

Is imidacloprid an effective alternative for controlling pyrethroid-resistant populations of *Triatoma infestans* (Hemiptera: Reduviidae) in the Gran Chaco ecoregion?

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The prevention of Chagas disease is based primarily on the chemical control of Triatoma infestans (Klug) using pyrethroid insecticides. However, high resistance levels, correlated with control failures, have been detected in Argentina and Bolivia. A previous study at our laboratory found that imidacloprid could serve as an alternative to pyrethroid insecticides. We studied the delayed toxicity of imidacloprid and the influence of the blood feeding condition of the insect on the toxicity of this insecticide; we also studied the effectiveness of various commercial imidacloprid formulations against a pyrethroid-resistant T. infestans population from the Gran Chaco ecoregion. Variations in the toxic effects of imidacloprid were not observed up to 72 h after exposure and were not found to depend on the blood feeding condition of susceptible and resistant individuals. Of the three different studied formulations of imidacloprid on glass and filter paper, only the spot-on formulation was effective. This formulation was applied to pigeons at doses of 1, 5, 20 and 40 mg/bird. The nymphs that fed on pigeons treated with 20 mg or 40 mg of the formulation showed a higher mortality rate than the control group one day and seven days post-treatment ($p < 0.01$). A spot-on formulation of imidacloprid was effective against pyrethroid-resistant T. infestans populations at the laboratory level.

Key words: *Triatoma infestans* - imidacloprid - formulation - neglected tropical disease - Gran Chaco ecoregion

Trypanosoma cruzi is the causative agent of Chagas disease, which is currently one of the most important parasitic diseases of the Americas (together with dengue and malaria). Chagas disease represents a serious health and social burden for most Latin American countries, where 10 million people are infected and more than 25 million people are at risk of infection (WHO 2012). The principal vector of this flagellated parasite is the kissing bug *Triatoma infestans* (Klug, 1834) (Hemiptera: Reduviidae), a blood-sucking triatomine that is usually associated with humans inhabiting poorly constructed dwellings. Moreover, the current intense migration to more developed countries contributes to spread and globalise such public health problems (Schmunis & Yadon 2010). In Latin America, this disease is responsible for annual productivity losses in the range of US\$ 1.2 billion. In addition, the medical costs for treating infected individuals who develop severe cardiac or digestive pathology are several times this amount (WHO 2012).

In 1991, several countries, including Argentina, Brazil, Chile, Paraguay and Uruguay, signed an agreement to pursue the campaign Southern Cone Initiative with the goal of interrupting the transmission of Cha-

gas disease, eliminating both domestic and peridomestic *T. infestans* populations, together with an improvement of the screening of blood donors (Dias et al. 2002, Dias 2007). The prevention of Chagas disease is primarily based on the chemical control of the vector using pyrethroid insecticides, which have been the preferred option since the 1980s (Zerba 1999). However, a series of non-exclusive control approaches such as biological control, genetic control, traps, housing improvement and health education have been shown to be effective (Dias 2007). For example, in the Gran Chaco ecoregion, the replacement of peridomestic enclosures serving as goat corrals reduced the incidence of triatomines (Gorla et al. 2013). Although housing improvements involving the replacement of the wall plaster or the thatched roofs have been effective and have produced a general improvement of living standards, this measure might be difficult to implement on a large scale. In this context, chemical control has been partially successful for the elimination of domestic triatomine infestation. In the past decade, however, high resistance levels to pyrethroids, correlated with control failures, have been detected in certain areas of Argentina and Bolivia (Picollo et al. 2005, Toloza et al. 2008, Germano et al. 2010, Gurevitz et al. 2012). Thus, new synthetic insecticides are needed to complement the various strategies for the control of *T. infestans*.

In a previous study, we investigated a wide range of insecticides with different modes of action against *T. infestans* and found that imidacloprid was effective against deltamethrin-resistant populations, emerging as a possible new option (Carvajal et al. 2012).

