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REVIEW ARTICLE

New Challenges in Insecticide Resistance

What happens when the insecticide does not kill? A review of sublethal toxicology and insecticide resistance in triatomines

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Abstract

Chagas disease is considered one of the most important human parasitosis in the United States. This disease is mainly transmitted by insects of the subfamily Triatominae. The chemical vector control is the main tool for reducing the incidence of the disease. However, the presence of triatomines after pyrethroids spraying has been reported in some regions, as in the case of *Triatoma infestans* in Argentina and Bolivia. The presence of insects can be explained by the colonization from neighbouring areas, the reduction of insecticide dose to sublethal levels due to environmental factors, and/or by the evolution of insecticide resistance. In the last two scenarios, a proportion of the insects is not killed by insecticide and gives rise to residual populations. This article focuses on the toxicological processes associated with these scenarios in triatomines. Sublethal doses may have different effects on insect biology, that is, sublethal effects, which may contribute to the control. In addition, for insect disease vectors, sublethal doses could have negative effects on disease transmission. The study of sublethal effects in triatomines has focused primarily on the sequence of symptoms associated with nervous intoxication. However, the effects of sublethal doses on excretion, reproduction and morphology have also been studied. *Rhodnius prolixus* and *T. infestans* and pyrethroids insecticides were the triatomine species and insecticides, respectively, mainly studied. Insecticide resistance is an evolutionary phenomenon in which the insecticide acts as a selective force, concentrating on the insect population's pre-existing traits that confer resistance. This leads to a reduction in the susceptibility to the insecticide, which was previously effective in controlling this species. The evolution of resistance in triatomines received little attention before the 2000s, but after the detection of the first focus of resistance associated with chemical control failures in *T. infestans* from Argentina in 2002, the study of resistance increased remarkably. A significant number of works have studied the geographical distribution, the resistance mechanisms, the biological modifications associated with resistance, the environmental

influences and the genetic of *T. infestans* resistant to pyrethroid insecticides. Currently, studies of insecticide resistance are gradually being extended to other areas and other species. The aim of this article was to review the knowledge on both phenomena (sublethal effects and insecticide resistance) in triatomines. For a better understanding of this article, some concepts and processes related to insect-insecticide interactions, individual and population toxicology and evolutionary biology are briefly reviewed. Finally, possible future lines of research in triatomine toxicology are discussed.

KEYWORDS
Chagas vectors, chemical control, insecticide resistance, pyrethroids, *Rhodnius prolixus*, sublethal effects, *Triatoma infestans*

INTRODUCTION

American trypanosomiasis, or Chagas disease, is considered one of the most important human parasitosis in the Americas. It is endemic in the American continent but is now an emerging health problem in Europe, Australia and Canada (WHO, 2023). It is currently estimated that between 6 and 7 million people in 44 countries are infected with the parasite, and 100 million are at risk of becoming infected (WHO, 2023). The disease is caused by the trypanosomatid protist *Trypanosoma cruzi*. Transmission of the parasite can occur through transfusion or ingestion of contaminated blood, from mother to child during pregnancy or childbirth, through laboratory accidents or through the faeces of triatomine insects. The transmission mechanism that involves insects, that is, the vector route, is one of the main routes causing 80% of the cases in Latin America (Pérez-Molina & Molina, 2018). This transmission way occurs naturally when faeces or urine of infected insects come into contact with mucous membranes or damaged areas of skin, and this occurs mainly when insects excrete/defecate while feeding on the mammalian host (Lent & Wygodzinsky, 1979).

As it is a disease for which there is no vaccine and no effective treatment for chronic forms, chemical vector control is the main tool used to reduce its incidence. Chemical control is based on the spraying of infested human dwellings homes and attached buildings (peridomestic structures) with residual insecticidal formulations (Salvatella et al., 2014). Currently, pyrethroids are the main insecticides used by Chagas control programmes in endemic countries. Its use, within the framework of regional intergovernmental control programmes, has led to the reduction of the distribution of triatomines and the consequent interruption/reduction of vector transmission of the disease in several zones of the endemic area (Salvatella et al., 2014; Schofield et al., 2006). However, in recent decades, the presence of triatomines after pyrethroids spraying has been reported in some zones, as in the case of *Triatoma infestans* in Argentina and Bolivia (Mougabure-Cueto & Picollo, 2015). Assuming that the control actions have been carried out correctly, the presence of insects can be explained by the colonization of insects from neighbouring areas (reinfestation), by rapid reduction of the insecticide dose to sublethal levels due to environmental degradation, and/or by the evolution of insecticide resistance in treated populations. In the last two scenarios, a proportion of the insects present prior to the insecticide application are not killed and give rise to residual populations.

In any chemical control strategy for an insect pest, an insecticide formulation is applied with a dose of insecticide that is lethal to most individuals of the species. However, the bioavailable amount of the applied product can be reduced by environmental factors such as sunlight, heat, rain and so forth. This means that the applied dose of insecticide may decrease over time to levels below the recommended application dose. In this scenario, individuals of the target species that come into contact with the applied insecticide will be exposed to doses that do not kill them, that is, sublethal doses. Sublethal doses can have different effects on the biology of insects (see below), that is, sublethal effects. Sublethal exposure is generally considered to be undesirable because control efforts have not produced the expected result. However, lethality may not be the only effect of an insecticide that could contribute to the control of a pest. For example, sublethal doses could negatively affect the population dynamics of the pest and contribute to its density being below damage thresholds (Stark & Banks, 2003). In addition, for insect disease vectors, sublethal doses could have negative effects on disease transmission by affecting the vector competence (Cohnstaedt & Allan, 2011). Thus, after insecticide application, insects that have not died and are in contact with the insecticide during its degradation are exposed to doses that may contribute to control. This situation typically occurs in the peridomestic structures of the rural human dwelling that host high densities of triatomines in association with domestic animals. There, the peculiarities of the construction and materials of these structures make it difficult to apply the insecticide uniformly and expose it to environmental degradation (Cecere et al., 2004).

Insecticide resistance is an evolutionary process in which the insecticide acts as a selection factor that concentrates, in the insect population, the genotypes that confer resistance to the toxicant. This selection leads to a reduction in the toxicological response of the population (i.e., the proportion of dead individuals) to the insecticide used, which was previously effective in controlling the pest (McKenzie, 1996). Obviously, the insecticide resistance evolution is undesirable since, like sublethal exposure, the control did not generate the expected result. Given early evidence of low genetic variation in triatomine populations (which was later shown not to be necessarily true) and their long ontogenetic development, it was long believed

that triatomine populations had a low probability of evolving resistance to insecticides (Guhl & Schofield, 1996; Marcet et al., 2008; Monteiro et al., 2001; Pérez de Rosas et al., 2007; Pizarro et al., 2008). The logical consequence of this assumption was to consider that, where chemical control of Chagas vectors was successful, it would be successful for a long time. However, resistance to insecticides evolved in triatomines, which was documented in the 70s for organochlorines and since the 90s for pyrethroids with high levels of resistance associated with ineffective control in the field (Mougabure-Cueto & Picollo, 2015; Pessoa et al., 2015).

The aim of this article is to review, for Chagas vector, the knowledge about the toxicological processes associated with the situations where the insecticide does not kill all the insects present; that is, exposure to sublethal doses and evolution of insecticide resistance. For this, first, a brief description of triatomines, its epidemiological significance and its control are presented. Then, toxicological concepts that allow framing the sublethal effects and resistance evolution are described. Finally, the two issues that are the focus of this review are developed: the effects of sublethal doses of insecticides and insecticide resistance evolution. For each, the basic theoretical concepts and the current knowledge in triatomines are presented. For a review of insecticide resistance in other vectors, we recommend comprehensive works such as Moyes et al. (2017) for *Aedes* vectors and Strode et al. (2014) for anopheline mosquitoes. Finally, we hope that this article will encourage the development of further reviews on these topics in other disease vectors in addition to triatomines and mosquitoes.

