin and beta-cyfluthrin. These pyrethroids are considerably more expensive per kg than the previously used insecticides, but they are applied at lower doses, end up being cost effective. They have the additional advantage of greater acceptability by householders and spraymen because they are odourless and leave no stains on the walls after application (Dias and Schofield, 1999; Zerba, 1999a,b; Dias et al., 2002). The pyrethroids are the first-choice insecticide used by the Chagas disease control programs of the endemic countries, and probably would still be if not for the evolution of the resistance to those insecticides in the populations of the insect vectors.

Independently of insecticide used, the traditional control programmes in Latin American countries were focused on the spraying of insecticides on houses and household annexes buildings. The insecticide formulations mainly used for Chagas disease were/are wettable powder, emulsifiable concentrate, and suspension concentrate (Zerba, 1999a). In this scenario, the elimination of vector-borne transmission is more feasible in areas with domestic vectors, like *T. infestans* and *R. prolixus*. The common pattern of the control programs follows several operational phases: 1) a preparatory phase for the mapping of the area to be treated, the general programming of activities and the estimation of resources; 2) an attack phase during consisting in a first massive insecticide spraying of houses, followed by a second spraying of re-infested houses no more than 6 months later, and 3) a surveillance phase for the detection of residual foci of triatomines after the objective of the attack phase, i.e. interruption of transmission, has been reached (Zerba, 1999a; Moncayo and Silveira, 2009). Following this action patterns, the reduction of Chagas disease had great progress through of large-scale vector control actions. Important advances in South America started in 1991 when the Initiative of the Southern Cone for the Elimination of Transmission of Chagas Disease was launched by the governments of Argentina, Brazil, Bolivia, Chile, Paraguay and Uruguay, and coordinated by the Pan American Health Organization – PAHO (WHO). Later, the Initiative of the Andean countries (Colombia, Ecuador, Peru y Venezuela) in 1997, the Initiative of the Central American countries (Belize, Costa Rica, El Salvador, Guatemala, Honduras, México, Nicaragua and Panamá) in 1997, and Initiative of the Amazon countries (Bolivia, Brazil, Colombia, Ecuador, French Guyana, Perú, Surinam and Venezuela) in 2004, were also launched. Thus, all endemic area for Chagas disease was covered with regional intergovernmental control programs whose common objectives were the elimination of all domestic populations of the main vectors in each region, screening of blood donors to reduce the risk of transfusional transmission, and maternal screening for infection and treatment of infected newborns (Schofield et al., 2006; Gurtler, 2009 Moncayo and Silveira, 2009). These control programs reduced the geographic range and infestation prevalence of major triatomine vectors leading to the interruption of transmission mediated by *T. infestans* in South America (Brazil, Chile, Uruguay, and provinces/departments from Argentina, Paraguay, Bolivia, and Peru), and decrease of infestations with *T. dimidiate* and important reduction of distribution of *R. prolixus* in Central America (Schofield et al., 2006; Gurtler, 2009 Maldonado et al., 2013; Salvatella et al., 2014).

4. Resistance to insecticides

Resistance has been defined as the development ability in a strain of insects to tolerate doses of toxicants which would prove lethal to the majority of individuals in a normal population of the same species (Anonymous, 1957). The developed ability by the population is the result of selecting individuals with a heritable capacity to withstand the toxicant. That heritable capacity is not caused by direct action of the toxicant on genetic material of insects, but is part of the genetic variability present in the popula-

tion prior to exposition to insecticide (Oppenoorth, 1985). So, part of the genetic variability that exists in a population of individuals generates phenotypically resistant insects to an insecticide. These individuals are selected for the insecticide, resulting through the generations in an increase in the proportion of resistant individuals in the population. In other words, the development of resistance is a microevolutionary process where the main factor of change in population genetic structure is the natural selection (Roush and McKenzie, 1987, Mougabure-Cueto, 2004).

The magnitude of the resistance, understood as population resistance, is a function of two factors: the frequency of resistant genotypes (i.e. the proportion of resistant individuals in the population) and the intensity of the resistant phenotype (i.e. the strength of the individual resistance which depends on resistance mechanisms) (ffrench-Constant and Roush, 1990). Interestingly, although the resistant phenotype has selective advantage over susceptible under pressure with an insecticide (resistant genotype has higher fitness), it is generally assumed that in the absence of insecticide, the resistant phenotypes have selective disadvantage in relation to the susceptible phenotypes (resistant genotype has lower fitness) remained resistant alleles at low frequency (Roush and Daly, 1990; McKenzie, 1996). Adaptive cost of resistance in an environment without insecticide is generally understood in terms of pleiotropy (McKenzie, 1996; Onstad and Guse, 2008). The pleiotropic effects of resistant alleles would result from the biochemical and physiological changes associated with the resistant phenotype, which ultimately affect some other functional process (see below).

Since the emergence of resistant individuals is unpredictable (given the random nature of the generation of genetic diversity), the development of pesticide resistance is a problem in which the solution begins after individual resistance is already present in the population. Thus, a strategy for managing the existing resistance is implemented. The main goal of this strategy is to detect the resistance at the lowest possible level (i.e. low frequency of resistant genotypes) and to avoid that selection process continues (Mougabure-Cueto et al., 2004). The named prevention strategies (e.g. rotation or mixture of insecticides) are in fact method that discontinues or delays the selection process preventing the increase of population resistance level; but this makes sense if the resistant genes are present in the population which will be maintained in low frequency, if the resistant genes or individuals are not present in the population, to rotate or not to rotate is the same because these methods do not prevent the occurrence of resistant mutations. Finally, a complete strategy of resistance management should include: toxicological monitoring of populations under chemical control, studies of resistance mechanisms, the development of alternative control strategies (i.e. alternatives insecticides and formulations, control strategies other than chemical control, etc.), and studies of biological process involved in the evolution of resistance (i.e. population dynamic, population structure, dispersion, reproduction, etc.) (Mougabure-Cueto, 2004).

5. Chronology of detection and distribution of the resistant foci

Early evidence of individuals surviving to dose of insecticides used in field was reported for insects from laboratories colonies exposed for 24 h to organophosphorus-impregnated papers, or for 48 h to organochlorinated-impregnated papers. Thus, Fox and Bayona (1966) reported survivors of *R. prolixus* from Colombia exposed to dieldrin, malathion and fenthion, and Correa et al. (1968) reported survivors of *T. infestans* and *Pastrongylus megistus* from Brazil exposed to dieldrin. However, the first evidence of field resistance in triatomines was provided by the studies carried out in Venezuela with *R. prolixus* during 1970s. Gonzalez Valdivieso et al. (1971) evaluated the response to dieldrin of *R. prolixus* from four states of Venezuela and reported tolerance (diminished susceptibility in field collected insect or F0, and similar susceptibilities to reference colony in the progeny of F0 survivors or F1, i.e. environmental induced tolerance) in the states of Cojedes and Portuguesa, and resistance (F0 and F1 showed diminished susceptibility, i.e. true resistance) in the states of Trujillo (Santo Domingo locality) and Yaracuy (La Yuyca y Guarataro localities). Furthermore, the insect from Trujillo were also resistant to lindane. This resistance to dieldrin and lindane in *R. prolixus* was the first report of evolution of insecticide resistance in a triatomine species. Later, the Santo Domingo strain was demonstrated to be resistant to the organophosphates fenthion, malathion and chlorpyrifos, and the carbamate propoxur (Cockburn, 1972; Nocerino, 1975). In addition, Nocerino (1975) also reported reduced susceptibility to dieldrin in field collected *R. prolixus* from eight localities of the state of Trujillo and three localities of the state of Tachira, and reduced susceptibility to lindane in field collected *T. maculata* from La Vera in the state of Falcon, but the authors have not confirmed whether it was real resistance or induced tolerance.

Those early studies were realized through contact bioassays where the insect (fifth instar) walks on papers impregnated with insecticides. However, Nelson and Colmenares (1979) used the technique of topical application to test insecticide activities and to evaluate resistance in *R. prolixus.* Similarly, Cockburn (1972) and Picollo et al. (1976) carried out preliminary topical assays on triatomines (without evaluate resistance), by the application of insecticide solution in acetone on dorsal abdomen of fifth instar. According to the authors, topical application is more practical and reliable, if droplet, size, instar and holding time after application are kept constant, and only the concentration of insecticides in solvent is varied. So, the application of organochlorine, organophosphorus, carbamate and pyrethroid insecticides against a dieldrin-resistant strain of *R. prolixus* (original Santo Domingo strain selected in laboratory with dieldrin for several generations) showed, compared to susceptible strain, high resistance ratios to lindane (RR > 1200) and dieldrin (RR > 550), low resistance to malathion (RR = 2.8), chlorphoxim (RR = 2.4) and bendiocarb (RR = 3.5), and susceptibility to all pyrethroids evaluated (Nelson and Colmenares, 1979).

Because the insecticide resistance in Chagas vectors was poorly documented and using different methodologies, in 1994 the Tropical Disease Research-World Health Organization financed a workshop realized in the Research Center of Pest and Insecticides (CIPEIN) from Argentina to develop a protocol of evaluation of insecticide susceptibility in *T. infestans* and *R. prolixus* (WHO/TDR Workshop in Insecticide Effect Evaluation in Triatomines) (WHO, 1994). This protocol determined the topical application as the best methodology for the bioassays, and defined the parameters of standardization of the biological material for toxicity measurement. The protocol was used in various Latin American countries in order to determine the base-line of susceptibility to insecticides used in each country for each triatomine, which served as reference for subsequent monitoring of susceptibility and resistance to insecticide in populations come from the field (Soto Vivas and Molina de Fernández, 2001; Reyes et al., 2007; Sonoda et al., 2009, 2010). The first systematic monitoring of resistance using the WHO protocol was realized on *T. infestans* and *R. prolixus* from different Latin American countries. The study determined resistance to deltamethrin and other pyrethroids in *R. prolixus* from Carabobo (Venezuela), and in *T. infestans* from Río Grande do Sul (Brazil), without evidences of unsatisfactory control activities in field. The resistance levels ranged from 3.4 to 7 for *T. infestans*, and from 4.5 to 12.4 for *R. prolixus*, and represented the first evidence of pyrethroid resistance in triatomines (Vassena et al., 2000). In 1997 started a monitoring program of resistance to deltamethrin in *T. infestans* in different provinces of Argentina. These studies demonstrated resistance in bugs from some departments of the provinces of San

Luis, La Rioja, Mendoza, Catamarca and Salta, but the resistance levels evolved (from 2.0 to 7.9) did not compromise the chemical control in the field (González-Audino et al., 2004; Santo-Orihuela et al., 2008). Later studies realized during 2002 detected high resistance levels to pyrethroids in four localities from province of Salta (El Chorro, La Toma, El Sauzal, and Salvador Mazza), in the northern Argentina, on the border with the Plurinational State of Bolivia (Picollo et al., 2005). Coincidently with these studies, the authorities of the Argentinian Ministry of Health reported the presence of insects after of the control campaigns with pyrethroid insecticides (>80% of infested houses) indicating the inefficacy of the spraying with those insecticides. The insects from those localities showed high resistance levels to deltamethirn (RRs from 51 to 133), beta-cypermethrin (RRs from 264 to 451), beta-cyfluthrin (RR from 60 to 668), and lambda-cyhalothrin (RRs from 65 to 289), but were susceptible to the organophosphorus fenitrothion (Picollo et al., 2005). Shortly after, serious levels of infestations after chemical control of field insects were reported in Yacuiba, in the southern Bolivia, on the border with the Argentina, by the Authorities of the Ministry of Health and Sports from Bolivia. The toxicological studies on *T. infestans* from rural areas of Yacuiba showed high resistance levels to deltamethrin (RR = 154) and susceptibility to fenitrothion and bendiocarb (carbamate), assuming the evolution of resistance to deltamethrin as a cause of control failures, (Santo-Orihuela et al., 2008).