Imidacloprid is a neonicotinoid insecticide, a class of neurotoxins with a unique mode of action differing from that of any other insecticide currently available for use

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in the field, vegetable or protected cropping systems. It acts as a nicotinic acetylcholine receptor agonist, altering the central nervous system (Horowitz et al. 2004). Imidacloprid was introduced in 1991 as the first commercially available product from the neonicotinoid class of insecticides and it has been found to be effective in a wide range of medical, veterinary, urban and agronomic systems (Tomizawa & Casida 2005).

As the study by Carvajal et al. (2012) implies, a complementary toxicological characterisation of imidacloprid, together with an evaluation of its effect when applied to various substrates, is required to gain a better understanding of this promising new alternative insecticide to control deltamethrin-resistant *T. infestans* populations.

In addition, two parameters should be considered: the time required for the insecticide to produce its effect at its site of action and the stability of the insecticide over a certain period of time. For example, Nasirian et al. (2006) demonstrated that the toxicity of fipronil against *Blattella germanica* (Dictyoptera: Blattellidae) increased after 72 h post-treatment and that its lethal effect then remained stable. In contrast, more than 85% of beetles *Harpalus pennsylvanicus* (Coleoptera: Carabidae) poisoned by contact exposure with imidacloprid recovered within four days (Kunkel et al. 2001). As both parameters, the time of action of the insecticide and the stability of its toxicological effects are intrinsic properties of the insecticide, the characterisation of the insecticide in terms of these parameters is essential.

The rate of penetration of the insecticide is dependent on the properties of the integument in which it is in contact (Fontán & Zerba 1987). Thus, the toxicological effect of the insecticide may vary according to the cuticular distension of the abdomen as a result of blood feeding (Hilberton 1978). A previous study of *T. infestans* by our laboratory showed a strong positive relationship between the penetration rate of topically applied DDT and the mortality occurring after feeding (Fontán & Zerba 1992).

The aims of the present study were to (i) evaluate the delayed toxicity of imidacloprid and the influence of the extent of abdominal distension on the toxicity of this insecticide and (ii) test the effectiveness of various commercial formulations of imidacloprid against *T. infestans*.

MATERIALS AND METHODS

Insects - Resistant population - Samples from the El Malá field population of *T. infestans* were collected in November 2010 from infested houses in the Chaco Province of Argentina (S25°56.077' W60°27.105'), where vector control using pyrethroid insecticides is considered ineffective by the authorities responsible for the Chagas Program of Chaco Province. Field-collected insects were transported to the Research Center of Pest and Insecticides (CIPEIN) laboratory and further generations of these insects were bred in the laboratory. The 50% lethal dose (LD_{50}) obtained for deltamethrin for this population is 134 ng/insect, with a high resistance ratio of 1,031 (Carvajal et al. 2012). A study performed at CIPEIN has shown that eight years after the most recent exposure to deltamethrin, the LD_{50} did not vary in a population from northern Argentina (Germano 2013).

Susceptible population - For comparison, we used a susceptible reference colony, NFS, derived from a domestic field population collected in December 2004 from Santiago del Estero, Argentina. Insects have been controlled successfully with deltamethrin in this area. This laboratory colony has been maintained without the introduction of new insects from external sources. A laboratory test with NFS has shown an LD_{50} of 0.13 (0.11-0.15) ng/I for deltamethrin. This value did not differ statistically from that of the traditional laboratory-susceptible strain maintained at CIPEIN (Roca Acevedo et al. 2011).

For rearing, each population was kept in enclosed boxes (30 x 30 x 30 cm) at $28 \pm 1^\circ\text{C}$ and 50-60% relative humidity with a photoperiod of 12:12 h (L:D). A pigeon was provided weekly as a blood meal source (WHO 1994).

Chemicals - Technical grade imidacloprid (98%) provided by Dr Ehrenstorfer (Augsburg, Germany) was used in topical application bioassays. Analytical grade acetone was purchased from JT Baker (San Pedro Xalostoc, Mexico).

In the surface assays and spot-on bioassays, the following formulations of imidacloprid were used: 35% emulsifiable concentrates (EC) (Mamboretá CONFÍ® and EC-Chemotécnica® (Argentina), 70% wettable granule (WG) (Bayer Confidor®, Argentina) and 10% spot-on (Bayer Advantage G®, Argentina). Moreover, 2.5% EC deltamethrin (Bayer K-Othrine®, Argentina) was used only in surface bioassays.

