Journal of Insect Physiology 97 (2017) 93–113

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Rhodnius prolixus intoxicated

Raúl A. Alzogaray *, Eduardo N. Zerba

UNIDEF, CITEDEF, CONICET, CIPEIN, Villa Martelli, Argentina
Instituto de Investigación e Ingeniería Ambiental (3IA), Universidad Nacional de San Martín, San Martín, Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 20 January 2016
Received in revised form 8 April 2016
Accepted 20 April 2016
Available online 22 April 2016

Keywords:
Vincent B. Wigglesworth
Rhodnius prolixus toxicology
Chagas disease vectors
Insecticides
Pest control

ABSTRACT

Rhodnius prolixus (Hemiptera: Reduviidae) is a hematophagous insect native from South America. By the end of the 20th century, it was one of the main vectors of Chagas disease in Venezuela, Colombia, several Central American countries and southern Mexico. The aim of the present article is to review the literature regarding *R. prolixus* toxicology. British entomologist Vincent B. Wigglesworth carried out the first studies on this subject over seventy years ago. A wide bibliographical search allowed to locate one hundred and thirty scientific articles describing the effects of different insecticides on *R. prolixus*. About one-third of these articles report the acute toxicity and/or sublethal effects produced by the main synthetic neurotoxic families of insecticides (organochlorines, organophosphates, carbamates and pyrethroids). Only a couple of these studies have regarded the toxicokinetics or toxicodynamics of these insecticides. Insect growth or development disruptors, such as juvenoids, chitin synthesis inhibitors, precocenes, azadirachtin and lignoids, have been thoroughly studied in *R. prolixus*. Important aspects on the mode of action of ureases were also described in this species. By the end of the 1960's, resistance to insecticides was detected in *R. prolixus* from Venezuela. Some years later, the existence of pyrethroid-resistant individuals was also reported. Control programmes for *R. prolixus* in countries where Chagas is endemic have only used synthetic neurotoxic insecticides. In 2011, Central America and southern Mexico were declared free of this insect. The recent sequencing of the *R. prolixus* genome will provide valuable information to understand the molecular basis of insecticide resistance in this species.

1. Insect toxicology

Insect toxicology studies the adverse effects of toxicants in insects. Although this includes the effects of any toxicant on any insect, insect toxicologists mainly specialize on the effects of insecticides on pest species. The development and rational use of products for controlling pest insects requires extensive knowledge on the physico-chemical properties of insecticides, insect biology and the interaction between them. Insect toxicology provides information on the latter.

Toxicological bioassays are the first step towards determining the toxicity of an insecticide on a species and comparing it to that of other substances. In these assays, the observation of intoxication symptoms and the application of specific inhibitors of enzymatic processes provide evidence regarding the mode of action and metabolism of the insecticides. Bioassays also allow determining sublethal effects.

In order to understand how an insecticide produces its toxic effects, it is necessary to know its toxicokinetics and toxicodynamics (Hodgson et al., 1998). Toxicokinetics refers to the movement and changes an insecticide undergoes inside an organism: absorption, distribution, metabolism and excretion. Toxicodynamics describes the physiological, biochemical and molecular effects of the compounds and the mechanisms in which they are involved. The study of these processes implies that insect toxicology is a multidisciplinary field, as it involves insect anatomy, biochemistry, behaviour, ecology, physiology, and genetics.

The foundation of insect toxicology is attributed to the North American chemist William M. Hoskins (1896–1993), who in 1929 taught the first course on that subject at the University of California, Berkeley (Casida and Quistad, 2001). At the time, the era of synthetic insecticides had still not begun and pest control was carried out using natural substances like pyrethrum, rotenone, nicotine, sulphur and arsenicals (Menn and Hollingworth, 1985). In 1939, the Swiss chemist Paul Müller (1899–1965) discovered the insecticide properties of 1,1′-(2,2,2-trichloroethane-1,1-diyl)bis (4-chlorobenzene) (also known as dichlorodiphenyltrichloroe thane or DDT) (Mellanby, 1992). This finding was a turning point in the history of pest control. It also stimulated the chemical and

* Corresponding author at: Centro de Investigaciones de Plagas e Insecticidas (CIPEIN-UNIDEF-CONICET), J.B. de La Salle 4397, (1603) Villa Martelli, provincia de Buenos Aires, Argentina.
E-mail address: ralzogaray@hotmail.com (R.A. Alzogaray).

http://dx.doi.org/10.1016/j.jinsphys.2016.04.004
0022-1910/

toxicological study of insecticides, initiating the "Golden Age of Insecticide Research" (Casida and Quistad, 1998).

Rhodnius prolixus (Hemiptera: Reduviidae) is an insect of medical importance in several countries of Latin American (Box 1). About eighty years ago, the British entomologist Vincent B. Wigglesworth (1899–1994) adopted it as a model for physiological studies. Since then, *R. prolixus* has been used by many other insect physiologists. The present review was requested to highlight Wigglesworth's contribution to insect toxicology and review the literature about *R. prolixus* toxicology. This is the first article aimed to review the whole literature regarding to this subject, therefore all the publications found on *R. prolixus* toxicology are cited here, from the pioneering studies by Wigglesworth (in the early 1940's) to the present (December 31, 2015).

Box 1. *R. prolixus*, a vector of Chagas disease

The blood-sucking bug *R. prolixus* (Hemiptera: Reduviidae) is a hemimetabolous insect that feeds exclusively on blood from mammals and other vertebrates throughout its entire life-cycle, which includes five nymphal stages (Buxton, 1930). In the mid-20th century, its geographical distribution included Venezuela, Colombia, Central America (except Panama, Belice, and Costa Rica) and south Mexico, but in the last years it seems to have been eliminated from Mexico and the Central American area (Dias, 2015).

Even though the presence of *R. prolixus* in Brazil has been reported sporadically, it has been suggested that these are misidentifications of morphologically similar species of the same genus (Dujardin et al., 1991; Gurgel-Gonalves et al., 2008). *R. prolixus* has also been detected in Guyana (Aguilar et al., 2007); its presence in Suriname and the French Guiana is doubtful (BÅrenger et al., 2009; Hiwat, 2014).

Together with *Triatoma infestans* and *Panstrongylus megistus*, *R. prolixus* is one of the main vectors of *Trypanosoma cruzi*, the protozoan that causes Chagas disease, also known as American trypanosomiasis (WHO, 2002). Chagas is the most severe parasitic chronic disease of the American continent. It affects around fifteen million people, and another eighty million are exposed to the infection (Coura and Dias, 2009). It is considered a neglected tropical disease (a disease with a high impact on the world's poorest people) (Feasy et al., 2009).

Triatomines ingest *T. cruzi* when they feed on an infected host (Rassi et al., 2010). Inside the insect's gut, the parasite goes through different developmental stages and reproduces (Kollien and Schaub, 2000). It is then transmitted to another host through the faeces deposited by the vector while it feeds (Rassi et al., 2010).

The English common name of *R. prolixus* is ''kissing bug". In Spanish, it is called ''pito" (Colombia), ''chipo" (Venezuela), ''chinche besucona" (Central America and Mexico), and ''chinche picuda" (Mexico). In both languages, these names are used when referring to any of the different species of triatomines indistinctly (Schofield and Galvâo, 2009).

Wigglesworth's first studies on physiology of *R. prolixus* were published in the early 1930's (Locke, 1992). In the following decades, he published nearly forty articles regarding different physiological aspects of this insect. It is said that Wigglesworth loved *R. prolixus* (Locke, 1996). In his laboratory he fed the colony on rabbits, but occasionally allowed the insects to feed on blood from his own arm (Locke, 1996). In *R. prolixus*, Wigglesworth discovered the key aspects of insect growth and development, excretion, development of the nervous and tracheal systems, formation of muscle and connective tissues, cuticle structure, antennae function, physiology of ecdysis, wound healing, and the role of haemocytes (Locke, 1992). His works, in addition to studies performed by other researchers, have made *R. prolixus* a classic model organism for insect physiology.

2. Vincent B. Wigglesworth and insect toxicology

The same year Müller discovered the insecticide properties of DDT, Wigglesworth published *The Principles of Insect Physiology* (Wigglesworth, 1939), a book which today is still one of the recommended references in courses on the subject. At that moment, Wigglesworth had already been working on *R. prolixus* for several years and carried out some of the many studies for which he would later be known as the "Father of Insect Physiology" (Locke, 1994).

Between 1923 and 1991, Wigglesworth published more than two hundred and eighty articles and books (Locke, 1992). His first work on a toxicological topic was published in 1941: *The effect of pyrethrum on the spiracular mechanism of insects* (Wigglesworth, 1941). Pyrethrum is a mixture of six insecticide molecules, called pyrethrins, that are extracted from flowers of the *Chrysanthemum* genus (Schleier and Peterson, 2011). Wigglesworth carried out this study to test his hypothesis that the cause of death from pyrethrum might be desiccation as the result of interference with spiracular control. Hence, he occluded the anus of adult *R. prolixus* with paraffin to prevent water loss by excretion. He then applied an extract of pyrethrum dissolved in paraffin to the antennae of a group of insects and recorded their weight loss during several days, comparing them to untreated insects. In another experiment, he applied the same treatment to the adult bedbugs *Cimex lectularius*. When the insects showed intoxication symptoms, he observed the frequency of spiracular opening under a transmitted light microscope. As he found no effects that could be attributed to the treatments, he dismissed his hypothesis.

His next toxicological article appeared the following year: *Some notes on the integument of insects in relation to the entry of insecticides* (Wigglesworth, 1942). This study was inspired on a recent investigation on the effect of non-toxic paraffin on the passage of insecticides through the insect cuticle (Hurst, 1940), and on Wigglesworth's own observations regarding the importance of the solvent oil/water partition coefficient on the speed of this passage (Wigglesworth, 1941). This time, he dissolved pyrethrum in solvents with different viscosity and applied it on fifth instar nymphs of *R. prolixus*. He then registered the time it took to observe intoxication symptoms. Wiggleswoth observed that the speed with which the symptoms appeared, and therefore the rate at which the substances entered the organisms, decreased as the viscosity of the solvents increased. With a histological examination of the integument, he discovered that oily substances penetrated faster through the base of the bristles that through other parts of the integument. He also observed that in adults soon after moulting, the rate of entry was higher through stretched than unstreched integuments. However, in old adults, the main entry point to the organism were the dermal gland ducts.

In a letter published in *Nature*, Wigglesworth described that when *R. prolixus* nymphs are made to walk over a surface treated with a finely sprayed coat of alumina (an inert dust) the loss of weight by dehydration increases (Wigglesworth, 1944). Using a silver staining technique, he obtained evidence that the inert dust destroys the layer of waxes covering the body of insects. Three years later he wrote a more extensive article on this matter, where he described the abrasion experiments he performed in five species of stored products beetles (Wigglesworth, 1947). He demonstrated that the species that elicit most activity are more damaged by abrasion than the less active ones. He also identified the parts of the body that are more susceptible to abrasion (places with soft cuticle, like the intersegmental articulations of the legs, the margins of the elytra and the terminal segment, and the articulations of the maxillary and labial palps).

Between 1961 and 1972, Wigglesworth published several articles on the effects of substances that mimic the activity of the juvenile

hormone (juvenoids). The first of these articles was published as an annex (written in English) to the article of another author (written in German) (Wigglesworth in Schmialek, 1961). In that work, Wigglesworth used farnesol, a substance recently isolated by his German colleague Peter Schmialek (1925?–1976) from yeasts and excrements of the *Tenebrio molitor* beetle (Schmialek, 1961). Farnesol is a natural sesquiterpenoid alcohol that in insects is a precursor of the juvenile hormone (Bede et al., 2001; Sen et al., 2003). Fifth instar nymphs of *R. prolixus* treated with farnesol developed into sixth instars (a nymph stage that does not naturally exist). Some decades before, Wigglesworth had observed the same phenomenon in his experiments on the implant of *corpora allata* (Wigglesworth, 1936).

To quantify juvenoid activity, Wigglesworth proposed a scoring system for the retention of juvenile characters (Wigglesworth, 1969). Normal adults are scored as 0 and supernumerary nymphs are scored as 19. A score of 10 indicates a "half-juvenalized" insect; in other words, an insect that still maintains 50% of its juvenile characteristics.

Wigglesworth studied different aspects of the biological activity of farnesol and other juvenoids in *R. prolixus*, such as the retention of nymphal characteristics in the moulting fifth instar nymphs and the induction of yolk formation in the oocytes of decapitated females (Wigglesworth, 1961), the juvenoid effects of a family of chemically-related-to-farnesol compounds (Wigglesworth, 1963), and the importance of geometrical isomerism, starvation and solvent properties on juvenoid activity (Wigglesworth, 1969, 1973).

The toxicological issues investigated by Wigglesworth are highly relevant in pest control. Understanding the relation between the physicochemical properties of solvents and their passage through the cuticle is indispensable for a rational development of insecticide formulations (Licastro et al., 1983; Stadler and Buteler, 2009). Cuticle damage is the phenomenon that underlies the use of insecticide products based on diatomaceous earth and other inert dusts (Buteler et al., 2015; Golob, 1997). Juvenoids have been used since the 1970's for controlling insect pests (Pener and Dhadialla, 2012).

Despite Wigglesworth's prolific scientific contribution, he only pursued purely toxicological topics on very few occasions. He explained the reason for this during a lecture he gave in London, invited by the Royal Society for the Encouragement of Arts, Manufactures and Commerce. There he mentioned how he had refused to take over a research unit for the physiological study of insecticide action at the Agricultural Research Council. "I'm afraid my reply was to the effect that I was not interested -Wigglesworth confessed to the audience-. Now I am fully convinced that insect physiology has a real contribution to make to the killing of insects by means of chemicals. But insect physiology is not an applied science, it is a fundamental science, and what is holding matters up is not our failure to know just how gamma-hexachlorobenzene kills the insect, but our lack of knowledge of the internal working of the insect as a whole" (Wigglesworth, 1956).

Although Wigglesworth only sporadically explored the insect toxicology territory, his physiological research paved the way to those studying the toxicokinetics and toxicodynamics of insecticides in insects.

3. *R. prolixus* toxicology

To write this review we tried to locate as many scientific articles as possible regarding toxicology of *R. prolixus*. The criteria used for this search was to include all studies describing the toxicokinetics, toxicodynamics, and lethal or sublethal effects of insecticides on this species, in field or laboratory conditions.

To identify the toxicological works carried out by Vincent B. Wigglesworth cited in Section 2, we consulted the complete bibliography of this author (Locke, 1992). The rest of the search was carried out online, using the following search engines: PubMed (www.ncbi.nlm.nih.gov/pubmed), ScienceDirect (www.sciencedirect.com) and Google (www.google.com). Different searches were carried out in each of these engines using the keywords "prolixus AND X", were "X" represents different words (general concepts such as "pesticides" or "toxicity", or specific names of insecticides like "pyrethroids", "carbamates", "azadirachtin", "juvenoids", among others). No time limits were specified in these searchs. All the results were screened by title to select only those related to the toxicology of *R. prolixus*.

Most of the bibliographical material was downloaded from the Biblioteca Electrónica de Ciencia y Tecnología, depending on the Ministerio de Ciencia, Tecnología e Innovación Productiva of Argentina, which was accessed via the Universidad Nacional de San Martín (Argentina). The open access Scientific Electronic Database (www.scielo.org) was also an important source of material. Some articles published in journals with limited diffusion, that are not indexed in databases, as well as unpublished World Health Organization (WHO) documents, were located by consulting the bibliography section of other articles. Twenty-five articles that were unavailable online were requested from other colleagues or organisms that, out of kindness to the authors of this review or as third party services, provided scans of the printed versions via e-mail (see "Acknowledgements").

In all, 130 scientific articles and 5 unpublished WHO documents reporting original results about some aspect of *R. prolixus* toxicology were gathered (Table 1; see also the section "References", where the gathered articles and documents are indicated by asterisk). Other WHO documents located in the searches were not taken into account after verifying that their results were also published in scientific journals. The unpublished WHO documents contain important information on *R. prolixus* toxicology, and are usually quoted in the bibliography on the subject. However, until very recently, most of them they were not available online (see "Acknowl edgments"). Five discussion papers focused on some aspect of *R. prolixus* toxicology (Azambuja and Garcia, 1991, 1992; Garcia et al., 1987, 1991; Rembold, 1987), and one book chapter (Garcia et al., 1989b) were also located.

Of the 135 scientific articles and WHO documents on *R. prolixus* toxicology, 41 (30.4%) report the results of studies with substances belonging to the four most important families of synthetic neurotoxic insecticides (organochlorines, organophophates, carbamates and pyrethroids). The insecticides that appear in the highest number of articles are azadirachtin and dieldrin (15 articles each, 11.3%), followed by lindane and precocene II (13 articles each, 9.8%).

Table 1
Articles on *R. prolixus* toxicology grouped according to their mode of action.

Mode of action and families or compounds	Number of articles	
Synthetic neurotoxic insecticides		42
Organochlorines, organophosphates, carbamates and pyrethroids	41[a]	
Ivermectin	1	
Insect growth or development disruptors		55
Juvenoids	16	
Chitin synthesis inhibitors	4	
Precocenes	13	
Azadirachtin and lignoids	22	
Synthetic insect repellents		7
Insecticides with other modes of action (including those non-specific or unknown)		31
Peptides	11	
Botanicals	8	
Fatty acids and aliphatic alcohols	2	
Inert dusts	4	
Sterilizants	6	
Total		135

[a] Including five unpublished WHO documents.

Most of the articles are in English (76.3%). The remaining are in Spanish (14.8%), Russian (6.7%), Portuguese (1.5%) or French (0.7%). The articles are authored by 216 researchers belonging to about 60 organisms from 15 countries.

4. *R. prolixus* intoxicated in the laboratory

4.1. Synthetic neurotoxic insecticides

Most bioassays evaluating synthetic neurotoxic insecticides on *R. prolixus* were carried out to quantify their acute toxicity (immediate mortality by a single exposure). The general aim of these works was to obtain potentially useful information for controlling this Chagas disease vector. Their specific objectives were at least one of the following five: (a) validating new methods for evaluating acute toxicity; (b) determining the acute toxicity of new insecticides (these assays may or may not include another insecticide with previously known toxicity with comparative aims), (c) assessing sublethal effects, (d) studying toxicokinetics and/or toxicodynamics processes in susceptible insects; and (e) establishing the existence and/or mechanisms of resistance to insecticides in natural or laboratory populations (Table 2).

The assays carried out to achieve objectives (a), (b) (c), and (d) used laboratory reared insect colonies. Both field and laboratory insects were used to assess objective (e), as in order to establish resistance it is necessary to compare the toxicity in both groups. This section reviews studies with aims (a), (b), (c), and (d). Publications on *R. prolixus* insecticide resistance are reviewed in Section 5.2.

4.1.1. Organochlorines, organophosphates, carbamates and pyrethroids

The family of organochlorines includes DDT, lindane, and cyclodienes. Organophosphates are esters of phosphoric acid; carbamates are derivatives of carbamic acid. The pyrethroid family consists of alpha-cyano and noncyano pyrethroids (molecules with and without a cyano group in the alpha carbon, respectively). The site of action of the synthetic neurotoxic insecticides are the voltage-gated sodium channels in nerve cell membranes (DDT and pyrethroids), the GABA receptors in gabaergic synapses (lindane and cyclodienes), and the enzyme acetylcholinesterase in cholinergic synapses (organophosphates and carbamates) (Casida and Durkin, 2013).

Organochlorines and some organophophates and carbamates are prohibited in several countries due to their effects on human health and/or environment (Camenzuli et al., 2015; Mostafalou and Abdollahi, 2013). Other organophosphates and carbamates, together with pyrethroids, are the most used active ingredients for controlling all types of insect pests. They account for three-quarters of the world's insecticide market (Casida, 2009).

Bioassays on *R. prolixus* generally use fifth instar nymphs, because it is the most tolerant stage to insecticides (Fox et al., 1966; Lent and Oliveira, 1944). However, according to the specific aims of each study, other stages are also used, including eggs if ovicidal activity needs to be determined.

Before the appearance of synthetic insecticides, it was common in bioassays to dissolve the studied substance in a mineral oil and apply the resulting solution on the insects as a mist using an "atomising gun" (Busvine and Barnes, 1948). During the 1940's, insects began to be exposed to filter papers impregnated with a solution of the insecticide dissolved in acetone or another organic solvent (letting the solvent evaporate before exposing the insects) (Busvine and Barnes, 1948). The toxicity of the organochlorines DDT, lindane and dieldrin in *R. prolixus* has been assessed almost exclusively with this method, exposing groups of insects to one or several concentrations of insecticide in each assay, but without

Table 2
Organochlorines, organophosphates, carbamates and pyrethroids tested on *R. prolixus*.