CHAGAS VECTORS AND THEIR CONTROL

All vectors of *T. cruzi* are insects belonging to the subfamily Triatominae of the family Reduviidae (Hemiptera: Heteroptera). The subfamily contains about 150 species classified into five tribes (Triatomini, Rhodniini, Alberproseniini, Bolboderini and Cavernicolini) and 15–17 genera (Lent & Wygodzinsky, 1979, Schofield & Galvão, 2009). They are distributed mainly in tropical and subtropical areas of America, from the northwest of the United States to southern Argentina (from latitude 42° N to 46° S), with the exception of *Triatoma rubrosfasciata*, which has a cosmopolitan distribution, six species of the genus Lynchosteous, which is distributed in several localities in India, and seven species of the rubrofasciata complex found in eastern Asia (Galvão et al., 2003; Lent & Wygodzinsky, 1979; Patterson et al., 2001).

Triatomines are hemimetabolous insects and their ontogenetic development includes egg, five immature stages and adult. Both nymphs and adults are obligate hematophages (Lent & Wygodzinsky, 1979). All species are probably capable of transmitting *T. cuzi* although relatively few are of epidemiological significance as vectors, for example, less than 20 species have been involved in the transmission to humans (Gürtler et al., 2021; Schofield, 1994). The relative importance of the triatomine species as vectors depends on behavioural and biological features, in particular the adaptation to domestic and peridomestic structures, degree of anthropophily and host preference, feeding and defecation times, and dispersal capabilities (Lent & 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 *T. infestans*, occurring in a wide area of southern of South America, *Rhodnius prolixus* in Colombia and Venezuela and *T. dimidiata* in Ecuador, Colombia and Venezuela, as well as in several Central American countries and Mexico (Gorla & Noireau, 2017; Lent & Wygodzinsky, 1979).

Chemical control of triatomines using synthetic insecticides began in the mid-20th century, stimulated by the success of the organochlorine dichlorodiphenyltrichloroethane (DDT) in controlling Malaria vectors. DDT was quickly discarded due to its low effectiveness against triatomines (Zerba, 2021). Metabolism mediated by DDT-dehydrochlorinase and DDT-hydroxylase and delayed penetration were described as mechanisms of the toxicological tolerance to DDT in *T. infestans* (Agosin, 1985; Fontán & Zerba, 1992; Zerba, 1999). After, in the late 1940s, other organochlorine insecticides were introduced in the Chagas vector control. The GAMA isomer of hexachlorocyclohexane (i.e., lindane) was the first synthetic insecticide used in the field to control triatomines and was used mainly in Argentina and Brazil. Dieldrin was introduced in Venezuela for the control of *R. prolixus*. Lindane and dieldrin were used until the late 1970s (Dias et al., 2002; Schofield, 1994; Zerba, 1999). The potential toxicological and ecotoxicological risks of organochlorine insecticides promoted their substitution by the less persistent carbamates and organophosphorus insecticides (Zerba, 1999). Field assays with propoxur were performed in Chile in the early 1970s and it became the first carbamate used to control triatomines. The phosphonothioates malathion and fenitrothion were introduced in 1975 into Chagas vector control programmes (Zerba, 1999). These insecticides have ovicidal activity and higher initial efficacy than lindane, but they have the disadvantage of leaving an unpleasant smell after application, which makes people reluctant to spray indoors (Picollo de Villar et al., 1980; Schofield, 1994).

The panorama of the chemical control of pest insects has been revolutionized by the development of the pyrethroid insecticides. Synthetic derivatives of natural pyrethrins found in the flowers of chrysanthemums (Casida & Quistad, 1995), pyrethroids quickly and successfully replaced their predecessors, mainly because of their high insecticidal activity and low mammalicidal activity, as well as their rapid environmental degradation (Zerba, 1999). The use of pyrethroids in the control of Chagas vectors has as a starting point the studies carried out in 1978 in Argentina that demonstrated the great toxic activity of deltamethrin on *T. infestans* (Zerba, 2021). Deltamethrin and cypermethrin have been successfully used in the field in Argentina and Brazil since 80s. Since the 1990s, the pyrethroids used were restricted to alpha-cyano-substituted pyrethroids such as deltamethrin, cypermethrin, beta-cypermethrin, lambda-cyhalothrin and beta-cyfluthrin (Zerba, 2021). Pyrethroids are currently the insecticide of first choice in Chagas disease control programmes, and would probably still be if it were not for the evolution of resistance to those insecticides in insect vector populations.

Regardless of the insecticide used, the triatomine control programmes in endemic countries have focused on the spraying of insecticide formulations on houses and household annexes buildings.

Wettable powders, emulsifiable concentrates and suspension concentrates were the main formulations used (Zerba, 1999). The reduction of Chagas disease achieved great progress through the deployment of large-scale control actions enhanced by the creation of intergovernmental initiatives coordinated by the Pan American Health Organization: Southern Cone in 1991 (Argentina, Brazil, Bolivia, Chile, Paraguay and Uruguay), Andean Pact in 1997 (Colombia, Ecuador, Peru y Venezuela), Central American countries in 1997 (Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, Panamá, and, later, Mexico) and Amazon Initiative in 2004 (Bolivia, Brazil, Colombia, Ecuador, French Guyana, Perú, Suriname and Venezuela) (Gürtler & Cecere, 2021). These control programmes reduced the geographical 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), *R. prolixus* in Central America (Costa Rica, El Salvador, Honduras, Guatemala y Nicaragua), and decreased the infestations with *T. dimidiata* (Gorla & Hashimoto, 2017; Gürtler et al., 2009; Salvatella et al., 2014; Schofield et al., 2006).

BASIC TOXICOLOGICAL CONCEPTS

The insect-insecticide interaction and the doses

The interaction between an insect and an insecticide begins with the exposure to the toxicant and involves a set of biochemical and physiological processes that determine the degree to which the toxicant can damage the organism (i.e., the toxicity) or, equally, the degree to which the organism is damaged by the toxicant (i.e., the individual susceptibility) (Mougabure-Cueto & Sfara, 2016). These processes generally are grouped into the toxicokinetic and toxicodynamic phases. The toxicokinetic phase includes the processes of absorption, distribution, biotransformation or metabolism, and excretion, and therefore determines the concentration of the insecticide in the target tissue. The toxicodynamic phase comprises the molecular interaction between the insecticide and the molecular target, that is, the primary site of action or receptor, and initiates a sequence of biochemical processes that triggers the intoxication process and results in the toxic effect observed (Hodgson & Levi, 1997; Yu, 2015). If the dose of insecticide is high enough, the observed effect is the death of the insect and this is a lethal dose. If the dose is lower, the insect does not die and it is likely to show some change in a biological process, then this is a sublethal dose (de França et al., 2017; Desneux et al., 2007; Stark & Banken, 1999).