During the following years, studies were focused on the Argentina–Bolivia border in order to know the extent of the resistant zone by the analysis of locations to the south and east from Salvador Mazza and north from Yacuiba. Thus, several studies covering the Chaco and Andean Valleys from Bolivia reported intermediate and high resistance to deltamethrin in insects from localities of Mataral (RR = 17.4, Department of Cochabamba), Sucre (RR = 31.3, Department of Chuquisaca), Entre Ríos (RR = 174), Tierras Nuevas (RR = 542), Villa El Carmen (RR = 438), El Palmar (RR = 300) y Villamontes (RR = 247) (Department of Tarija) (Toloza et al., 2008; Germano et al., 2010b, 2012). Lardeux et al. (2010) reported different levels of resistance to deltamethrin in several localities from four departments of Bolivia, La Paz (RRs from 3.7 to 5.8), Cochabama (RR = 14), Chuquisaca (RRs from 5 to 323) y Tarija (RRs from 30 to 491). In agreement with the studies cited above, the resistant insects from four evaluated departments were susceptible to bendiocarb and malathion. Few localities showed susceptible insects or low levels of resistance to deltamethrin, these belonging to the majority of evaluated localities of La Paz and some localities of Chuquisaca. (Lardeux et al., 2010). Depeckire et al. (2012) reported resistance to deltamethirn in five localities of Department of Santa Cruz (Estancia Bacilio, Kuatrienda, San Silvestre, Rancho Nuevo and Tamachindi, RRs from 3.8 to 8.3) and one locality of Villamontes, Department of Tarija (Taiguati, RR = 818). Recently, high resistance to deltamethrin was reported again in Villamontes (RR = 129.1) and low resistance in Departments of Cochabamba and Santa Cruz (RR from 4.2 to 8.5) (Gomez et al., 2014). All studies cited above showed the highest levels of resistance to pyrethroid insecticides in southern Bolivia, in the Department of Tarija. Some studies evaluated the insecticide susceptibility in sylvatic insects but they will be discussed in the corresponding section of this review.

The studies carried out on insects from Argentinean localities covered several provinces mostly belonging to Gran Chaco ecoregion. Santo-Orihuela et al. (2008) reported medium resistance to deltamethrin in Cuatro Esquinas (RR = 14) and San Antonio (RR = 21) (Province of La Rioja) Germano et al. (2010b) reported low resistance levels to deltamethrin in Taco Pozo (Province of Chaco), rural areas from Capital city of Santiago del Estero province, Palo Blanco (Province of Catamarca), and San Carlos (Province of Salta) (RRs from 3.7 to 8.4). The highest resistance level determined in that study was for Banda Sur (Province of Salta, RR = 39), close to the high resistance focus from the Argentina–Bolivia border (Germano et al., 2010b). Later, Germano et al. (2012) and Roca-Acevedo et al. (2013) reported resistance to deltamethrin in Acambuco (RR = 32.5) and Campo Largo (RR = 1108) villages (Salta) respectively. In 2011, the authorities of the ministry of health reported the presence of *T. infestans* after the deltamethrin-based control campaigns in two localities (El Malá and La Esperanza) from the Argentinean province of Chaco, in the rural area around the J.J. Castelli city. The toxicological studies conducted on insects from those localities demonstrated very high resistance levels to deltamethrin, RR 1031 for El Malá and RR 233 for La Esperanza (Carvajal et al., 2012; Germano et al., 2013). In a nearby locality, Pampa del Indio, Gurevitz et al. (2012) observed reduced mortality in diagnostic dose assays in eleven of fourteen *T. infestans* populations, and reported low resistance level (RR = 7.2) in a residual population that survived four deltamethrin sprays. Currently, the studies and monitoring are focused in the area around the Castelli focus, in the heart of the Argentinean Gran Chaco ecoregion, main area where the elimination of *T. infestans* has failed. In the context of this monitoring, recently the highest resistance level detected to date was reported in insects from a circa-La Esperanza locality (RR > 2000) (unpublished data).

Yon et al. (2004) using impregnated paper with discriminant dose showed reduced susceptibility to pyrethroids in *T. infestans* from Vitor locality (department of Arequipa) and *Panstrongylus herreri* from El Triunfo and Filadelfia localities (department of Cajamarca) of Perú. Recently, *T. sordida* from Conego Marinho, Montalvania, Monte Azul, and Porteirinha municipalities of the state of Minas Gerais, Brazil, showed slight decreased susceptibility to deltamethrin (RR ranged from 2.02 to 3.94) (Pessoa et al., 2014), although the authors stablished that all *T. sordida* populations from center-west region of Brazil evaluated by Obara et al. (2011) would be resistant considering the reference strain used in their study, evidencing the importance of adopting a single reference population of each triatomine species for all groups investigating insecticide susceptibility/resistance (see below).

It should be noted that the chronology described above do not necessary reflect the actual sequence in which the different foci of detected resistance have evolved. A resistant population could have been detected in a time close to when the selection process started, or when a long time has elapsed since that beginning. These scenarios are associated with the frequency that entomological evaluations and control activities were carried out (which allow evaluate the effectiveness of insecticides in the field) which have been highly variable between the endemic countries and between regions within each country. It should also be pointed out that perhaps other resistant foci exist, but are not known because were not monitored all endemic areas (and have not been evidenced through control failures). Thus, conducting toxicological monitoring in the species of epidemiological importance, covering all the endemic areas is a priority; this will give a complete picture of susceptibility/resistance in the vectors of Chagas.

As was demonstrated by the preceding paragraphs, insecticide resistance in triatomines is a growing problem, where new focus are detected in different areas, both with sustained or sporadic history of chemical control. Even more so, the resistance to pyrethroids in *T. infestans* is evidenced as a complex problem both at the biochemical–physiological (i.e. resistance mechanisms) and population levels (i.e. resistance profiles of populations and geographical distribution).

6. Mechanisms of resistance

The interaction of an insect with an insecticide (in fact any organism with any toxicant) begins with the exposition to the toxicant, and involves a set of biochemical and physiological pro-

cesses that will determine the degree to which the toxicant can damage to organism (i.e. the toxicity) or, what is the same, the degree to which the organism is damaged by the toxicant (i.e. the susceptibility). Those processes generally are grouped in the toxicokinetic and toxicodynamic phases. Toxicokinetics describes what happens to the toxicant when it makes contact with the organism and once inside it, and specifically involves the absorption, distribution, metabolism and excretion processes. Toxicodynamics describe the molecular interaction between the toxicant and the molecular target, also known as site of action, which will trigger the toxic response (Hogdson and Levi, 1997). The genetic alterations that affect qualitatively and quantitatively the aforementioned processes, from exposition to interaction with the site of action, will modify the effect level of the toxicant on the individual compared with other conspecific individuals; in other words, the individual susceptibility to the toxicant will be modified. In the context of insect control, if those alterations result in reduction of the susceptibility to a degree that the individuals survive the dose used for control in the field, these individuals will be resistant individuals and will be selected beginning the process of evolution of resistance. Thus, the modified toxicokinetic/toxicodynamic processes conferring individual resistance are named mechanisms of resistance, being the most relevant and the most documented in insects the reduced penetration (decreasing the entry of the insecticide), enhanced metabolism (increasing the degradation rate of insecticide), and modified site of action (decreasing the probability of binding with the insecticide) (ffrench-Constant and Roush, 1990; McKenzie, 1996).

The study of the mechanisms of resistance can involve toxicological bioassays to identify cross-resistance patterns between different insecticides which are indicative of the presence of some processes that determine the lower toxicity of those insecticides (e.g. increased activity of enzymes shared by the metabolic pathways of the insecticides). The inhibitors of detoxifying enzymes, and the modification of the toxicity after their administration, are tools that allow inferences about the role of those enzymes in the observed resistance. More directly, biochemical and molecular studies allow the detection and description at phenotypic and genetic level of the resistance mechanisms, such as the increased activity of detoxifying enzymes or the presence of mutations in the target site of the insecticide (ffrench-Constant and Roush, 1990; McKenzie, 1996). This section focuses on the diversity of studies and of resistance mechanisms that were described in the *T. infestans* populations. The differences between resistant *T. infestans* from different geographical areas will be discussed in the next section.

The toxicological studies showed cross resistance pattern mostly restricted to pyrethroid insecticides and susceptibility to insecticides belonging to other chemical groups. Vassena et al. (2000) showed that *T. infestans* from Rio Grande do Sud (Brazil) were resistant to pyrethroids while *R. prolixus* from Carabobo (Venezuela) were resistant to all evaluated pyrethroids and to the organochlorine dieldrin. In Argentina and Bolivia, the insects from different localities showed cross resistance to all evaluated pyrethroids but were susceptible to possible alternative insecticides as the organophosphorus fenitrothion and malathion, and carbamate bendiocarb (Picollo et al., 2005; Santo-Orihuela et al., 2008; Toloza et al., 2008; Lardeux et al., 2010; Carvajal et al., 2012; Depickère et al., 2012; Germano et al., 2012, 2013). These results evidenced the presence of toxicokinetic/toxicodynamic process shared by all pyretroids but not by organophosphorus and carbamate insecticides as resistance mechanism. The studies for identify these mechanisms were focused mainly on degradative enzymes and site of action, although also the penetration through the cuticle was evaluated.

When a toxicant enters the internal milieu of insect is substrate of a battery of detoxifying enzymes (e.g. P450 enzymes, esterases, glutathione-S-transferases) that partially determine how much toxicant will reaches the site of action. Increased detoxication means less amount of insecticide exerts its toxic action conferring resistance. The studies were focused on P450 enzymes and esterases due they are the major metabolic mechanisms responsible for pyrethroid resistance (Oppenoorth, 1984; ffrench-Constant and Roush, 1990 ffrench-Constant and Roush, 1990). The use of enzymatic inhibitors such as piperonyl butoxide (PBO) for P450 complex (cytochrome P450 monooxigenases) and triphenyl phosphate (TPP) for esterases, revealed that only PBO led to slight reduction in levels of resistance in *T. infestans* indicating that P450 enzymes (i.e. oxidative metabolism) are partially involved as resistance mechanism in Argentina, and suggesting that other mechanisms should be present (Vassena et al., 2000; Picollo et al., 2005). Lardeux et al. (2010) reported diminution in LD_{50} of resistant insect from Bolivia after PBO treatment but their interpretation (i.e. the oxidative metabolism is involved partially in resistance) is inconsistent due to the authors did not determined nor considered the effect of PBO on susceptible reference strain (i.e. LD_{50} of reference strain after PBO treatment) overestimating the actual role of these enzymes on pyrethroid resistance. The role of enhanced detoxication was confirmed through biochemical assays. In general, these assays measured the fluorescence emitted by 7-hidroxycoumarin (7-OHC) produced by the enzymatic activity on the corresponding substrate, 7-Ethoxycoumarin, reveling monooxigenase activity, or 7-Coumaryl permethrate, reveling permethrate esterase activity. Using these methodologies, several studies confirmed that both P450 enzymes and esterases (mainly pyrethroid-esterases), depending of *T. infestans* populations, are involved in the resistance to pyretroids in *T. infestans* from Argentina (González-Audino et al., 2004; Picollo et al., 2005; Santo-Orihuela et al., 2008; Germano et al., 2012) (Table 1). Recently, Santo-Orihuela et al. (2013) reported no significant differences in glutathione-S-transferases activity between reference strain and pyrethroid resistant populations from Bolivia.

The partial reversion of resistance levels using inhibitors or the slightly significant differences in enzymes activities revealed that the enhanced metabolism evolved as resistance mechanism, but do not fully explain the resistance to pyrethroids in *T. infestans*. During several years another mechanism was speculated to occur, especially one that could explain the very high levels of resistance: altered site of action. Pyrethroids exert their toxic action on the nervous system by modifying the open-close dynamic of the voltage-gated sodium channel (Soderlund, 1995). Mutations and amino acidic substitutions that negatively alter the binding of the insecticide, but relatively not change the functionality of the channel, will be resistance-conferring mutations. Most resistance-mutations were found in domain II of that large membrane protein, particularly in the region comprised between transmembrane segments IV and VI, and were reported in several pest insects' species (Soderlund and Knipple, 2003; Dong, 2007). Recently, two point mutations in the sodium channel gene associated to resistance, equivalent to L1014F and L925I from sodium channel of *Musca domestica*, were described in two permethrin-resistant populations of *T. infestans* (Fabro et al., 2012; Capriotti et al., 2014). The L104F substitution was found in Madrejones, a locality near the focus of high resistance detected in northern Salta, which showed a resistance level of 35.7. The L925I substitution was detected in El Malá, a locality from Chaco province in the heart of Argentinean Gran Chaco region (Table 1). It is interesting to note that the resistance level of populations carrying the L925I mutation is much higher than the ones carrying L104F mutations, and meanwhile the L104F mutation was reported in species belonging to several orders (Dipetera, Phthiraptera, Lepidoptera, etc) the L925I has been found only in pyrethorid resistant populations of Hemiptera (Fabro et al., 2012; Capriotti et al., 2014). In addition, these authors developed simple

Table 1
Resistance mechanisms determined in triatomines resistant to insecticides.