Topical application bioassays - *T. infestans* first instars (5-7 days old) that had been starved since eclosion were selected for toxicity tests according to the World Health Organization protocol (WHO 1994). The bioassays consisted of the topical application of 0.2 μL of the insecticide diluted in acetone on the dorsal abdomen of the first instar using a 10 μL Hamilton syringe equipped with an automatic dispenser. In the evaluation of blood feeding, nymphs were previously fed to repletion on a pigeon and the insecticide was applied immediately after feeding. The control groups received only pure acetone. The final concentrations of imidacloprid tested ranged from 0.0025-0.5 mg/mL. All concentrations were replicated at least three times with a minimum of 10 insects per replicate. To calculate the LD values at $p < 0.05$, a minimum of 30 insects per concentration was required and mortality values between 10-90% were observed (Robertson et al. 2007). All treatments were performed on different days. Mortality was evaluated after 24 h and also after 48 h and 72 h in the delayed toxicity assays, by placing the insects on a circular piece of filter paper (11 cm diameter) and observing their ability to walk. Only nymphs that were able to walk from the centre of the filter paper to the border were considered to be alive (Picollo et al. 2005).

Evaluation of formulated insecticides - On glass - Insecticides were applied to a square area (96 cm^2) using a 1 mL pipette and a constant flow to achieve uniform impregnation. The treated surface was dried for 24 h. Each replicate consisted of a negative control group (water), a positive control group [EC 2.5% deltamethrin in water at 25 mg active ingredient (ai)/ m^2] and one or more doses of

formulated imidacloprid, ranging from 1,000-5,000 mg ai/m². Three replicates were conducted for each formulation.

Groups of 10 nymphs (3rd-instar nymphs aged 10-20 days, starved since last moult) were confined in glass rings and exposed for 1 h to the treated surfaces. After exposure, the insects were placed in clean flasks with filter paper and were maintained under the laboratory conditions described previously. Mortality was recorded after 24 h (Germano et al. 2014).

On filter paper - Circular disks of Whatman N° 1 filter paper (5.5 cm diameter) were used and placed in plastic Petri dishes (5.5 cm diameter). The papers (area 23.75 cm²) were homogeneously impregnated with 0.1 mL of the formulated insecticide. The tested imidacloprid concentrations ranged from 1-100 mg/mL. The tested deltamethrin concentration was 10 mg/mL. The control groups were exposed to filter paper homogeneously impregnated with 0.1 mL of pure water. After 1 h, when the solvent had evaporated, the insects (10 1st-instar nymphs per group selected as in topical application bioassays) were held in contact with the treated surface for 1 h. After this period, live insects were placed in clean flasks and were maintained under the laboratory conditions described earlier. Mortality was recorded after 24 h (Rojas de Arias & Fournet 2002).

On pigeons - Selected pigeons were treated with various doses of imidacloprid Advantage G®. The average weight of the pigeons was 252.7 ± 39.4 g. Twenty-one pigeons were used. The experimental design included five groups (4 treated and 1 control) with a minimum of three pigeons per group. Prior to the application of the spot-on, we removed several feathers from the pigeon to produce a "blind spot" or arena where the insects could feed. The insecticide was applied to the base of the neck with a needle-less syringe. The insects were then exposed in the area where the spot-on formulation was applied. We have previously determined that this area was the most feasible site because the insects were removed easily without any additional disturbance to the treated pigeon.

The pigeon groups were treated with 1, 5, 20 and 40 mg/ai of the formulation (i.e., = 4, 20, 80 and 160 mg/ai per kg of animal). The control group was manipulated similarly, without the addition of any insecticide, but water.

Synchronised insects (1st and 3rd-instar), previously starved for 15-20 days, were allowed to feed for 30 min on the treated area of the pigeons. The residual effect of the drug was studied on feeding nymphs at different intervals of time (1, 7, 14 and 21 days post spot-on application) after the administration of the drug. Each nymph fed once and was then removed. Each test was replicated three-six times. The tests were performed by allowing the insects housed in jars containing 10-30 insects to feed on the pigeon. A total of 1,054 and 717 first and third-instar nymphs were used, respectively. The fed insects were transferred to clean flasks with filter paper and kept under the laboratory conditions described previously. Mortality was recorded after 24 h.