Insecticide	Study objective[c] (stage used)	Mode of application	Reference
DDT (OC)	Evaluating acute toxicity (unspecified nymphs, adult)	Dust sprinkled on the bottom of Petri dishes In a pigeon blood meal	Lent and Oliveira (1944)
DDT (OC) Lindane (OC)	Validating exposure to films (fourth and fifth instar, adult)	Film on filter paper	Busvine and Barnes (1948)
Dieldrin (OC)	Using xenointoxication for determining the presence of insecticide in dog blood (unspecified nymphs)	In a dog blood meal	Carrillo and Vicente (1955)
Dieldrin (OC)	Evaluating acute toxicity in laboratory conditions and in the field (in laboratory: egg, unspecified nymphs, adults; in field: wild population)	Film on filter paper Sprayed on houses	Peñalver and Villagran (1955)
Dieldrin (OC)	Using xenointoxication for determining the presence of insecticide in human blood (unspecified nymphs)	In a human blood meal	Blazquez and Bianchini (1957)
DDT (OC) Dieldrin (OC) Fenthion (OP) Malathion (OP)	Evaluating acute toxicity (first to fifth instar, adult)	Film on filter paper	Fox et al. (1966)
Propoxur	Evaluating effectivity in laboratory conditions and in the field (in laboratory: egg; in field: unspecified nymphal stage[d])	Film on different surfaces Sprayed on houses	Valdivieso and Diaz (1968)
Aldrin (OC) Alpha-, beta-, and delta-hexachlorocyclohexane (OC) DDT (OC) Chlordane (OC) Dieldrin (OC) Endosulfan (OC) Endrin (OC) Heptachlor (OC) Isodrin (OC) Lindane (OC) Methoxychlor (OC) TDE (OC) Toxaphene (OC)	Evaluating effect on diuresis (fifth instar)	Injection into the haemolymph Topical application	Casida and Maddrell (1970)

Table 2 (*continued*)

Insecticide	Study objective[c] (stage used)	Mode of application	Reference
EPN (OP) Malathion (OP) Schradan (OP) Carbaryl (CA) Carbofuran (CA) Pirimicarb (CA)			
Amino mexacarbate (CA) Mexacarbate (CA)	Evaluating the effect on diuresis (fifth instar)	Injection into the haemolymph Topical application	Maddrell and Casida (1971)
Dieldrin (OC) Lindane (OC)	Monitoring insecticide resistance in several Venezuelan localities (fifth instar and other unspecified stages)	Film on filter paper	Valdivieso et al. (1971)
Dieldrin (OC) Lindane (OC) Propoxur (CA)	Monitoring insecticide resistance in the state of Trujillo (Venezuela) (third to fifth instar)	Topical application	Cockburn (1972)
Dieldrin (OC) Fenthion (OP) Malathion (OP)	Evaluating acute toxicity (fifth instar, adult)	Film on filter paper	Kul'kova and Fedder (1972)
Dieldrin Lindane Propoxur	Laboratory selection of a dieldrin-resistant colony	Film on filter paper	Nocerino (1972)
Dieldrin (OC) Lindane (OC) Fenthion (OP)	Monitoring insecticide resistance in several Venezuelan localities (unspecified nymphs, adult)	Film on filter paper	Nocerino (1976)
Fenthion (OP) Malathion (OP) Pirimifos methyl (OP) Jodfenphos (OP) Propoxur (CA)	Evaluating effectivity in the field (wild population, and unspecified stage[d])	Sprayed on houses	Nocerino et al. (1976)
Dieldrin (OC) Fenitrothion (OP) Fenthion (OP) Propoxur (CA)	Monitoring insecticide resistance in several Venezuelan localities (fifth instar)	Film on filter paper	Nelson and Colmenares (1979a)
Dieldrin (OC) Lindane (OC) Bromophos (OP) Chlorphoxim (OP) Fenitrothion (OP) Fenthion (OP) Jodfenphos (OP) Malathion (OP) Phoxim (OP) Pirimiphos ethyl (OP) Pirimiphos metil (OP) Bendiocarb (CA) Propoxur (CA) Bioallethrin (PY) S-bioallethrin (PY) Bioresmethrin (PY) Cypermethrin (PY) Decamethrin[a] (PY) Permethrin (PY) Tetramethrin (PY) Sumithrin (PY) 2-Z[b] (PY)	Validating topical application on susceptible and resistant insects (fifth instar)	Topical application	Nelson and Colmenares (1979b)
Dieldrin (OC)	Evaluating sublethal effects on oxygen consumption, moulting time, eggs laid and survivorship (first instar, adult)	Film on filter paper	Arends and Rabinovich (1980)
Bioallethrin (PY) Bioresmethrin (PY) Decamethrin[a] (PY)	Studying the effect on the pattern of *corpora cardiaca* electrical activity (fifth instar)	Topical application on the *corpora cardiaca* after removing an area of overlying cuticle	Orchard (1980)
Fenthion (OP)	Evaluating ovicidal activity (egg)	Film on filter paper	Kul'kova (1981a)
Lindane (OC)	Evaluating knockdown and recovery (fifth instar, adult)	Film on cardboard	Kul'kova (1981b)
DDT (OC)	Field testing of toxicity (wild population)	Sprayed on houses	Williams et al. (1981a)
Fenitrothion (OP)	Evaluating effectivity in the field of different application equipments (wild population)	Sprayed or aerosolized on houses	Williams et al. (1981b)
DDT (OC) Dieldrin (OC) Lindane (OC) Bromophos (OP) Chlorphoxim (OP) Fenitrothion (OP) Fenthion (OP)	Laboratory, semi-field and field testing of toxicity (in laboratory: fifth instar; in semi-field: fifth instar[d]; in field: wild population, and fifth instar[d])	Film on the bottom of Petri dishes Fumigant Sprayed on houses	Nelson et al. (1983)

(*continued on next page*)

Table 2 (*continued*)

Insecticide	Study objective[c] (stage used)	Mode of application	Reference
Jodfenphos (OP) Pirimiphos ethyl (OP) Pirimiphos methyl (OP) Bendiocarb (CA) Propoxur (CA) Cypermethrin (PY) Deltamethrin (PY)			
Bendiocarb (CA)	Evaluating acute toxicity (egg, fourth and fifth instar)	Film on filter paper and samples of earthen walls	Sherlock et al. (1983)
Permethrin (PY)	Evaluating repellency (adult)	Skin-treated rabbit	Buescher et al. (1985)
Dieldrin (OC) Lindane (OC) Bromophos (OP) Fenitrothion (OP) Fenthion (OP) Jodfenphos (OP) Malathion (OP) Pirimiphos-methyl (OP) Dioxacarb (CA) Propoxur (CA)	Evaluating acute toxicity (fifth instar)	Film on filter paper	Nocerino and Hernández (1985)
Permethrin (PY)	Evaluating repellency (adult)	Skin-treated rabbit	Buescher et al. (1985)
Lindane (OC) Fenitrothion (OP) Malathion (OP)	Evaluating acute toxicity of the three compounds. Evaluating the penetration rate, acetylcholinesterase inhibition and metabolism of malathion (first instar)	Film on filter paper Topical application	Villar et al. (1990)
DDT (OC) Lindane (OC) Malathion (OP) Trichlorfon (OP)	Validating a lethal bait (first to fifth instar, adult)	In a sheep blood meal	Lima et al. (1991)
Lindane (OC)	Validating a lethal bait (first to fifth instar, adult)	In a distilled water meal	Lima et al. (1992)
Dichlorvos (OP) Malathion (OP) Cypermethrin (PY)	Comparing different application methods (wild population)	Film of paint on houses and peridomestic structures Fumes delivered by fumigant cans inside houses	Filho (1996)
Alpha-cypermethrin (PY) Cyfluthrin (PY) Deltamethrin (PY) Lambda-cyhalothrin (PY)	Evaluating acute toxicity (fifth instar)	Topical application	Filho (1999)
Beta-cyfluthrin (PY) Beta-cypermethrin (PY) Cypermethrin (PY) Deltamethrin (PY) Lambda-cyhalothrin (PY)	Determining the presence of insecticide resistance in individuals from the state of Carabobo (Venezuela) (first instar)	Topical application	Vassena et al. (2000)
Beta-cyfluthrin (PY) Beta-cypermethrin (PY) Deltamethrin (PY) Lambda-cyhalothrin (PY)	Quantifying hyperactivity, incoordination, recovery and mortality (third instar)	Film on filter paper Topical application	Alzogaray and Zerba (2001b)
Fenitrothion (OP) Pirimiphos methyl (OP) Propoxur (CA) Deltamethrin (PY) Cyfluthrin (PY) Lambda-cyhalothrin (PY)	Monitoring insecticide resistance in several Venezuelan localities (first instar)	Topical application	Fernández and Vivas (2001)
Malathion (OP) Deltamethrin (PY)	Evaluating acute toxicity (first and fifth instar)	Film on filter paper Topical application	Sandoval (2001)
Fenitrothion (OP) Pirimiphos methyl (OP) Propoxur (CA) Deltamethrin (PY) Lambda-cyhalothrin (PY)	Evaluating acute toxicity (first and fifth instar)	Topical application	Vivas and Fernández (2001)
Lambda-cyhalothrin (PY)	Laboratory and field assessment of toxicity (fifth instar[d])	Film on palm leaves	Mazariego-Arana et al. (2002)
Beta-cyfluthrin (PY) Deltamethrin (PY)	Evaluate efectiveness (wild population)	Film on houses and peridomestic structures	Nakagawa et al. (2003)
Deltamethrin (PY) Lambda-cyhalothrin (PY)	Monitoring insecticide resistance in several Venezuelan localities (fifth instar)	Topical application	Fernández et al. (2004)

CA, carbamate; EPN, ethyl 4-nitrophenyl phenylphosphonothioate; OC, organochlorine; OP, organophoshate; PY, pyrethroid; TDE, tetrachlorodiphenylethane.

[a] Later called deltamethrin.

[b] Unidentified pyrethroid.

[c] Performed in laboratory, except when stated otherwise.

[d] After houses were sprayed, laboratory insects were experimentally exposed to the treated surfaces.

calculating toxicological parameters. In some cases, the papers treated with insecticides were provided by the WHO as requested by the researchers.

Although exposure to filter papers is still used, at the end of the 1970's topical application began to be used as well. This consists in applying a minute drop of insecticide solution on each insect using a microsyringe provided with a dispenser. Applying different doses to different groups of insects, dose-response relationships are obtained which are then used to calculate the median lethal dose (LD50).

In a protocol developed for evaluating insecticides in *R. prolixus*, WHO recommends both exposure to treated filter papers and topical application (WHO, 2001). The results of the first method can be used to calculate the median lethal concentration (LC50), while the results of the second method are used to calculate LD50 values. These toxicological parameters allow comparing the toxicity of different substances in a very precise way. They are widely used in studies with all types of living beings.

After the Second World War, on account of its incredible results in pest control, DDT was considered for controlling Chagas vectors. However, this idea was discarded almost immediately after proving that, unlike what happens in most insects, the toxicity of DDT is very low in triatomines (Gualtieri et al., 1985). The cause of this was discovered years later. *Triatoma infestans*, the main Chagas disease vector in Argentina and limiting countries, biotransforms DDT to polar metabolites that are rapidly excreted (Agosin et al., 1964). Additionally, the passage of DDT through the cuticle of *T. infestans* is slow (Fontán and Zerba, 1992). The toxicity of DDT in *R. prolixus* is also lower compared to other insecticides (Fox et al., 1966). Although it has still not been determined whether the cause of this low toxicity is the same as in *T. infestans*, it is highly probable based on their taxonomical proximity.

Bioassays with DDT, lindane, and dieldrin on *R. prolixus* were interrupted towards the end of the 1970's and beginning of the 1980's, as these insecticides fell in disuse throughout the world.

Table 3
Toxicity of some representative insecticides on *R. prolixus*.

Insecticide	Family or structure (mode of action)	Toxicity (stage used and effect evaluated)	References
		Exposure (2 h) to films on filter paper	
Lindane	Organochlorine (neurotoxic)	LC50 = 0.002 mg per cm^2 (unspecified stage, mortality)	Busvine and Barnes (1948)
Pyrethrins	A mixture of six esters of chrysanthemic and pyrethric acids (neurotoxic)	LC50 = 0.009 mg per cm^2 (unspecified stage, mortality)	Busvine and Barnes (1948)
DDT	Organochlorine (neurotoxic)	LC50 = 10 mg per cm^2 (unspecified stage, mortality)	Busvine and Barnes (1948)
		Oral application (ad libitum in a blood meal)	
Azadirachtin A	A triterpenoid limonoid (feeding deterrent; molting inhibitor)	EC50 = 0.04 μg per ml (fourth instar, moult inhibition)	Garcia et al. (1984)
Azadirachtin A	A triterpenoid limonoid (feeding deterrent; molting inhibitor)	EC50 = 25 μg per ml (fourth instar, feeding inhibition)	Garcia et al. (1984)
Pinoresinol	Lignoid (ecdysis disruptor)	EC50 = 60 μg per ml (fourth instar, ecdysis inhibition)	Garcia et al. (2000)
Pinoresinol	Lignoid (ecdysis disruptor)	EC50 > 100 μg per ml (fourth instar, feeding inhibition)	Garcia et al. (2000)
		Topical application	
Lambda-cyhalothrin	Pyrethroid (neurotoxic)	$LD50_{(72\ h)}$ = 40 ng per insect (fifth instar, mortality)	Filho (1999)
Fenitrothion	Organophosphorus (neurotoxic)	$LD50_{(48\ h)}$ = 100 ng/insect (fifth instar, mortality)	Vivas and Fernández (2001)
Deltamethrin	Pyrethroid (neurotoxic)	$LD50_{(72\ h)}$ = 150 ng per insect (fifth instar, mortality)	Filho (1999)
Propoxur	Carbamate (neurotoxic)	$LD50_{(48\ h)}$ = 690 ng per insect (fifth instar, mortality)	Vivas and Fernández (2001)
Triflumuron	Benzoylphenylurea (chitin synthesis inhibitor)	$LD50_{(24\ h)}$ < 50 μg per insect (fifth instar, mortality)	Mello et al. (2008)
Pyriproxyfen	Juvenoid (mimics juvenile hormone)	EC50 = 150 ng per insect (fifth instar, supernumerary molt)	Langley et al. (1990)
Methoprene	Juvenoid (mimics juvenile hormone)	EC50 = 4.2 μg per insect (fifth instar, supernumerary molt)	Langley et al. (1990)
Precocene II	Precocenes (damage *corpora allata*)	EC50 40 μg per insect (third instar, induction of precocious metamorphosis)	Azambuja et al. (1996)
		Exposure to vapors	
Dichlorvos	Organophosphorus (neurotoxic)	KT50 = 3.6 min continuous exposure to 390 μg per cm^2 (first instar, knockdown)	Moretti et al. (2015)
Thymol	Monoterpene (neurotoxic)	KT50 = 78.9 min continuous exposure to 3,900 μg per cm^2 (first instar, knockdown)	Moretti et al. (2013)
Eugenol	Monoterpene (neurotoxic)	KT50 = 89.8 min continuous exposure to 3,900 μg per cm^2 (first instar, knockdown)	Moretti et al. (2013)
Eucalyptol	Monoterpene (probably neurotoxic)	KT50 = 42.6 min continuous exposure to 3,900 μg per cm^2 (first instar, knockdown)	Moretti et al. (2015)

Notwithstanding, two studies published in the 1990's evaluated the oral toxicity of DDT and lindane in *R. prolixus* by using baits with insecticides in suspension (Box 2).

Box 2. *R. prolixus* xenointoxicated

Xenointoxication occurs when a parasite is intoxicated after feeding on a host that has been treated with a pesticide. Already in the 1940's, it was observed that *R. prolixus* died when it fed on pigeons that had previously ingested capsules containing DDT (Lent and Oliveira, 1944).

Before the appearance of analytical equipment able to detect minimal quantities of an insecticide in biological samples, xenointoxication of *R. prolixus* nymphs was used as a way of determining the presence of dieldrin in dog blood (Carrillo and Vicente, 1955). It was also used to determine its presence in the blood of workers exposed daily to the insecticide (Blazquez and Bianchini, 1957).

Oral intoxication of triatomines with baits containing insecticides was also evaluated. Nymphs of *R. prolixus* and another six species of triatomines were fed on suspensions of DDT, lindane, or the organophosphates malathion and trichlorfon (1 g/l) in defibrinated sheep blood contained in latex bags (Lima et al., 1991). As lindane produced the best results (between 94% and 100% mortality a week after feeding), it was used in a second experiment. There it was offered to the insects as a water suspension at room temperature and similar results were obtained (Lima et al., 1992). The authors suggested that this type of baits could be an economical way of controlling triatomines. However, there are no indications of these traps being tested in field experiments, where they most certainly would not have been as effective in attracting triatomines, having to compete with the physical and chemical cues emitted by human beings and other hosts.

More recently, xenointoxication of *T. infestans* was assessed using experimental chickens, goats and dogs (Amelotti et al., 2010, 2012; GÏrtler et al., 2009; Juan et al., 2013).

Each family of insecticides is composed by substances with very different toxicity. In addition, a wide variety of experimental has been used for evaluating their effects. For this reason, it is very difficult to make accurate comparisons regarding the toxicity of different families or molecules on *R. prolixus* (Table 3). In general, lindane is more toxic than dieldrin and equally or more toxic than organophosphates and carbamates (depending on which of the members of the family is used to compare it with). Some, but not all, organophosphates are more toxic than carbamates. Pyrethroids are by far the most effective, with a toxicity five orders of magnitude higher than that of other families of insecticides. Among pyrethroids, those with an alpha-cyano group are more toxic than those without.

Susceptibility to insecticides varies throughout the life cycle of *R. prolixus*. For example, the organophosphate pirimiphos-methyl and the carbamate propoxur are fifty-four times more toxic on first than on fifth instar nymphs, and the pyrethroid deltamethrin is one hundred and eighty times more toxic (Fernández and Vivas, 2001). The difference in size between both stages only partially justifies this difference in toxicity, hence there must also be some toxicokinetic differences.

Like other insects, when intoxicated with neurotoxic insecticides, *R. prolixus* intoxicated shows characteristic symptoms that indicate malfunctioning of the nervous system: hyperactivity, tremors, incoordination, leg paralysis (starting with the third pair), and proboscis extension (Alzogaray and Zerba, 2001b; Lima et al., 1992; Osborne, 1985).

Hyperactivity is the first observable symptom of intoxication with pyrethroids in insects (Alzogaray and Zerba, 2001a,b; Alzogaray et al., 1997; Gammon, 1978). The locomotor activity of third instar nymphs of *R. prolixus* exposed to filter papers treated with alpha-cyanopyrethroids increased lineally as a function of the logarithm of concentration (Alzogaray and Zerba, 2001b).

Hyperactivity has a practical application in the Chagas vector control. Triatomines spend most of the day hidden in the crevices of mud walls and thatched roofs in rural households (Jurberg and Galvão, 2006). In Argentina, the operators of control campaigns apply the pyrethroid tetramethrin in aerosol to determine whether a house is infested with *T. infestans* (Gürtler et al., 1993). Hyperactive insects abandon their hideouts and are thus spotted by the operators. This phenomenon, called flushing-out, allows to determine whether a household is infested or not. In Venezuela, pyrethrum has been used to flushing-out *R. prolixus* (Nelson and Colmenares, 1979b; Williams et al., 1981a).

Knockdown is a fast and reversible paralysis that is manifested in insects intoxicated with pyrethrum or pyrethroids (Alzogaray and Zerba, 1997; Sawicki, 1962; Scott and Georghiou, 1984). In third instar nymphs of *R. prolixus* treated topically with α-cyanopyrethroids, the values of the mean effective dose (ED50) calculated at different times decreased during the first hours following the treatment, it then remained constant for about four days and finally increased (Alzogaray and Zerba, 2001b). This variation of ED50 along time indicates nymph recovery. Individuals that at one point were knocked-down, then recovered and began acting normally again. When piperonyl butoxide, an inhibitor of microsomal mixed-function oxidases (MMFO), was applied simultaneously with the insecticides, no recovery was observed. This suggests that the recovery was due to the degradation of the insecticides by MMFOs.

Exposure to neurotoxic insecticides induces abdominal distension in triatomines. Both contact and ingestion of DDT in a blood meal induced this symptom in *T. infestans*, *P. megistus*, *T. sordida* and *R. prolixus* (Lent and Oliveira, 1944). The same symptom was observed in *T. infestans* nymphs exposed to the pyrethroid *cis*-permethrin (Alzogaray, 1996), and in different stages of *R. prolixus* treated with lethal concentrations of dieldrin (Peñalver and Villagran, 1955), lindane (Lima et al., 1992) or the botanical monoterpene eucalyptol (Moretti et al., 2015).

The appearance of intoxicated triatomines with abdominal distension is similar to that of individuals that have had *ad libitum* access to food. *R. prolixus* nymphs can ingest up to seven times their weight in a single blood meal (Beckel and Friend, 1964; Garcia et al., 1975). This is possible by the plasticization of the abdominal cuticle (a physico-chemical change controlled via the nervous system, that allows the cuticle to stretch its surface up to four times its size). Plasticization is triggered by the contact of the proboscis with a warm source (Ianowski et al., 1998).