Resistance mechanism	Component involved	Triatomine species, locality (province/state, country)
Reduced penetration	Epicuticular and total hydrocarbons	*T. infestans* from Salvador Mazza (Salta, Argentina)[a]
	Thickness of the cuticle	*T. infestans* from Salvador Mazza (Salta, Argentina)[a]
Enhanced metabolism	Esterases/Pyrethroid-esterases	*T. infestans* from Salvador Mazza[b], El Chorro[c], La Toma[c], El Sauzal[c], Acambuco[d] (Salta, Argentina), Cuatro Esquinas[c], San Antonio[c] (La Rioja, Argentina)
	P450 enzymes	*R. prolixus* from n/l (Carabobo, Venezuela)[e]
		T. infestans from n/l (Rio Grande do Sul Brazil)[e], Salvador Mazza (Salta, Argentina)[b,f]
Altered site of action	Mutations in sodium channel: L1014F	*T. infestans* from Madrejones (Salta, Argentina)[g]
	L925	*T. infestans* from El Malá (Chaco, Argentina)[h]

[a] Pedrini et al. (2009).
[b] González Audino et al. (2004).
[c] Santo-Orihuela et al. (2008).
[d] Germano et al. (2012).
[e] Vassena et al. (2000).
[f] Picollo et al. (2005).
[g] Fabro et al. (2012).
[h] Capriotti et al. (2014).

and sensible molecular methods for the identification of the two resistant mutations in field insects in order to detect resistant foci in early phase of the selection process.

The insect cuticle is the first barrier against the penetration of exogenous compounds. Insects with cuticular modifications that reduce the entry rate of insecticides will tolerate higher dose than insects without those modifications. The reduced penetration is generally described as a contributive mechanism less specific than the two mechanisms presented above (McKenzie, 1996). Studying resistant *T. infestans* from Salvador Mazza (Salta province, Argentina), Pedrini et al. (2009) showed no qualitative difference in hydrocarbon components compared with susceptible insects, but showed significantly higher values of the following parameters: the per insect weight, the epicuticle/total hidrocarbons ratio, the amount of hydrocarbons per unit surface, and the amounts of hydrocarbons extracted from the fourth-instar exuviae (Table 1). The authors concluded that due that cuticle free of hydrocarbons of *T. infestans* were more permeable to chemicals penetration than intact cuticles, the cuticle enriched in hydrocarbons probably restricts deltamethrin penetration in the resistant insects. In addition, scanning electron microscopy revealed that the cuticle of resistant insects was significantly thicker than that of susceptible insects (Pedrini et al., 2009)

In summary, in triatomines, particularly in *T. infestans*, the resistance to pyrethroids shows certain degree of complexity at biochemical–physiological level where the three most relevant resistance mechanisms have been described, even same population (e.g. Salvador Mazza) (Table 1). As was suggested, the highest levels of resistance would be determined by the alterations in the site of action, and the increased metabolism and decreased penetration would be involved as contributive mechanisms or would be associated to low levels of resistance.

7. Macro and microgeographical differences

The resistance to insecticides can be detected in several areas of the geographical distribution of an insect species, i.e. resistant foci. The problem that means the geographical amplitude of resistance is obvious; however the problem is exacerbated by the possibility that the foci have different resistance profiles depending of the type and amount of resistance mechanisms evolved in each. The evolution of resistance to insecticides is a microevolutionary process, i.e. a change occur in the gene frequency in a population. The dynamics and structure of the population and the gene flow between groups of individuals will determine the maximum geographical spread of each process of evolution of resistance. The occurrence of several resistance foci might be due the scattering of an original or ancestral focus (i.e. a single selection process and subsequent dispersion) or the development of independent selection processes which could involve different resistance mechanisms for each. This disquisition is of practical relevance in resistance management because the actions to be taken depend on the evolutionary scenario. If each focus had an independent origin, each will probably have a different resistance profile (although not necessarily) and therefore will probably require a particular strategy mainly depending on the resistance mechanism evolved. If all foci are descendants of an ancestral resistant population, all will have the same resistance profile and the same strategy could be applied to each.

Effectively, the resistance to pyrethroids in *T. infestans* has been detected in several areas of the geographical distribution of the species, spaced from a few tens of kilometers to over a thousand kilometers across two countries, Argentina and Bolivia (Picollo et al., 2005; Santo-Orihuela et al., 2008; Toloza et al., 2008; Lardeux et al., 2010; Germano et al., 2010b, 2012, 2013, 2014; Roca Acevedo et al., 2013 Depickère et al., 2012; Gomez et al., 2014) (Fig. 1). *T infestans* resistant to pyrethroids was reported in the Bolivian Andean Valleys (Departments of Cochabamba, Chuquisaca and La Paz), Bolivian Chaco (Departments of Tarija and Santa Cruz), and Argentinean Chaco (Provinces of Salta and Chaco). Does the geographical spread of the resistance in *T. infestans* could be explained by the dispersion of an ancestral focus or by independent evolutionary events? While independent origins do not necessarily imply different resistance profiles, different resistance profiles involves independent evolutionary processes. This was suggested by Germano et al. (2012) who described three different insecticide-resistant profiles (i.e. the susceptibility to insecticides other than pyrethroids, the susceptibility of the eggs, the resistance mechanisms described) in *T. infestans* from Argentina and Bolivia: the Ti-R1 profile in Acambuco (Salta, Argentina), the Ti-R2 profile in Entre Ríos (Tarija, Bolivia), and the Ti-R3 profile in Mataral (Cochabamba, Bolivia). Adding to this description the results of the other studies cited above, the following pattern can be outlined. All populations were resistant to deltamethrin and other pyrethroids insecticides and susceptible to organophosphates insecticides such as fenitrothion and malathion, but only the Bolivian populations were resistant to phenylpyrazol insecticide fipronil (see below) (Picollo et al., 2005; Toloza et al., 2008; Lardeux et al., 2010; Germano et al., 2012). The populations from Argentina (e.g. Salvador Mazza, Acambuco, Campo largo) expressed high levels of deltamethrin resistance in eggs (RR from 29 to 1193) while in some populations from Bolivia (e.g. Mataral,

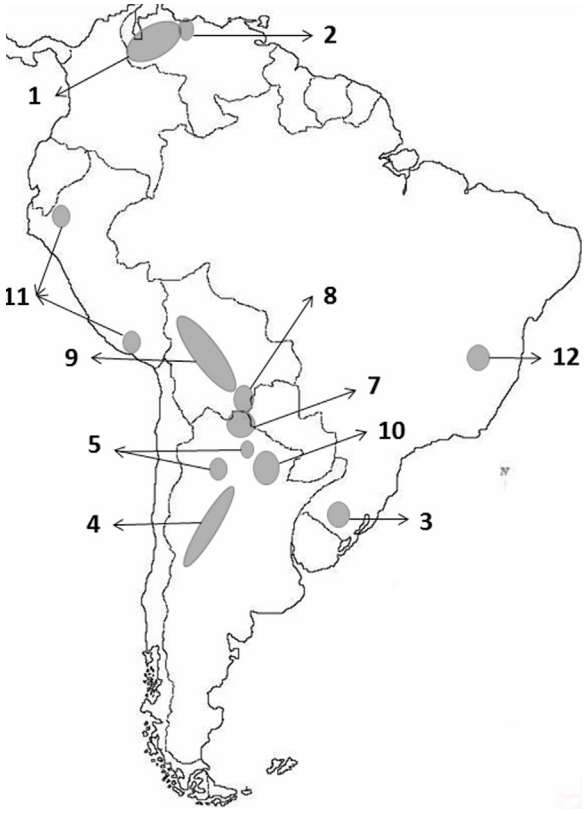

Fig. 1. Location of the foci of resistant triatomines reported to date (or. 1) *Rhodnius prolixus* resistant to organochlorines (and others), localities of the states of Trujillo, Yaracuy and Tachira, Venezuela (1970s); 2) *R. prolixus* resistant to pyrethroids, state of Carabobo (locality is not reported), Venezuela (1990s); 3) *Triatoma infestans* resistant to pyrethroids, states of Rio Grande do Sul (locality is not reported), Brazil (1990s); 4) *T. infestans* resistant to pyrethroids, localities of the provinces of Catamarca, La Rioja, Mendoza, San Luis y Santiago del Estero, Argentina (1990s); 5) *T. infestans* resistant to pyrethroids, localities of southern and eastern of the province of Salta, Argentina (2000s); 7) *T. infestans* resistant to pyrethroids, Salvador Mazza and other localities of northern of the province of Salta, Argentina (2000s); 8) *T. infestans* resistant to pyrethroids, Yacuiba and other localities of the department of Tarija, Bolivia (2000s); 9) *T. infestans* resistant to pyrethroids, localities of the departments of Chuquisaca, Cochabamba and La Paz Bolivia (2000s); 10) *T. infestans* resistant to pyrethroids, localities of the province of Chaco, Argentina (2010s); 11) *T. infestans* and *Panstrongylus herreri* resistant to pyrethroids, localities of the departments of Arequipa and Cajamarca respectively; 12) *T. sordida* resistant to deltamethrin, localities of the states of Minas Gerais, Brazil (2010s).

Sucre and Yacuiba) the eggs were not resistant or expressed low resistance (RR from 0.56 to 8.4) (Toloza et al., 2008; Germano et al., 2012). Enhanced activity of detoxifying enzymes (mainly pyrethroid-esterases) was found in insects from Argentina, in the northern Salta, but was not detected in the insects from Bolivian Chaco or Andean Valleys (González-Audino et al., 2004; Picollo et al., 2005; Santo-Orihuela et al., 2008; Germano et al., 2012) (Table 1). On the other hand, the mutations in sodium channel seems too show a geographical distribution with the L104F substitution distributed around the Salvador Mazza–Yacuiba focus and the L925I substitution distributed in the Chaco Province around the J.J. Castelli focus (Fabro et al., 2012; Capriotti et al., 2014) (Table 1). Thus, the differences in the resistance profiles between the different resistant foci of *T. infestans* suggest the occurrence of independent evolutionary processes. Deepening this hypothesis and considering the high degree of population structure in *T. infestans*, the different resistance profiles could be the consequence of the different regimes of selection with insecticides occurred in the different endemic areas acting on the different genetic backgrounds of the populations.

It is noteworthy the observed difference in the toxic response to fipronil between Argentinean and Bolivian populations (Toloza et al., 2008; Germano et al., 2010b; Germano et al., 2012; Toloza et al., 2008; Germano et al., 2010b, 2012; Roca Acevodo et al., 2011). Insects from Entre Ríos, Villa El Carmen, El Palmar (Department of Tarija), Mataral (Department of Cochabamaba) and Sucre (Department of Chuquisaca) showed resistant levels ranged from 12 to 590. The fipronil has not been used for chemical control of *T.infestans* so that the lower susceptibility would not be the outcome of the selection with this insecticide. Moreover, the fact that the site of action of deltamethrin is the voltage-gated sodium channels, and that of fipronil is γ-aminobutyric acid gated chloride channels implies that a cross-resistance phenomenon between these insecticides as a result of altered action site is not possible. Cross-resistance mediated by increased detoxification may be possible due both deltamethrin and fipronil are metabolized by P450 complex, however the Bolivian insects did not show increased activity of P450 enzymes. Thus, it seems unlikely that the reduced susceptibility to fipronil is associated to deltamethrin resistance. More plausibly, as the site of action of fipronil is the same to that of the organochlorine lindane and dieldrin, the previous use of these compounds in Bolivia against triatomines could have resulted in a cross-resistance phenomenon to fipronil (Germano et al., 2010b; Toloza et al., 2008). Finally, it is possible to consider the hypothesis that this toxic response to fipronil is not a resistance phenomenon but actually a tolerance or low natural susceptibility, i.e. the low susceptibility is not consequence of the selection with fipronil but its evolution was independent of the presence of insecticide (see below the discussion about concepts of tolerance). These hypotheses can also explain the low susceptibility and knockdown resistance to formulated fipronil in *T. infestans* reported by Rojas de Arias et al. (2011).

At microgeograhical level (i.e. houses within a locality), the resistance foci do not appear to be toxicologically homogeneous. Germano et al. (2013) studied the susceptibility to deltamethrin of *T. infestans* from different dwellings of the locality of La Esperanza (Argentinean province of Chaco). The authors demonstrated that the insects from different dwellings present different toxicological phenotype to deltamethrin showing that some houses host resistant insects and other houses host susceptible insects, and that there was not association between the toxicological status and the original location of the insects within dwellings (i.e. peridomestic or domestic structures). According to the authors, these differences highlight the complex micro-spatial distribution of toxicological phenotypes of insects that persist after chemical control actions and suggest a high degree of population structure at micro-level on which the insecticide exerts its selective pressure. Regarding the later, a significant genetic difference between *T. infestans* from different houses in the same locality was reported through the analysis of microsatellite loci, and genetic structure within the same house dwellings (e.g. domestic vs peridomestic area) was suggested (Marcet et al., 2008; Pérez de Rosas et al., 2008). Microgeographic structuring in *T. infestans* was also demonstrated by morphometry studies which showed significant differences between habitats of dwellings in the Chaco region of Argentina (Hernández et al., 2011; Gaspe et al., 2012). As was exposed above, the different toxicological profiles of *T. infestans* from different locations from Argentina and Bolivia have been regarded as independently originated. This microgeographical pattern reinforce that hypothesis and extend it to the small geographic scale, as insects from relatively close dwellings have been shown to develop different susceptibility to deltamethrin, including resistance, after the exposure to similar levels of treatment (Germano et al., 2013).