Statistical analysis - In the topical application and surface bioassays, mortality data were analysed using

POLO Plus software v.2.0. Dose-mortality data were subjected to a probit analysis to estimate the LD (ng/insect) required to kill 50% of the treated individuals (LD₅₀).

In the spot-on bioassays, all mortality data were corrected for control mortality with Abbott's equation (Abbott 1925). The percentage mortality was determined and transformed to arcsine square-root values for an ANOVA. Treatment means were compared and separated with a Duncan test at $p < 0.05$. A 0.05 significance level was chosen as the criterion for biological significance among related treatments.

RESULTS

Evaluation of delayed toxicity and influence of the blood feeding state - The LD₅₀ values for the susceptible (S) and resistant (R) populations were 5.2 (3.4-7.8) and 9.2 (7.4-11.2) ng/insect, respectively, and did not differ up to 72 h after the initial topical application.

We also studied the variation in the toxic effects of imidacloprid relative to the blood feeding condition of the insect because the cuticular distention resulting from blood feeding facilitates the penetration of the insecticide. The blood feeding condition (starvation/feeding) of the insects had no significant influence on the insecticidal activity of the imidacloprid in either population (Table).

Formulation-Surfaces - Four formulations of imidacloprid were tested against first and third-instar *T. infestans* on two different surfaces: filter paper and glass.

The ECs Chemotécnica® and Mamboretá CONFÍ® and the WG Confidor® showed no effects, either on glass against third-instar nymphs (at 1,000-5,000 mg ai/m²) or on filter paper against first-instar nymphs (at a 100 mg/mL dose).

The spot-on Advantage G® could not be tested on glass because it did not form a film after 24 h of drying. On filter paper, Advantage G® was effective, with a mortality of 100% with a 100 mg/mL dose in both susceptible and resistant populations. The LC₅₀ obtained for Advantage G® on filter paper was 22.84 (14.94-36.89) mg/mL.

In contrast, a 10 mg/mL dose of deltamethrin on filter paper caused 100% mortality in the susceptible population, whereas no mortality (0%) was found in the resistant population.

Spot-on on pigeons - A first evaluation of formulations of imidacloprid on glass and filter paper showed that only the spot-on formulation was effective. Accordingly, we analysed the effect of the spot-on formulation of imidacloprid by applying different doses of the insecticide to pigeons.

The applied dose of 1 mg/ai showed no lethal effects against first and third-instar *T. infestans* ($p > 0.05$).

Twenty-four hours after the application of 5 mg/ai to pigeons, nymphs that had fed on the pigeons showed a higher mortality rate (49.8 ± 1% and 40.5 ± 18% for first and third-instar, respectively) than the control group ($p < 0.01$). Nymphs fed seven days after a spot-on application did not show significant differences in mortality between the treated and control groups ($p > 0.05$).

Nymphs fed 14 and 21 days after spot-on application did not show significant differences in mortality be-

TABLE
Insecticidal activity of imidacloprid over starved and fed nymphs I of *Triatoma infestans* at 24 h

Population	Blood feeding state	LD ₅₀ (ng/insect)	Confidence limits (ng/insect)
Susceptible	Starved	5.2	3.4-7.8
	Fed	4	2.4-7
Resistant	Starved	9.2	7.4-11.2
	Fed	10.8	6.4-19

LD: lethal dose.

tween the treated and control groups at any studied dose or nymphal stage ($p > 0.05$).

Both first and third-instar nymphs fed on pigeons that had been treated with 20 mg or 40 mg of the formulation showed a higher mortality rate than the control group one and seven days post-treatment ($p < 0.01$). The residual effect (7 days after treatment) was higher for 40 mg than for 20 mg ($p < 0.01$).