The individual susceptibility and its population distribution

Individual susceptibility to an insecticide is the expression of multiple biochemical and physiological processes that occur during the toxicokinetic and toxicodynamic phases of the insect-insecticide interaction (see above), each determined by genetic and environmental factors. As any other phenotypic character, the individual susceptibility varies and is randomly distributed among the individuals of a population due to variation in the genetic and/or environmental determinants (Futuyma, 1998; Hewlett & Plackett, 1978). Specifically for toxicological susceptibility, genetic and environmental variation may generate individual differences in toxicokinetic processes (e.g., rate of biotransformation) and/or toxicodynamic parameters (e.g., affinity of action site) generating population variation (Mougabure-Cueto & Sfara, 2016). Variation in susceptibility implies that different individuals in the same population require different doses to show the same effect (e.g., death). This variation is evidenced in the dose–response (D-R) curves that emerge from toxicological bioassays with quantal response. Thus, when groups of individuals are treated with a range of doses of toxicant and the proportion of responding individuals are registered (i.e., toxicological bioassay), a variation in the proportion of the response will occur as a function of the dose (i.e., D-R curve). Higher doses are expected to produce a greater response and a symmetric sigmoid curve is usually obtained when the proportion of responding individuals (y axis) is plotted against the log-dose (x axis); that is, the cumulative normal distribution of susceptibility (Hewlett & Plackett, 1978; Robertson et al., 2007). This susceptibility distribution determines the population effect of the doses of an insecticide applied. If the applied dose is higher than the maximum dose of the susceptibility distribution of the exposed population, this dose will kill 100% of the individuals. Obviously, the doses recommended for the use of insecticides in the field meet this requirement. On the other hand, if the dose applied is within the susceptibility distribution of the exposed population, this dose will be lethal to the most susceptible individuals and sublethal to the less susceptible individuals of the distribution that survive.

EFFECTS OF SUBLETHAL DOSES

Sublethal doses can cause various effects on the physiology and development of exposed individuals and on the properties of populations (de França et al., 2017; Desneux et al., 2007; Stark & Banken, 1999).

Sublethal effects at the individual level

These effects include the wide range of biological changes that occur in individuals as a result of their exposure to levels of insecticide that do not kill them. Some changes may be the inevitable or necessary result of the presence of the insecticide and its interaction with the biochemistry and physiology of the insect, other changes may be traits that have evolved through natural selection because they allow the toxic effect of insecticides to be reduced. In the first case, the insecticide-target interaction, that is, the primary site of action (e.g., the voltage-gated sodium channel for pyrethroids), triggers the

intoxication process which is manifested as a sequence of symptoms associated with the progressive deterioration of the affected physiological system (e.g., the nervous system for pyrethroids) (Soderlund, 1995; Yu, 2015). If the dose is sublethal, the final event in this sequence is not death but a preceding symptom (e.g., hyperactivity for pyrethroids) which in turn may affect other processes, such as flight or walking (Alzogaray & Zerba, 2001a). Furthermore, insecticides have secondary sites of action (e.g., voltage-gated calcium and chloride channels, GABA receptors for pyrethroids) (Soderlund et al., 2002). If the dose is sublethal, physiological alterations may also be triggered by the interaction of the insecticide with the secondary sites of action. Finally, at sublethal doses, the insecticide may modify the physiology of the organism due to toxicokinetic processes, which is explained by two mechanisms: (1) the biochemical and physiological systems involved in toxicokinetic processes will not be available for other processes in which they also act (e.g., the enzymes that metabolize the insecticide will not be available for the synthesis of a hormone) and (2) toxicokinetic processes use energy that will not be available for other physiological processes. The alterations just described are the ones that have received the most attention in the literature, so the notion of sublethal effects has been closely associated with this type of effect at the individual level (Desneux et al., 2007; Haynes, 1988; Lee, 2000; Müller, 2018). On the other hand, the insecticides can act as environmental factors that exert selective pressure due to their toxic action, thus promoting a process of natural selection that results in adaptation (Futuyma, 1998; McKenzie, 1996; Mougabure-Cueto & Picollo, 2015). In the context of the toxic action of a molecule, an adaptation would be a phenotypic character that minimizes the damage caused by the toxicant. However, this adaptive character can be considered as a sublethal effect if it acquires its functional conformation and acts only after the insect is exposed to the insecticide; that is, the insecticide promotes or induces the process that minimizes its toxic effect. These effects would include the toxicokinetic processes that begin to operate in the presence of the toxicant, such as the detoxifying enzymes induced by insecticides (Agosin, 1985; Feyereisen, 2006; Yu, 2015).

Sublethal dose effects at the population level

The individual level is essential to define doses as lethal or sublethal. Lethal doses kill the individual and sublethal doses do not. However, sublethal doses can also affect population properties such as population dynamics or structure. For example, sublethal doses of insecticides may affect individual survival and fecundity of individuals and thus the intrinsic rate of population growth (Ahmadi, 1983; Stark & Wennergren, 1995). Although the latter is derived from effects at the individual level, this effect can be located at the population level because the insecticide effectively modifies an emergent property at the population level. These effects are mostly addressed by demographic toxicology, an ecotoxicological approach that incorporates life table parameters in the context of toxicology and provides a measure of the effect on population growth rate (Lashkari et al., 2007; Stark & Banks, 2003; Stark & Wennergren, 1995). On the other hand, the sublethal doses of an insecticide could modify the distribution of phenotypes and genotypes in the population through a process of natural selection. This occurs because individual toxicological susceptibility varies in the population (see the section The individual susceptibility and its population distribution) and the dose could be sublethal for some individuals and lethal for others. Since individual susceptibility and its variation is partly genetically determined (see the section The individual susceptibility and its population distribution), the differential toxic action of the insecticide will, over generations, increase the proportion of individuals with low susceptibility in the population. This is a way of explaining the evolution of insecticide resistance (see the section Resistance to insecticide) (McKenzie, 1996; Mougabure-Cueto & Picollo, 2021; Mougabure-Cueto & Sfara, 2016), and allows insecticide resistance to be interpreted as a sublethal effect at the population level. In addition, genes that determine low individual susceptibility to insecticide may have pleiotropic effects on biological processes other than toxicological processes (Kliot & Ghanim, 2012). In this way, the individuals selected by the sublethal doses would not only express the low susceptibility but could also express a particular state of some other phenotypic characteristic, which will increase their frequency in subsequent generations (Mougabure-Cueto & Picollo, 2015).

RESISTANCE TO INSECTICIDE

Insecticide resistance is a microevolutionary process in which the main factor altering the genetic structure of a population is based on the natural selection caused by the toxic effects of an insecticide on individuals with varying degrees of susceptibility to the insecticide (Roush & McKenzie, 1987). Considering what has been seen in previous paragraphs (see the sections The individual susceptibility and its population distribution and Sublethal dose effects at the population level), this process can be easily understood as follows. Individuals in a population have naturally different toxicological susceptibilities to insecticides, so a dose of an insecticide may kill some individuals and leave others alive. As the variation in individual toxicological susceptibility is, in part, genetically determined, surviving individuals (the least susceptible or resistant) will produce descendance with low susceptibility. Thus, repetition of the insecticide application-differential survival-reproduction sequence will result in an increase in the population proportion of individuals genetically with low susceptibility, generating a population resistant to the applied insecticide. In terms of the D-R curve (see the section The individual susceptibility and its population distribution), the consequence of this selective process is a shift of the D-R curve towards higher doses, that is, to the right (Mougabure-Cueto & Picollo, 2021). In terms of pest control, insecticide resistance is a problem as it renders a successful chemical control strategy inefficient.

Individual resistance is a consequence of genetic alterations that qualitatively or quantitatively affect the toxicokinetic/toxicodynamic processes that occur during insecticide-insect interaction and, therefore, modify the individual susceptibility to the toxicant. Specifically, in the resistant individuals those alterations reduce the susceptibility to a degree that the individuals survive the dose used for control in

the field. So, 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 & Roush, 1990; McKenzie, 1996). At the same time, the resistant mutation can have effects over other physiological processes with possible consequences at ecological and evolutionary levels (Kliot & Ghanim, 2012). This can occur through the modification of processes in which the gene that carries the resistant mutation is also involved or through the energetic cost of the resistant phenotype that decreases the energy available for other processes (Lobbia et al., 2018). These pleiotropic effects can have negative, positive or neutral consequences on the adaptation of resistant insects to the natural environment (i.e., without insecticide) in relation to susceptible insects (Mougabure-Cueto & Picollo, 2015; Rivero et al., 2010). The first case is defined as biological or adaptive costs of resistance, it is the most likely to occur and explain the low frequency of resistant individuals in natural populations (Kliot & Ghanim, 2012). However, if the consequence is positive, the modification constitutes an adaptation and determines a high frequency of individuals with low susceptibility in natural populations (i.e., tolerant populations) (Lobbia et al., 2018). This difference has a great impact on resistance management strategies since, if the insecticide is stopped, the frequency of resistant insects will decrease if they have costs, while it will not decrease if the effect is adaptively positive (Mougabure-Cueto & Picollo, 2015). In addition, in insects that act as disease vectors, the pleiotropic effects can have direct consequences on the transmission of the disease by modifying the insect's vectorial capacity (Rivero et al., 2010).