In summary, the pyrethroid resistance in *T. infestans* seems to have evolved independently at both macro and microgeographical level. This does not exclude the possibility of dispersal processes

that have spread resistance to nearby areas in which there were only susceptible insects, which probably explains the local extension of some foci (e.g. Salvador Mazza and surrounding areas in Northern Province of Salta). Despite the differences in the profiles of resistance in *T. infestans* from different geographical areas, all populations studied did not show variation in susceptibility to organophosphates (e.g. malathion and fenitrothion), or concerning a reference susceptible colony nor between them (i.e. all were susceptible to organophosphates). Thus, these insecticides, which are approved by regulatory organisms and has been used in the past against triatomines, could be alternatives to pyrethroids insecticides when resistance evolved (see below).

8. Genetic basis of resistance

The development of resistance cannot be fully understood without studies analyzing the evolutionary dimension of the phenomenon, for which the genetic basis of resistance is central. These studies would allow understanding the origin of a particular resistant focus, the dynamic of the selection process, and propose future scenario including resistance management strategy. The description of genetic basis of the resistance involves knowing the number of genes involved in the resistant phenotype, and the inheritance and heritability of resistance.

It is argued that high levels of resistance that lead to field control failures evolve by one or few genes that contribute to the resistant phenotype while polygenic resistance is typically obtained from laboratory selection experiments. The individual susceptibility (or more precisely the individual tolerance) is distributed randomly among the individuals of a population because many genetic and environmental factors, those involved in all toxicological processes, determine its expression (see below). If the insecticide selects individuals within of this distribution, such as in typical laboratory studies where a dose that let live a percentage of treated individuals is used, the resistance is expected to be polygenic because the selected individuals are in the tail of a distribution in part determined polygenically. On the other hand, if the dose of insecticide is higher than the maximum dose of the distribution, such as in field control regimen where dose that ensures 100% mortality is used, the resistance is expected to be determined by one or few genes because the selected individuals (if they are present) probably carry rare mutations which places them outside the susceptibilities distribution of the original population (i.e. individuals with exceptionally low susceptibility) (Roush and McKenzie, 1987; McKenzie, 1996; ffrench-Constant, 2013). These assumptions have generated selection models that predict the evolution of the system and allow the comparison of different strategies of resistance management. One or two major genes have been found in a high percentage of cases of resistance (Onstad and Guse, 2008). However, many other cases of resistance in field could not be modeled under these assumptions. Although the action of a major gene has been demonstrated, the action of minor genes is commonly present (polygenic resistance) (Firko and Hayes, 1990). The analysis from toxicological responses of intercross and backcross progenies of *T. infestans* highly resistant to deltamethrin from Argentinean locality of Salvador Mazza rejected the hypothesis of a single gene being responsible for resistance; even more, the estimated minimum number of independent segregation genes was three (Cardozo et al., 2010). This agrees with the toxicological, biochemical and molecular studies described above, which showed that different resistance mechanism (two families of detoxifying enzymes, two mutations in the sodium channel, alterations in the cuticle) evolved in *T. infestans* even within the same population, demonstrating that resistance to pyrethroids in *T. infestans* is determined by more than one gene.

The evolution of resistance and resistance management also depend greatly on the dominance relation in the genes involved. In general, the dose–response assays performed on resistant, susceptible and hybrid strains allow identifying dominance relations regarding inheritance. Different relationships were reported for different mechanisms of resistance: resistance to pyrethroids and DDT by modified site of action was showed to be recessive or semi-recessive, resistance to organophosphorus, carbamate, and cyclodiene by modified site of action was dominant or co-dominant, and most metabolic resistance was showed to be dominant (Roush and Daly, 1990; Onstad and Guse, 2008). Furthermore, applying a specific dose (e.g. that used in field) the dominance relation can be determined for the fitness (i.e. effective dominance) and this is dependent on the dose (McKenzie, 1996). Cardozo et al. (2010) and Germano et al. (2010a) in similar studies analyzing *T. infestans* resistant to deltamethrin from Salvador Mazza showed that the response of SR and RS hybrids to deltamethrin was intermediate between the highly resistant and the susceptible parent colonies, indicating some degree of semi-dominance for resistance. Moreover, lack of significant differences between the LD_{50} and resistance ratios of the reciprocally mated insects indicated no maternal effects or sex linkage on this trait. These results, in addition to degree of dominance and effective dominance calculations, suggested that resistance to deltamethrin in this colony of *T. infestans* was controlled by semi-dominant, autosomally inherited factors. However, the effective dominance (at level of discriminant dose in Germano et al. (2010a) and at level of field dose in Cardozo et al. (2010)) showed values higher than 0.6 indicating a higher prevalence of resistant individuals at doses that are lethal for susceptible individuals.

9. Biological costs of resistance

As already stated, although the resistant individuals have selective advantage over susceptible ones when are exposed to lethal doses of insecticide, it is generally assumed that in an environmental without insecticide the resistant genotype present an adaptive cost which leads to a selective disadvantage in relation to the susceptible individuals (however, the resistant individual not necessarily carries a biological cost, see Section 10). Adaptive cost of resistance is generally understood in terms of pleiotropy (McKenzie, 1996; Onstad and Guse, 2008). The pleiotropic effects of resistant alleles would occur in two ways. On one side, through the modification of biochemical and physiological processes in which the resistant allele's products are normally involved (i.e. processes in which those genes are functionally involved, even in its wild type condition), which ultimately affect the fitness of the individual. For example, enzymes detoxifying insecticides such as P450 enzymes are involved in developmental processes, and hormones and pheromones metabolism (Feyereisen, 1999, 2006), thus, the enhanced activity of these enzymes could negatively modify the ontogenetic development or the success in finding couple. Furthemore, through the energy cost arises from maintaining of resistant phenotype, e.g. the enhanced metabolism or a thick cuticle, which may require a trade-off with other traits such as reproduction. Actually, the only study was performed by Germano and Picollo (2015), who investigated the reproductive and developmental costs of deltamethrin resistance in *T. infestans* from Argentinean Gran Chaco eco-region, locality of Aguaray, Province of Salta. The study demonstrated a reproductive cost expressed as a lower fecundity, and developmental costs expressed as shorted second and third nymph stage duration and an extension of the fifth stage. In addition, the authors suggest a maternal effect as these biological costs were identified in resistant females and their progeny independently of the mated male's deltamethrin response.

10. Resistance and tolerance

As was exposed above, the unexpected low susceptibility to the insecticide fipronil in some *T. infetans* populations (Rojas de Arias et al., 2011; Toloza et al., 2008) raised the particular discussion about if it is not a resistance phenomenon but a tolerance or low natural susceptibility. However, this discussion can be extended to one more general about whether all difference in susceptibility to insecticides should be interpreted in terms of resistance, and whether the natural susceptibility of *T. infestans* to insecticides varies along the geographical distribution of the species.

It is important to clarify concepts due there is confusion between the terms of tolerance and resistance applied to insect toxicology. Resistance is not conflicting term. It has a population and individual meaning, both already seen in this review. The population sense refers to the incremented proportion of resistant individuals in a population as consequence of the selection with the insecticide. The individual sense refers to the resistant individual, who was or will be selected by the insecticide, and to the factors that determine the resistant phenotype (i.e. the resistance mechanisms and their genetic substrate). Tolerance is a more conflicting term. It also has population and individual meanings. The population sense can refer to: a) diminished susceptibility as synonym of resistance (Hodgson and Levi, 1997), or b) a low natural susceptibility, not as a result of insecticide selection (see below). The individual sense can be refer to: a) a change in the susceptibility to a toxicant over the life of an individual, i.e. induced tolerance, generally as a consequence of the activation of a biochemical process following the previous exposition to the toxicant (e.g enzymatic induction) (Hodgson and Levi, 1997), b) the dose of a drug that is just insufficient to show the quantal or all-or-none response concerned (e.g. dead) (Hewlett and Plackett, 1956). The latter concept is very important due that explain the toxicological response of a population exposed to an insecticide or other selective factor (i.e. the population senses of resistance and tolerance terms). Briefly, the difference in the response observed (e.g. proportion of dead insects) to different doses (i.e. the dose–response curve) emerges from the individual variation of biological characters that determine the individual tolerance, or more simply, from the variation of individual tolerances (Hewlett and Plackett, 1978). The tolerance varies randomly among the individuals of a population (showing a normal distribution in log–dose) due to genetic and/or environmental factors just as any other phenotypic characters (e.g. height or body weight), and it is on this genetic variation that selection with an insecticide operate, shifting the dose–response curve to higher doses (and this variation, as part of the genetic background of a population, in turn too varies between different structured populations of *T. infestans* allowing the different selection regimes as was discussed above).

In this section, the discussion is focused on the population meanings. The tolerance at population level refer to low natural susceptibility, low respect to a reference susceptible colony (i.e. colony without contact with insecticides), and natural as not a result of selection with the insecticide but evolved in independent way of insecticide application. Thus, the ability of survive high doses of insecticide would be the toxicological phenotype naturally or ancestrally preponderant in the population. Tolerance and resistance are historical processes, and both originate similar populations in terms of response to an insecticide. The difference lies in how was reached that toxicological situation: with or without insecticide (i.e. some variables of the natural environmental acted as selection factor). While the effect of both phenomena on chemical control is the same (i.e. control becomes poor) and the immediate actions are the same (e.g. change the insecticide), the evolutionary difference expressed above is important in terms of long-term planning of chemical control. On the one hand, to know whether the reduced susceptibility is resistance or tolerance is allowed to speculate on the ability of response to selection with insecticide by the populations of a particular species of insect. Furthermore, the existence of tolerant populations evidences the population variation of the toxicological response to insecticides, i.e. insecticide susceptibility varies across the geographic range of the species. Finally, the tolerance character of tolerance should have necessarily evolved in concert with the rest of the traits since the entire phenotype (phenotype toxicological joined the rest) was the subject of selection for the natural environment (i.e. without insecticide). In this case, it is clear that the tolerant individual, contrary to what is assumed for the resistant, does not have an adaptive cost in the absence of insecticide simply because it was selected in the environment without insecticide. By contrast, in the case of resistance, having a new environment (the environment with insecticide) which exerts a drastic selection (kills insects adapted to the natural environment, which are the majority), the character that determines survival is exclusively the resistant character. In this situation, the new emerged character, which allows the survival of the phenotype of which is part, is not necessarily tightly arranged with other phenotypic traits (not necessarily means that anyway can be), so that the resistant individual have not necessarily the same fitness than the susceptible individual in the natural environment. This is the evolutionary rationale of generally assumed adaptive cost of resistance, but is also the explanation of that resistance could not be associated with such adaptive cost. This difference between resistance and tolerance is very important due to the frequency of tolerant insects is not expected to decrease when the chemical control is finished as is expected to occur for resistant insects, if they carry a biological cost.