Abdominal distention in intoxicated insects was attributed to the formation of gas inside the insects (Lent and Oliveira, 1944; Lima et al., 1992; Peñalver and Villagran, 1955). Still it has not been explained why this happens. It would be interesting determine if the cuticle of intoxicated insects suffering abdominal distension is plasticized as in feeding insects, and how this occurs in the absence of external stimuli.

Apart from intoxication symptoms, other sublethal effects of synthetic neurotoxic insecticides in *R. prolixus* have hardly been studied. DDT, lindane and members of the other families of synthetic neurotoxic insecticides induce secretion in the Malpighian tubules of *R. prolixus* (Casida and Maddrell, 1970). This causes an increase in the volume of rectal fluids at the expense of the hemolymph. Although the mechanism producing this effect has not been entirely uncovered, there is evidence that it is caused by the release of a factor from the mesothoracic ganglionic mass into

the hemolymph (Maddrell and Casida, 1971). This factor could be the diuretic hormone. This is a plausible hypothesis, as insecticides alter the electrical activity of neurosecretory cells in *R. prolixus* and other insects (Orchard, 1980; Orchard and Osborne, 1979) and induce the release of neurohormones (Granett and Leeling, 1972; Samaranayaka, 1974).

Exposure of *R. prolixus* to a sublethal concentration of dieldrin (7.2×10^{-5} g/day) extended the time of moulting and slowed down the rate of oviposition, but did not affect the number of moulting individuals nor the total amount of eggs laid (Arends and Rabinovich, 1980). Dieldrin also increased life expectancy when applied eight days after feeding, but decreased it when applied fifteen or more days later. To explain this last result, the authors suggested that insects that have fed recently have a higher metabolic rate that allows biotransforming the insecticides more efficiently than insects subjected to prolonged fasting. As an alternative explanation, they considered hormoligasis. The bioavailability of insecticides would be lower in insects that have fed recently because they present high levels of lipids and lipoproteins where insecticides are stored.

4.1.2. Ivermectin

Ivermectin is a semi-synthetic macrocyclic lactone derived from the microbial toxins avermectin B1a and B1b (both are biosynthesized by the bacterium *Streptomyces avermitilis*) (Ōmura, 2008). It is a broad-spectrum anti-parasitic drug currently used against onchocerciasis, lymphatic filariasis and strongyloidiasis (Ōmura and Crump, 2014).

Feeding *R. prolixus* nymphs and adults on mices previously treated with a commercial product based on ivermectin (0.2 mg/kg of body weight) produced high mortality (Azambuja et al., 1985). The surviving nymphs did not moult and egg production was lower in the surviving adult females.

4.2. Insect growth or development disruptors

Insect development and metamorphosis are regulated by two hormones, ecdysone and juvenile hormone. Ecdysone, a steroid secreted by the prothoracic gland, is a pro-hormone that is converted into 20-hydroxyecdysone in several tissues (Riddiford, 2012). The latter, also known as "moulting hormone", induces moulting.

The existence of juvenile hormones was first proposed by Wigglesworth (1936). They are a group of sesquiterpenoids that regulate metamorphosis and insect reproduction (Jindra et al., 2013). Juvenile hormones are secreted by the *corpora allata* gland. They prevent metamorphosis in immature stages and control ovarian development in adult females.

Various groups of substances interfere negatively with the growth or development of insects: juvenile hormone mimics (juvenoids), chitin synthesis inhibitors, anti-juvenile hormone compounds (precocenes), and ecdysteroid agonists (Pener and Dhadialla, 2012).

Many substances, both synthetic and natural, act like ecdysone agonists (Pener and Dhadialla, 2012), but there seem to no publications describing their effect on *R. prolixus*. On the contrary, there are many studies describing the effects of other growth or development disruptors in this insect.

4.2.1. Juvenoids

When a hemimetabolous insect is in the last nymphal stage, the production of juvenile hormone is physiologically interrupted and the next moult produces an adult. However, if the insect is treated with a juvenoid, metamorphosis does not take place and the insect moults to another juvenile stage (Devillers, 2013). The end result depends on the dose of juvenoid administered. Insects receiving a high dose will moult to juvenile individuals (supernumerary nymphs). Lower doses will produce individuals that are similar to adults, but that still have juvenile features (adultoids).

About one hundred and thirty juvenoids were evaluated in *R. prolixus* (Grove et al., 1974; Kul'kova et al., 1983; Patterson, 1973; Patterson and Schwarz, 1977; Pridantseva et al., 1978; Wigglesworth, 1963, 1969, 1973). The activity of these molecules was mainly quantified using the scoring system proposed by Wigglesworth (see Section 2).

Topical application of juvenile hormone from the moth *Hyalophora cecropia* on fifth instar nymphs of *R. prolixus* gave a score of 10 at a dose of 0.015 μg per insect (Wigglesworth, 1973). Juvenoids produced the same effect at doses between 0.0024 and more than 800 μg per insect.

The effect of a juvenoid strongly depends on its chemical structure. Among several farnesene derivatives, *trans,trans*-farnesyl methyl ether is the most active in *R. prolixus* (0.32 μg per insect produces a score of 10) (Wigglesworth, 1963). Farnesene derivatives with polar groups (–OH, $–NH_2$, –COOH) are less active than those without them; derivatives with an epoxy group in the $C_{6–7}$ position are less active than those that have it in the C_{10-11} position (Wigglesworth, 1969).

Other terpenoids that elicit an important effect in *R. prolixus* are: those with a methyl ester and an epoxide group at opposite ends of the molecule; *meta* substituted aryl terpenoids; aryl terpenoid amines with an ester substituted in the *para* position of the aromatic ring, and a methyl ester and an epoxy group in the positions 6,7 in the terpenoid side chain, respectively; and aryl terpenoid amines with two chlorines substituted in positions 2 and 5 of the aromatic ring (Patterson and Schwarz, 1977).

A divinyl cyclohexanone, that is a compound chemically unrelated to the juvenile hormone, showed very good juvenoid activity on larvae of the mosquito *Culex pipiens quinquefasciatus* (Desmarchelier and Fukuto, 1974). However, when it and other molecules belonging to the same group were tested on *R. prolixus*, juvenoid activity was not observed (Shekhter et al., 1978).

Geometric isomerism has an important effect on juvenoid activity. The juvenile hormone from *H. cecropia* has three chiral carbons. The most active isomeric configuration in *R. prolixus* is *trans, trans, cis* (Wigglesworth, 1969). It is four hundred and fifty-five times more effective than the synthetic form *cis, cis, cis*. Other isomeric configurations present intermediate activities. *Trans*-isomers of cyclohexenones are also more active than *cis*-isomers (Grove et al., 1974).

Juvenile hormones are quickly degraded by hydrolysis of the ester group (White, 1972). Therefore, the effect of juvenoids with this chemical group is limited by their rapid metabolism. Applying 1 μl/insect/day of juvenoids with an ester group is twelve times more effective that a single dose of 5 μl/insect one day after feeding (Patterson, 1973).

Another factor limiting the activity of juvenoids in *R. prolixus* is that this species is susceptible to these substances only during the ten days after feeding in the last nymphal stage (Wigglesworth, 1969). This time-frame represents only a small fraction of the insect's life-cycle, which takes at least four months to reach the adult stage (Arévalo et al., 2007).

Juvenoids also affect *R. prolixus* embryogenesis. The most powerful inhibitors of metamorphosis are also the ones with the highest activity on embryos (although there are some exceptions) (Patterson and Schwarz, 1979). However, prevention of eclosion only occurs if eggs are treated immediately after oviposition.

As evidenced by two-dimensional electrophoretic analysis, embryos treated with the juvenoid fenoxycarb present a pattern of proteins that is different to non-treated embryos (Kelly and Huebner, 1986, 1987). This suggests the presence of alterations in molecular events accompanying development.

The juvenoid pyriproxyfen elicits a good activity in *R. prolixus* (Langley et al., 1990). Its ED50 for inducing supernumerary nymphs is 0.15 μg per insect (twenty-eight times more effective than the juvenoid methoprene, used as positive control). Applying 10 μg of pyriproxyfen per egg caused 100% unviability, and young adult females treated with 10 μg produced non-viable eggs. Exposure of female adults to filter papers impregnated with 0.15 mg of pyriproxyfen in oil per cm^2 reduced their egg viability to zero.

Retinoids are sesquiterpenes derived from retinol (vitamin A) that are structurally related to juvenile hormones (Němec et al., 1993). In holometabolous insects, these substances participate in morphogenesis and produce a juvenilizing effect when applied on last instar nymphs. Injection of several retinoids (120 pmol per insect) to fourth instar nymphs of *R. prolixus* caused morphological abnormalities and death during moulting (Nakamura et al., 2007). Retinoids, methoprene and juvenile hormones also decreased phenoloxidase activity, which is involved in the insect immune response, suggesting that these compounds may have some effect on the insect's immune system.

4.2.2. Chitin synthesis inhibitors

Chitin is one of the main components of the insect cuticle (Cohen, 2010). It is a polymer of N-acetyl-D-glucosamine, and chitin synthase is the enzyme involved in the construction of the polymer chain. Molecules of the benzoylphenylurea family inhibit chitin synthesis (Pener and Dhadialla, 2012). Insects treated with these substances die during or after the following moult. The effects of two benzoylphenylureas, triflumuron and lufenuron, were tested in *R. prolixus.*

Exposure of *R. prolixus* fifth instar nymphs to cotton materials treated with triflumuron produced abnormalities in the external morphology and mortality during moulting (Vásquez et al., 2002). Further experiments on the same nymphal stage showed that topical, oral or continuous applications of triflumuron were all effective (Mello et al., 2008). In oral applications, it was added to a human blood meal; in continuous applications, insects were exposed to films of triflumuron placed on the bottom of Petri dishes. The lowest dose/concentration applied in each case (0.05 mg per insect in topical applications, 0.05 mg per ml in oral applications, and 0.007 mg per cm^2 in continuous applications) produced a high mortality rate and inhibited moulting in the surviving insects.

Beauveria bassiana is an entomopathogenic fungi commercialized as a biological control agent for certain insects (Skinner et al., 2014). Different combinations of this fungus and triflumuron were tested in *R. prolixus* and *R. pallescens* (another Chagas vector) (Saldarriaga et al., 2005). The activity of some of these combinations on insect mortality was slightly higher than the activity of each agent on its own. However, the heterogeneity of the results did not allow reaching a clear conclusion.

As chitin is a component of *R. prolixus* eggs, lufenuron affects oogenesis and egg laying (Mansur et al., 2010). Injections of 7.5 or 15 μg of lufenuron in females reduced the size of oocytes, the number of chorionated oocytes and the polymerization of N-acetylglucosamine in ovaries. It also induced oosorption (reabsorption of oocytes in ovaries). Compared to the control group, this substance reduced the number of eggs laid by 30 to 50%, and altered their shape and colour (pink at first, but then turning abnormally grey). Only 1% of the eggs laid by treated females hatched, whereas 98% of hatching was observed in the controls.

4.2.3. Precocenes

Precocenes I and II are naturally occurring 2H-chromenes produced by species of the plant genus *Ageratum* (Bowers, 1976). Both interrupt the secretion of juvenile hormone by damaging the *corpora allata* (Azambuja and Garcia, 1991). In the absence of juvenile hormone, immature stages moult to small sterile adults called adultoids (Bowers, 1976). As juvenile hormones, they also control ovarian development, therefore newly emerged females treated with precocenes are sterile (Azambuja and Garcia, 1991). There are no commercial products based on precocenes due to their low effectivity, and because one of its metabolites is a potentially carcinogenic epoxide (Coats, 1994; Pener, 2002).

Studies performed with *R. prolixus* during the 1980's contributed to understand the mode of action of precocenes. The results of these works were summarized and discussed in two discussion papers (Azambuja and Garcia, 1991; and Garcia et al., 1987). Briefly, contact, oral, and topical application of precocene I, II or their synthetic analogues delayed moulting and induced the formation of adultoids in all stages of *R. prolixus* (Azambuja and Garcia, 1987; Azambuja et al., 1981a,b, 1982, 1984; Garcia et al., 1984a,b, 1988; Jurberg et al., 1984; Pridantseva et al., 1981; Tarrant and Cupp, 1978; Tarrant et al., 1982). The greatest effect was observed when these compounds were orally applied. Blood meals containing 10–300 μg/ml of precocene II delayed ecdysis and induced precocious metamorphosis, all in a concentration-dependent manner (Azambuja et al., 1981a). A single oral application of precocene II (1, 5 or 10 μg/ml of blood) to adult females prevented yolk deposition, reduced the number of mature oocytes and decreased egg production in a dose-dependent manner (Azambuja and Garcia, 1987).

Two-(2-ethoxyethoxy)ethyl furfuryl ether, a furanyl-containing compound, showed a high precocene activity (Bowers et al., 1995). When tested on *R. prolixus*, its toxicity was similar to that of precocene II (ED50 values for the induction of precocious metamorphosis were 55 and 40 μg per insect, respectively) (Azambuja et al., 1996; Jurberg et al., 1997).

4.2.4. Azadirachtin and lignoids

Azadirachtin is a triterpenoid limonoid found in the seed of the neem tree, *Azadirachta indica* (Mordue et al., 2005). It is a strong insect feeding deterrent, and also induces delays in the development of juvenile stages, incomplete ecdysis, malformations during metamorphosis, sterility in eggs, and reduces fertility in adults (Morgan, 2009). The mode of action of azadirachtin is not exactly known, but it has been suggested that its effects on development could be caused by blocking microtubule formation in actively dividing cells (Morgan, 2009).

With fifteen articles reporting its toxicokinetics and diverse effects on *R. prolixus*, azadirachtin is one of the most studied insecticide in this insect. Most of these studies were published in the 1980's and early 1990's, and have already been summarized and discussed in several discussion papers (Azambuja and Garcia, 1992; Garcia et al., 1987, 1991; Rembold, 1987), so only a brief description of their main results is given below.

Azadirachtin presents good insecticidal activity on fourth instar nymphs of *R. prolixus*. ED50 values for the inhibition of feeding and ecdysis were 25 and 4×10^{-4}–4×10^{-2} μg per ml of blood, respectively (Garcia and Rembold, 1984; Garcia et al., 1984b). Injection of 10 ng per insect prevented ecdysis in 100% of the treated nymphs (Garcia et al., 1986). Ingestion of azadirachtin in a blood meal produced immunodepression in insects previously inoculated with *Enterobacter cloacae* (Azambuja et al., 1991). Azadirachtin decreases the title of prothoracicotropic hormone in hemolymph (Garcia et al., 1990b). It also decreases oocyte growth and egg production in adult *R. prolixus* females (Feder et al., 1988; Garcia et al., 1990a; Moreira et al., 1994).

One of the few and most complete studies on insecticide toxicokinetics in *R. prolixus* was carried out with azadirachtin (Garcia et al., 1989c). When orally administered, [22,23-$^3H_2$] dihydroazadirachtin crosses the gut wall into the haemolymph.

Following a peak 24 h after feeding, its level in the haemolymph gradually decreases as it is excreted unmetabolized.

Although azadirachtin is not toxic to *T. cruzi*, the causative agent of Chagas disease, it does prevent the proliferation of this protozoan in the gut of *R. prolixus* (Box 3).

Box 3. Azadirachtin and lignoids prevent the proliferation of *T. cruzi* in the gut of *R. prolixus*

Oral administration of azadirachtin before, with or after a blood meal containing *T. cruzi*, decreased the gut population of this parasite in *R. prolixus* and other triatomines to almost undetectable levels in a few weeks (Garcia et al., 1989a; Gonzalez and Garcia, 1992; Rembold and Garcia, 1989). However, these results are not due to the toxic effect of azadirachtin, as *T. cruzi* develops normally and does not loose its infectious capacity when cultured *in vitro* in the presence of this insecticide (Rembold and Garcia, 1989). The lignoids burchellin and NDGA also inhibited the establishment of *T. cruzi* in the gut of *R. prolixus* when applied before or together with the parasite (Cabral et al., 1999a).

Once in the midgut of *R. prolixus*, *T. cruzi* becomes attached to the perimicrovillar membrane of epithelial cells (Alves et al., 2007; Nogueira et al., 2007). This attachment seems crucial for the reproduction of the parasite. Taking this into account, it has been suggested that the effect of azadirachtin on triatomine gut populations of *T. cruzi* could be explained by the changes the insecticide produces on the epithelial cells of the midgut (Cortez et al., 2012).

The surface of normal midgut cells has a homogeneous distribution of densely packed microvilli, with well organized layers of extracellular membranes covering them. Inside the microvilli there are bundles of microfilaments. After the ingestion of azadirachtin, the microvilli are found clustered, giving the cell surface a wrinkled appearance. Furthermore, the extracellular membrane layers are disorganized and microfilaments are lost (Nogueira et al., 1997). Head transplantation from untreated donors and oral treatment with ecdysone re-establish both the morphology of gut epithelial cells and *T. cruzi* development (Cortez et al., 2012, Gonzalez et al., 1999). These results suggest that the modifications produced by azadirachtin in gut epithelial cells might be incompatible with the viability of *T. cruzi* in the gut of *R. prolixus*.

Lignoids are dimers of phenylpropanoid units biosynthesized by plants from more than seventy families (Pan et al., 2009). Some of these substances cause acute mortality, feeding deterrence and/or ecdysis disruption in different insects (Garcia and Azambuja, 2004). The activity of the lignoids burchellin, licarin A, nordihydroguaiaretic acid (NDGA), pinoresinol, podophyllotoxin and sesamin was studied in fourth instar nymphs of *R. prolixus*. The effects of lignoids on insects were reviewed some years ago (Garcia and Azambuja, 2004). The studies related to *R. prolixus* are briefly summarized below.

Podophyllotoxin was the most toxic lignoid, followed by licarin A (90% and 30% of acute mortality, respectively, after ingesting 100 μg per ml of a blood meal) (Cabral et al., 2000a). At first, it was thought that pinoresol did not have anti-moulting activity (Cabral et al., 1995); however, subsequent studies showed that both this substance and NDGA inhibit ecdysis (with ED50s lower than 20 μg per insect) (Cabral et al., 1999b; Garcia et al., 2000). Podophyllotoxin, burchellin and, to a lesser degree, licarin A, reduce the production of urine (Cabral et al., 2000b; Garcia et al., 2000). Burchellin seems to prevent the secretion of the diuretic hormone and/or induce the release of antidiuretic compounds (Cabral et al., 2000b). This lignoid is biotransformed to piperonyl alcohol and other metabolites in the hemolymph of *R. prolixus*, (Cabral et al., 2008).

As in the case of azadirachtin, some lignoids prevent the proliferation of *T. cruzi* in the gut of *R. prolixus* (Box 3).

4.3. Synthetic insect repellents

An insect repellent has been defined as "something that causes insects to make oriented movements away from its source" (White and Moore, 2015). Repellents are detected by the insects' olfactory sense (Bohbot and Dickens, 2010; Kain et al., 2013; Zermoglio et al., 2015). N,N-diethyl-3-methylbenzamide (DEET) is the more popular insect repellent. It is effective against all groups of biting arthropods, and has been used as a commercial product for more than fifty years (White and Moore, 2015).

There are very few studies on repellents in *R. prolixus*. During the 1980's, the first works showed what would be then confirmed in later investigations on this species and other Chagas vectors: triatomines are less sensitive to repellents than mosquitoes and other blood-sucking arthropods (Buescher et al., 1985, 1987). Another tendency observed in triatomines and other insects is that natural substances originated from plants are less effective than DEET (although there are some exceptions) (Alzogaray et al., 2000, 2011; Lucía et al., 2009; Rajendran and Sriranjini, 2008; Rice and Coats, 1994a,b).

Of eighteen experimental and commercial repellents tested by application on shaved rabbit skin, the pyrethroid permethrin, the experimental compound RH-398 and DEET showed the highest repellent activity on *R. prolixus* adults (Buescher et al., 1985). The median effective concentration (EC50) values of these three compounds were 7, 390, and 443 μg per cm^2, respectively. Indalone and dimetil-phtalate, repellents with confirmed efficiency in other arthropods, were ineffective against *R. prolixus*.

In a comparative study using twelve blood-sucking species of arthropods and the same methodology as described above, DEET proved to be as good a repellent as permethrin on eight of the species (Buescher et al., 1987). It showed a very low activity on *R. prolixus*, the mosquito *Ae. tueniorhynchus*, the flea *Xenopsylla cheopis*, and the tick *Ornithodoros parkeri*.

Although DEET has been used worldwide for more than five decades, its mode of action on a molecular level has only recently been studied (Corbel et al., 2009; Kain et al., 2013; Leal, 2014; Xu et al., 2014). There is evidence that it blocks the perception of the host's odours (Ditzen et al., 2008; McIver, 1981), and that itself can also be perceived by insects as an odour (Alzogaray et al., 2000; Alzogaray, 2015). In *R. prolixus*, it was shown that DEET and piperidine produce repellency in both the presence and absence of human odours, but icaridine only produces repellency when the host is absent (Zermoglio et al., 2015).