The level of resistance of a population depends on the proportion of resistant individuals and the intensity of individual resistance, which depends on the resistance mechanisms (see above) (ffrench-Constant & Roush, 1990). This level is quantified by comparing the D-R curves (see the section The individual susceptibility and its population distribution) of a resistant population with a susceptible reference population. The lethal dose 50 (LD_{50}) statistics is typically obtained from the D-R curves, which is the dose that kills 50% of the exposed individuals. Resistant populations show D-R curves and LD_{50}s shifted towards higher doses in relation to the susceptible population. The level of resistance measures this relative displacement and the simplest calculation is the ratio LD_{50} of the resistant population/LD50 of the susceptible population, that is, the resistance ratio (RR). It is commonly said that by obtaining the D-R curve and the LD_{50} of a population to an insecticide, the susceptibility of that population to the insecticide is determined.

SUBLETHAL EFFECT OF INSECTICIDES IN TRIATOMINES

In the context of chemical control of triatomines, the exposure of insects to sublethal doses of insecticides is a very likely scenario, especially in the peridomicile of human dwelling. Peridomiciliary structures house domestic animals that promote the development of high densities of triatomines. For this reason, insecticidal formulations are applied both in the domicile and in the peridomiciliary structures. The complexity in the construction and the type of materials of these structures prevent a uniform application of insecticides while leaving them exposed to environmental degradation, determining a variation in doses in space and time. Thus, the surviving insects that live there, or those that arrive from other houses or from the wild environment, will be exposed for a time to sublethal doses of the applied insecticide.

The first reports on the sublethal effects of insecticides on triatomines came from a series of studies carried out at the University of Chile that focused on the effects of DDT on the biochemical systems of *T. infestans*. In these studies, third instar nymphs, fifth instar nymphs and/or male adults were exposed by topical application to acetone solutions of DDT (mainly 300 μg/insect), and the effects were recorded on the same exposed instars.

Thus, Agosin et al. (1961) studied the effects on glycolysis and glycolytic and pentose phosphate enzymes and showed that DDT inhibited the pyruvate production in males and third instar nymphs (22 h of exposure), the alpha-glycerophosphate production in males (48 h of exposure), the glycolytic and pentose phosphate enzymes in males, and aldolase and glucose-6-phosphate dehydrogenase in nymphs. Agosin and Dinamarca (1963) studied the effect on the levels of oxidized and reduced pyridine nucleotides and showed that DDT increased the total adenine dinucleotide phosphate (NADP) content in nymphs, and the total nicotinamide adenine dinucleotide (NAD) content and the NAD/NAD + hydrogen (H) (NADH) ratio in males. Agosin et al. (1965) demonstrated that DDT stimulated the protein biosynthesis in vivo and in vitro only in nymphs. Agosin et al. (1967) reported increased activity and induction of NAD kinase by DDT exposure in fifth instar nymphs. Ilivicky and Agosin (1967) showed that DDT increased total glutathione levels and glutathione turnover in third instar nymphs, but not in males. Litvak et al. (1968) reported that DDT induced the synthesis of messenger RNA in fifth instar nymphs.

Later, several other studies continued to address the effects of sublethal dose of insecticides on the biochemistry and molecular biology of triatomines. Juárez (1995) demonstrated that sublethal doses of different insecticides inhibited lipid synthesis in nymphs of the fifth instar of *T. infestans*. Specifically, the pyrethroid d-phenothrin reduced incorporation of the precursors acetate and propionate, the organophosphate fenitrothion inhibited propionate incorporation and fatty acid synthetase (FAS) activity of the fat body and integument, and the carbamate propoxur inhibited integumentary FAS activity. Sivori et al. (1997) showed that the organophosphorus fenitrothion (0.5 μg/i), salithion (0.125 μg/i) and malathion (0.5 μg/i), and pyrethroid tetramethrin (0.35 μg/i) increased cytosolic glutathione-S-transferase activity in adult males of *T. infestans*. Grosso et al. (2016, 2018) studied the effect of deltamethrin on the components of the P450 complex (system responsible for the oxidative metabolism of insecticides) in the fat body of the fifth nymphal instar of *T. infestans*. The authors demonstrated that topical application of LD_{50} of deltamethrin (dose not

reported) increased the mRNA levels of three cytochrome P450 genes (*CYP4EM7*, *CYP3085B1* and *CYP3092A6*) in insects from resistant and susceptible strains, and increased the mRNA levels of NADPH cytochrome P450 reductase gene in insects from susceptible strain. Dulbecco et al. (2018) showed that the LD_{50} of deltamethrin (dose not reported) induced member of *CYP4EM* and *CYP3093A* subfamilies of cytochrome P450 genes in the integument of fourth instar nymphs of pyrethroids resistant *T. infestans*. Traverso et al. (2022) studied the modulation of gene expression due to topical application of deltamethrin (0.2 ng/i) in first instar nymphs of *T. infestans* with low resistant level to pyrethroids. The results showed that 77 genes changed their expression levels after treatment, 61 upregulated and 16 downregulated. The modified expression was observed in genes associated with transcription and translation processes, rearrangement of the cuticle, transmembrane transport (ATP-biding cassette transporters), chemoreception (odorant biding proteins and chemosensory proteins), protein homeostasis (heat-shock proteins) and energetic metabolism. This study did not detect activation of detoxification-related gene families, and the authors speculated that this induction could occur at a later phase of the pyrethroid intoxication process.

Another important body of knowledge on sublethal effects in triatomines was obtained through a series of studies carried out in Argentina that focused on the symptoms associated with the poisoning process caused by pyrethroids. Alzogaray and Zerba (1993, 1997) and Alzogaray et al. (1997) studied the symptoms and the dynamic of hyperactivity, incoordination, paralysis and recovery of third instar nymphs of *T. infestans* exposed to pyrethroids. Insects were exposed to insecticides using insecticide-impregnated filter papers and/or topical application, and concentrations/doses varied between experiments (see cited studies for details). The symptoms were similar to those described previously for other insects: hyperactivity, tremors, moving backward or to the sides, contraction of legs, elevation of legs, convulsions, paralysis of hind legs, slow movements and lack of movements. The hyperactivity (i.e., increased locomotor activity) depended on temperature and application site: hyperactivity was recorded at 26 and 36°C but not at 16°C and application to the head was more effective than to the abdomen. In addition, no hyperactivity was observed when nymphs were pretreated with N-ethylmaleimide (sulphhydryl reagent) suggesting that chemoreception could be an underlying mechanism. The prostration (after cited as incoordination by the same authors, i.e., from tremors to paralysis of hind legs) also depended on the temperature: the potency toxic was greater (i.e., lower EC_{50}) at 16 than 28°C but the symptoms occurred more quickly at 28 than 16°C, and the symptoms appeared when insect was moved from 28 at 16°C and disappeared rapidly when were moved from 16 to 28°C. The authors showed also that incoordination was faster with deltamethrin and paralysis occurred first with cis-permethrin. In addition, the two insecticides showed equivalent incoordination power, but exposure to deltamethrin showed a faster recovery and in greater amounts than exposure to cis-permethrin. Finally, recovery from incoordination was inhibited by piperonyl butoxide suggesting that P450 enzymes (oxidative metabolism) are involved in the process of recovery. Alzogaray and Zerba (2001a) studied the hyperactivity and repellency (see below) produced by pyrethroids impregnated in filter papers (0.69–690 μg/cm^2) on fifth instar nymphs of *T. infestans*. Hyperactivity was greater for cyanopyrethroids than for non-cyano pyrethroids, hyperactivity was inhibited by preexposure to *N*-ethylmaleimide, and neither pyrethroid produced repellency. Alzogaray and Zerba (2001b) studied also the symptoms and lethality of α-cyanopyrethroids (β-cypermethrin, β-cyfluthrin, λ-cyhalothrin, and deltamethrin) on third instar nymph of *R. prolixus*. Exposure to the insecticide was carried out using impregnated filter papers (0.1–7.0 μg/cm^2) or by topical application (0.0004–3.3 ng/i). In agree with previous studies on *T. infestans*, the insects exposed to all pyrethroids (λ-cyhalothrin was the most effective) showed increased locomotor activity, incoordination, recovery from incoordination, and inhibition of recovery when piperonyl butoxide was applied, suggesting that biotransformation by P4540 enzymes is involved in the recovery process.