The toxicological response of field populations that were not subjected to chemical control could provide valuable information to discriminate between both phenomena (i.e resistance and tolerance). Roca-Acevedo et al. (2011) studied the toxicological profiles of *T. infestans* collected from sylvatic habitats from Cochabamba (Mataral, Ilicuni, and 20 de Octubre) and Potosí (Kirus-Mayu), Bolivia. Those profiles, which included the susceptibility to deltamethrin and fipronil, were different among the Bolivian populations and between Argentinean reference colony and Bolivian populations, the latter showing lower susceptibility to both insecticide (RR to deltamethrin from 1.9 to 11.9, and RR to fipronil from 11.8 to 96.9). Similarly, Depickère et al. (2012) and Gomez et al. (2014) showed reduced susceptibility to deltamethrin in sylvatic *T. infestans* from Potosí (Julo Grande, 96% mortality at DD) and Cochabamba (Cotapachi and Mataral, RR=2.9 and 4.2 respectively), in Bolivian Andes. While, the only sylvatic evaluated by Lardeux et al. (2010) was susceptible to deltamethrin. The sylvatic populations had not been targeted by chemical control interventions so that the reduced susceptibility in these insects could the results of any of the following processes: a) the evolution of tolerance; b) the evolution of resistance by exposition to insecticides used to other insect pests (e.g. agriculture or other sanitary pest), c) migratory flow from domestic resistant populations to nearby sylvatic populations (Germano et al., 2010; Depickère et al., 2012 Depickère et al., 2012). Considering the specifications in doses, formulations, and application methods that allow the effective control of triatomines, which are very different to the standardized specifications for other pest, and considering too that some studied sylvatic populations are not close to a village subject to control or to a farmland (e.g. Mataral) (Depickère et al., 2012), is unlikely that insecticides used in agriculture or against other disease vectors (e.g. malaria vectors) may have a sufficient level of toxic impact on the sylvatic insect to select resistant insects. In addition, current evidence does not support a consistent flow of Andean *T. infestans* from Bolivia between sylvatic refuges and domestic habitats with control history (Richer et al., 2007; Noireau, 2009). In conse-

quence, the toxicological profiles showed by sylvatic populations are unlikely to result from selection pressure with insecticide or gene flow from controlled areas, but those profiles would represent natural or wild toxicological phenotypes which determine tolerance to deltamethrin and fipronil (Roca-Acevedo et al., 2011). Even more, these authors speculate that as the Andean valleys in Bolivia are believed to represent the center of origin and dispersal of *T. infestans* (although there is alternative hypothesis (Torres-Pérez et al., 2011), it is possible that the toxicological profiles of the sylvatic populations from Cochabamba could represent the ancestral toxicological profile of *T. infestans.* Thus, this scenario support the hypothesis that *T. infestans* from different geographic areas have different toxicological phenotypes (see above), even in absence of insecticide selection, determining differences in the susceptibility to insecticides. As was discussed in the previous paragraphs, the susceptibility and biochemical differences may relate to the high genetic structure of the *T. infestans* populations from Argentina and Bolivia evidenced both at macrogeographical and microgeographical levels (Pérez de Rosas et al., 2007, 2008; Marcet et al., 2008; Pizarro et al., 2008). Furthermore, Monteiro et al. (1999) and Piccinali et al. (2009) demonstrated that Bolivian and Argentinean populations are part of different haplotype clusters. Thus, it is possible to speculate that these different genetic backgrounds determine in part the different toxicological responses to insecticides, including alleged natural differences, found both between and within the two countries (Germano et al., 2010b; Roca-Acevedo et al., 2011). However, another scenario could occur in other regions as was suggested in a study carried out in the Paraguayan Chaco according to which the current sylvatic triatomines could have been stablished and adapted in the wild by dispersing from rural communities after massive spraying with insecticides (Rojas de Arias et al., 2011). The question remains open about of the historical events that led to the current geographical variation of the susceptibility to insecticides in *T. infestans,*

The geographical variation of the natural susceptibility highlights a practical problem for monitoring of susceptibility/resistance to insecticides: the reference susceptible strain. If there is not variation, the optimum is to have a single reference strain that would be used by all laboratories studying susceptibility to insecticides (Pessoa et al., 2014). If there is variation, the use of a single reference has the problem that the tolerant insects in an area would be taken as resistant; in this case, susceptible insects from that area should be the reference colony (i.e. insects collected in localities with poor history of chemical control or insects bred for several generations without contact with insecticides). In this way, baseline susceptibility for each area to be monitored for resistance should be a necessity. However, even with this discussion in mind, the use of a single strain of reference for all the evaluated areas (e.g. CIPEIN strain) remains relevant because it is the only way to determine the existence of such variation in natural susceptibility to a given insecticide.

11. Is there any association between the persistence of *T. infestans* in the Gran Chaco region and the evolution of resistance?

In spite of sustained and intensive vector control activities at macro-scale, at the center of the Gran Chaco eco-region the rates of domiciliary infestation by *T. infestans* remain high (Gürtler, 2009; Moncayo and Silveira, 2009). The reasons for the persistence of *T. infestans* in this region, which coincide with the center of its current distribution (Fig. 2), are poorly understood, although must be sought considering the environmental, biological, cultural, political and economic particularities of Gran Chaco region.

The Gran Chaco extends for over Argentina, Bolivia, and Paraguay (1.3 million km^2), present wide climatic gradients determining a humid and a dry sub-region, and the rural human population is clustered in villages sparsely distributed and with difficult access by dirt roads (Gürtler, 2009). The rural human dwelling, the habitat of *T. infestans*, is constituted by domiciliary zone (bedrooms and structures attached) and peridomicilliary zone separated from the previous and composed by common courtyard and several scattered structures (corrals, coops, shed, etc.). Although there are differences between areas of the Chaco, the typical building materials include mud-and-stick, adobe, brick and wood for the walls, and straw and cane for the roofs. The interaction of *T. infestans* and humans occurs mostly in the domicile; however the peridomicile have great epidemiological significance since they are considered the main source of reinfestation and/or invasion of domiciles by *T. infestans* and other triatomines that come from sylvatic environment. This is because the peridomicilliary structures house domestic animals that favor the development of high densities of triatomines, and elimination of these insects is underprivileged due to the structural features of the building hinder the uniform application of the insecticide while leaving it exposed to environmental degradation (Cecere et al., 2004). In addition, the countries in which extends the Gran Chaco were characterized by political and economic instability during much of the twentieth century, including military governments and neoliberal economic politics, which negatively affected the actions of the State in the most sensitive areas of society (health, education, etc). In this context, the discontinuity in time and space of the control actions; the peridomestic structures housing domestic animals; and the limited effectiveness of insecticides in such peridomestic structures can be considered as possible causes of persistence of *T. infestans*. However, in light of studies carried out in recent years, reviewed in this paper, the reduced susceptibility to insecticides (resistance or tolerance) undoubtedly is imposed as one main explanation for the failures of the control actions in this geographical region.

As was described in the previous paragraphs, the resistance emerges as consequence of the continued use, in time, of insecticides. This is the evolutionary scenario more probable in some zones such as Salvador Mazza focus and its surroundings in Argentina (Zaidemberg, 2012). However, the discontinuity of the control actions in vast areas of Gran Chaco allows speculate that this kind of hard selection might not be the most likely explanation for the decreased susceptibility detected in villages located in such areas. So, some other hypothesis can be built to explain the variation of the toxicological phenotype in *T. infestans*: 1) even within the context of selection with insecticide, other scenario can be considered in which some environmental variables decreases the field doses of formulated insecticide in a way that causes less of 100% of mortality; in other words, this field effective dose would allow survive individuals with the higher tolerances within the variation of individual tolerances of the population (see above). As, in the same way that other phenotypic characters, the variation of tolerance is due to genetic and environmental variation, the high tolerance (or less susceptibility) of survivors is genetically based. Thus, the field doses would select genetically less susceptible individuals, beginning the process of evolution of resistance (McKenzie, 1996). This process could occur in the peridomilliary structures where the insecticides are rapidly degraded decaying the effective dose to below of 100% effectivity. 2) Some environmental variables could select individuals with high tolerance without, or before of, the presence of the insecticide. This process explains the existence of populations with low natural susceptibility. In this case, the individuals with high tolerance (resistant individuals) could be selected by: a) the toxicological phenotype, i.e. part of the phenotype involved in the interaction with the insecticide and that determine the resistant status; in other words, the selective

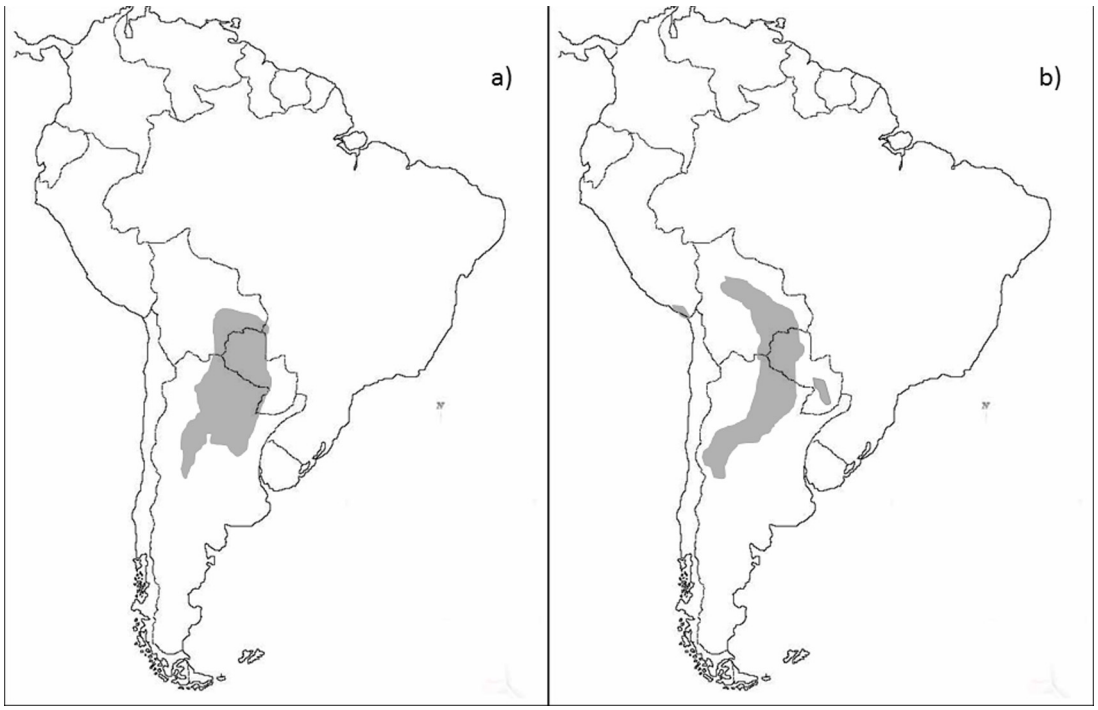

Fig. 2. a) Geographical location of Gran Chaco region, b) Apparent current geographical distribution of *Triatoma infestans*.

environmental variable would be equivalent to an insecticide; or b) other phenotypic attributes not involved with the interaction with the insecticide but associated to, or determined by, the resistant genes; in other words, the resistant genes could have pleiotropic effects (determining other phenotypic features than toxicological phenotype) by which the individuals could be selected. 3) Recently, Panzera et al. (2014) proposed that pyrethroid-resistant populations of *T. infestans* from the Argentinean–Bolivian border are most likely the result of recent secondary contact between the Andean and non-Andean lineages. The main evidence that support the hypothesis is the detection of pyrethroid-resistant insects in a hybrid zone between both chromosomal groups, which showed high frequency of chromosome fragments, suggesting a correlation between this kind of genomic variability and insecticide resistant populations. The authors emphasize that the elimination of *T. infestans* by pyrethroid insecticides in Brazil, Chile, Uruguay and parts of Argentina and Paraguay, clearly indicates that pyrethroid resistance was very uncommon in non-Andean regions, while in the Bolivian Andes, pyrethroid resistant populations appear to be much more frequent. This suggests that Andean populations have particular genetic backgrounds that enable the development of resistance to pyrethroid insecticides more rapidly than non-Andean populations. This is in according with Roca-Acevedo et al. (2011), Depickère et al. (2012), and Gomez et al. (2014) who showed varying degrees of tolerance to deltamethrin and fipronil in the sylvatic *T. infestans* from Andean valleys of Bolivia. However, as has been seen in this review, the resistance to pyrethroid insecticides evolved in the Argentinean Chaco (i.e. non-Andean region); even more, these insects showed the highest resistance levels ever determined for triatomines. Thus, although the different genetic backgrounds probably determine the different natural susceptibilities to insecticides and different responses to selection with insecticides, the hypothesis that claims that the cross between Andean and non-Andean individuals was the historical event that explains the evolution of pyrethroid resistance in *T. infestans* is not supported or at least is restricted only to resistant focus of the Argentina–Bolivian border.

12. Resistance management

The evolution of insecticide resistance imposes the need for a change in the control strategy adopted so far. This change has been conceptualized as Resistance Management Plan (RMP), Pesticide or Insecticide Resistance Management (PRM or IRM), and related names, which include recommendations of practices and strategies to be implemented. If no change, the selection process continues and the resistance level increases. The change may involve from a toxicological monitoring of low resistance population (i.e. even without compromise in the field control) to the change of the insecticide and the implementation of alternative control strategies (e.g. biological control or behavior- modifying strategies) as a complement to the chemical control or directly replacing it. Before the resistance cause control weaknesses in field, resistance prevention tactics (such as mixtures or rotation of insecticides belonging to unrelated chemical classes, refuges to susceptible individuals, etc) can be implemented to delay the increase in the frequency of resistance genes (Roush and Tabashnik, 1990; Onstad and Guse, 2008). However, the severity of the diseases transmitted for vector insects and the relatively few insecticides/formulations available for their control (i.e. with high effective insecticides, formulated in appropriate and effective way, and approved by governmental regulatory organisms) determine that the resistance management in disease vectors cannot be nourished of the complete battery of methods and tactics that emerges from evolutionary theory.