The fact that DEET is perceived by smell was supported by the capacity this molecule has to promote adaptation, a phenomenon associated with stimulus-receptor interaction (Dolzer et al., 2003). After different times of continuous exposure to DEET, the behavioural response of *R. prolixus* to this repellent decreased in a time-dependent way (Sfara et al., 2011). However, the response was recovered after interrupting the exposure. A similar decrease in the behavioural response was obtained when exposing *R. prolixus* to the natural monoterpene repellents menthyl acetate and geraniol (Lutz et al., 2014). Continuous exposure to one monoterpene decreased the repellency produced by the other. This last result might indicate that these substances share at least part of the olfactory pathway from the chemoreceptor to the central nervous system.

Nitric oxide is involved in the olfaction transduction pathway in insects (Bicker, 1998; Davies, 2000). It activates soluble guanylyl cyclase, leading to the synthesis of cGMP (Müller, 1997). The treatment of *R. prolixus* antennae with S-nitroso-acetyl-cysteine (SNAC), a nitric oxide donor, reduced DEET, menthyl acetate and geraniol

repellency (Lutz et al., 2014; Sfara et al., 2008). These results are consistent with the participation of nitric oxide in the sensorial perception of the repellents tested in *R. prolixus*.

Compared to DEET, the synthetic repellent IR3535 was ineffective in *R. prolixus* adults (Buescher et al., 1985). However, its lowest observed effect level in fifth instar nymphs was similar to DEET (74 μg/cm^2) when applied in the absence of a host-related stimuli (Alzogaray, 2015).

The response of *R. prolixus* to carbon dioxide, a cue associated with host localization, is influenced by age and physiological state (Bodin et al., 2009). However, DEET and IR3535 repellency was not modified by nymphs age (between 1 and 3 h to 99 days from last ecdysis).

The toxicity of DEET and IR3535 in insects has been scarcely studied. They both showed very poor insecticidal activity in *R. prolixus* (Alzogaray, 2015). A dose of 750 μg of DEET per insect produced only 40% mortality seven days after topical application. Simultaneous application of DEET and pyperonilbutoxide, an inhibitor of MFMO, doubled the mortality. These results suggest that MFMO are involved in the metabolism of DEET in this species. No symptoms of intoxication were observed after topical application of 750 μg per insect of IR3535, either with or without pyperonil butoxide.

4.4. Insecticides with other modes of action (including those non-specific or unknown)

4.4.1. Peptides

δ-Endotoxins are parasporal crystalline inclusions biosynthesized by the Gram positive bacterium *Bacillus thuringiensis* at the onset of sporulation and during the stationary phase (Palma and Berry, 2016; Palma et al., 2014). After being ingested by insects, δ-endotoxins are dissolved in the midgut and proteolitically activated by enzymes. Activated toxins bind to receptors located on midgut cell membranes, leading to cell disruption followed by insect death (Bravo et al., 2011).

In vitro studies showed that the 27×10^3 M_r δ-endotoxin from *B. thuringiensis* var. *israeliensis* alters the function of Malpighian tubules isolated from *R. prolixus* (Maddrell et al., 1988). Concentrations higher than 1 μg per ml modified both basal and luminal microvilli. They also dissociated intercellular junctions and induced cytoplasmic vacuolization. These microstructural alterations caused the microtubules to stop secreting fluids. δ-Endotoxin produced a rapid collapse in the basal membrane potential and increased membrane permeability to sucrose (Maddrell et al., 1989). These effects are consistent with the formation of pores in cell membranes.

Both these studies were published when the mode of action of δ-endotoxin was still unknown. Although Malpighian tubules are not the primary target of these toxins, their results showed that δ-endotoxins form aggregates that lead to the formation of pores, as now is known to occur in the gut epithelium.

Canatoxin is a protein initially isolated from the seeds of the jackbean *Canavia ensiformis* (Carlini and Guimarães, 1981). It is toxic for both mammals and insects (Carlini et al., 1984, 1997). Third instar nymphs of *R. prolixus* feeding on blood with canatoxin showed a decrease in post-feeding weight loss and a mortality peak 72–96 h after the meal (LD50 was 0.4–0.8 μg/mg of insect body weight) (Carlini et al., 1997). Canatoxin was similarly toxic in fourth instar nymphs of *R. prolixus*, but produced no mortality in adult males (Ferreira-DaSilva et al., 2000).

At the beginning of the 21st century, it was determined that canatoxin is an urease isoform (Follmer et al., 2001) which led to investigate whether other ureases from *C. ensiformis* showed insecticidal activity. Ureases are nickel-dependent enzymes biosynthesized by bacteria, fungi and plants (Modolo et al., 2015). They catalyse the hydrolysis of urea to ammonia and carbon dioxide. A review on insect toxicity by ureases was published a few years ago (Stanisçuaski and Carlini, 2012). The next paragraph briefly summarizes its effects on *R. prolixus*.

The toxicity of urease isoforms canatoxin and jack bean urease in insects is due to a 10-kDa peptide released by a cathepsin-like activity in the midgut (Carlini et al., 1997). Canatoxin, jack bean urease, the isoform JBURE-IIb and the recombinant peptide jaburetox-2Ec (equivalent to the product of canatoxin hydrolysis), inhibit serotonin-stimulated secretion of fluid in isolated Malpighian tubules (Mulinari et al., 2011; Stanisçuaski et al., 2010; Tomazetto et al., 2007). The effect of jack bean urease is eicosanoid metabolite- and calcium ion-dependent. On the other hand, the effect of jaburetox-2Ec is cGMP-dependent (Stanisçuaski et al., 2009). Neither jack bean urease nor jaburetox-2Ec are agonists of the antidiuretic hormone receptor RhorpCAPA-2 (Paluzzi et al., 2012).

More recently, the properties of jack bean urease with chemically modified lysine residues or carboxylic groups were evaluated (Real-Guerra et al., 2013). Chemical modification of lysine residues altered antidiuretic activity *in vitro*. When acidic residues were modified, the peptide was not hydrolysed by the digestive enzymes. Although these modifications did not altered the urease activity, they did decrease its toxicity in the cotton stainer bug *Dysdercus peruvianus* (toxicity in *R. prolixus* was not assessed).

Destruxins are a family of about forty cyclic hexadepsipeptides biosynthesized by *Metarhizium anisopliae* and other entomopathogenic fungi (Liu and Tzeng, 2012). They are toxic to viruses, bacteria, fungi and insects. Destruxin A has a strong toxic effect on isolated Malpighian tubules of *R. prolixus* stimulated with serotonin or cAMP (Ruiz-Sanchez et al., 2010). The median inhibitory concentration (IC50) of fluid secretion in tubules stimulated by the diuretic neurotransmisor serotonin was 3×10^{-7} M. A concentration of 10^{-6} M altered the transepithelial potential, disrupting the second and third phase of the triphasic response to serotonin. Destruxin A increased the pH of the secreted fluid, but did not affect Na^+ or K^+ concentrations nor ATP content in the tubules. These results support the hypothesis that the target of destruxin A in the Malpighian tubules could be related to the inhibition of the apical V-type H+ ATPase of tubule cells. V-ATPase proton pumps are located in the endomembranes of eukaryotic cells (Cotter et al., 2015). Among other functions, they provide energy for intracellular transportation across membranes. Similar effects of destruxins were reported in other biological models like yeast and human tumours (Liu and Tzeng, 2012).

4.4.2. Botanicals

Essential oils are complex mixtures of phenols, and mono- and sesquiterpenes, produced as secondary metabolites by aromatic species belonging to the families Myrtaceae, Lauraceae, Lamiaceae, Asteraceae, among others (Regnault-Roger et al., 2012). They are volatile, liposoluble and rarely coloured liquids with characteristic smells and tastes (Bakkali et al., 2008). Due to their low toxicity to mammals and low environmental residual activity, their insecticidal properties have been the object of a great number of studies in the last decades (Isman, 2010). The activity of several essential oils and some of their monoterpene components was studied in *R. prolixus* (for a review about toxicity of essential oils in triatomines, see Sainz et al., 2012).

Topical application of crude essential oils from the leaves of the Brazilian shrub *Policarpus spicatus* (0.5 and 1 μg per insect) and the American tree *Zanthoxylum caribaeum* (0.5–5 μg per insect) produced paralysis and high mortality on fifth instar nymphs of *R. prolixus* (Mello et al., 2007; Nogueira et al., 2014). Oral application of the essential oil from *P. spicatus* (5 μl per ml of blood) prolonged the moulting period for 48 h compared to controls. Only 5%

of the insects survived moulting. Nymphs continuously exposed to essential oil from *Z. caribaeum* (5 μl per cm^2 on filter paper) exhibited malformations in legs and wings.

Commercial essential oils from eucalyptus, geranium, lavender, mint, and orange applied as fumigants on first instar nymphs were between 6.7 and more than 16.8 times less toxic than dichlorvos, an organophosphate with renowned fumigant activity (Sfara et al., 2009). At the same stage, eighteen monoterpenes were between 21.9 and more than 116.7 times less toxic than dichlorvos (Moretti et al., 2013, 2015; Sfara et al., 2009). The most effective essential oils were thymol, eugenol, and eucalyptol.

Nine essential oils from plants of the genera *Artemisia*, *Mentha*, *Satureja*, and *Thymus* produced a repellent effect similar to that of dimethyl phtalato (positive control) (Sainz et al., 2012). Lavender and mint essential oils showed good repellent activity on first instar nymphs of *R. prolixus* (Sfara et al., 2009). Most monoterpenes tested on first instar nymphs showed repellence (Moretti et al., 2013, 2015; Sfara et al., 2009). The repellent effect of carvacrol, eugenol and geraniol was as good as DEET.

As described in Section 4.1.1, hyperactivity is one of the first visible symptoms in insects intoxicated with pyrethroids and other insecticides. Some monoterpenes present in essential oils also produce this effect. Of eleven monoterpenes studied in first instar *R. prolixus* nymphs, ten increased locomotor activity (Moretti et al., 2013, 2015). Hyperactivity was evidenced using concentrations of at least two orders of magnitude greater than that required by deltamethrin, a powerful insect hyperactivant, to produce the same effect. Carvacrol was the best hyperactivant while eugenol showed no effect.

The only publication found on the effects of monoterpenes on the life cycle of *R. prolixus* reports that exposure to citral or ruda vapours delayed moulting from fifth instar nymphs to adults by almost a month (Abramson et al., 2007).

The sites of action of only a few monoterpenes have been discovered. Eugenol and α-terpineol inhibit the octopaminergic receptor of the cockroach *Blattella germanica* (Enan, 2001); thymol and carvacrol inhibit the tyramine receptor of *Drosophila melanogaster* (Enan, 2005). The site of action of eucalyptol is unknown. In *R. prolixus*, it produces similar symptoms to those observed in insects intoxicated with neurotoxic insecticides such organophosphates and pyrethroids (Table 4) (Moretti et al., 2015). These symptoms suggest that the site of action of eucalyptol is located in the nervous system.

Only two studies were found on the insecticidal activity of plant extracts on *R. prolixus*. Ethanol extracts from the seeds of *Annona muricata*, *Mammea americana*, *Melia azedarach* and *Ricinus communis* produced acute toxicity when applied topically on fourth instar nymphs and eggs (Parra-Henao et al., 2007). The LC50 values ranged between 1.02% and 4.33% p/p (one and two orders of magnitude less toxic than the positive controls nicotin and deltamethrin, respectively). All the extracts produced repellency but only those from *A. muricata*, *M. azedarach* and *R. communis* showed ovicidal activity (25% or less when applied at a dose of 2.5–3% p/p).

Topical application of an ethanol extract of the giant star potato tree *Solanum macranthus* produced a LD50 of 172 mg per ml in *R. prolixus* (Carvajal et al., 2010). The main components of the extract were alkaloids, coumarins, terpenic lactoses and flavonoids.

Table 4
Visible symptoms of intoxication with eucalyptol observed in first instars of *T. infestans* and *R. prolixus*.

First phase (minor to moderate)	Second phase (severe)
Abnormal rest position: ventral contact of full body with support surface (at normal rest position, the body remains suspended over the support surface)	Reverse walk with elevated abdomen or normal walk with leaning abdomen
Reverse or lateral walk	Slow vertical oscillation of body over antero-posterior axis
Flexion of antennal flagella 90° outwards	"Praying mantis" position: forelegs flexed beneath elevated thorax
Proboscis extension (in absence of phagostimulants)	Unable to return to normal position after accidentally getting in dorsal decubitus position
Paralysis of hind legs	Total immobility
Antennas aligned forward	

Source: Moretti et al. (2015). Reproduced under license from John Wiley and Sons.

4.4.3. Fatty acids and aliphatic alcohols

The discovery that certain fatty acids are teratogenic in the house fly (Quraishi, 1971) lead to further investigations to determine whether they produced similar effects in *R. prolixus* (Gomez, 1985). Two short-chain unsaturated fatty acids, octinoic and undecylenic acid, produced malformations in legs and mouthparts in all the juvenile stages. Topical application on eggs (20 μl of octionoic acid per egg or 5 μl of undecylenic acid per egg) produced 100% of mortality before eclosion. Treatment of juvenile stages with doses between 0.1 and 2 μg per insect produced low mortality and malformations which were manifested in the stages following the one receiving the treatment. Undecylenic acid produced greater mortality and lower teratogenicity.

With the previous knowledge that aliphatic alcohols (C_2–C_{18}) present insecticidal properties in mosquitoes and human head lice (Sinniah, 1983; Cueto et al., 2002), the toxicity of 1-dodecanol was studied in *R. prolixus* (Cueto et al., 2005). This alcohol was 623,333 times more toxic in teneral first instar nymphs than in post-teneral ones (LC50 values were 0.03 and 18,700 ng/nymph, respectively). Notwithstanding, it presented similar toxicity in both groups when injected. This evidenced the fact that its passage through the cuticle has an important role in the toxicity of this molecule. Another effect of 1-dodecanol was manifested in the tanning of cuticle: 24 h-old nymphs treated 1-4 h after hatching looked as red as recently hatched nymphs. The mechanism underlying these effects has yet to be determined.

4.4.4. Inert dusts

Inert dusts exert their insecticide effect physically, causing abrasions to the insect cuticle (Golob, 1997; Wigglesworth, 1944, 1947). Without the protection of their cuticle, insects exposed to inert dusts become easily dehydrated. Moreover, the entry rate of insecticides to the organism increases when the insect cuticle is damaged. Inert dusts are mainly used for protecting stored products (Subramanyam and Roesli, 2000).

Exposure to a mixture of amorphous silica, ammonium fluorosilicate and pyrethrum during 2–15 min produced between 87.9% and 100% mortality in fifth instar nymphs and adults of both sexes of *R. prolixus* after 24 h (Kul'kova, 1975). Additionally, 100% of the insects lost their capacity to feed immediately after an exposure of 10 min.

R. prolixus adults were more susceptible than fifth instar nymphs when exposed to a mixture of silica aerogel and ammonium fluorosilicate, but no differences were found due to nymph sex (Kul'kova, 1983).

4.4.5. Sterilizants

Some alkylating agents are known to induce sterility in insects (Labrecque and Fye, 1978). Treatment of fifth instar nymphs of *R. prolixus* with 1-[bis(2-methyl-1-aziridinyl)phosphoryl]-2-methyla ziridine (metepa) sterilized in a dose-dependent manner the adults emerging in the following moult (Feliciangeli et al., 1972). Sterilization was quantified by registering the percentage of eclosion. A dose of 200 μg per insect completely sterilized all the treated males. Females were more tolerant, as a dose of 400 μg per insect only induced an average of 70% sterility. In addition to its

chemosterilant effect, metepa increased locomotor activity and reduced longevity in insects of both sexes (Feliciangeli, 1972). In similar experiments, but using individuals fed on mice infected with *T. cruzi*, faeces examination showed that metepa does not seem to affect the viability of the protozoan in the insect's gut (Feliciangeli et al., 1972).

Exposure to ionizating radiaton is another way of sterilizing insects. This led to the concept of the sterile insect technique (Lance and McInnis, 2005). This technique consists in sterilizing males and then releasing them in large quantities in the environment. The objective is for sterile males to mate with wild females who then produce sterile eggs, thus reducing the insect population. The sterile insect technique is used successfully for controlling dipterans of agricultural importance, especially the Mediterranean fruit fly, *Ceratitis capitata* (Shelly and McInnis, 2015).

One hundred per cent of sterility was observed mating normal females with males irradiated with gamma-rays (20,000 R) (Gómez et al., 1962; Gómez-Núñez et al., 1964). In other experiment, the application of 6,800 R produced an average fertility of 23.9% (Maudlin, 1976). Cytogenetic examination showed that infertility was related to chromosomal abnormalities. *R. prolixus* has holocentric chromosomes and a high rate of translocations were observed in the F1 progeny.

X-ray irradiation of male fifth instar nymphs produced sterile adults after moulting, but the sterilizing dose interfered with mating (Baldwin and Shaver, 1963). Radiation of adults affected mating to a lesser degree. In an experimental population where males treated with a dose of 17,500 R were introduced, there was an important decrease in viable eggs produced by non irradiated females. However, the sterile males died prematurely.

Beyond these results, the sterile insect technique is not a recommendable strategy for controlling domiciliary populations of *R. prolixus*, as it would imply releasing into the homes a massive amount of hematophagous insects that are vectors of an infectious disease.

5. *R. prolixus* intoxicated in the field

5.1 Past and present of R. prolixus control

The control of *R. prolixus* and other Chagas vectors mainly depends on sociocultural, political and economical factors (Dias et al., 1994). Chemical control of vectors and close surveillance of blood banks (to avoid reception from infected donors) are the main actions taken to interrupt the transmission of the disease (Moncayo, 1999).

Chemical control strategies are strongly influenced by insect toxicology. Studies in this area are essential when deciding what insecticides to apply. Insect toxicology also detects if there is insecticide resistance in insect populations. By studying the biological mechanisms involved in the resistance, it provides the necessary information to determine the best way to manage this problem.

The chemical control of *R. prolixus* began halfway through the last century and to the present day has been based on the application of synthetic neurotoxic insecticides. This is due to their high efficiency, the rapidity with which they elicit their effect and their adequate residual activity.

Vector-borne diseases are a problem that affects public health. Therefore, and as proclaimed by the WHO Constitution based on international and local treatises, the control of these diseases is the responsibility of the governments (WHO, 2014). The first governmental programme for controlling *R. prolixus* was organized in Venezuela in the mid-20th century (Feliciangeli, 2009). After proving the low toxicity of DDT in *R. prolixus* and the low residuality of lindane, the initial successful field trials carried out in this country in 1949 used dieldrin. This insecticide was chosen based on results obtained in a semi-field experience, where structures imitating the rural households of the area were sprayed with a wettable powder formulation of dieldrin (Gualtieri et al., 1985). A massive application of 1 g of dieldrin per m^2 was then initiated (Carrillo, 1954). Between 1952 and 1955, the treatment of some 300,000 homes reduced triatomine infestation in Venezuela by 95% (Aché and Matos, 2001). In 1966, the Venezuelan Program for the Control of Chagas Disease adopted a centralized and vertical structure, with a minimum decentralization of activities via local headquarters in the endemic area (Aché and Matos, 2001). This strategy reduced the levels of *R. prolixus* infestation even further.

In 1974, coinciding with an important increase in the rentability of petroleum, the Venezuelan programme became even more centralized (Briceño-León, 2006). A few years later, the price of petrol began to fall while the external debt of the country increased. This caused an economical crisis that led to the decentralization of the Chagas vector control programme and the health services were transferred to the states (Briceño-León, 2006).

In Colombia, there was no national policy for controlling Chagas disease before the 1980's (Guhl and Vallejo, 1999). It was only in 1995 that the prevalence of the disease became known and the following year the first national program for controlling Chagas vectors was initiated. Actions against Chagas were at first centralized but then became decentralized (Guhl et al., 2006). Decentralization was strongly criticized due to the important delays in transferring funds that resulted in defunded programmes, inability to access remote communities and the lack of national policies to direct, coordinate and audit activities.

In Central America, the first vector-control strategies against the two main vectors, *R. prolixus* and *T. dimidiata*, were also conceived as a vertical structure. In general, scarce resources were assigned to this activity and by the end of the 20th century, vector-control activities with trained personnel had almost disappeared from the region (Nakagawa et al., 2006).

Towards the end of the 80's, vector-control programmes for *R. prolixus* and other Chagas vectors began to use pyrethroids. The cyanopyretroids deltamethrin, alpha-cypermethrin, cyfluthrin, beta-cyfluthrin, and lambda-cyhalothrin were chosen for their high triatomicidal activity (Zerba, 1999). In some countries of the Southern Cone, beta-cypermethrin was also used. The recommended concentrations for these insecticides ranged between 25 and 50 mg per m^2.