Several other biological processes were studied. Spiller (1966) showed that the DDT (20 μg/i) affected the oxygen consumption in fasting and fed fifth-instar nymphs of *R. prolixus*. Maddrell and Casida (1970) observed an induction of secretion from the Malpighian tubules of *R. prolixus* exposed to sublethal doses of organochlorines. Arends and Rabinovich (1980) studied the effects of the exposure to vapours of a sublethal dose of dieldrin (filter paper impregnated with 0.4% of the toxicant) on oxygen consumption, weight loss, survival, moulting, fecundity and fertility of *R. prolixus*. The insecticide prolonged moulting times, modified survival according to nutritional status, and decreased oviposition in the first weeks after feeding and increased oviposition in the following weeks but without modifying the total number of eggs laid per female. Alzogaray and Zerba (1996) showed an increase in the time to moult of first instar nymphs of *T. infestans* exposed to cloth treated with sublethal doses of deltamethrin in acetone (14 ng/cm^2). Rolandi et al. (2020) reported an increase in the CO2 production rate of first instar nymphs of *T. infestans* previously exposed by topical application to sublethal dose (LD_{20}) of deltamethrin, without differences between pyrethroids susceptible (LD_{20}: 0.096 ng/i) and resistant (LD_{20}: 14.65 ng/i) insects.

Recently, a series of studies addressed the effects of sublethal doses of insecticides on external morphology. Nattero et al. (2021) showed that *T. infestans* adults that were exposed by topical application to a sublethal dose of deltamethrin (LD_{50}: 1 ng/i) in the first nymphal instar had larger, less symmetrical and less canalized wings than control insects. Nattero et al. (2022) studied the morphology of the wings and cuticle of pyrethroid-resistant and susceptible *T. infestans* adults exposed by topical application to sublethal doses of deltamethrin (1 ng/i and 10 ng/i for susceptible and resistant, respectively) in the nymphal stages. The authors reported that adults exposed to the insecticide presented larger wings and shape changes in the proximal and distal parts of the wings in both toxicological phenotypes, and thicker cuticles only in the resistant insects. Both effects increased with repeated exposure throughout ontogeny. The authors speculated that wing modifications could affect flight dispersal and, therefore, house invasion and reinfestation dynamics; and the

modification in the cuticle could be a resistance mechanism that works through induction by the insecticide itself.

Pyrethroids also have a repellent effect at low doses. This phenomenon, called excito-repellency, is associated with an increase in the insect's locomotor activity, which causes the insect to move away from the source of the substance, as has been observed in haematophagous insects such as mosquitoes and Triatominae bugs (Diotaiuti et al., 2000). This process, a sublethal effect associated with neurotoxicity rather than a true repellent action (i.e., mediated by the sensory system, typically olfactory) (Alzogaray, 2016; Boné et al., 2019), has a practical application in the control actions of triatomines since it helps determine the infestation of the households. In Argentina, during the entomological evaluation of dwellings, control campaign operators apply the pyrethroid tetramethrin in aerosol on walls, ceilings and other places that serve as refuge for *T. infestans*. Hyperactive insects abandon their shelters, evidencing the infestation of the homes (Alzogaray & Zerba, 2017; Gürtler et al., 1993). It has been shown that pyrethroids also exert repellency mediated by olfactory receptors (Boné et al., 2019). In the present review, the action of non-insecticidal repellents (e.g., N,N-diethyl-3-methylbenzamide or DEET) has been excluded since it is not an effect promoted by doses of insecticides that do not kill.

INSECTICIDE RESISTANCE IN TRIATOMINES

In the last 10 years, several reviews have been published on the evolution of insecticide resistance in triatomines. Mougabure-Cueto and Picollo (2015) and Pessoa et al. (2015) presented the first detailed reviews that, despite some obvious overlaps, each delved into different theoretical aspects so they are complementary and together offer an excellent perspective on the topic. Then, Mougabure-Cueto and Picollo (2021) presented an update on the topic since the 2015 reviews, focusing on new areas of study that have emerged in recent years, and Mougabure-Cueto and Lobbia et al. (2021) presented a historical review focused on *T. infestants* of Argentina, with emphasis on toxicological monitoring. We recommend reading these reviews to gain a deeper understanding of what is presented, in less detail, in this manuscript.

Detection of insecticide resistance

Although Fox and Bayona (1966) and Correa et al. (1968) reported the survival of insects from laboratory colonies (*R. prolixus*, *T. infestans* and *P. megistus*) exposed to field doses of dieldrin, malathion and fenthion, the first reports of the evolution of insecticide resistance in the field in triatomines was the resistance to dieldrin and lindane in *R. prolixus* from the states of Trujillo and Yaracuy of Venezuela (Gonzalez-Valdivieso et al., 1971). Nocerino (1975) reported reduced susceptibility to dieldrin in *R. prolixus* collected from the field in the states of Trujillo and Táchira, and to lindane in *T. maculata* from the field in the state of Falcón, Venezuela. These early studies used contact bioassays where fifth instar nymphs walked on filter papers impregnated with insecticides. When the topical application bioassay was used, *R. prolixus* from Trujillo showed high levels of resistance 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 pyrethroids (Nelson & Colmenares, 1979). In 1994, with financing of the Special Programme for Research and Training in Tropical Diseases of the World Health Organization, a protocol of evaluation of insecticide susceptibility and resistance for *T. infestans* and *R. prolixus* was developed (WHO, 1994). The protocol determined the topical application as the best methodology to evaluate resistance in laboratory conditions. This protocol was/is used in various Latin American countries to determine the base-line of susceptibility and monitoring of resistance in these species to the insecticides used in each country, and was adapted to other species such as *T. sordida*, *T. mazzotti* and *T. longipennis* (Davila-Barboza et al., 2018; Lardeux et al., 2010; Pessoa et al., 2014; Picollo et al., 2005; Reyes et al., 2007; Sonoda et al., 2009, 2010; Soto & Molina de Fernández, 2001; Vassena et al., 2000). So, Vassena et al. (2000) reported the first evidence of resistance to pyrethroids in triatomines by determining resistance in *R. prolixus* from the Carabobo state of Venezuela (RR from 4.5 to 12.4) and in *T. infestans* from the Río Grande do Sul state of Brazil (RR from 3.4 to 7), without evidences of unsatisfactory control in the field. González-Audino et al. (2004) detected *T. infestans* with low levels of resistance (RR from 2 to 7.9) in the Argentine provinces of San Luis, Mendoza, Catamarca and Salta, again without compromising control in the field. After the Ministry of Health of Argentina reported the presence of triatomines after spraying with pyrethroids in the north of the province of Salta, on the border with Bolivia, Picollo et al. (2005) determined high levels of resistance to pyrethroids in *T. infestans* from four villages in that area (El Chorro, La Toma, El Sauzal, and Salvador Mazza). These insects showed high resistance to deltamethirn (RR from 51 to 133), beta-cypermethrin (RR from 264 to 451), beta-cyfluthrin (RR from 60 to 668), and lambda-cyhalothrin (RR from 65 to 289), but were susceptible to the organophosphorus fenitrothion. Similarly, after the Ministry of Health and Sports from Bolivia reported high levels of infestation with triatomines after control actions in the south of the department of Tarija (Yacuiba), on the border with Argentina, high resistance to deltamethrin (RR = 154) and susceptibility to fenitrothion and the carbamate bendiocarb was demonstrated in *T. infestans* from that area (Santo-Orihuela et al., 2008). Based on this evidence, studies on the evolution of resistance to pyrethroids in *T. infestans* in Argentina and Bolivia increased significantly during the following years. Over two decades, these studies allowed access to solid, although still incomplete, knowledge about different aspects of pyrethroid resistance in *T. infestans*, such as geographical distribution of resistance, resistance levels, differences between resistant foci, the cross-resistance patterns, the resistance mechanisms, the biological modifications associated with the resistant phenotype, the inheritance of resistance, and the environmental variables associated with the distribution of resistance.