In this context, the management of insecticide resistance in triatomines, or *T. infestans* in particular, is determined and conditioned by the status of the insect as disease vector, and by biological (e.g. genetic variation, susceptibility to different insecticides, etc), operational (e.g. availability of insecticides and formulations, regulatory norms, etc), and cultural (structural characteristics of rural human dwellings, receptivity of people to chemical treatment of the dwellings, etc) particularities associated to the control of Chagas diseases vectors. So, some typical recommendations or tactics proposed by expert committees and international organisms to carry out insecticide resistance management programs cannot

implement to Chagas vectors. Measures such as insecticide rotation or mixtures of insecticides are hampered by scarce variety of insecticides and formulations available (see below), or directly the lack of formulated (e.g. mixtures), to the control of Chagas vectors. The use of refuges, or similar strategies, in order to maintain susceptible individuals in the populations is hindered for being disease vectors. Moreover, so far they have not been developed and implemented on a large scale, effective alternatives to spraying of houses with residual insecticides, both within the chemical control and other control strategies (e.g. biological control, behavior modification, etc). The shortage of alternatives is not due to lack of creativity or effort in researches, since there are many original and rigorous works conducted by several laboratories in Latin America that have explored different strategies and techniques in order to improve, supplement or replace the traditional spraying of dwellings with synthetic insecticides, unfortunately none of these strategies have shown the effectiveness and safety profile needed to be established or recommended as routine control measures by national or supranational health organisms. The purpose of this section is not address to these investigations, but rather to briefly present the most relevant techniques that showed promising results at least in field conditions and could be effective to control pyrethroid resistant insects, requirements that allow supposing that through further research and evaluations could eventually be implemented in control programs.

In order to study the triatomicide activity of insecticides, and eventually to find a substitute for pyrethroids, several non-pyrethroid neurotoxic insecticides from different chemical groups with different sites of action were evaluated in susceptible and deltamethrin-resistant *T. infestans*. Topical application of insecticides diluted in acetone was the methodology used in these studies. Fipronil (GABA gated chloride channel blocker) showed high activity against resistant populations from Argentina, but, as was discussed above, *T. infestans* from Bolivia were tolerant/resistant to this insecticide (Toloza et al., 2008; Germano et al., 2010b). Amitraz (octopamine receptor agonist), flubendiamide (ryanodine receptor modulator), ivermectin (chloride channel activator), indoxacarb (voltage-dependent sodium channel blocker) and spinosad (nicotinic acetylcholine receptor allosteric activator) did not show lethal activity in susceptible and resistant populations at the high applied doses (LD_{50} > 200 ng/insect) (Carbajal et al., 2012). Fenitrothion, malathion, and bendiocarb (acetylcholinesterase inhibitors) and imidacloprid (nicotinic acetylcholine receptor agonist) were effective against both populations (Picollo et al., 2005; Santo-Orihuela et al., 2008; Lardeux et al., 2010). Organophosphates (mainly fenitrothion) and bendiocarb are the current alternative to control the resistant foci in Argentina and Bolivia (see below). The high toxic potency of imidacloprid, comparable to fenitrothion, allows further development of adequate formulations both in laboratory and field assays. It is surprising the low toxicity in *T. infestans* of several molecules which exhibit high toxic potency against many pest insects and excellent performance in different programs of chemical control. This reminds the already mentioned tolerance of *T. infestans* to DDT, however the mechanisms of this alleged multiple tolerance are not known yet. Just like the laboratory toxicological studies, field test and field evaluations of medium and long term showed that fenitrothion, malathion and bendiocarb formulations (wettable powder or emulsifiable concentrate) were highly effective to control the resistant foci in Argentina and Bolivia. Particularly, the circa–Castelli focus and the circa–Salvador Mazza focus from Argentina were mainly controlled through the spraying with the fenithrotion or malathion formulation (Zaidemberg, 2012; Gurevitz et al., 2012; Germano et al., 2014), while the bendiocarb formulation was mainly used in Bolivia (Programa Nacional de Chagas, 2009).

Respect to the alternative techniques to the traditional spraying of dwellings, the insecticide treatment of domestic animals which are important feeding source for triaomines was one of the most evaluated alternatives. The strategy is based on the insect intoxication when they fed on vertebrate, either by direct contact with the contaminated skin or by ingesting blood which contains the insecticide, and is focalized mainly in the peridomiciliary area. A number of studies have been carried out on different vertebrates (e.g. goats, dogs, cats, hens, chickens, pigeons) treated with different insecticides (e.g. fipronil, ivermectin, imidacloprid, deltamethirn, cypermethrin, β-cypermethrin, insect grow regulators) via different formulations/application methods (e.g. pour-on, spot-on, collars) under different experimental conditions (e.g. laboratory, semi-field, field) (Gentile et al., 2004; Reithinger et al., 2005, 2006; Gürtler et al., 2009; Amelotti et al., 2009a,b, 2012; Juan et al., 2013; Dadé et al., 2014; Carvajal et al., 2014). Some results are contradictory even with the same insecticide (e.g. fipronil) (Gentile et al., 2004; Gürtler et al., 2009), however, in general the studies showed some degree of effectiveness, although with remarkable variation in residual effect, showing its potential as complementary tool to the traditional spray mainly in highly infested peridomestic environments. The insecticidal paints are other promising alternative tools for the control of triatominae including the resistant foci. The new insecticidal paints are microencapsulated formulations containing organophosphates (diazinon and chlorpyrifos) or pyrethroids (α-cypermethrin and d-alethrin), and an insect growth regulator (pyriproxifen). This paints showed high mortality and long residual activity against *T. infestans* both in treated surfaces and in rural houses from Bolivia (Dias and Jemmio, 2008; Amelotti et al., 2009b; Alarico et al., 2010; Maloney et al., 2013). In the context of biological control, the entomopathogenic fungi *Beauveria bassiana* has received attention since a couple of decades. Several strains were evaluated against several of triatomine species (e.g. *T. infestans*, *T sordida*, *R. prolixus*, *Meccus pallidipennis*) (Luz et al., 1999, 2004; Pedrini et al., 2009; Forlani et al., 2011; Zumaquero-Rios et al., 2014). Results from laboratory and field studies using powder formulation or mineral-oil base formulation, confirm that *B. bassiana* is a promising candidate to control *T. infestans* (Luz et al., 1999; Pedrini et al., 2009). An interesting strategy is the development of an attracting trap containing a powder formulation in order to attract insects to facilitate close contact with *B. bassiana* (i.e. the attraction–infection trap) (Pedrini et al., 2009; Pedrini et al., 2009). However, to improve the effectivity, to stabilize the infectivity, and to integrate this tool in control programs, more investigations on formulation, application and evaluation under field conditions are necessary.

In this context, actually it is only possible to count, considering effectiveness and approval, with pyrethroid insecticides, mainly deltamethrin formulated as suspension concentrate, the organophosphate insecticides fenitrothion as wettable powder and malathion as emulsifiable concentrate, and carbamates insecticides, mainly bendiocarb as wettable powder. Considering toxic potency, toxicological risk, quality of the formulations and receptivity of formulations by residents of endemic areas, the rotation between pyrethroids and organophosphates/carbamates (i.e. between deltamethrin and fenitrothion) is not recommended. Consequently, spraying houses with pyrethroids following the corresponding national protocols is the option to use while not resistant foci commit control field are detected. Then, the toxicological (i.e. following the evolution of resistance ratio) and genetic (i.e. determining the field frequency of resistant alleles) monitoring in the populations subjected to chemical control are necessary actions that allows the detection of resistance in early stages of evolution and change the scenario on time, i.e. to be attentive to the slightest change in susceptibility or frequency of resistant genes or to minimum failures in control and change the insecticide.

Finally, housing improvement, environmental and host management, without replace the chemical control but complementing it and acting all tools in an integrated way (i.e. integrated pest management or IPM), and respecting the variety of cultural practices developed throughout the Americas, are non-renounceable practices necessary for a sustainable vector control strategy (Cecere et al., 2002; Zeledón et al., 2008; Gurevitz et al., 2011; Gorla et al., 2013; Bustamante et al., 2014; Gürtler and Yadon, 2015). The tremendous positive impact that this environmental improvement would have on the health of rural human populations imposes the need to always emphasize their priority.

13. Conclusions

The evolution of insecticide resistance (or tolerance), mainly pyrethroid resistance, in triatomines is a process that negatively affects the progress and goals achieved in the control of these vectors, and consequently in Chagas disease management, by national and regional intergovernmental control programs. The resistance/tolerance imposes a new scenario that challenges to the involved actors (i.e. the population affected by the disease, health workers and professionals, scientists and professionals involved in the design and implementation of programs of control) to change routines and develop new practices adjusted to the specific difficulties that presents the Chagas vector control. The geographical distribution of foci, the heterogeneity between foci, the multiplicity of physiological–biochemical mechanisms, and the different evolutionary scenarios that would explain the different foci, reveal the level of complexity of the phenomenon which determine the need to implement strategies that are suited to such complexity. Toxicological continuous monitoring of population under chemical control, including molecular monitoring for resistant mutations, and studies of the resistance mechanisms and biological processes linked to resistance evolution are of primary importance to understand the phenomenon and to design the appropriate resistance management program. The current alternative to control the pyrethroids resistant insects, the spraying with organophoshpates or carbamates insecticides, sooner or later will be ineffective by the evolution of resistance to those insecticides. In this probable situation, if there are not new effective and reliable tools could not be controlled the new resistant insects, unless the reversion of resistance to pyrethroids had happened. Thus, it is necessary to continue the evaluation of new insecticides and the development of suitable formulations for optimal effectiveness and application, while allowing easy handling and ensure minimal toxicological risk to the operators performing applications. The existence of different toxicological profiles in populations from different areas has high impact because that would imply the need of different insecticides/doses in different areas. So, the determination of baseline of susceptibility to different insecticides in each area would be a previous and necessary study before the implementation of a regional chemical control strategy. However, to design a long-term resistance management program is imperative to develop new techniques and strategies that complement to the spraying of dwellings with insecticides. The entomopathogenic fungi *B. bassiana*, the paints containing insecticides, and the treatment of domestic animals with insecticides are the strategies that should be considered but need to obtain clear results about effectiveness in field, residual effect of product and treatment (i.e. duration of effectiveness of the product and the time taken for re-infestation), and toxicological safety for humans and domestic animals. The improvement of human rural dwelling including perdidomestic structures, which would hinder the colonization by triatomines, is not only the best partner for any control strategy but in itself a control tool; the first tool both historical order and priority. Finally, the susceptibility to insecticides in triatomine has not been evaluated in vast sectors of endemic area for Chagas disease; thus, the toxicological monitoring covering all endemic area, studying the species of epidemiological importance of each region, is a priority that should be promoted and addressed by the national or regional health organisms.

References

Agosin, M., 1985. Role of microsomal oxidations in insecticide degradation. In: Kerkut, G.A., Gilbert, C.I. (Eds.), Comprehensive Insect Physiology, Biochemistry and Pharmacology, vol. 12. Pergamon Press, pp. 529–603.

Alarico, A.G., Romero, N., Hernández, L., Catalá, S., Gorla, D., 2010. Residual effect of a micro-encapsulated formulation of organophosphates and piriproxifen on the mortality of deltamethrin resistant *Triatoma infestans* populations in rural houses of the Bolivian Chaco region. Mem. Inst. Oswaldo Cruz 105, 752–756.

Amelotti, I., Catalá, S.S., Gorla, D.E., 2009a. Response of *Triatoma infestans* to pour-on cypermethrin applied to chickens under laboratory conditions. Mem. Inst. Oswaldo Cruz 104, 481–485.

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

Amelotti, I., Catalá, S.S., Gorla, D.E., 2012. The residual efficacy of a cypermethrin pour-on formulation applied on goats on the mortality and blood intake of *Triatoma infestans*. Mem. Inst. Oswaldo Cruz 107, 1011–1015.

Anonymous, 1957. 7th report. In: Who Technical Reports Series No. 125. World Health Organization Committee on Insecticides.

Bustamante, D.M., de Urioste-Stone, S.M., Cruz, J.G., Pennington, P.M., 2014. Ecological, social and biological risk factors for continued transmission by *Triatoma dimidiata* in Guatemala. PLoS One 9, e104599.