At the same time, the countries of the Southern Cone started to discuss the need to perform a joint and sustained regional effort to interrupt the transmission of Chagas disease. The activities proposed were aimed at controlling insect vectors and monitoring blood banks. In order to achieve continuity in the intervention and vigilance of the disease, the discussion was focused on acquiring a sustained political compromise from the countries in the region. Based on these premises, the Initiative of the Southern Cone Countries (including Argentina, Bolivia, Brazil, Chile, Paraguay and Uruguay) was created in 1991 (Schofield and Dias, 1999). In these countries, that are outside the distribution area of *R. prolixus*, *T. infestans* is the main Chagas vector (or was, as in some cases the transmission has been interrupted). This initiative was followed by others countries from different regions of Latin America. In all the cases, insect-vector control was based on the use of cyanopyrethroids. Due to the high triatomicidal activity of these compounds, it was not necessary to use insecticides from other chemical families.

At the XIIIth Meeting of the Central American Health Sector held in Belize in 1997, a resolution was approved establishing that the control of Chagas disease should be a priority for the countries of that subcontinent (Ponce, 2007). In that same year, the Initiative of Central America and Mexico was launched, aiming to eliminate *R. prolixus* from the region, reduce domiciliary infestation of *T. dimidiata*, and interrupt transmission via blood transfusions (Ponce, 2007). It was supported by important donations, most of

which were provided by large Non-Governmental Organizations such as the Japan International Cooperation Agency. This initiative generated good results: by August 2011, Central America and Mexico were certified free of Chagas disease transmission by *R. prolixus* (Hashimoto and Schofield, 2012). Chagas still remains present in these countries due to the presence of other triatomine disease vectors, but the eradication of *R. prolixus* implied a significant decrease in its transmission.

The fast elimination of *R. prolixus* in Central America is attributed to the fact that this species is not autochthonous to the region, but was introduced accidentally at the beginning of the last century (Zeledón, 2004). It is believed that the *R. prolixus* individuals that accidentally entered San Salvador were originated from insects collected in La Guaira (Venezuela) in 1912. This sample was taken to Paris to study its application in the xenodiagnosis of Chagas disease. A sample of this colony was then taken to a laboratory in San Salvador, the capital city of El Salvador, where it was accidentally released in 1913. From there they dispersed to neighbouring countries. This would explain the discontinuous distribution of *R. prolixus* before it was eliminated from Central America. It was present in Venezuela and Colombia and in the area between Nicaragua and south Mexico, but was never found in Panama nor in central and south Costa Rica. *R. prolixus* was detected in north Costa Rica in 1953, but was rapidly eliminated applying lindane (Ruiz, 1953).

The sample collected in La Guaira and the fraction sent from the Parisian colony to Central America involved drastic bottle-necks, so the group of insects that arrived in San Salvador had very low genetic variability (Dujardin et al., 1998). This could explain the important differences found between the Central American and South American populations of the species. South American *R. prolixus* are larger and inhabit both sylvatic and domiciliary habitats; Central American *R. prolixus* are found exclusively in domiciliary habitats. It has been suggested that this latter characteristic contributed largely to its rapid elimination (Dujardin et al., 1998). In Colombia and Venezuela, the control of *R. prolixus* is more difficult because both countries have high rates of *R. prolixus* infestation in palm trees next to the households. The migration of these insects to the houses keeps Chagas vector transmission active despite the vector-control measures taken (Angulo et al., 2012; Gómez-Núñez, 1969; Zeledón and Rabinovich, 1981). Direct sequencing and microsatellite analysis confirmed that sylvatic *R. prolixus* colonize homes (Fitzpatrick et al., 2008).

The Andean Countries Initiative for controlling Chagas disease (including Venezuela, Colombia, Ecuador and Peru) was also created in 1997 within the framework of the Hipólito Unanue Agreement (Moncayo, 1999). It would then become partially superposed by the Initiative of the Amazon Countries (including Bolivia, Brazil, Colombia, Ecuador, Guyana, French Guiana, Peru, Surinam and Venezuela) (Coura et al., 2014).

The objective of the Initiative of the Andean Countries was to interrupt the transmission of Chagas disease via domiciliary vector control and serological monitoring. With the coordination of the Pan-American Health Organization, a substantial improvement was achieved in blood bank surveillance, especially in Colombia (Guhl, 2007). However, the progress in control activities has been slower because some of the countries involved do not have organized national programmes of domiciliary vector-control (Salvatella and Schofield, 2006).

Despite the difficulties for controlling *R. prolixus* in these countries, the X Meeting of the Intergovernmental Commission of the Andean Initiative for Controlling Vector and Transfusion Transmission and Medical Attention for Chagas Disease, held in Bogota in 2014, certified the interruption of *T. cruzi* vector transmission in ten municipalities from four endemic departments in Colombia (OPS/OMS, 2014). Venezuela has still not interrupted vector transmission in its endemic states.

Therefore, *R. prolixus* is still an important Chagas vector in some areas of Venezuela and Colombia. Due to chemical control, it is no longer present in Mexico or Central America. However, Chagas disease is still a problem in these countries due to the presence of other triatomine vectors.

5.2. Resistance to insecticides

Pesticide resistance is a "genetically based decrease in susceptibility to a pesticide" (Tabashnik et al., 2014). Along the generations of insect populations, alleles conferring insecticide resistance appear spontaneously. The application of insecticides is a strong selective force that determines the elimination of susceptible individuals and survival of resistant specimens. Insecticide resistance is mainly conferred by changes in the cuticle that reduce the entry of the insecticide into the organism (reduced penetration resistance), changes in enzymatic activity that enhance insecticide biotransformation (metabolic resistance), and changes in the site of action that reduce binding to the insecticide (target site resistance) (Tabashnik et al., 2014). The knowledge of the toxicological characteristics and biological mechanisms of insecticide resistance is indispensable for managing this problem when it is manifested in the field. Two reviews were recently published on the history, evolution and management of triatomine insecticide resistance (Mougabure-Cueto and Picollo, 2015; Pessoa et al., 2015). Below is a brief description of this subject regarding *R. prolixus*.

The first cases of triatomine insecticide resistance were detected in *R. prolixus* populations from Venezuela towards the end of the 1960's. By then, the Venezuelan Anti-Chagas campaigns had been using dieldrin for about a decade (Aché and Matos, 2001). Upon reports suggesting that this insecticide was not generating the expected results, the government implemented a programme to investigate the cause. Bioassays carried out in 1969 showed that individuals from the states of Cojedes and Trujillo survived dieldrin concentrations that were lethal to the laboratory reared susceptible colony (Valdivieso et al., 1971). The highest survival rates were observed in insects from Santo Domingo and other communities in the state of Trujillo. This is considered the first report of resistance to dieldrin in triatomines (Nocerino, 1976). Based on these results, dieldrin was replaced by the organophosphate fenthion or the carbamate propoxur in the affected communities (Nocerino, 1976). Two years later, a lower susceptibility to fenthion and propoxur was reported in insects from Santo Domingo (Nocerino, 1976; Nelson and Colmenares, 1979a). Meanwhile, due to toxicological and cost reasons, dieldrin began to be replaced by lindane in Venezuela. A few years later, some cases of lower susceptibility to this insecticide were reported.

From a group of insects collected at a household in Trujillo in 1969, a colony was reared under laboratory conditions. After exposure to sublethal concentrations of dieldrin during several generations, it became increasingly resistant to this organochlorine (Nelson and Colmenares, 1979a; Nocerino, 1972, 1976). This might apparently be the only case of laboratory-selected resistance to insecticides in *R. prolixus*.

The first bioassays looking for dieldrin resistance had been carried out exposing insects to filter papers impregnated with three concentrations of insecticide, 0.8, 0.16 and 4%, that in the susceptible colonies produced 40%, 86% and 100% mortality, respectively (Valdivieso et al., 1971). The WHO then recommended topical application of a higher number of doses (Nelson and Colmenares, 1979b). This allowed calculating LD50 values and with them, the Resistance Ratio coefficient (RR) = LD50 in field insects/LD50 in laboratory susceptible insects. The RR indicates the amount of insecticide needed to produce the same mortality in field insects as in laboratory susceptible insects.

The RR values allowed quantifying the resistance of the aforementioned laboratory-selected colony of *R. prolixus* (Nelson and Colmenares, 1979b). Selection with dieldrin during various generations made the colony highly resistance to this insecticide, with RR values over 550 (killing 50% of the resistant insects required over 550 times more dieldrin than used for the susceptible colony).

The next published resistance study in *R. prolixus* was carried out at the end of the last century. Bioassays were performed using descendants of individuals collected in the Venezuelan state of Carabobo. The insecticides tested were dieldrin and five pyrethroids. The RR values for dieldrin was 3, while the values for the pyrethroids varied between 4.5 (lambda-cyhalothrin) and 12.4 (cypermethrin) (Vassena et al., 2000). In the state of Carabobo, triatomines were controlled using dieldrin, lindane and the organophosphate fenitrothion, but no pyrethroids had been applied. The results were interpreted as cross-resistance, a phenomenon that is manifested when insects selected with a determined insecticide become resistant to insecticides to which they have not been exposed (Tabashnik et al., 2014). This can happen when insecticides belonging to different families share a same metabolic pathway or act on the same primary target. Another explanation proposed was that the population of *R. prolixus* might have been exposed to the intensive use of pyrethroids for agriculture in the state of Carabobo.

Studies on the mechanisms of triatomine resistance to insecticides have only recently begun. Most of these have been carried out with *T. infestans*, where metabolic and target site factors involved in resistance have been identified (Mougabure-Cueto and Picollo, 2015). Deltamethrin resistance in *R. prolixus* individuals from Carabobo was attributed to an increase in MFMO activity, because the inhibition of these enzymes with pyperonil butoxide decreased resistance (Vassena et al., 2000).

The only report of insecticide resistance in *R. prolixus* outside Venezuela is from Colombia, where low values of RR to deltamethrin were detected in two samples of *R. prolixus* from the department of Casanare (Reyes et al., 2009).

6. Conclusions

The four main families of neurotoxic synthetic insecticides (organochlorines, organophosphates, carbamates and pyrethroids) are the substances that have been used the most for controlling pests in the last seventy years. In particular, they are the only insecticides that have been used for controlling *R. prolixus* in countries where this insect is a Chagas disease vector. These chemicals have received a lot of attention by insect toxicologists and therefore their interaction with insects is well known. Nevertheless, there are very few studies on their toxicokinetics and toxicodynamics in *R. prolixus*. This is probably due to the fact that *R. prolixus* is a pest that affects a few economically disadvantaged countries, where it is the vector of a neglected disease. The insects of medical and domestic importance used as models for studying the metabolism and mode of action of the main insecticides are mosquitoes, the housefly, and the American and German cockroaches. These species are cosmopolitan and produce an enormous impact on human health and goods.

Many details on the mode of action of insect growth or development disruptors (juvenoids, chitin synthesis inhibitors, precocenes, azadirachtin, lignoids) have been revealed in *R. prolixus*. However, based on their particular mode of action, these insecticides have not been used and probably will never be used for controlling Chagas vectors (Zerba, 1999). The application of insect growth or development disruptors produces delayed ecdysis and abnormalities after moulting. Therefore, the effects of these insecticides on triatomine populations would be slow compared to the fast mortality obtained when applying organophosphates or pyrethroids that drastically and immediately reduce the risk of becoming infected with Chagas. Despite the high selectivity and effectivity of some experimental and commercial juvenoids in triatomines, *R. prolixus* is only susceptible to these insecticides for a few days after feeding in the last nymphal stage. This represents a minute fraction of a life cycle that lasts several months.

It is only less than ten years ago that the mode of action of insect repellents has began to be understood and it is a practically unexplored subject in triatomines. One of the great questions that awaits to be answered is why substances like DEET or IR3535, that are so effective in mosquitoes, flies and other hematophagous insects, present such low activity in *T. infestans* and *R. prolixus*.

The recent sequencing of the *R. prolixus* genome will have a great impact on the study of this insect (Mesquita et al., 2015). Regarding toxicology, this advance provides researchers with the sequences of the genes encoding detoxification enzymes and the action sites of all the insecticides. This information will be very useful for understanding the molecular basis of resistance to these chemicals (Schama et al., 2015). Genomics will allow performing large-scale comparisons between the genomes of susceptible and resistant individuals. These sort of studies carried out in *Musca domestica* led to the identification of genes that were not suspected of being related to insecticide resistance (Pedra et al., 2004).

Using genomic information, new potential molecular targets could be identified for developing new insecticides. But the history of Chagas disease vectors control indicates that the probability that these findings lead to the development of new commercial tools for controlling triatomines is practically null. The development of new insecticides is an activity carried out by large international companies seeking short term results. These companies use faster means to identify new insecticides, such as random synthesis, and the evaluation of natural compounds and their semi-synthetic derivates.

It is highly likely that the future of toxicology of *R. prolixus* and other triatomines continues to be dedicated to providing information for Chagas vectors control programmes. Bioassays with new insecticides and insecticide resistance monitoring will be priority studies.

Acknowledgments

R.A.A. and E.N.Z. are members of the Carrera del Investigador Científico del CONICET. Salaries were provided by CITEDEF, CONICET, and UNSAM. Authors research mentionated in this review was supported by several subsidies from the Agencia Nacional de Promoción Científica y Tecnológica of Argentina (ANPCyT), CONICET, and WHO. The authors thanks to Guest Editor Claudio Lazzari for his invitation to write this review, Catriona Kirkwood for translating the manuscript into English, Sofia Mengoni for preparing the Graphical Abstract, Milán Szőri (University of Szeged, Hungary) for the information he provided on P. Schmialek, and two anonymous reviewers for their valuable comments. For locating, scanning and e-mailing to authors the bibliographical material that was unavailable online, thanks are also due to the following people and organisms: Sandra Barbazán y Florencia Provenzano (UNSAM, San Martín, Argentina), Gabriela Diessler (IBYME, Buenos Aires, Argentina), Darjaniva Molina de Fernández (IAE-CEESA, Maracay, Venezuela), Sonia Pirotzky (Biblioteca de la SEA, La Plata, Argentina), Marlene Reyes Jerez (CINTROP-UIS, Piedecuesta, Colombia), Eve Watson (Royal Society of Arts, London, UK), National Library of Russia (Moscow). By request of the authors, WHO Institutional Repository for Information Sharing kindly uploaded to the web several unpublished WHO documents about *R. prolixus* toxicology. Now they can be downloaded for free at <apps.who.int/iris>.

References

*Abramson, C.I., Aldana, E., Sulbaran, E., 2007. Exposure to citral, cinnamon and ruda disrupts the life cycle of a vector of chagas disease. Am. J. Environ. Sci. 3, 7–8.

Aché, A., Matos, A.J., 2001. Interrupting Chagas disease transmission in Venezuela. Rev. Inst. Med. Trop. Sao Paulo 43, 37–43.

Agosin, M., Morello, A., Scaramelli, N., 1964. Partial characterization of the *in vivo* metabolites of DDT-C^{14} in *Triatoma infestans*. J. Econ. Entomol. 57, 974–977.

Aguilar, H.M., Abad-Franch, F., Dias, J.C.P., Junqueira, A.C.V., Coura, J.R., 2007. Chagas disease in the Amazon region. In: Mem. Inst. Oswaldo Cruz 102 (Suppl. I), 47–55.

Alves, C.R., Albuquerque-Cunha, J.M., Mello, C.B., Garcia, E.S., Nogueira, N.F., Bourguingnon, S.C., Souza, W., Azambuja, P., Gonzalez, M.S., 2007. *Trypanosoma cruzi*: attachment to perimicrovillar membrane glycoproteins of *Rhodnius prolixus*. Exp. Parasitol. 116, 44–52.

Alzogaray, R.A., 1996. Caracterización de la toxicidad de piretroides en *Triatoma infestans* (Klug) (Ph.D. dissertation). Universidad de Buenos Aires.

*Alzogaray, R.A., 2015. Behavioral and toxicological responses of *Rhodnius prolixus* (Hemiptera: Reduviidae) to the insect repellents DEET and IR3535. J. Med. Entomol. http://dx.doi.org/10.1093/jme/tjv194.

Alzogaray, R.A., Fontán, A., Zerba, E.N., 1997. Evaluation of hyperactivity produced by pyrethroid treatment on third instar nymphs of *Triatoma infestans* (Hemiptera: Reduviidae). Arch. Insect Biochem. Physiol. 35, 323–333.

Alzogaray, R.A., Fontán, A., Zerba, E.N., 2000. Repellency of deet to nymphs of *Triatoma infestans*. Med. Vet. Entomol. 14, 6–10.

Alzogaray, R.A., Lucia, A., Zerba, E.N., Masuh, H., 2011. Insecticidal activity of essential oils from eleven *Eucalyptus* spp and two hybrids: lethal and sublethal effects of their major components on *Blattella germanica*. J. Econ. Entomol. 104, 595–606.

Alzogaray, R.A., Zerba, E.N., 1997. Incoordination, paralysis and recovery after pyrethroids treatments on third instars of *Triatoma infestans*. Mem. Inst. Oswaldo Cruz 92, 431–435.

Alzogaray, R.A., Zerba, E.N., 2001a. Behavioral response of fifth instar nymph of *Triatoma infestans* (Hemiptera: Reduviidae) to pyrethroids. Acta Trop. 78, 51–57.

*Alzogaray, R.A., Zerba, E.N., 2001b. Third instar nymphs of *Rhodnius prolixus* exposed to alpha-cyanopyrethroids: from hyperactivity to death. Arch. Insect Biochem. Physiol. 46, 119–126.

Amelotti, I., Catalá, S.S., Gorla, D.E., 2010. The effects of cypermethrin pour-on and piperonyl butoxide on *Triatoma infestans* under laboratory conditions. J. Med. Entomol. 47, 1135–1140.

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.

Angulo, V., Esteban, L., Luna, K., 2012. *Attalea butyracea* próximas a las viviendas como posible fuente de infestación domiciliaria por *Rhodnius prolixus* (Hemiptera: Reduviidae) en los Llanos Orientales de Colombia. Biomédica 32, 277–285.

*Arends, A., Rabinovich, J.E., 1980. Effects of sublethal dose exposure to dieldrin on *Rhodnius prolixus* (Hemiptera: Reduviidae) under laboratory conditions. Acta Trop. 37, 5–15.

Arévalo, A., Carranza, J.C., Guhl, F., Clavijo, J.A., Vallejo, G.A., 2007. Comparación del ciclo de vida de *Rhodnius colombiensis* Moreno, Jurberg & Galvão, 1999 y *Rhodnius prolixus* Stål, 1872 (Hemiptera, Reeduviidae, Triatominae) en condiciones de laboratorio. Biomédica 27 (Suppl. 1), 119–129.

*Azambuja, P., Furtado, A.F., Garcia, E.S., 1984. Effects of juvenile hormone analogue on ecdysis prevention induced by precocene in *Rhodnius prolixus* (Hemiptera: Reduviidae). Mem. Inst. Oswaldo Cruz 79, 419–423.

*Azambuja, P., Garcia, E.S., 1987. Short- and long-term effects of proallatotoxin (ethoxyprecocene II) on *Rhodnius prolixus* females. Mem. Inst. Oswaldo Cruz 82, 247–251.

Azambuja, P., Garcia, E.S., 1991. Effects of proallatotoxins (precocenes) on the development and reproduction of *Rhodnius prolixus*: some data. In: Mem. Inst. Oswaldo Cruz 86 (Suppl. II), 113–115.

Azambuja, P., Garcia, E.S., 1992. Effects of azadirachtin on *Rhodnius prolixus*. Immunity and trypanosoma interaction. In: Mem. Inst. Oswaldo Cruz 87 (Suppl. V), 69–72.

Azambuja, P., Garcia, E.S., Bowers, W.S., 1996. Biological activity of a furanyl anti-juvenile hormonal compound on triatomine larvae. Pestic. Sci. 47, 159–164.

*Azambuja, P., Garcia, E.S., Furtado, A.F., 1981a. Action du précocène II sur le retard de la mue et de la métamorphose chez *Rhodnius prolixus* (Heteroptère). C. R. Acad. Sci. 292, 1173–1175.

*Azambuja, P., Garcia, E.S., Ratcliffe, N.A., Warthen Jr., J.D., 1991. Immune-depression in *Rhodnius prolixus* induced by the growth inhibitor, azadirachtin. J. Insect Physiol. 37, 771–777.

*Azambuja, P., Garcia, E.S., Ribeiro, J.M.C., 1981b. Effects of ecdysone on the metamorphosis and ecdysis prevention induced by precocene II in *Rhodnius prolixus*. Gen. Comp. Endocr. 45, 100–104.

*Azambuja, P., Gomes, J.E.P.L., Lopes, F., Garcia, E.S., 1985. Efficacy of ivermectin against the bloodsucking insect, *Rhodnius prolixus* (Hemiptera, Triatominae). Mem. Inst. Oswaldo Cruz 80, 439–442.