The resistance to pyrethroids in *T. infestans* is high and widespread in Bolivia, where it was reported in six of the country's nine departments. Different levels of resistance (from low to very high)

were reported in the departments of Chuquisaca (RR from 5 to 323), Cochabamba (RR from 14 to 17.4), La Paz (RR from 3.7 to 5.8), Potosí (RRs from 25.6 to 54.7), Santa Cruz (RR from 3.8 to 8.3) and Tarija (RR from 30 to 818) (Depickère et al., 2012; Espinoza Echeverria et al., 2018; Germano et al., 2010, 2012; Gomez et al., 2014; Lardeux et al., 2010; Toloza et al., 2008). Espinoza Echeverria et al. (2018) reported toxicological variation at microgeographical level by showing different resistance levels in insects from different peridomiciliary structures of the same dwelling (Potosí, Bolivia). In agreement with the studies cited above, when the response to carbamates (e.g., bendiocarb) and organophosphates (e.g., malathion) was evaluated, pyrethroid-resistant insects showed susceptibility to these insecticides (Lardeux et al., 2010). In summary, these studies showed that the highest levels of pyrethroid resistance evolved in southern Bolivia, in the Andean and Chaco Valleys of the departments of Tarija and Chuquisaca. In Argentina, the pyrethroid resistance in *T. infestans* seems to be geographically more restricted but is very high. Since 2013, the Ministry of Health of Argentina, through the National Chagas Program, has carried out sustained national monitoring of susceptibility and resistance to pyrethroids in *T. infestans* (Mougabure-Cueto & Lobbia, 2021). Until the establishment of this sustained and routine monitoring, the evaluation of resistance in *T. infestans* in Argentina was framed in a first and ephemeral monitoring attempt at the end of the 1990s and, later, was focused in areas where it was reported that sprayings with insecticides did not have the expected effect. Thus, different levels of resistance to pyretroids in *T. infestans* were reported in the provinces of La Rioja (RR from 14 to 21), Santiago del Estero (RR = 3.8), Catamarca (RR = 4.5), Salta (RR from 8.4 to 1108) and Chaco (RR from 3.06 to >2000) (Carvajal et al., 2012; Fronza et al., 2016; Gaspe et al., 2021; Germano et al., 2010, 2012, 2013, 2014; Gurevitz et al., 2012; Roca-Acevedo et al., 2013; Santo-Orihuela et al., 2008; Sierra et al., 2016; Toloza et al., 2008). Again, pyrethroid-resistant insects were not resistant to other classes of insecticides, for example, organophosphates such as malathion and fenitrothion, carbamates such as bendiocarb, and phenylpyrazoles such as fipronil. So, these studies established that in Argentina there were two foci of high resistance to pyrethroids associated with a deficiency in chemical control: one in the north of the province of Salta in an area of the department of General San Martín that includes the towns of Salvador Mazza (in the border with Bolivia), Aguaray and surrounding areas, and another in the northwest of the province of Chaco in an area of the department of General Güemes that includes the town of J. J. Castelli and surrounding areas. Little was known about the rest of the endemic area, except for some provinces with places with low resistance without compromising chemical control. The monitoring currently underway has considerably expanded the knowledge about the geographical distribution of susceptibility and resistance to deltamethrin in *T. infestans* showing that (data until 2021) (1) the evolution of high levels of resistance associated with deficiencies in chemical control is limited to the two foci already described (north of Salta and northwest of Chaco), (2) in the foci of high resistance there are also villages with susceptible insects, and (3) outside the two foci of high resistance, the majority of the villages in 10 provinces have susceptible insects (89% of the sites evaluated) with very few sites with insects with low or medium resistance and without deficiencies in chemical control (Mougabure-Cueto & Lobbia, 2021). Fronza et al. (2016) studied the Chaco focus in detail and showed that susceptibility to pyrethroids was highly variable with villages with insects with high resistance (36%), villages with insects with low resistance (41%) and villages with susceptible insects (23%). There, high-resistant populations showed less genetic variability than susceptible or low-resistant populations (Piccinali et al., 2020). Toxicological variation was also observed within a highly resistant village (La Esperanza, Chaco) with houses harbouring resistant *T. infestans* and houses harbouring susceptible insects (Germano et al., 2013). However, this is not necessarily the case since in other resistant sites (e.g., Acambuco and Pampa Argentina) there were no toxicological differences between insects from different dwellings (Germano et al., 2013; Remón et al., 2020).

Outside the context of high resistance with a commitment to field control in *T. infestans* from Argentina and Bolivia, reports of insecticide resistance in other areas and species are scarce. Yon et al. (2004) using impregnated paper with a 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. Pessoa et al. (2014) reported slight decreased susceptibility to deltamethrin in *T. sordida* from Conego Marinho, Montalvania, Monte Azul and Porteirinha municipalities of the state of Minas Gerais, Brazil (RR from 2 to 3.9), although the authors established that all *T. sordida* populations from centre-west region of Brazil evaluated by Obara et al. (2011) would be resistant considering the reference strain used in their study. Torres et al. (2013) reported moderate (RR <100) and low (RR <10) levels of resistance to lambda-cyhalothrin and fenitrothion, respectively, in a field population of *Panstrongylus geniculatus* from Capitanejo municipality of the department of Santander, Colombia. Davila-Barboza et al. (2018) showed moderate resistance to deltamethrin in *T. mazzoti* from the state of Guerrero (RR = 26.9) and low resistance to permethrin in *T. longipennis* from the state of Jalisco (RR = 2.9) and Guanajuato (RR = 3.2), Mexico. Calderón et al. (2020) reported deltamethrin resistance in *R. prolixus* collected from oil palms in the department of Casanare, Colombia (RR = 5). Finally, indications of resistance evolution from the presence of insects after spraying with insecticides (deltamethrin or fenitrothion) were reported for *T. dimidiata* from Colombia (Reyes et al., 2007) and *R. prolixus* from Colombia and Venezuela (Angulo et al., 2006; Sanchez, 2006).