Capriotti, N., Mougabure-Cueto, G., Rivera-Pomar, R., Ons, S., 2014. L925I mutation in the *para*-type sodium channel is associated with pyrethroid resistance in *Triatoma infestans* from the Gran Chaco region. PLoS Negl. Trop. Dis. 8, e2659, http://dx.doi.org/10.1371/journal.pntd.0002659

Cardozo, R.M., Panzera, F., Gentile, A.G., Segura, M.A., Pérez, R., Díaz, R.A., Basombrío, M.A., 2010. Inheritance of resistance to pyrethroids in Triatoma infestans, the main Chagas disease vector in South America. Infect. Genet. Evol. 10, 1174–1178.

Carvajal, G., Mougabure-Cueto, G., Toloza, A.C., 2012. Toxicity of non-pyrethroid insecticides against *Triatoma infestans* (Hemiptera: Reduviidae). Mem. Inst. Oswaldo Cruz 107, 675–679.

Carvajal, C., Picollo, M.I., Toloza, A.C., 2014. Is imidacloprid an effective alternative for controlling pyrethroid-resistant populations of *Triatoma infestans* (Hemiptera: Reduviidae) in the Gran Chaco ecoregion? Mem. Inst. Oswaldo Cruz 109, 761–766.

Cecere, M.C., Gürtler, R.E., Canale, D.M., Chuit, R, Cohen, J.E., 2002. Effects of partial housing improvement and insecticide spraying on the reinfestation dynamics of *Triatoma infestans* in rural northwestern Argentina. Acta Trop. 84, 101–116.

Cecere, M., C, Vazquez-Prokopec, G.M., Gürtler, R.E., Kitron, U., 2004. Spatio-temporal analysis of reinfestation by *Triatoma infestans* (Hemiptera: Reduviidae) following insecticide spraying in a rural community in northwestern argentina. Am. J. Trop. Med. Hyg. 71, 803–810.

Cockburn, J.M., 1972. Laboratory Investigations Bearing on Possible Insecticide Resistance in Triatomid Bugs. WHO/VBC/72.359.

Correa, R.R., de Lima, A.R., da Rocha e Silva, E.O., 1968. Resistencia e susceptibilidade do Triatoma infestans e de outros Triatomineos transmissores da doença de Chagas, ao dieldrin e ao lindane. In: Anais XVII Congresso Brasileiro de Higiene, Salvador, Bahia, pp. 45–46.

Dadé, M.M., Daniele, M.R., Marín, G.H., Silvestrini, M.I., Mestorino, N., 2014. Ivermectin efficacy against *Triatoma infestans* in vivo using Hen model. J. Pharm. Pharmacol. 2, 353–358.

Depickère, S., Buitrago, R., Siñani, E., Baune, M., Monje, M., Lopez, R., Waleckx, E., Chavez, T., Brenière, S.F., 2012. Susceptibility and resistance to deltamethrin of wild and domestic populations of *Triatoma infestans* (Reduviidae: Triatominae) in Bolivia: new discoveries. Mem. Inst. Oswaldo Cruz 107, 1042–1047.

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.

Dias, J.C.P., Jemmio, A., 2008. Sobre uma pintura inseticida para o controle de *Triatoma infestans*, na Bolívia. Rev. Soc. Bras. Med. Trop. 41, 79–81.

Dong, K., 2007. Insect sodium channels and insecticide resistance. Invertebr. Neurosci. 7, 17–30.

Fabro, J., Sterkel, M., Capriotti, N., Mougabure-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.

Feyereisen, R., 1999. Insect P450 enzymes. Annu. Rev. Entomol. 44, 507–533.

Feyereisen, R., 2006. Evolution of insect P450. Biochem. Soc. Trans. 34, 1252–1255.

ffrench-Constant, R.H., Roush, R.T., 1990. Resistance detection and documentation: the relative roles of pesticidal and biochemical assay. In: Roush, R.T.,