*Azambuja, P.D., Bowers, W.S., Ribeiro, J.M.C., Garcia, E.S., 1982. Antifeedant activity of precocenes and analogs on *Rhodnius prolixus*. Experientia 38, 1054–1055.

Bakkali, F., Averbeck, S., Averbeck, D., Adomar, M., 2008. Biological effects of essential oils. A review. Food Chem. Toxicol. 46, 446–475.

*Baldwin, W.F., Shaver, E.L., 1963. Radiation induced sterility in the insect *Rhodnius prolixus*. Can. J. Zool. 41, 637–648.

Beckel, W.E., Friend, W.G., 1964. The relation of abdominal distension and nutrition to molting in *Rhodnius prolixus* (Stål) (Hemiptera). Can. J. Zool. 42, 71–78.

Bede, J.C., Teal, P.E.A., Goodman, W.G., Tobe, S.S., 2001. Biosynthetic pathway of insect juvenile hormone III in cell suspension cultures of the sedge *Cyperius iria*. Plant Physiol. 127, 584–593.

Bérenger, J.M., Pluot-Sigwalt, D., Pagès, F., Blanchet, D., Aznar, C., 2009. The triatominae species of French Guiana (Heteroptera: Reduviidae). Mem. Inst. Oswaldo Cruz 104, 1111–1116.

Bicker, G., 1998. NO news from insect brains. Trends Neurosci. 21, 349–355.

*Blazquez, J., Bianchini, C., 1957. Estudio de los primeros casos de intoxicación por dieldrin y aplicación de la xenodeterminación en humanos. Bol. Oficina Sanit. Panam. 43, 504–511.

Bodin, A., Vinauger, C., Lazzari, C.R., 2009. State-dependency of hostseeking in *Rhodnius prolixus*: the post-ecdysis time. J. Insect Physiol. 55, 574–579.

Bohbot, J.D., Dickens, J.C., 2010. Insect repellents: modulators of mosquito odorant receptor activity. PLoS One 5, e12138. http://dx.doi.org/10.1371/journal.pone.0012138.

Bowers, W.S., 1976. Discovery of insect antiallatotropins. In: Gilbert, L.I. (Ed.), The Juvenile Hormone. Plenum Press, New York, pp. 394–408.

Bowers, W.S., Unnithan, G.C., Fukushima, J., Toda, J., Sugiyama, T., 1995. Synthesis and biological activity of furanyl anti-juvenile hormonal compounds. Pestic. Sci. 43, 1–11.

Bravo, A., Likitvivatanavong, S., Gill, S.S., Soberón, M., 2011. *Bacillus thuringiensis*: a story of a successful bioinsecticide. Insect Biochem. Mol. Biol. 41, 423–431.

Briceño-León, R., 2006. Enfermedad de Chagas, descentralización y renta petrolera en Venezuela. In: Yadón, Z.E., Gürtler, R.E., Tobar, F., Medici, A.C. (Eds.), Descentralización y Gestión del Control de las Enfermedades Transmisibles en América Latina. Organización Panamericana de la Salud, Buenos Aires, pp. 325–342.

*Buescher, M.D., Rutledge, L.C., Wirtz, R.A., 1987. Studies on the comparative effectiveness of permethrin and deet against bloodsucking arthropods. Pestic. Sci. 21, 165–173.

*Buescher, M.D., Rutledge, L.C., Wirtz, R.A., Nelson, J.H., 1985. Laboratory repellent tests against *Rhodnius prolixus* (Heteroptera: Reduviidae). J. Med. Entomol. 1, 49–53.

*Busvine, J.R., Barnes, S., 1948. Observations on mortality among insects exposed to dry insecticidal films. Bull. Entomol. Res. 38, 81–90.

Buteler, M., Sofie, S.W., Weaver, D.K., Driscoll, D., Muretta, J., Stadler, T., 2015. Development of nanoalumina dust as insecticide against *Sitophilus oryzae* and *Rhyzopertha dominica*. Int. J. Pest Manage. 61, 80–89.

Buxton, A.M., 1930. The biology of a blood-sucking bug, *Rhodnius prolixus*. Trans. Ent. Soc. Lond. 78 (Part II), 227–256.

*Cabral, M.M.O., Azambuja, P., Gottlieb, O.R., Garcia, E.S., 1999a. Neolignans inhibit *Trypanosoma cruzi* infection of its triatomine insect vector, *Rhodnius prolixus*. Parasitol. Res. 85, 184–187.

*Cabral, M.M.O., Azambuja, P., Gottlieb, O.R., Garcia, E.S., 2000a. Effects of some lignans and neolignans on the development and excretion of *Rhodnius prolixus*. Fitoterapia 71, 1–9.

*Cabral, M.M.O., Garcia, E.S., Gottlieb, O.R., Kelecom, A., 2008. Degradation of the neolignan, burchellin in the hemolymph of the bloodsucking insect *Rhodnius prolixus*. Fitoterapia 79, 59–63.

*Cabral, M.M.O., Garcia, E.S., Kelecom, A., 1995. Lignanes from the Brazilian *Melia azedarach*, and their activity in *Rhodnius prolixus* (Hemiptera, Reduviidae). Mem. Inst. Oswaldo Cruz 90, 759–763.

*Cabral, M.M.O., Kelecom, A., Garcia, E.S., 1999b. Effects of the lignan, pinoresinol on the moulting cycle of the bloodsucking bug *Rhodnius prolixus* and of the milkweed bug *Oncopeltus fasciatus*. Fitoterapia 70, 561–567.

*Cabral, M.M.O., Kollien, A.H., Kleffmann, T., Azambuja, P., Gottlieb, O.R., Garcia, E.S., Schaub, G.A., 2000b. *Rhodnius prolixus*: effects of the neolignan burchellin on *in vivo* and *in vitro* diuresis. Parasitol. Res. 86, 710–716.

Camenzuli, L., Scheringer, M., Hungerbühler, K., 2015. Local organochlorine pesticide concentrations in soil put into a global perspective. Environ. Pollut. http://dx.doi.org/10.1016/j.envpol.2015.08.028.

Carlini, C.R., Gomes, C.B., Guimarães, J.A., Markus, R.P., Sato, H., Trolin, G., 1984. Central nervous effects of the convulsant protein canatoxin. Acta Pharmacol. Toxicol. 54, 161–166.

Carlini, C.R., Guimarães, J.A., 1981. Isolation and characterization of a toxic protein from *Canavalia ensiformis* (jack bean) seeds, distinct from convanavalin-A. Toxicon 19, 667–676.

*Carlini, C.R., Oliveira, A.E.A., Azambuja, P., Xavier-Filho, J., Wells, M.A., 1997. Biological effects of canatoxin in different insect models: evidence for a proteolytic activation of the toxin by insect cathepsinlike enzymes. J. Econ. Entomol. 90, 340–348.

Carrillo, S.J., 1954. El empleo del dieldrin en Venezuela. Bol. Oficina Sanit. Panam. 37, 76–81.

*Carrillo, S.J., Vicente, J.B., 1955. Xenodeterminación toxicológica de dieldrin en la sangre. Bol. Oficina Sanit. Panam. 39, 296–299.

*Carvajal, J.E.H., Orjuela, Y.F., Vallejo, G.A., 2010. Insecticidal activity assessment of *Solanum macranthum* (Dunal) on IV and V stages nymphs of *Rhodnius pallescens*, *Rhodnius prolixus*, *Rhodnius colombiensis*. Rev. Cubana Farm. 45, 71–78.

Casida, J., Durkin, K.A., 2013. Neuroactive insecticides: targets, selectivity, resistance, and secondary effects. Annu. Rev. Entomol. 58, 99–117.

Casida, J.E., 2009. Pest toxicology: the primary mechanisms of pesticide action. Chem. Res. Toxicol. 22, 609–619.

*Casida, J.E., Maddrell, S.H.P., 1970. Diuretic hormone release on poisoning *Rhodnius* with insecticide chemicals. Pestic. Biochem. Physiol. 1, 71–83.

Casida, J.E., Quistad, G.B., 1998. Golden age of insecticide research: past, present, or future? Annu. Rev. Entomol. 43, 1–16.

Casida, J.E., Quistad, G.B., 2001. Introduction. Insect Toxicology 2000. Pest Manag. Sci. 57, 875–876.

Coats, J.R., 1994. Risks from natural versus synthetic insecticides. Annu. Rev. Entomol. 39, 489–515.

*Cockburn, J.M., 1972. Laboratory investigations bearing on possible insecticide resistance in triatomid bugs. WHO/VBC/72.359 http://apps.who.int/iris/bitstream/10665/201700/1/WHO_VBC_72.359_eng.pdf.

Cohen, E., 2010. Chitin biochemistry: synthesis, hydrolysis and inhibition. Adv. Insect Physiol. 38, 5–74.

Corbel, V., Stankiewicz, M., Pennetier, C., Fournier, D., Stojan, J., Girard, E., Dimitrov, M., Molgó, J., Hougard, J.M., Lapied, B., 2009. Evidence for inhibition of cholinesterases in insect and mammalian nervous systems by the insect repellent deet. BMC Biol. 5 (7), 47. http://dx.doi.org/10.1186/1741-7007-7-47.

*Cortez, M.R., Provençano, A., Silva, C.E., Mello, C.B., Zimmermann, L.T., Schaub, G.A., Garcia, E.S., Azambuja, P., Gonzalez, M.S., 2012. *Trypanosoma cruzi*: effects of azadirachtin and ecdysone on the dynamic development in *Rhodnius prolixus*. Exp. Parasitol. 131, 363–371.

Cotter, K., Stransky, L., McGuire, C., Forgac, M., 2015. Recent insights into the structure, regulation, and function of the V-ATPases. Trends Biochem. Sci. 40, 611–622.

Coura, J.R., Dias, J.C.P., 2009. Epidemiology, control and surveillance of Chagas disease – 100 years after its discovery. Mem. Inst. Oswaldo Cruz 104 (Suppl. I), 31–40.

Coura, J.R., Viñas, P.A., Junqueira, A.C.V., 2014. Ecoepidemiology, short history and control of Chagas disease in the endemic countries and the new challenge for non-endemic countries. Mem. Inst. Oswaldo Cruz 109, 856–862.

Cueto, G.M., Audino Gonzalez, P., Vassena, C.V., Picollo, M.I., Zerba, E.N., 2002. Toxic effect of aliphatic alcohols against susceptible and permethrin-resistant *Pediculus humanus capitis* (Anoplura: Pediculidae). J. Med. Entomol. 39, 457–460.

*Cueto, G.M., Zerba, E., Picollo, M.I., 2005. Biological effect of 1-dodecanol in teneral and post-teneral *Rhodnius prolixus* and *Triatoma infestans* (Hemiptera: Reduviidae). Mem. Inst. Oswaldo Cruz 100, 59–61.

Davies, S., 2000. Nitric oxide signalling in insects. Insect Biochem. Mol. Biol. 30, 1123–1138.

Desmarchelier, J.M., Fukuto, T.R., 1974. Toxicological effects produced by some 1,3-benzodioxoles, catechols, and quinones in *Culex* mosquito larvae. J. Econ. Entomol. 67, 153–158.

Devillers, J., 2013. Juvenile hormones and juvenoids. A historical survey. In: Devillers, J. (Ed.), Juvenile Hormones and Juvenoids. CRC Press, Boca Raton, London, New York, pp. 1–14.

Dias, J.C.P., 2015. Evolution of Chagas disease screening programs and control programs. Global Heart 10, 193–202.

Dias, J.C.P., Briceño-Leon, R., Storino, R., 1994. Aspectos sociales, económicos, políticos, culturales y psicológicos. In: Storino, R., Milei, J. (Eds.), Enfermedad de Chagas. Doyma, Buenos Aires, pp. 527–548.

Ditzen, M., Pellegrino, M., Vosshall, L.B., 2008. Insect odorant receptors are molecular targets of the insect repellent DEET. Science 319, 1838–1842.

Dolzer, J., Fischer, K., Stengl, M., 2003. Adaptation in pheromone-sensitive trichoid sensilla of the hawkmoth *Manduca sexta*. J. Exp. Biol. 206, 1575–1588.

Dujardin, J.P., Garcia-Zapata, M.T., Jurberg, J., Roelants, P., Cardozo, L., Panzera, F., Dias, J.C.P., Schofield, C.J., 1991. Which species of *Rhodnius prolixus* is invading houses in Brazil? T. R. Soc. Trop. Med. H. 85, 679–680.

Dujardin, J.P., Muñoz, M., Chavez, T., Ponce, C., Moreno, J., Schofield, C., 1998. The origin of *Rhodnius prolixus* in Central America. Med. Vet. Entomol. 12, 113–115.

Enan, E.E., 2001. Insecticidal activity of essential oils: octopaminergic sites of action. Comp. Biochem. Physiol. Part C 130, 325–337.

Enan, E.E., 2005. Molecular response of *Drosophila melanogaster* tyramine receptor cascade to plant essential oils. Insect Biochem. Mol. Biol. 35, 309–321.

Feasy, N., Wansbrough-Jones, M., Mabey, D.C.W., Solomon, A.W., 2009. Neglected tropical diseases. Br. Med. Bull. 93, 179–200.

*Feder, D., Valle, D., Rembold, H., Garcia, E.S., 1988. Azadirachtin-induced sterilization in mature females of *Rhodnius prolixus*. Z. Naturforsch. 43c, 908–913.

*Feliciangeli, M.D., 1972. Chemosterilization of adult *Rhodnius prolixus* by metepa. J. Med. Entomol. 9, 139–143.

Feliciangeli, M.D., 2009. Control de la enfermedad de Chagas en Venezuela. Logros pasados y retos presentes. Interciencia 34, 393–399.

*Feliciangeli, M.D., Hübsch, R., Chiechi, N., 1972. Esterilidad individual en *Rhodnius prolixus* Stål tratados con metepa (óxido de tris (1-(2-metil) azaridinil) fosfuro), y acción del mismo sobre *Trypanosoma cruzi*. Rev. Saúde Públ. Sao Paulo 6, 79–83.

*Fernández, D.M., Vivas, A.S., 2001. Resistencia a insecticidas en cepas de campo de *Rhodnius prolixus* en Venezuela. In: RELCOT – Red Latinoamericana de Control de Triatominos. Monitoreo de la resistencia a insecticidas en triatominos en América Latina, Serie Enfermedades Transmisibles, Publicación Monográfica 1. Fundación Mundo Sano, Buenos Aires, pp. 33–37.

*Fernández, D.M., Vivas, A.S., Barazarte, H., 2004. Susceptibilidad a insecticidas piretroides en cepas de campo de *Rhodnius prolixus* Stål (Hemiptera: Reduviidae) de Venezuela. Entomotropica 19, 1–5.

*Ferreira-DaSilva, C.T., Gombarovits, M.E.C., Masuda, H., Oliveira, C.M., Carlini, C.R., 2000. Proteolytic activation of canatoxin, a plant toxic protein by insect cathepsin-like enzymes. Arch. Insect Biochem. Physiol. 44, 162–171.

*Filho, A.M.O., 1996. Use of new tools for controlling triatomines in different entomologic situations in the American continent. Rev. Soc. Bras. Med. Trop. 30, 41–46.

*Filho, A.M.O., 1999. Differences of susceptibility of five triatomine species to pyrethroid insecticides – Implications for Chagas disease vector control. Mem. Inst. Oswaldo Cruz 94 (Suppl. I), 425–428.

Fitzpatrick, S., Feliciangeli, M.D., Sanchez-Martin, M.J., Monteiro, F.A., Miles, M.A., 2008. Molecular genetics reveal that silvatic *Rhodnius prolixus* do colonise rural houses. PLoS Negl. Trop. Dis. 2, e210. http://dx.doi.org/10.1371/journal.pntd.0000210.

Follmer, C., Barcellos, G.B.S., Zingali, R.B., Machado, O.L.T., Alves, E.W., Barja-Fidalgo, C., Guimarães, J.A., Carlini, C.R., 2001. Canatoxin, a toxic protein from jack beans (*Canavalia ensiformis*), is a variant form of urease (EC 3.5.1.5): biological effects of urease independent of its ureolytic activity. Biochem. J. 360, 217–224.

Fontán, A., Zerba, E., 1992. Influence of the nutritional state of *Triatoma infestans* over the insecticidal activity of DDT. Comp. Biochem. Physiol. 101C, 589–591.

*Fox, I., Bayona, I.G., Orozco, H.I., 1966. Toxicity of DDT, dieldrin, malathion and fenthion to *Rhodnius prolixus* in the laboratory. Bull. World Health Organ. 35, 974–976.

Gammon, D.W., 1978. Neural effects of allethrin on the free walking cockroach *Periplaneta Americana*: an investigation using defined doses at 15 and 32 °C. Pestic. Sci. 9, 79–91.

*Garcia, E.S., Rembold, H., 1984. Effects of azadirachtin on ecdysis of *Rhodnius prolixus*. J. Insect Physiol. 30, 939–941.

*Garcia, E.S., Azambuja, P., 2004. Lignoids in insects: chemical probes for the study of ecdysis, excretion and *Trypanosoma cruzi*–triatomine interactions. Toxicon 44, 431–440.

*Garcia, E.S., Azambuja, P., Bowers, W.S., 1984a. Comparison of structurally analogous allatotoxins on the molting and morphogenesis of *Rhodnius prolixus*, and the reversal of ecdysial stasis by ecdysone. Arch. Insect Biochem. Physiol. 1, 367–373.

*Garcia, E.S., Azambuja, P., Feder, D., Bowers, W.S., 1988. Inhibition of ecdysteroid production in nymphs of *Rhodnius prolixus* treated with ethoxyprecocene II. Arch. Insect Biochem. Physiol. 8, 127–134.

*Garcia, E.S., Azambuja, P., Forster, H., Rembold, H., 1984b. Feeding and molt inhibition by azadirachtins A, B and 7-acetyl azadirachtin A in *Rhodnius prolixus* nymphs. Z. Naturforsch. 39c, 1155–1158.

*Garcia, E.S., Cabral, M.M.O., Schaub, G.A., Gottlieb, O.R., Azambuja, P., 2000. Effects of lignoids on a hematophagous bug, *Rhodnius prolixus*: feeding, ecdysis and diuresis. Phytochemistry 55, 611–616.

*Garcia, E.S., Feder, D., Gomes, J.E.L., Rembold, H., 1990a. Short- and long-term effects of azadirachtin A on development and egg production of *Rhodnius prolixus*. Mem. Inst. Oswaldo Cruz 85, 11–15.

Garcia, E.S., Feder, D., Gomes, J.E.P.L., Azambuja, P., 1987. Effects of precocene and azadirachtin in *Rhodnius prolixus*: Some data on development and reproduction. Mem. Inst. Oswaldo Cruz 82 (Suppl. III), 67–73.

Garcia, E.S., Garcia, M.L.M., Macarini, J.D., Ubatuba, F.G., 1975. Influência do número de hemácias no volume de sangue sugado por larvas de *Rhodnius prolixus*. Rev. Brasil. Biol. 35, 207–210.

Garcia, E.S., Gonzalez, M.S., Azambuja, P., 1991. Effects of azadirachtin in *Rhodnius prolixus*: Data and hypotheses. Mem. Inst. Oswaldo Cruz 86 (Suppl. I), 107–111.

*Garcia, E.S., Gonzalez, M.S., Azambuja, P., Rembold, H., 1989a. Chagas' disease and its vector. Effect of azadirachtin A on the interaction of a triatomine host (*Rhodnius prolixus*) and its parasite (Trypanosoma cruzi). Z. Naturforsch. 44c, 317–322.

Garcia, E.S., Gonzalez, M.S., Rembold, H., 1989b. Curing effect of azadirachtin A in the triatomine host, *Rhodnius prolixus*, from its parasite, *Trypanosoma cruzi*. In: Borobsky, D., Spielman, A. (Eds.), Host Regulated Developmental Mechanisms in Vector Arthropods. University of Florida, Vero Beach, pp. 263–269.

*Garcia, E.S., Luz, N., Azambuja, P., Rembold, H., 1990b. Azadirachtin depress the release of prothoracicotropic hormone in *Rhodnius prolixus* larvae: evidence from head transplantations. J. Insect Physiol. 36, 679–682.

*Garcia, E.S., Subrahmanyam, B., Müller, T., Rembold, H., 1989c. Absorption, storage, organ distribution, and excretion of dietary [22,23–3H2]dihydroxiazadirachtin A in the blood-feeding bug, *Rhodnius prolixus*. J. Insect Physiol. 35, 743–749.

*Garcia, E.S., Uhl, M., Rembold, H., 1986. Azadirachtin, a chemical probe for the study of moulting process in *Rhodnius prolixus*. Z. Naturforsch. 41c, 771–775.

Golob, P., 1997. Current status and future perspectives for inert dusts for control of stored product insects. J. Stored Prod. Res. 33, 69–79.

*Gomez, I., 1985. Efecto teratogénico y tóxico de ácidos grasos de cadena corta insaturados, en *Rhodnius prolixus*. Mem. Inst. Oswaldo Cruz 80, 375–385.