Resistance mechanisms

There are several techniques for identifying resistance mechanisms. These include toxicological bioassays to identify patterns of cross-resistance between different insecticides that indicate the presence of processes that determine the lower toxicity of these insecticides (e.g., increased activity of enzymes that are common to the metabolic pathways of the insecticides). The modification of the toxicity after

administration of enzyme inhibitors is an indirect way of identifying the role of detoxifying enzymes in the observed resistance. On the other hand, biochemical and molecular studies allow the direct detection and description of the resistance mechanisms at the phenotypic and genetic level, such as the increased activity of detoxifying enzymes or the presence of mutations in the target site of the insecticide (ffrench-Constant & Roush, 1990; McKenzie, 1996). Resistant insects may exhibit more than one resistance mechanism simultaneously (McKenzie, 1996), and the three main mechanisms (reduced penetration, enhanced detoxification and target-site modification) have been described for triatomine pyrethroid-resistant populations (see Mougabure-Cueto & Picollo, 2015 for a complete review). This section summarizes the diversity of studies on resistance mechanisms described in triatomine populations, focusing on recent research.

Reduced penetration

Cuticle modifications can reduce the rate at which toxic compounds enter the insect's body. These modifications prevent or delay the achievement of toxic levels of insecticide molecules at the target site. In addition, the reduced rate of penetration gives detoxification enzymes more time to act, multiplying their effectiveness in the breaking down of the toxic compound (Roush & Tabashnik, 1990). Pedrini et al. (2009) studied *T. infestans* fourth instar nymphs from Salta and Chaco provinces (Argentina) and provided evidence of resistance-related cuticle differences. Electron microscopy revealed a thicker cuticle in pyrethroid-resistant bugs. Capillary gas chromatography coupled with mass spectrometry analyses revealed greater amounts of surface hydrocarbons in this population compared to susceptible ones, suggesting that this enrichment would delay the uptake of pyrethroid through the cuticle. Later, molecular and bioinformatic approaches proposed the role for the insect integument in detoxification events (Dulbecco et al., 2018, 2021). New members of the cytochrome P450 gene family (*CYP*), belonging to the highly genome-wide expanded *CYP3093A* and *CYP4EM* subfamilies, have been described in the integument of *R. prolixus* and *T. infestans*. A set of CYP4-clan genes was overexpressed in the integument of deltamethrin-resistant *T. infestans* nymphs, (*CYP3093A11* and *CYP4EM10*), as was evidenced by the biochemical determination of increased of ethoxycoumarin-O-deethylase activity. This active P450-dependent detoxification in the integument of *T. infestans* suggests a potential role in metabolic resistance.

Enhanced detoxification

Pyrethroids are cleaved mainly by monooxygenase activity and esterase-mediated hydrolysis. Monooxygenase or P450 enzyme activity can be involved in the metabolism of virtually all insecticides. Esterases hydrolyze ester bonds present in chemicals used extensively in vector control programmes (McKenzie, 1996; Roush & Tabashnik, 1990). For some insects, detoxification is so active that the insecticide does not reach its molecular target before being metabolized by the enzymes. Metabolic resistance caused by esterases and cytochrome P450 monooxygenases has been characterized in *T. infestans* populations from Argentina and Bolivia (Dulbecco et al., 2018; Fronza et al., 2020; Grosso et al., 2016; Roca-Acevedo et al., 2013, 2015; Santo-Orihuela et al., 2008, 2013, 2017; Traverso et al., 2017). There was significantly increased activity of esterases in field populations of *T. mazzottii* and *T. longipennis* from Mexico compared with the reference strain (Davila-Barboza et al., 2018) and increased activity of oxidases, esterases and transferases in *R. prolixus* from Colombia (Calderón et al., 2020). Grosso et al. (2016), Traverso et al. (2017), and Dulbecco et al. (2018) showed overexpression of P450 genes belonging to the CYP4 clade in deltamethrin-resistant *T. infestans* from Argentina. Similarly, RNA interference of NADPH-Cytochrome P450 increased deltamethrin susceptibility in a resistant strain (Varela et al., 2024). However, it is worth noting that both inhibition of the detoxifying enzymes and RNAi-mediated gene silencing of CYP4s did not fully affect the susceptibility, suggesting another primary mechanism promoting resistance to deltamethrin (Dulbecco et al., 2018; Picollo et al., 2005).

Target-site modifications

Pyrethroids exert their insecticidal action on the insect nervous system by altering the normal function of voltage-gated sodium channels in the membranes of neurons. Knockdown resistance (kdr) is the reduction in the sensitivity to pyrethroids caused by point mutations in the sodium channel gene (Soderlund, 2008). Two point mutations associated with pyrethroid resistance, corresponding to L1014F and L925I from the sodium channel of *Musca domestica* Linnaeus (Diptera: Muscidae), have been reported in *T. infestans* (Capriotti et al., 2014; Fabro et al., 2012). The first of these mutations (L1014F) was identified in populations from the Bolivian-Argentine border, while the second mutation (L925I) appears to be associated with the Argentine Chaco (Sierra et al., 2016), in some populations with the highest levels of resistance found (Fronza et al., 2020). A study in Mexico revealed the presence of two other kdr mutations in field-resistant triatomines exposed to deltamethrin and permethrin (Davila-Barboza et al., 2018). The A943V mutation was detected in sequences from *T. mazzottii* individuals, although the K964R mutation has been described in *T. longipennis*, highlighting the importance and geographical spread of this mechanism for pyrethroid resistance in Chagas vectors.

In summary, the coexistence of the three major resistance mechanisms described above adds complexity to the chemical control of *T. infestans*.

Biological modification linked to resistance

Insecticide-based selection can have pleiotropic effects on biological processes other than the toxicological processes involved in resistance mechanisms. These effects may affect the adaptation of the resistant insect to the natural environment and/or may have direct

consequences on the epidemiology of the disease by modifying biological processes with epidemiological relevance (e.g., the vectorial capacity, reproductive potential, etc.) (Kliot & Ghanim, 2012; Lobbia et al., 2018; McKenzie, 1996). In this sense, studies have been carried out to compare the performance of different biological processes between pyrethroid-susceptible and pyrethroid-resistant *T. infestans*. Ontogenetic development and reproductive potential of resistant and susceptible insects were compared in Germano and Picollo (2015), Lobbia et al. (2019a, 2021) and Maza et al. (2023). These studies showed reproductive costs, lower autogenic capacity and developmental changes. Lobbia et al. (2018) showed changes in the excretion/defecation pattern of resistant *T. infestans*. Resistant *T. infestans* started defecating later, defecated less and had a lower proportion of defecating individuals in the first hour after feeding compared with susceptible insects. Other studies showed the effects of resistance on the dispersal capacity by walking of *T. infestans* (Lobbia et al., 2019b; Lobbia & Mougabure-Cueto, 2020). Here, resistant insects showed a lower number of dispersal events, a lower proportion of dispersed individuals, and fewer exit and entry events to/from experimental shelters. Guanuco et al. (2022) showed that pyrethroid-resistant *T. infestans* nymphs ingested less blood, took longer to defecate, and ingested and defecated more *T. cruzi* but defecated fewer parasites per parasite ingested and a lower proportion of metacyclic trypomastigotes than susceptible insects. Hernández et al. (2023) showed that resistant insects had smaller wings and heads and a different shape compared with susceptible ones. In this study, resistant phenotypes were considered compatible with lower dispersal potential by flight, mainly at short distances, and different active dispersal behaviour, and the antennae phenotype also revealed sensory simplification in resistant insects. The above-mentioned changes or effects related to resistance could be associated with adaptation costs and could also reduce the vectorial capacity of insects. On the other hand, some studies suggest positive consequences of pleiotropic effects. For example, when studying the effect of resistance on reproductive efficiency after dispersal in *T. infestans* (Lobbia et al., 2019a), dispersal-resistant females showed higher reproductive efficiency than dispersal-susceptible females and compared with non-dispersal-resistant females. May-Concha et al. (2020) showed that the faeces of resistant insects were more attractive than those of susceptible insects, also suggesting a benefit for the resistant ones. Another example is the study of cuticle thickness and wing size and shape in pyrethroid-susceptible and resistant *T. infestans* exposed to sublethal dose of deltamethrin (Nattero et al., 2022). The results of this study showed that resistant insects had thicker cuticles and evidenced a greater increase in wing sizes and cuticle thickness after insecticide exposure than susceptible insects, and suggested that morphological change induced by sublethal exposure may have evolutionary and epidemiological consequences.