Tabashnik, B.E. (Eds.), Pesticide Resistance in Arthropods. Chapman and Hall, New York and London, pp. 4–38.
ffrench-Constant, R.H., 2013. The molecular genetics of insecticide resistance. Genetics 194, 807–815.
Firko, M.J., Hayes, J.L., 1990. Quantitative genetic tools for insecticide resistance risk assessment: estimating the heritability of resistance. J. Econ. Entomol. 83, 647–654.
Forlani, L., Pedrini, N., Juárez, M.P., 2011. Contribution of the horizontal transmission of the entomopathogenic fungus *Beauveria bassiana* to the overall performance of a fungal powder formulation against *Triatoma infestans*. Res. Rep. Trop. Med. 2, 135–140.
Fontán, A., Zerba, E., 1992. Influence of the nutritional state of *Triatoma infestans* over the insecticidal activity of DDT. Comp. Biochem. Physiol. C: Comp. Pharmacol. Toxicol. 101, 589–591.
Fox, I., Bayona, I.G., 1966. Toxicity of DDT, dieldrin, malathion and fenthion to *Rhodnius prolixus* in the laboratory. Bull. World. Health Org. 35, 974–976.
Gascón, J., Albajar, P., Canas, E., Flores, M., Prat, J.G., Herrera, R.N., Lafuente, C.A., Luciardi, H.L., Moncayo, A., Molina, L., Muñoz, J., Puente, S., Sanz, G., Treviño, B., Sergio-Salles, X., 2007. Diagnosis, management and treatment of chronic Chagas' heart disease in areas where *Trypanosoma cruzi* infection is not endemic. Rev. Esp. Cardiol. 60, 285–293.
Gaspe, M.S., Schachter-broide, J., Gurevitz, J.M., Kitron, U., Gürtler, 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.
Gentile, A.G., Sartini, J.L., Campo, M.C., Sánchez, J.F., 2004. Eficacia del Fipronil en el control del ciclo peridomiciliario de *Triatoma infestans* en un área con resistencia a la deltametrina. Cad Saú de Pública 20, 1240–1248.
Germano, M.D., Vassena, C.V., Picollo, M.I., 2010a. Autosomal inheritance of deltamethrin resistance in field populations of *Triatoma infestans* (Heteroptera: Reduviidae) from Argentina. Pest Manage. Sci. 66, 705–708.
Germano, M.D., Acevedo, G.R., Cueto, G.A.M., Toloza, A.C., Vassena, C.V., Picollo, M.I., 2010b. New findings of insecticide resistance in *Triatoma infestans* (Heteroptera: Reduviidae) from the Gran Chaco. J. Med. Entomol. 47, 1077–1081.
Germano, M.D., Santo Orihuela, P., Roca Acevedo, G., Toloza, A.C., Vassena, C., Picollo, M.I., Mougabure 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., Picollo, M.I., Mougabure-Cueto, G., 2013. Microgeographical study of insecticide resistance in *Triatoma infestans* from Argentina. Acta Trop. 128, 561–565.
Germano, M.D., Picollo, M.I., Spillmann, C., Mougabure-Cueto, G., 2014. Fenitrothion: an alternative insecticide for the control of deltamethrin-resistant populations of *Triatoma infestans* in Northern Argentina. Med. Vet. Entomol. 28, 21–25, http://dx.doi.org/10.1111/mve.12014
Germano, M.D., Picollo, M.I., 2015. Reproductive and developmental costs of deltamethrin resistance in the Chagas disease vector *Triatoma infestans*. J. Vector Ecol. 40, 1–7.
Gomez, M.B., Pessoa D'Avila, G.C., Garcia Orellana, A.L., Rojas Cortez, M., Rosa, A.C.L., Noireau, F., Gonçalves Diotaiuti, L., 2014. Susceptibility to deltamethrin of wild and domestic populations of *Triatoma infestans* of the Gran Chaco and the Inter-Andean Valleys of Bolivia. Parasite Vectors 7, 497.
González-Audino, P., Vassena, C., Barrios, S., Zerba, E., Picollo, M.I., 2004. Role of enhanced detoxication in a deltamethrin-resistant population of *Triatoma infestans* (Hemiptera, Reduviidae) from Argentina. Mem. Inst. Oswaldo Cruz 99, 335–339.
Gonzalez-Valdivieso, F.E., Sanchez, B., Diaz, B., Nocerino, F., 1971. Susceptibility of *R. prolixus* to Chlorinated Hydrocarbon Insecticides in Venezuela. WHO/VBC/71.264.
Gorla, D.E., Abrahan, L., Hernández, M.L., Porcasi, X., Hrellac, H.A., Carrizo, H., Catalá, S.S., 2013. New structures for goat corrals to control peridomestic populations of *Triatoma infestans* (Hemiptera: Reduviidae) in the Gran Chaco of Argentina. Mem. Inst. Oswaldo Cruz 108, 352–358.
Guhl, F., Schofield, C.J., 1996. Population genetics and control of Triatominae. Parasitol. Today 12, 169–170.
Gurevitz, J.M., Ceballos, L.A., Gaspe, M.S., Alvarado-Otegui, J.A., Enríquez, G.F., Kitron, U., Gürtler, R.E., 2011. Factors affecting infestation by *Triatoma infestans* in a rural area of the humid Chaco in Argentina: a multi-model inference approach. PLoS Negl. Trop. Dis. 5, e1349, http://dx.doi.org/10.1371/journal.pntd.0001349
Gurevitz, J.M., Gaspe, M.S., Enríquez, G.F., Vassena, C., Alvarado-Otegui, J.A., Provecho, Y., Mougabure-Cueto, G., Picollo, M.I., Kitron, U., Gürtler, R.E., 2012. Unexpected failures to control Chagas disease vector with pyrethroid spraying in Northern Argentina. J. Med. Entomol. 49, 1379–1386.
Gürtler, R.E., 2009. Sustainability of vector control strategies in the Gran Chaco region: current challenges and possible approaches. Mem. Inst. Oswaldo Cruz 104, 52–59.
Gürtler, R.E., Ceballos, L.A., Stariolo, R., Kitron, U., Reithinger, R., 2009. Effects of topical application of fipronil spot-on on dogs against the Chagas disease vector *Triatoma infestans*. Trans. R. Soc. Trop. Med. Hyg. 103, 298–304.
Gürtler, R.E., Yadon, Z.E., 2015. Eco-bio-social research on community-based approaches for Chagas disease vector control in Latin America. Trans. R. Soc. Trop. Med. Hyg. 109, 91–98.
Hewlett, P.S., Plackett, R.L., 1956. The relation between quantal and graded responses to drugs. Biometrics 12, 72–78.
Hewlett, P.S., Plackett, R.L., 1978. The Interpretation of Quantal Responses in Biology. Arnold, London, UK.
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.
Hodgson, E., Levi, P., 1997. A Textbook of Modern Toxicology. Appleton & Lange.
Lardeux, F., Depickère, S., Duchon, S., Chavez, T., 2010. Insecticide resistance of *Triatoma infestans* (Hemiptera, Reduviidae) vector of Chagas disease in Bolivia. Trop. Med. Int. Health 15, 1037–1048.
Juan, L.W., Seccacini, E.A., Zerba, E.N., Canale, D., Alzogaray, R.A., 2013. Triatomicidal effect of new spot-on formulations applied to poultry in semi-field conditions. Parasitol. Res. 112, 155–161, http://dx.doi.org/10.1007/s00436-012-3119-z
Lent, H., Wygodzinsky, P., 1979. Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas disease. Bull. Am. Mus. Nat. Hist. 163, 123–520.
Luz, C., Silva, I., Magalhães, B., Cordeiro, C.T., Tigano, M., 1999. Control of *Triatoma infestans* (Klug) (Reduviidae: Triatominae) with *Beauveria bassiana* (Bals.) Vuill.: preliminary assays on formulation and application in the field. An. Soc. Entomol. Bras. 28, 101–110.
Luz, C., Rocha, L.F.N., Nery, G.V., Magalhães, B.P., Tigano, M.S., 2004. Activity of oil-formulated *Beauveria bassiana* against *Triatoma sordida* in peridomestic areas in Central Brazi,l. Mem. Inst. Oswaldo Cruz 99, 211–218.
Maldonado, M., Rojas de Arias, A., Vera de Bilbao, N., Martínez, J., Schinini, A., Carpinelli de Tomassone, M.M., 2013. Clinical and epidemiological characterization of *Trypanosoma cruzi* infected patients attending a referral center of Chagas disease in Paraguay, preliminary results. Rev. Patol. Trop. 42, 403–416.
Maloney, K.M., Ancca-Juarez, J., Salazar, R., Borrini-Mayori, K., Niemierko, M., Yukich, J.O., Naquira, C., Keating, J.A., Levy, M.Z., 2013. Comparison of insecticidal paint and deltamethrin against *Triatoma infestans* (Hemiptera: Reduviidae) feeding and mortality in simulated natural conditions. J. Vector Ecol. 38, 6–11, http://dx.doi.org/10.1111/j.1948-7134.2013.12003.x
Marcet, P.L., Mora, M.S., Cutrera, A.P., Jones, L., G^{3}rtler, 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.
McKenzie, J.A., 1996. Ecological and Evolutionary Aspects of Insecticide Resistance. Academic Press, Inc., California, USA.
Molina, I., Prat, J., Salvador, F., Treviño, B., Sulleiro, E., Serre, N., Pou, D., Roure, S., Cabezos, J., Valerio, L., Blanco-Grau, A., Sánchez-Montalvá, A., Vidal, X., Pahissa, A., 2014. Randomized trial of posaconazole and benznidazole for chronic Chagas' Disease. New Engl. J. Med. 370, 1899–1908.
Moncayo, A., Silveira, A.C., 2009. Current epidemiological trends for Chagas disease in Latin America and future challenges in epidemiology, surveillance and health policy. Mem. Inst. Oswaldo Cruz 104, 17–30.
Monteiro, F.A., Perez, R., Panzera, F., Dujardin, J.P., Galvao, C., Rocha, D., Noireau, F., Schofield, C., Beard, C.B., 1999. Mitochondrial DNA variation of *Triatoma infestans* populations and its implication on the specific status of *T. melanosoma*. Mem. Inst. Oswaldo Cruz 94 (Suppl. 1), 229–238.
Monteiro, F.A., Escalante, A.A., Beard, C.B., 2001. Molecular tools and triatomine systematics: a public health perspective. Trends Parasitol. 17, 344–347.
Mougabure-Cueto, G., 2004. Caracterización de la resistencia a insecticidas piretroides en *Pediculus humanus capitis* De Geer 1778 (Phthiraptera: Pediculidae): estudio comparativo entre estados embrionarios y post-embrionarios. In: Ph.D. Thesis. University of Buenos Aires, 125 pp.
Ministerio da Saude Brazilian, 2005. Consensus on Chagas disease. Rev. Soc. Bras. Med. Trop. 38 (Suppl. 3), 7–29.
Nelson, M.J., Colmenares, P., 1979. Tropical Application of Insecticides to *Rhodnius prolixus* (Reduviidae: Triatominae) a Chagas' Disease Vector. WHO/VBC/79.737.
Nocerino, F., 1975. Insecticide Susceptibility of *Rhodnius prolixus* and *Triatoma macúlala* in Venezuela. WHO/VBC/75.565.
Noireau, F., 2009. Wild *Triatoma infestans*, a potential threat that needs to be monitored. Mem. Inst. Oswaldo Cruz 104, 60–64.
Obara, M.T., Otrera, V.C.G., Gonçalves, R.G., Santos, J.P., Santatucia, M., da Rosa, J.A., de Almeida, P.S., Barata, J.M.S., 2011. Monitoramento da suscetibilidade de populações de *Triatoma sordida* Stal, 1859 (Hemiptera:Reduviidae) ao inseticida deltametrina, na região Centro-Oeste do Brasil. Rev. Soc. Bras. Med. Trop. 44, 206–212.
Onstad, D.W., Guse, C.A., 2008. Concepts and complexities of population genetics. In: Onstad, D.W. (Ed.), Insect Resistance Management. Academic Press, pp. 69–88.
Oppenoorth, F.J., 1985. Biochemistry and genetics of insecticide resistance. In: Kerkut, G.A., Gilbert, C.I. (Eds.), Comprehensive Insect Physiology, Biochemistry and Pharmacology, vol 12. Pergamon Press, pp. 731–773.
Panzera, F., Ferreiro, M.J., Pita, S., Calleros, L., Pérez, R., Basmadjián, Y., Guevara, Y., Brenière, S.F., Panzera, Y., 2014. Evolutionary and dispersal history of *Triatoma infestans*, main vector of Chagas disease, by chromosomal markers. Infect. Genet. Evol. 27, 105–113.
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, http://dx.doi.org/10.1371/journal.pntd.0000434
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.
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.
Pessoa, G.C.D., Dias, L.S., Diotaiuti, L., 1859. Deltamethrin pyrethroid susceptibility characterization of *Triatoma sordida* Stål, 1859 (Hemiptera: Reduviidae) populations in the northern region of Minas Gerais, Brazil. Rev. Soc. Bras. Med. Trop. 47, 426–429.
Piccinali, R.V., Marcet, P.L., Noireau, F., Kitron, U., Gürtler, R.E., Dotson, E.M., 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., Wood, E.J., Zerba, E.N., de Licastro, S.A., Rúveda, M.A., 1976. Mútodos de laboratorio para medir la toxicidad de insecticidas en *Triatoma infestans*, Klug. Acta Bioquimica Clénica Latinoamericana 10, 67–70.
Picollo de Villar, M.I., Wood, E.J., Zerba, E., de Licastro, A.S., Casabe, N., 1980. Cholinesterases and eserine-resistant esterases in the developing embryo of *Triatoma infestans* and its role as targets for inhibition in the ovicide action of parathion. Comp. Biochem. Physiol. 67, 55–59.
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.
Pizarro, J.C., Gilligan, L.M., Stevens, L., 2008. Microsatellites reveal a high population structure in *Triatoma infestans* from Chuquisaca, Bolivia. PLoS Negl. Trop. Dis. 2, e202.
Programa Nacional de Chagas, 2009. Anuario 2008. Programa Nacional de Chagas, Ministerio de Salud y Deportes, Estado Plurinacional de Bolivia, 36pp.
Reithinger, R., Ceballos, L., Stariolo, R., Davies, C.R., Gürtler, R.E., 2005. Chagas disease control: deltamethrin-treated collars reduce Triatoma infestans feeding success on dogs. Trans. R. Soc. Trop. Med. Hyg. 99, 502–508.
Reithinger, R., Ceballos, L.A., Stariolo, R., Davies, C.R., Gürtler, R.E., 2006. Extinction of experimental *Triatoma infestans* populations following continuous exposure to dogs wearing deltamethrin treated collars. Am. J. Trop. Med. Hyg. 74, 766–771.
Reyes, M., Angulo, V.M., Sandoval, C.M., 2007. Efecto tóxico de β-cipermetrina, deltametrina y fenitrotión en cepas de *Triatoma dimidiata* (Latreille, 1811) y *Triatoma maculata* (Erichson, 1848) (Hemiptera, Reduviidae). Biomédica 27, 75–82.
Richer, W., Kengne, P., Cortez, M.R., Perrineau, M.M., Cohuet, A., Fontenille, D., Noireau, F., 2007. Active dispersal by wild *Triatoma infestans* in the Bolivian Andes. Trop. Med. Int. Health 12, 759–764.
Roca-Acevedo, G., Mougabure Cueto, G., Germano, M., Santo Orihuela, P., Rojas Cortez, M., Noireau, F., Picollo, M.I., Vassena, C., 2011. Susceptibility of sylvatic *Triatoma infestans* from andeans valleys of Bolivia to deltamethrin and fipronil. J. Med. Entomol. 48, 828–835.
Roca-Acevedo, G., Picollo, M.I., Santo-Orihuela, P., 2013. Expression of insecticide resistance in immature life stages of *Triatoma infestans* (Hemiptera: Reduviidae). J. Med. Entomol. 50, 816–818.
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., Rolón, M., Vega, M.C., Gómez, A., Román, F., Sánchez, H., Acosta, C., Villalba, C., Cecere, C., Marcet, P., Dotson, E., 2011. Molecular epidemiology and ecosystem approach of the reinfestation process by *Triatoma infestans* in rural communities of the Paraguayan Chaco. Biomódica 31, 153–156.
Roush, R.T., McKenzie, J.A., 1987. Ecological genetics of insecticide and acaricide resistance. Annu. Rev. Entomol. 32, 361–380.
Roush, R.T., Daly, J.C., 1990. The role of population genetics in resistance research and management. In: Roush, R.T., Tabashnik, B.E. (Eds.), Pesticide Resistance in Arthropods. Chapman and Hall, New York and London, pp. 97–152.
Roush, R.T., Tabashnik, B.E., 1990. Pesticide Resistance in Arthropods. Chapman and Hall, New York and London.
Salvatella, R., Irabedra, P., Castellanos, L.G., 2014. Interruption of vector transmission by native vectors and the art of the possible. Mem. Inst. Oswaldo Cruz 109, 122–130.
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.
Santo-Orihuela, P.L., Carvajal, G., Picollo, M.I., Vassena, C.V., 2013. Toxicological and biochemical analysis of the susceptibility of sylvatic *Triatoma infestans* from the Andean Valley of Bolivia to organophosphate insecticide. Mem. Inst. Oswaldo Cruz 108, 790–795.
Schofield, C.J., 1994. Triatominae: Biología y Control. Eurocommunica Publications, West Sussex, United Kingdom.
Schofield, C.J., Jannin, J., Salvatella, R., 2006. The future of Chagas disease control. Trends Parasitol. 21, 583–588.
Schofield, C.J., Galvao, C., 2009. Classification, evolution, and species groups within the Triatominae. Acta Trop. 110, 88–100.
Soderlund, D., 1995. Mode of action of pyrethrins and pyrethroids. In: Casida, J.E., Quistad, G.B. (Eds.), Pyrethrum Flowers, Production, Chemistry, Toxicology, and Uses. Oxford university press, Inc., Oxford, pp. 217–232.
Soderlund, D.M., Knipple, D.C., 2003. The molecular biology of knockdown resistance to pyrethroid insecticides. Insect Biochem. Mol. Biol. 33, 563–577.
Sonoda, I.V., Pessoa, G.C.D., Rojas Cortez, M., Dias, J.C.P., Romanha, A.J., Diotaiuti, L., 2009. Susceptibility of *Triatoma infestans* to deltamethrin in Rio Grande do Sul Brazil. Mem. Inst. Oswaldo Cruz 104, 668–670.
Sonoda, I.V., Dias, L.T., Bezerra, C.M., Dias, J.C.P., Romanha, A.J., Diotaiuti, L., 2010. Susceptibility of *Triatoma brasiliensis* from state of Ceará, Northeastern Brazil, to the pyrethroid deltamethrin. Mem. Inst. Oswaldo Cruz 105, 348–352.
Soto Vivas, A., Molina de Fernández, D., 2001. Toxicidad de cinco insecticidas en una cepa de laboratorio de *Rhodnius prolixus* Stal 1859 (Hemiptera, Reduviidae) de Venezuela. Entomotropica 16, 187–190.
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.
Torres-Pérez, F., Acuna-Retamar, M., Cook, J.A., Bacigalupo, A., García, A., Cattan, P.E., 2011. Statistical phylogeography of Chagas disease vector Triatoma infestans: testing biogeographic hypotheses of dispersal. Infect. Genet. Evol. 11, 167–174.
Vassena, C., Picollo, M.I., Zerba, E., 2000. Insecticide resistance in Brazilian *Triatoma infestans* and Venezuelan *Rhodnius prolixus*. Med. Vet. Entomol. 14, 51–55.
WHO, 1994. Protocolo de evaluación de efecto insecticida sobre triatominos. Acta Toxicológica Argentina 2, 29–32.
Yon, C., Balta, R.L., García, N.A., Troyes, M.A., Cumpa, H.O., Valdivia, A., 2004. Susceptibilidad y Resistencia de *Triatoma infestans* y *Panstrongylus herreri* a los insecticidas piretroides, perú 2001. Rev. Med. Exp. Salud Píblica 21, 179–182.
Zaidemberg, M., 2012. Evolución de la infestación en un área de triatominos resistentes a piretroides Salvador Mazza Salta Argentina. Revista Argentina de Zoonosis y Enfermedades Infecciosas Emergentes VII, 3–13.
Zeledón, R., Rojas, J.C., Urbina, A., Cordero, M., Gamboa, S.H., Lorosa, E.S., Alfaro, S., 2008. Ecological control of *Triatoma dimidiata* (Latreille, 1811): five years after a Costa Rican pilot project. Mem. Inst. Oswaldo Cruz 103, 619–621.
Zerba, E.N. 1999a. Past and present of Chagas vector control and future needs. Available from: whqlibdoc.who.int/hq/1999/WHO_CDS_WHOPES_GCDPP_99.1
Zerba, E., 1999b. Susceptibility and resistance to insecticides of Chagas disease vectors. Medicina 59, 41–46.
Zumaquero-Rios, J.L., López-Tlacomulco, J.J., Rojas, G.R., Sansinenea, E., 2014. Lethal effects of a Mexican *Beauveria bassiana* (Balsamo) strain against *Meccus pallidipennis* (Stal). Braz. J. Microbiol. 45, 551–557.