*Gómez, J.C., Gallimore, J., Fernández, M., y Gross,, A., 1962. El efecto de las radiaciones ionizantes sobre la biología y la ecología de *Rhodnius prolixus*, vector principal de *Schizotrypanum cruzi* en Venezuela. Acta Cient. Venez. 13, 46–52.

Gómez-Núñez, J.C., 1969. Resting places, dispersal and survival of Co^{60}-tagged adult *Rhodnius prolixus*. J. Med. Entomol. 6, 83–86.

*Gómez-Núñez, J.C., Gross, A., Machado, C., 1964. Las radiaciones gamma y el comportamiento reproductivo del *Rhodnius prolixus* macho. Acta Cient. Venez. 15, 97–104.

*Gonzalez, M.S., Garcia, E.S., 1992. Effect of azadirachtin on the development of *Trypanosoma cruzi* in different species of triatomine insect vectors: long-term and comparative studies. J. Inverteb. Pathol. 60, 201–205.

*Gonzalez, M.S., Nogueira, N.F.S., Mello, C.B., Souza, W., Schaub, G.A., 1999. Influence of brain and azadirachtin on *Trypanosoma cruzi* development in the vector, *Rhodnius prolixus*. Exp. Parasitol. 92, 100–108.

Granett, J., Leeling, N.L., 1972. A hyperglycemic agent in the serum of DDT-prostrate American cockroaches, *Periplaneta americana*. Ann. Entomol. Soc. Am. 65, 299–302.

*Grove, J.F., Jennings, R.C., Johnson, A.W., White, A.F., 1974. Synthesis of some juvenile hormone mimics and their biological activity on *Rhodnius prolixus*. Chem. Ind. London 8, 346–347.

Gualtieri, J., Nelson, M., Cichero, J., 1985. Present and perspectives of the chemical control. In: Carcavallo, R., Rabinovich, J., Tonn, R. (Eds.), Factores Biológicos y Ecológicos en la Enfermedad de Chagas, tomo II. Ministerio de Salud y Acción Social de la Nación, Buenos Aires, pp. 319–329.

Guhl, F., 2007. Chagas disease in Andean countries. Mem. Inst. Oswaldo Cruz 102 (Suppl. I), 29–37.

Guhl, F., Robles, J.L., Cepeda, I., 2006. Impacto de la descentralización de los programas de control de las enfermedades transmisibles: el caso de la enfermedad de Chagas en Colombia. In: Yadón, Z.E., Gürtler, R.E., Tobar, F., Medici, A.C. (Eds.), Descentralización y Gestión del Control de las Enfermedades Transmisibles en América Latina. Organización Panamericana de la Salud, Buenos Aires, pp. 255–278.

Guhl, F., Vallejo, G.A., 1999. Interruption of Chagas disease transmisión in the Andean countries: Colombia. Mem. Inst. Oswaldo Cruz 94 (Suppl. I), 413–415.

Gurgel-Gonçalves, R., Abad-Franch, F., Ferreira, J.B.C., Santana, D.B., Cuba, C.A., 2008. Is *Rhodnius prolixus* (Triatominae) invading houses in central Brazil? Acta Trop. 107, 90–98.

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*. T. R. Soc. Trop. Med. H. 103, 298–304.

Gürtler, R.E., Schweigmann, N.J., Cecere, M.C., Chuit, R., Wisnivesky-Colli, C., 1993. Comparison of two sampling methods for domestic populations of *Triatoma infestans* in north-west Argentina. Med. Vet. Entomol. 7, 238–242.

Hashimoto, K., Schofield, C.J., 2012. Elimination of *Rhodnius prolixus* in Central America. Parasit. Vectors 5, 45. http://dx.doi.org/10.1186/1756-3305-5-45.

Hiwat, H., 2014. Triatomine species of Suriname (Heteroptera: Reduviidae) and their role as vectors of Chagas disease. Mem. Inst. Oswaldo Cruz 109, 452–458.

Hodgson, E., Mailman, R.B., Chambers, J.E. (Eds.), 1998. Dictionary of Toxicology. Macmillan Reference, London.

Hurst, H., 1940. Permeability of insect cuticle. Nature 145, 462–463.

Ianowski, J.P., Manrique, G., Núñez, J.A., Lazzari, C.R., 1998. Feeding is not necessary for triggering plasticization of the abdominal cuticle in haematophagous bugs. J. Insect Physiol. 44, 379–384.

Isman, M.B., 2010. Botanical insecticides, deterrents, repellents and oils. In: Singh, B. P. (Ed.), Industrial Crops and Uses. Fort Valley State University, Fort Valley, pp. 433–445.

Jindra, M., Palli, S.R., Riddiford, L.M., 2013. The juvenile hormone signaling pathway in insect development. Annu. Rev. Entomol. 58, 181–204.

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.

*Jurberg, J., Costa, J.M., Gonçalves, T.C.M., Garcia, E.S., Azambuja, P., 1984. Efeitos morfogénicos de precoceno II em ninfas de *Rhodnius prolixus* (Stål, 1859) (Hemíptera – Triatominae). Mem. Inst. Oswaldo Cruz 79, 397–407.

Jurberg, J., Galvão, C., 2006. Biology, ecology, and systematics of triatominae (Heteroptera, Reduviidae), vectors of Chagas disease, and implications for human health. Denidia 19, 1096–1116.

*Jurberg, J., Galvão, C., Bowers, W.S., Garcia, E.S., Azambuja, P., 1997. Biomorphological alterations induced by an anti-juvenile hormonal compound, 2-(2-ethoxyethoxy)ethyl furfuryl ether, on three species of triatominae larvae (Hemiptera: Reduviidae). Mem. Inst. Oswaldo Cruz 92, 263–268.

Kain, P., Boyle, S.M., Tharadra, S.K., Guda, T., Pham, C., Dahanukar, A., Ray, A., 2013. Odour receptors and neurons for DEET and new insect repellents. Nature 502, 507–512.

*Kelly, G.M., Huebner, E., 1986. The effects of the insect growth regulator fenoxycarb on *Rhodnius prolixus* (Insecta, Hemiptera) embryogenesis. Can. J. Zool. 64, 2425–2429.

*Kelly, G.M., Huebner, E., 1987. Juvenoid effects on *Rhodnius prolixus* embryogenesis. Insect Biochem. 17, 1079–1083.

Kollien, A.H., Schaub, G.A., 2000. The development of *Trypanosoma cruzi* in Triatominae. Parasitol. Today 16, 381–387.

*Kul'kova, T.A., 1975. Toxicity of the preparation drayon for the bug *Rhodnius prolixus* Stål (order Hemiptera, family Triatomidae). Med. Parazitol. 44, 63–65 (in Russian).

*Kul'kova, T.A., 1981a. Action of Baytex on the eggs of *Rhodnius prolixus* Stål bugs. Med. Parazitol. 50, 83–84 (in Russian).

*Kul'kova, T.A., 1981b. Effect of the knockdown caused by the gamma isomer of hexachlorocyclohexane on the feeding, copulation and fertility capacity of *Rhodnius prolixus* Stål bugs. Med. Parazitol. 50, 33–38 (in Russian).

*Kul'kova, T.A., 1983. Effect of the desiccant Dry-Die-67 on *Rhodnius prolixus* Stål bugs. Med. Parazitol. 52, 57–60 (in Russian).

*Kul'kova, T.A., Fedder, M.L., 1972. Sensitivity of *Rhodnius prolixus* Stål bugs to dieldrin, phenthion and malathion. Med. Parazitol. 41, 108–110 (in Russian).

*Kul'kova, T.A., Pridantseva, E.A., Drabkina, A.A., Shekhter, O.V., Tsizin, Y.S., 1983. Action of altozar, altozide SR-10 and derivatives of farnesylic and 3,11-dimethyl-2-dodecenoic acids on *Rhodnius prolixus* Stål bugs. Med. Parazitol. 52, 46–51 (in Russian).

Labrecque, G.C., Fye, R.L., 1978. Cytogenetic and other effects of the chemosterilants tepa, metepa, apholater and hempa in insects (a review). Mutat. Res. 47, 99–113.

Lance, D.R., McInnis, D.O., 2005. Biological basis of the sterile insect technique. In: Dyck, V.A., Hendrichs, J., Robinson, A.S. (Eds.), Sterile Insect Technique: Principles and Practice in Area-wide Integrated Pest Management. Springer, Dordrecht, pp. 69–994.

*Langley, P.A., Howl, V., Oouchi, H., 1990. Regulation of reproduction in *Rhodnius prolixus* by the juvenile hormone mimic pyriproxyfen. Entomol. Exp. Appl. 57, 271–279.

Leal, W., 2014. The enigmatic reception of DEET – the gold standard of insect repellents. Curr. Opin. Insect Sci. 6, 93–98.

*Lent, H., Oliveira, S.J., 1944. Nota preliminar sobre a ação do DDT (dicloro-difenil-tricloroetana) em insetos transmissores de doença de Chagas. Rev. Brasil. Biol. 4, 329–331.

Licastro, S.A., Zerba, E.N., Casabé, N., 1983. The relation between viscosity and penetration of some diethyl *p*-substituted phenyl phosphorothionates and oil carriers into the cuticle of *Triatoma infestans*. Pest. Biochem. Physiol. 19, 53–59.

*Lima, M.M., Rey, L., Mello, R.P., 1991. Lethality of triatomines (Hemiptera: Reduviidae), vectors of Chagas' disease, feeding on blood baits containing synthetic insecticides, under laboratory conditions. Rev. Inst. Med. Trop. 33, 427–433.

*Lima, M.M., Rey, L., Mello, R.P., 1992. Lethal effect of a bait for *Rhodnius prolixus* (Hemiptera: Reduviidae), the vector of Chagas' disease, containing hexachlorocyclohexane (HCH) under laboratory conditions. Rev. Inst. Med. Trop. 34, 295–301.

Liu, B.L., Tzeng, Y.M., 2012. Development and applications of destruxins: a review. Biotechnol. Adv. 30, 1242–1254.

Locke, M., 1992. Professor Sir Vincent B. Wigglesworth. Biography and contributions to insect morphology and embryology for the 1992 Distinguished International Award. Int. J. Insect Morphol. Embryol. 21, 101–115.

Locke, M., 1994. Obituary. Professor Sir Vincent B. Wigglesworth C.B.E., M.D., F.R.S. (1899–1994). J. Insect Physiol. 40, 823–826.

Locke, M., 1996. Sir Vincent Brian Wigglesworth, CBE 17 April 1899–12 February 1994. Biogr. Mem. Fellows R. Soc. 42, 540–553.

Lucía, A., Licastro, S., Zerba, E., González Audino, P., Masuh, H., 2009. Sensitivity of *Aedes aegypti* (Diptera: Culicidae) to the vapors of *Eucalyptus* essentials oils. Bioresour. Technol. 100, 6083–6087.

*Lutz, A., Sfara, V., Alzogaray, R.A., 2014. Repellence produced by monoterpenes on *Rhodnius prolixus* (Hemiptera: Reduviidae) decreases after continuous exposure to these compounds. J. Insect Sci. 14, 254. http://dx.doi.org/10.1093/jisesa/ieu116.

*Maddrell, S.H.P., Casida, J.E., 1971. Mechanism of insecticide-induced diuresis in *Rhodnius*. Nature 231, 55–56.

*Maddrell, S.H.P., Lane, N.J., Harrison, J.B., Overton, J.A., Moreton, R.B., 1988. The initial stages in the action of an insecticidal -endotoxin of Bacillus thuringiensis var. israeliensis on the epithelial cells of Malpighian tubules in the insect, *Rhodnius prolixus*. J. Cell Sci. 90, 131–144.

*Maddrell, S.H.P., Overton, J.A., Ellar, D.J., Knowles, B.H., 1989. Action of activated 27,000 M_r toxin from *Bacillus thuringiensis* var. *israelensis* on Malpighian tubules of the insect, *Rhodnius prolixus*. J. Cell Sci. 94, 601–608.

*Mansur, J.F., Figueira-Mansur, J., Santos, A.S., Santos-Junior, H., Ramos, I.B., Medeiros, M.N., Machado, E.A., Kaiser, C.R., Muthukrishnan, S., Masuda, H., Vasconcellos, A.M.H., Melo, A.C.A., Moreira, M.F., 2010. The effect of lufenuron, a chitin synthesis inhibitor, on oogenesis of *Rhodnius prolixus*. Pestic. Biochem. Physiol. 98, 59–67.

*Maudlin, I., 1976. The inheritance of radiation induced semi-sterility in *Rhodnius prolixus*. Chromosoma 58, 285–306.

*Mazariego-Arana, M.A., Ramírez-San, Juan E., Alejandre-Aguilar, R., Nogueda-Torres, B., 2002. Activity and residual effect of two formulations of lambdacyhalothrin sprayed on palm leaves to *Rhodnius prolixus*. Mem. Inst. Oswaldo Cruz 97, 353–357.

McIver, S.B., 1981. A model for the mechanisms of action of the repellent DEET on *Aedes aegypti*. J. Med. Entomol. 18, 357–361.

Mellanby, K., 1992. The DDT Story. The British Crop Protection Council, Farnham.

*Mello, C.B., Mendonça-Lopes, D., Feder, D., Uzeda, C.D., Carneiro, R.M., Rocha, M.A., Gonzalez, M.S., 2008. Laboratory evaluation of the effects of triflumuron on the development of *Rhodnius prolixus* nymph. Mem. Inst. Oswaldo Cruz 103, 839–842.

*Mello, C.B., Uzeda, C.D., Bernardino, M.V., Mendonça-Lopes, D., Kelecom, A., Fevereiro, P.C.A., Guerra, M.S., Oliveira, A.P., Rocha, L.M., Gonzalez, M.S., 2007. Effects of the essential oil obtained from *Pilocarpus spicatus* Saint-Hilaire (Rutaceae) on the development of *Rhodnius prolixus* nymphae. Braz. J. Pharmacogn. 17, 514–520.

Menn, J.J., Hollingworth, R.M., 1985. Introduction. In: Kerkut, G.A., Gilbert, L.I. (Eds.), Comprehensive Insect Physiology, Biochemistry and Pharmacology, Insect Control, vol. 12. Pergamon Press, Oxford, New York, Toronto, Sydney, Paris, Frankfurt, pp. 1–8.

Mesquita, R.D., Vionette-Amaralc, R.J., Lowenbergerd, C., Rivera-Pomar, R., Monteiro, F.A., et al., 2015. Genome of *Rhodnius prolixus*, an insect vector of Chagas disease, reveals unique adaptations to hematophagy and parasite infection. Proc. Natl. Acad. Sci. U.S.A. 112, 14936–14941.

Modolo, L.V., Souza, A.X., Horta, L.P., Araujo, D.P., Fátima, A., 2015. An overview on the potential of natural products as ureases inhibitors: a review. J. Adv. Res. 6, 35–44.

Moncayo, A., 1999. Progress towards interruption of transmission of Chagas disease. Mem. Inst. Oswaldo Cruz 94 (Suppl. I), 401–404.

Mordue, A.J., Morgan, E.D., Nisbet, A.J., 2005. Azadirachtin, a natural product in insect control. In: Gilbert, L.I., Gill, S.S. (Eds.), Comprehensive Molecular Insect Science, Elsevier, vol. 6. Amsterdam, Boston, pp. 117–135.

*Moreira, M.F., Garcia, E.S., Masuda, H., 1994. Inhibition by azadirachtin of phospholipid transfer from lipophorin to the oocytes in *Rhodnius prolixus*. Arch. Insect Biochem. Physiol. 27 (287), 299.

*Moretti, A.N., Zerba, E.N., Alzogaray, R.A., 2013. Behavioral and toxicological responses of *Rhodnius prolixus* and *Triatoma infestans* (Hemiptera: Reduviidae) to 10 monoterpene alcohols. J. Med. Entomol. 50, 1046–1054.

*Moretti, A.N., Zerba, E.N., Alzogaray, R.A., 2015. Lethal and sublethal effects of eucalyptol on *Triatoma infestans* and *Rhodnius prolixus*, vectors of Chagas disease. Entomol. Exp. Appl. 154, 62–70.

Morgan, E.D., 2009. Azadirachtin, a scientific gold mine. Bioorgan. Med. 17, 4096–4105.

Mostafalou, S., Abdollahi, M., 2013. Pesticides and human chronic diseases: evidences, mechanisms, and perspectives. Toxicol. Applied. Pharmacol. 268, 157–177.

Mougabure-Cueto, G., Picollo, M.I., 2015. Insecticide resistance in vector Chagas disease: evolution, mechanisms and management. Acta Trop. 149, 70–85.

*Mulinari, F., Becker-Ritt, A.B., Demartini, D.R., Ligabue-Braun, R., Stanisçuaski, F., Verli, H., Fragoso, R.R., Schroeder, E.K., Carlini, C.R., Grossi-de-Sá, M.F., 2011. Characterization of JBURE-IIb isoform of *Canavalia ensiformis* (L.) DC urease. Biochim. Biophys. Acta 1814, 1758–1768.

Müller, U., 1997. The nitric oxide system in insects. Progr. Neurobiol. 51, 363–381.

*Nakagawa, J., Cordón-Rosales, C., Juárez, J., Itzep, C., Nonami, T., 2003. Impact of residual spraying on *Rhodnius prolixus* and *Triatoma dimidiata* in the Department of Zacapa in Guatemala. Mem. Inst. Oswaldo Cruz 98, 277–281.

Nakagawa, J., Kojima, M., Zúñiga, C., Ota, K., Serpas, M., 2006. Modalidades de descentralización y control de la enfermedad de Chagas: análisis comparativo entre Guatemala, Honduras y El Salvador. In: Yadón, Z.E., Gürtler, R.E., Tobar, F., Medici, A.C. (Eds.), Descentralización y Gestión del Control de las Enfermedades Transmisibles en América Latina. Organización Panamericana de la Salud, Buenos Aires, pp. 231–254.

*Nakamura, A., Stiebler, R., Fantappié, M.R., Fialho, E., Masuda, H., Oliveira, M.F., 2007. Effects of retinoids and juvenoids on moult and on phenoloxidase activity in the blood-sucking insect *Rhodnius prolixus*. Acta Trop. 103, 222–230.

*Nelson, M.J., Colmenares, P., 1979a. Insecticide susceptibility of vectors of Chagas' disease in Venezuela. WHO/VBC/79.736 http://apps.who.int/iris/handle/10665/201702.

*Nelson, M.J., Colmenares, P., 1979b. Topical application of insecticides to *Rhodnius prolixus* (Reduviidae: Triatominae) a Chagas' disease vector. WHO/VBC/79.737 http://apps.who.int/iris/handle/10665/188270.

*Nelson, M.J., Williams, J.O., Nocerino, F., Arata, A.A., Tonn, R.J., 1983. Laboratory and field testing of insecticides against *Rhodnius prolixus* (Reduviidae, Triatominae), the vector of Chagas' disease in Venezuela. WHO/VBC/83.886 http://apps.who.int/iris/handle/10665/201703.

Němec, V., Kodrík, D., Matolín, S., Laufer, H., 1993. Acid in insect metamorphosis, embryogenesis and reproduction. J. Insect Physiol. 39, 1083–1093.

*Nocerino, F., 1972. Selección de una cepa de *R. prolixus* resistente al dieldrin. Bol. Inf. Malar. Saneam. Amb. 12, 210–216.

*Nocerino, F., 1976. Susceptibilidad de *R. prolixus* y *T. maculata* a los insecticidas en Venezuela. Bol. Inf. Dir. Malariol. San. Amb. 16, 47–52.

*Nocerino, F., Hernández, A., 1985. Nueva línea básica de susceptibilidad a insecticidas de *Rhodnius prolixus*. Bol. Oficina Sanit. Panam. 98, 261–268.

*Nocerino, F., Rodriguez, H., Diaz, B.S., Valenzuela, J.V., Otero, M.A., Tonn, R.J., 1976. Field trials using fenthion, propoxur, malathion, pirimifos metil and jodfenphos for the control of *Rhodnius prolixus* in Venezuela. WHO_VBC_76.606 http://apps.who.int/iris/handle/10665/204125.

*Nogueira, J., Mourão, S.C., Dolabela, I.B., Santos, M.G., Mello, C.B., Kelecom, A., Mexas, R., Feder, D., Fernandes, C.P., Gonzalez, M.S., Rocha, L., 2014. *Zanthoxylum caribaeum* (Rutaceae) essential oil: Chemical investigation and biological effects on *Rhodnius prolixus* nymph. Parasitol. Res. 113, 4271–4279.

*Nogueira, N.F.S., Gonzalez, M., Garcia, E.M., Souza, W., 1997. Effect of azadirachtin A on the fine structure of the midgut of *Rhodnius prolixus*. J. Inverteb. Pathol. 69, 58–63.