Resistance and environment

According to the traditional definition, resistance is a population process resulting from the interaction of two factors: the presence of individuals with the heritable ability to survive the insecticide, and the continued selective pressure exerted by the insecticide (McKenzie, 1996). Thus, the insecticide plays a dominant role and the environment in which it operates could influence the selective pressure exerted.

Peridomestic ecotopes reduce the residual lethality of the insecticide because they are exposed to environmental conditions such as extremes of temperature, exposure to sunlight, wind and rain (Vazquez-Prokopec et al., 2002). Thus, insecticide is less effective in peridomestic habitats, allowing residual populations of *T. infestans* to persist (i.e., insect that survived sublethal doses) and, perhaps, the evolution of resistance by low doses (Gürtler et al., 2007; Mougabure-Cueto & Picollo, 2021). In intradomicile, the insecticide lasts longer at its optimal dose and would only select individuals located outside the natural distribution of susceptibilities of the population (see above), if they were present, promoting the evolution of resistance by high doses. Thus, this variation between intra and peridomestic environments (and between villages) and the consequent non-homogeneous insecticide pressure, could contribute to heterogeneity in deltamethrin resistance levels (Fronza et al., 2019; Lardeux et al., 2010; Mougabure-Cueto & Picollo, 2021). Germano et al. (2013) studied *T. infestans* susceptibility to deltamethrin at the microgeographic level in La Esperanza locality (Chaco Province, Argentina). They found that some houses harboured resistant insects, while others harboured susceptible insects, with no association between the toxicological status and the location of the insects within the houses (domestic vs. peridomestic). This highlights the complex spatial distribution of toxicological phenotypes of insects that persist after chemical control actions.

Natural conditions could be involved in the evolution of tolerance to insecticides, an evolutionary process associated with resistance. Environmental variables might select for individuals carrying phenotypic traits that are not related to the interaction with the insecticide but are determined by genes that simultaneously confer individual resistance or are linked to resistant genes. In this situation, the frequency of resistant insects in the natural environment would increase in the absence of the insecticide, resulting in a population with low natural susceptibility (a tolerant population) (Mougabure-Cueto & Picollo, 2015). This phenomenon has been suggested as an explanation for the low toxicological susceptibility of sylvatic *T. infestans* from Bolivia, which has never been exposed to pyrethroids and the phenylpyrazole fipronil (Depickère et al., 2012; Gomez et al., 2014; Roca-Acevedo et al., 2011).

Recently, more attention has been paid to studying the influence of the environment on resistance. The methodological framework is similar to that used in studies analysing the influence of environmental and spraying factors on response variables such as distribution of *T. infestans*, dwelling infestation rates or home invasion (Brito et al., 2017; Espinoza et al., 2014; Espinoza Echeverria et al., 2017; Gorla, 2002). In a bibliographic search of 24 articles reporting on pyrethroid resistance in Argentina and Bolivia, Bustamante et al. (2016) found a correlation between the lethal doses that kills 50% of *T. infestans* evaluated population (LD_{50}) and five environmental variables by using a generalized linear model. The bimodal distribution of

LD_{50} suggested two groups concentrated in the area where populations of the intermediate cytogenetic group were found (Panzera et al., 2014). This might reflect the spatial heterogeneity of the genetic variability of *T. infestans*, which appears to be the cause of the insecticide resistance in the zone, even for sylvatic populations that have never been exposed to pyrethroids.

The first study of environmental influences on *T. infestans* resistance from toxicological bioassays was conducted by Fronza et al. (2019). The authors evaluated the association of deltamethrin resistance levels in populations from the resistance focus of General Güemes with several explanatory variables, such as climate, village size and some constructed from information on control programmes over 11 years. Spraying was spread over the entire period analysed, suggesting a homogeneous selective pressure. Models including temperature and precipitation indicators provided 65% of the explanation, and the explanatory power reached 70% when village size variables were added. Pyrethroid efficacy is higher at low temperatures and when residual activity is not reduced by environmental factors such as excessive rain (Alzogaray & Zerba, 1993; Vazquez-Prokopec et al., 2002). These effects, combined with the higher proportion of sprayed houses observed in small villages, would result in a strong selective pressure in the study area.

Recently, the same associative study was extrapolated to the Argentine *T. infestans* endemic zone to understand if there are any environmental variables that influence resistance distribution (Fronza et al., 2024). Presence/absence models were the most accurate, with precipitation, distance from the capital city, and land use as the main predictors. The inclusion of the latter suggests that agricultural pesticides are involved in the differential pressure selection. The authors discuss the intricate and unintended consequences that the exposure of non-target organisms to agrochemicals used in crop production could have on life story traits and their potential to promote insecticide-resistant phenotypes, as has been demonstrated in mosquitoes (e.g., Baglan et al., 2018; Bara et al., 2014; Bataillard et al., 2020; Riaz et al., 2009). Information on these relationships could contribute to *T. infestans* control strategies in future scenarios characterized by changes in land use and precipitation.

CONCLUSIONS AND FUTURE RESEARCH QUESTIONS

In summary, chemical control can be very successful in achieving its objective of killing and controlling vector populations, but it can also have unwanted and unpredictable effects if the insecticides used do not have the intended effect. Insecticide resistance in triatomines, mainly in *T. infestans*, is a complex process that operates at different geographical scales, can have pleiotropic effects on biological processes and can be promoted by at least the three physiological mechanisms described. Insecticides may act as environmental factors exerting selective pressure, thereby promoting a process of natural selection leading to adaptation to their toxicity. In addition, at sublethal doses, physiological systems and energy available for toxicokinetic processes may be occupied, promoting changes in exposed individuals and also in population dynamics. To add complexity to the phenomena described, climatic variables such as temperature and precipitation appear to be involved in modulating insecticide activity in *T. infestans* populations exposed to pyrethroids. In this context, future research will address several aspects. To better understand resistance, it is important to continue and expand toxicological monitoring in the *T. infestans* endemic zone, as well as other *T. infestans* endemic zones throughout the Americas and endemic zones of other Chagas vectors. If new resistant foci are detected, elucidate the physiological mechanisms involved, the association with environmental variables and the possibility of incorporating control alternatives. Various control strategies (with or without insecticides) have been studied and proposed as alternatives to the current chemical control of Chagas vectors, none of which has yet been implemented on a large scale in the field (for a review: Alzogaray & Zerba, 2017; Mougabure-Cueto & Picollo, 2015; Gorla & Hashimoto, 2017). With regard to sublethal doses, further studies on the different possible physiological and population effects of pyrethroids and other insecticides are needed to determine the true contribution to vector control and disease epidemiology. Based on the recent study by Lobbia et al. (2023), which showed that *T. cruzi* infection increases the susceptibility of *T. infestans* to pyrethroids but does not modify the level of resistance, a particular interest emerges in studies on the various interactions between the toxicological processes discussed in this article (i.e., sublethal effects and pleiotropic effects of resistance) and *T. cruzi* infection. This type of study, which is conspicuously lacking, is highly relevant to the epidemiology of the disease, as it would address the possible interactions between the effects of vector control on the parasite, its development and transmission. Understanding the processes that occur when insecticides fail to kill could therefore help to improve the planning of chemical control of the triatomine vectors of Chagas disease.

AUTHOR CONTRIBUTIONS

Gastón Mougabure-Cueto: Conceptualization; writing – original draft; writing – review and editing; supervision. **Georgina Fronza:** Conceptualization; writing – original draft; writing – review and editing. **Julieta Nattero:** Conceptualization; writing – original draft; writing – review and editing.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

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