Nogueira, N.F.S., Gonzalez, M.S., Gomes, J.E., Souza, W., Garcia, E.S., Azambuja, P., Nohara, L.L., Almeida, I.C., Zingales, B., Coll, W., 2007. *Trypanosoma cruzi*: Involvement of glycoinositolphospholipids in the attachment to the luminal midgut surface of *Rhodnius prolixus*. Exp. Parasitol. 116, 120–128.

Ōmura, S., 2008. Ivermectin: 25 years and still going strong. Int. J. Antimicrob. Agents 31, 91–98.

Ōmura, S., Crump, A., 2014. Ivermectin: panacea for resource-poor communities? Trends Parasitol. 30, 445–455.

OPS/OMS – Organización Panamericana de la Salud/ Organización Mundial de la Salud, 2014. X Reunión de la Comisión Intergubernamental (CI) de la Iniciativa Andina de Control de la Transmisión Vectorial y Transfusional y la Atención Médica de la Enfermedad de Chagas (IIPA) http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&gid=26260&Itemid.

*Orchard, I., 1980. The effects of pyrethroids on the electrical activity of neurosecretory cells from the brain of *Rhodnius prolixus*. Pest. Biochem. Physiol. 13, 220–226.

Orchard, I., Osborne, M.P., 1979. The action of insecticides on neurosecretory neurons in the stick insect, *Carausius morosus*. Pest. Biochem. Physiol. 10, 197–202.

Osborne, M.P., 1985. DDT, -HCH and the cyclodienes. In: Kerkut, G.A., Gilbert, L. (Eds.), Comprehensive Insect Physiology, Biochemistry and Pharmacology, Insect Control, vol. 12. Pergamon Press, Oxford, New York, Toronto, Sydney, Paris, Frankfurt, pp. 131–182.

Palma, L., Berry, C., 2016. Understanding the structure and function of *Bacillus thuringiensis* toxins. Toxicon 109, 1–3. http://dx.doi.org/10.1016/j.toxicon.2015.10.020.

Palma, L., Muñoz, D., Berry, C., Murillo, J., Caballero, P., 2014. *Bacillus thuringiensis* toxins: an overview of their biocidal activity. Toxins 6, 3296–3325.

*Paluzzi, J.P.V., Young, P., Deferrari, M.S., Orchard, I., Carlini, C.R., O'Donnell, M.J., 2012. Investigation of the potential involvement of eicosanoid metabolites in anti-diuretic hormone signaling in *Rhodnius prolixus*. Peptides 34, 127–134.

Pan, J.Y., Chen, S.L., Yang, M.H., Wu, J., Sinkonnen, J., Zou, K., 2009. An update on lignans: natural products and synthesis. Nat. Prod. Rep. 26, 1251–1292.

*Parra-Henao, G., Pajón, C.M.G., Torres, J.M.C., 2007. Actividad insecticida de extractos vegetales sobre *Rhodnius prolixus* y *Rhodnius pallescens* (Hemiptera: Reduviidae). Bol. Malariol. San. Amb. 42, 125–137.

*Patterson, J.W., 1973. The effect of the persistence of juvenile hormone mimics on their activity on *Rhodnius prolixus*. J. Insect Physiol. 19, 1631–1637.

*Patterson, J.W., Schwarz, M., 1977. Chemical structure, juvenile hormone activity and persistence within the insect of juvenile hormone mimics for *Rhodnius prolixus*. J. Insect Physiol. 23, 121–129.

*Patterson, J.W., Schwarz, M., 1979. The activity of juvenile hormone mimics for the eggs of *Rhodnius prolixus*. J. Insect Physiol. 25, 399–404.

Pedra, J.H., McIntyre, L.M., Scharf, M.E., Pittendrigh, B.R., 2004. Genome-wide transcription profile of field- and laboratory-selected dichlorodiphenyltrichloroethane (DDT)-resistant *Drosophila*. Proc. Natl. Acad. Sci. U.S.A. 101, 7034–7039.

*Peñalver, L.M., Villagran, E., 1955. Experimentos con el insecticida dieldrin en la lucha antitriatomidea. Bol. Oficina Sanit. Panam. 38, 127–140.

Pener, M.P., 2002. Insect growth regulators. In: Pimentel, D. (Ed.), Encyclopedia of Pest Management. CRC Press, Boca Raton, London, New York, pp. 398–401.

Pener, M.P., Dhadialla, T.S., 2012. An overview of insect growth disruptors; applied aspects. Adv. Insect Physiol. 43, 1–162.

Pessoa, G.C.D., Viñas, P.A., Rosa, A.C.L., Diotaiuti, L., 2015. History of resistance of triatominae vectors. Rev. Soc. Bras. Med. Trop. 48, 380–389.

Ponce, C., 2007. Current situation of Chagas disease in Central America. Mem. Inst. Oswaldo Cruz 102 (Suppl. 1), 41–44.

*Pridantseva, E.A., Shekhter, O.V., Sergovskaya, N.L., Popova, N.A., Tsizin, Iu.S., 1978. Action of insect development inhibitors on *Aedes aegypti* L. mosquitoes and on *Rhodnius prolixus* Stål bug. II. The juvenile activity of methoprene and structurally close compounds. Med. Parazitol. 47, 65–68 (in Russian).

*Pridantseva, E.A., Kornienko, N.I., Tsizin, Iu.S., 1981. Action of development inhibitors on *Aedes aegypti* L. mosquitoes and *Rhodnius prolixus* Stål bugs. IV. The activity of precocenes. Med. Parazitol. 50, 46–51 (in Russian).

Quraishi, M.S., 1971. Toxic and teratogenic effects of saturated and unsaturated fatty acids on house fly larvae. J. Econ. Entomol. 64, 787–792.

Rajendran, S., Sriranjini, V., 2008. Plant products as fumigants for stored-product insect control. J. Stored Prod. Res. 43, 126–135.

Rassi Jr., A., Rassi, A., Marin-Neto, J.A., 2010. Chagas disease. Lancet 375, 1388–1402.

*Real-Guerra, R., Carlini, C.R., Stanisçuaski, F., 2013. Role of lysine and acidic amino acid residues on the insecticidal activity of Jackbean urease. Toxicon 71, 76–83.

Regnault-Roger, C., Vincent, C., Arnason, J.T., 2012. Essential oils in insect control: low-risk products in a high-stakes world. Annu. Rev. Entomol. 57, 405–424.

Rembold, H., 1987. The azadirachtins – potent insect growth inhibitors. Mem. Inst. Oswaldo Cruz 82 (Suppl. III), 61–66.

*Rembold, H., Garcia, E.S., 1989. Azadirachtin inhibits *Trypanosoma cruzi* infection of its triatomine insect host, *Rhodnius prolixus*. Naturwissenschaften 76, 77–78.

Reyes, M., Luna, L.M., Angulo, V.M., 2009. Susceptibilidad y/o resistencia a deltametrina en cepas silvestres de Rhodnius prolixus de Colombia. In: Comité Organizador de la FLAP (Ed.), Libro de Resúmenes del XIX Congreso Latinoamericano de Parasitología. Sociedad Científica del Paraguay, Asunción, Paraguay, p. 187.

Rice, P.J., Coats, J.R., 1994a. Insecticidal properties of monoterpenoids derivatives to the house fly (Diptera: Muscidae) and red flour beetle (Coleoptera: Tenebrionidae). Pest. Sci. 41, 195–202.

Rice, P.J., Coats, J.R., 1994b. Insecticidal properties of several monoterpenoids to house fly (Diptera: Muscidae), red flour beetle (Coleoptera: Tenebrionidae), and southern corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 87, 1172–1179.

Riddiford, L.M., 2012. How does juvenile hormone control insect metamorphosis and reproduction? Gen. Comp. Endocr. 179, 477–484.

Ruiz, H., 1953. *Rhodnius prolixus* en Costa Rica. Rev. Biol. Trop. 1, 239–240.

*Ruiz-Sanchez, E., Orchard, I., Lange, A.B., 2010. Effect of the cyclopeptide mycotoxin destruxin A on the Malpighian tubules of *Rhodnius prolixus* (Stål). Toxicon 55, 1162–1170.

Sainz, P., Sanz, J., Burillo, J., González-Coloma, A., Bailén, M., Martínes-Díaz, R.A., 2012. Essential oils for the control of reduviid insects. Phytochem. Rev. 11, 361–369.

*Saldarriaga, Y., Vásquez, C.A., Calle, J.J., 2005. Virulencia de *Beauveria bassiana* y triflumuron sobre *Rhodnius prolixus* y *R. pallescens* (Hemiptera: Reduviidae). Rev. Colomb. Entomol. 31, 177–182.

Salvatella, R., Schofield, C.J., 2006. Enfermedad de Chagas. Iniciativas para su control en Latinoamérica. Biomedicina 1, 36–46.

Samaranayaka, M., 1974. Insecticide induced release of hyperglycaemic and adipokinetic hormones of *Schistocerca gregaria*. Gen. Comp. Endocr. 24, 424–436.

*Sandoval, C.M., 2001. Actividad insecticida del malatión y la deltametrina en una cepa colombiana de Rhodnius prolixus (Hemiptera: Reduviidae). In: RELCOT - Red Latinoamericana de Control de Triatominos (Ed.), Monitoreo de la

resistencia a insecticidas en triatominos en América Latina, Serie Enfermedades Transmisibles, Publicación Monográfica 1. Fundación Mundo Sano, Buenos Aires, pp. 27–32.
Sawicki, R.M., 1962. Insecticidal activity of pyrethrum extract and its four insecticidal constituents against house flies. III. Knock-down and recovery of flies treated with pyrethrum extract with and without piperonyl butoxide. J. Sci. Food Agric. 13, 283–292.
Schama, R., Pedrini, N., Juárez, M.P., Nelson, D.R., Torres, A.Q., Valle, D., Mesquita, R. D., 2015. *Rhodnius prolixus* supergene families of enzymes potentially associated with insecticide resistance. Insect Biochem. Mol. Biol. http://dx.doi.org/10.1016/j.ibmb.2015.06.005, pii: S0965-1748(15)30008-4.
Schleier, J.J., Peterson, R.K.D., 2011. Pyrethrins and pyrethroid insecticides. In: López, O., Fernández-Bolaños, J.G. (Eds.), Green Trends in Insect Control. RSC Publishing, Cambridge, pp. 94–131.
*Schmialek, P., 1961. Die identifizierung zweier im Tenebriokot und in hefe vorkommender substanzen mit juvenilhormonwirkung. Z. Naturforschg. 16b, 461–464.
Schofield, C., Dias, J.C.P., 1999. The Southern cone initiative against Chagas. Dis. Adv. Parasitol. 42, 1–27.
Schofield, C.J., Galvão, C., 2009. Classification, evolution, and species groups within the Triatominae. Acta Trop. 110, 88–100.
Scott, J.G., Georghiou, G.P., 1984. Influence of temperature on knockdown, toxicity, and resistance to pyrethroids in the housefly, *Musca domestica*. Pestic. Biochem. Physiol. 21, 53–62.
Sen, S.E., Sperry, A.E., Childress, M., Hannemann, D.E., 2003. Juvenile hormone biosynthesis in moths: synthesis and evaluation of farnesol homologs as alternate substrates of farnesol oxidase. Insect Biochem. Mol. Biol. 33, 601–607.
*Sfara, V., Mougabure-Cueto, G., Zerba, E.N., Alzogaray, R.A., 2011. Adaptation of the repellency response to DEET in *Rhodnius prolixus*. J. Insect Physiol. 57, 1431–1436.
*Sfara, V., Zerba, E.N., Alzogaray, R.A., 2008. Decrease in DEET repellency caused by nitric oxide in *Rhodnius prolixus*. Arch. Insect Biochem. Physiol. 67, 1–8.
*Sfara, V., Zerba, E.N., Alzogaray, R.A., 2009. Fumigant insecticidal activity and repellent effect of five essential oils and seven monoterpenes on first instar nymphs of *Rhodnius prolixus*. J. Med. Entomol. 46, 511–515.
*Shekhter, O.V., Pridantseva, E.A., Tsizin, Iu.S., 1978. Action of insect growth inhibitors on Aedes aegypti *L. mosquitoes* and *Rhodnius prolixus* Stål bugs. III. The activity of diarylidene cyclonones. Med. Parazitol. 47, 69–72 (in Russian).
Shelly, T., McInnis, D., 2015. Sterile insect technique and control of tephritid fruit flies: Do species with complex courtship require higher overflooding ratios? Ann. Entomol. Soc. Am. 1–11. http://dx.doi.org/10.1093/aesa/sav101.
*Sherlock, I.A., Piesman, J., Guitton, N., 1983. Action of bendiocarb against triatomine (Hemiptera: Reduviidae) vectors of Chagas' disease. J. Med. Entomol. 20, 440–445.
Sinniah, B., 1983. Insecticidal effect of aliphatic alcohols against aquatic stages of *Aedes* mosquitoes. Trans. R. Soc. Trop. Med. H. 77, 35–38.
Skinner, M., Parker, B.L., Kim, J.S., 2014. Role of entomopathogenic fungi in integrated pest management. In: Abrol, P. (Ed.), Integrated Pest Management. Elsevier, Amsterdam, Boston, Heidelberg, London, New York, Oxford, Paris, San Diego, San Francisco, Singapore, Sydney, pp. 169–1961.
Stadler, T., Buteler, M., 2009. Modes of entry of petroleum distilled spray-oils into insects: a review. Bull. Insectol. 62, 169–177.
Stanisçuaski, F., Carlini, C.R., 2012. Plant urease and related peptides: understanding their entomotoxic properties. Toxins 4, 55–67.
*Stanisçuaski, F., Brugge, V.T., Carlini, C.R., Orchard, I., 2009. *In vitro* effect of *Canavalia ensiformis* urease and the derived peptide Jaburetox-2Ec on *Rhodnius prolixus* Malpighian tubules. J. Insect Physiol. 55, 255–263.
*Stanisçuaski, F., Brugge, V.T., Carlini, C.R., Orchard, I., 2010. Jack bean urease alters serotonin-induced effects on *Rhodnius prolixus* anterior midgut. J. Insect Physiol. 56, 1078–1086.
Subramanyam, Bh., Roesli, R., 2000. Inert dusts. In: Subramanyam, Bh., Hagstrum, D. W. (Eds.), Alternatives Pesticides in Stored-Product IPM. Kluwer Academic Publishers, Boston, pp. 321–380.
Tabashnik, B.E., Mota-Sanchez, D., Whalon, M.E., Hollingworth, R.M., Carrière, Y., 2014. Defining terms for proactive management of resistance to Bt crops and pesticides. J. Med. Entomol. 107, 496–507.
*Tarrant, C., Cupp, E.W., Bowers, W.S., 1982. The effects of precocene II on reproduction and development of triatomine bugs (Reduviidae: Triatominae). Am. J. Trop. Med. Hyg. 31, 416–420.
*Tarrant, C.A., Cupp, E.W., 1978. Morphogenetic effects of precocene II on the immature stages of *Rhodnius prolixus*. T. R. Soc. Trop. Med. H. 72, 666–668.
*Tomazetto, G., Mulinari, F., Stanisçuaski, F., Settembrini, B., Carlini, C.R., Ayub, M.A. Z., 2007. Expression kinetics and plasmid stability of recombinant E. coli encoding urease-derived peptide with bioinsecticide activity. Enzyme Microb. Technol. 41, 821–827.
*Valdivieso, F.G., Diaz, B.S., 1968. Ensayo de campo de la acción del insecticida OMS 33 (Bayer 39007, Baygon) sobre *R. prolixus*. Bol. Inf. Dir. Malariol. San. Amb. 8, 358–364.
*Valdivieso, F.G., Diaz, B.S., Nocerino, F., 1971. Susceptibilidad de *R. prolixus* a los insecticidas clorados en Venezuela. Bol. Inf. Dir. Malariol. San. Amb. 11, 47–52.
*Vásquez, C.A., Saldarriaga, Y., Gómez, W.A., Pineda, F., 2002. Susceptibilidad de *Rhodnius prolixus* (Hemiptera: Reduviidae) a la acción del triflumuron. Rev. Colomb. Entomol. 28, 13–16.
*Vassena, C.V., Picollo, M.I., Zerba, E.N., 2000. Insecticide resistance in Brazilian *Triatoma infestans* and Venezuelan *Rhodnius prolixus*. Med. Vet. Entomol. 14, 51–55.
*Villar, M.I.P., Fontan, A., Wood, E., Zerba, E., 1990. The biochemical basis of tolerance to malathion in *Rhodnius prolixus*. Comp. Biochem. Physiol. 96C, 361–365.
*Vivas, A.S., Fernández, D.M., 2001. Toxicidad de cinco insecticidas en una cepa de laboratorio de *Rhodnius prolixus* (Stål), 1859 (Hemiptera: Reduviidae) de Venezuela. Entomotropica 16, 187–190.
White, A.F., 1972. Metabolism of the juvenile hormone analogue methyl farnesoate 10,11-epoxide in two insect species. Life Sci. 11, 201–210.
White, G.B., Moore, S.J., 2015. Terminology of insect repellents. In: Debboun, M., Frances, S.P., Strickman, D.A. (Eds.), Insect Repellent Handbook, 2nd Ed. CRC Press, Boca Raton, London, New York, pp. 3–30.
WHO – World Health Organization, 2001. Protocolo de evaluación de efecto insecticida en Rhodnius prolixus. In: RELCOT – Red Latinoamericana de Control de Triatominos (Ed.), Monitoreo de la resistencia a insecticidas en triatominos en América Latina, Serie Enfermedades Transmisibles, Publicación Monográfica 1. Fundación Mundo Sano, Buenos Aires, pp. 61–67.
WHO – World Health Organization, 2002. Control of Chagas disease. WHO, Geneva.
WHO – World Health Organization, 2014. Basic Documents. WHO, Geneva.
Wigglesworth, V.B., 1936. The function of the *corpus allatum* in the growth and reproduction of *Rhodnius prolixus* (Hemiptera). Q. J. Microsc. Sci. 79, 91–121.
Wigglesworth, V.B., 1939. The Principles of Insect Physiology. Methuen, London.
*Wigglesworth, V.B., 1941. The effect of pyrethrum on the spiracular mechanism of insects. Proc. R. Entomol. Soc. Lond. A 16, 11–14.
*Wigglesworth, V.B., 1942. Some notes on the integument of insects in relation to the entry of contact insecticides. Bull. Entomol. Res. 33, 205–218.
*Wigglesworth, V.B., 1944. Action of inerts dusts on insects. Nature 153, 493–494.
*Wigglesworth, V.B., 1947. The site of action of inert dusts on certain beetles infesting stored products. Proc. R. Entomol. Soc. Lond. A 22, 65–69.
Wigglesworth, V.B., 1956. Insect physiology in relation to insecticides. J. R. Soc. Arts 104, 426–438.
*Wigglesworth, V.B., 1961. Some observations on the juvenile hormone effect of farnesol in *Rhodnius prolixus* Stål (Hemiptera). J. Insect Physiol. 7, 73–78.
*Wigglesworth, V.B., 1963. The juvenile hormone effect of farnesol and some related compounds: quantitative experiments. J. Insect Physiol. 9 (105), 119.
*Wigglesworth, V.B., 1969. Chemical structure and juvenile hormone activity: comparative tests on *Rhodnius prolixus*. J. Insect Physiol. 15, 73–94.
*Wigglesworth, V.B., 1973. Assays on *Rhodnius prolixus* for juvenile hormone activity. J. Insect Physiol. 19, 205–211.
*Williams, J.O., Tonn, R.J., Dias, B.S., Ortega, R., Castillo, C., 1981a. DDT como un insecticida en el control de *Rhodnius prolixus*, una prueba limitada de campo. Bol. Dir. Malariol. San. Amb. 21, 40–44.
*Williams, J.O., Tonn, R.J., Dias, B.S., Ortega, R., Castillo, C., 1981b. Comparación de tres diferentes tipos de equipos para aplicar insecticidas en el control de los vectores de la enfermedad de Chagas con OMS-0043. Bol. Dir. Malariol. San. Amb. 19, 66–76.
Xu, P., Choo, Y.M., De La Rosa, A., Leal, W.S., 2014. Mosquito odorant receptor for DEET and methyl jasmonate. Proc. Natl. Acad. Sci. U.S.A. 111, 16592–16597.
Zeledón, R., 2004. Some historical facts and recent issues related to the presence of *Rhodnius prolixus* (Stål, 1859) (Hemiptera: Reduviidae) in Central America. Entomol. Vect. 11, 233–246.
Zeledón, R., Rabinovich, J.E., 1981. Chagas disease: an ecological appraisal with special emphasis on its insect vectors. Annu. Rev. Entomol. 26, 101–133.
Zerba, E., 1999. Susceptibility and resistance to insecticides of Chagas disease vectors. Position paper. Medicina 59, 41–46.
*Zermoglio, P.F., Martin-Herrou, H., Bignon, Y., Lazzari, C.R., 2015. *Rhodnius prolixus* smells repellents: Behavioural evidence and test of present and potential compounds inducing repellency in Chagas disease vectors. J. Insect Physiol. 81, 137–144.