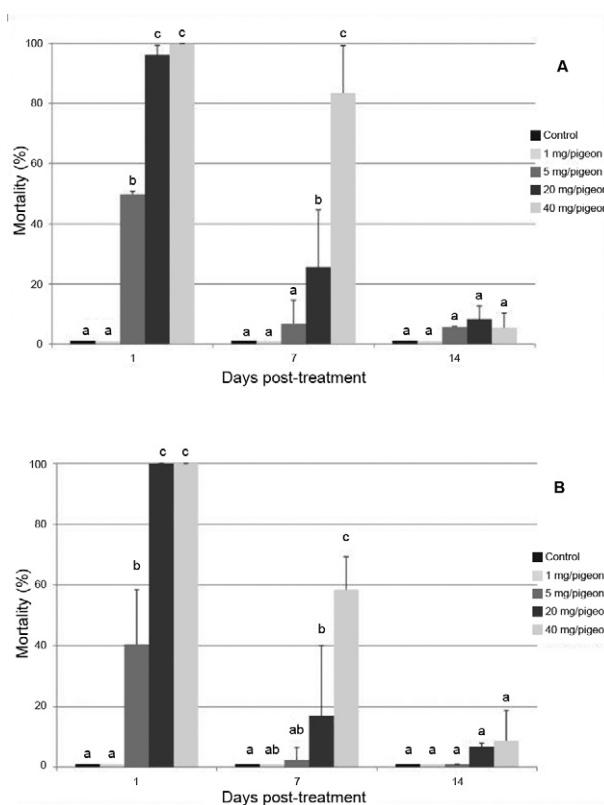
The lethal effect was similar against first and third-instar nymphs at all doses and time intervals (A, B in Figure).

DISCUSSION

Although the pyrethroids deltamethrin and λ -cyhalothrin are available, the only insecticides approved by the Health Service of Argentina for use in the field control of *T. infestans* are the organophosphates fenitrothion and malathion. These insecticides were used to control *T. infestans* in the 70's, but because of their high toxicity in mammals, strong odour and tendency to leave stains on the walls after application they were replaced by pyrethroids in the 80's (Schofield & Dias 1999). Fenitrothion has been shown to be effective against several deltamethrin-resistant populations under laboratory and field conditions (Picollo et al. 2005, Toloza et al. 2008, Carvajal et al. 2012, Santo-Orihuela et al. 2013, Germano et al. 2014). In the past decade, the development of pyrethroid-resistant populations has led to the re-utilisation of either malathion or fenitrothion against triatomines by the health authorities of Argentina and Bolivia.

This is the first study of the efficacy of several formulations of imidacloprid on different surfaces against susceptible and pyrethroid-resistant *T. infestans*.

In an attempt to characterise the toxicology of imidacloprid against *T. infestans*, we studied the variation in mortality through time after topical application and the influence of the blood feeding condition of the insect on this toxicity. Our results showed that the LD₅₀ did not vary significantly up to 72 h after the initial topical application. This result could indicate that the toxicological effects of the imidacloprid remain stable through time. These results differ from that obtained by Kunkel et al. (2001), who found a decrease in the toxicity of imidacloprid through time in the ground beetle *H. pennsylvanicus* (Coleoptera: Carabidae) following contact exposure.



Mortality of *Triatoma infestans* fed at different intervals after the spot-on application. A: first-instar nymphs; B: third-instar nymphs. Bars with different letters are significantly different ($p \leq 0.05$) within each day.

The blood feeding condition (starvation/feeding) of the insects had no significant influence on the insecticidal activity of the imidacloprid. Thus, the rate of penetration associated with physicochemical modifications of the cuticle after blood feeding appears not to alter the toxic effects after topical application.

We also analysed various types of commercial formulations of imidacloprid on two different surfaces. The formulation of an insecticide is of vital importance in its effectiveness. Thus, the first step, focusing on the field application of the insecticide, is to find a correct formulation of the insecticide. We found that neither the EC nor the WG formulations were effective against *T. infestans* nymphs. However, the spot-on formulation was highly effective on resistant insects. Although most surfactants and polymers are biologically inert when applied to insects, these chemicals can profoundly affect the biological activity of the pesticide when used as part of a formulation (Scher 1988).

Traditional spraying is highly effective inside domiciles, but it usually leaves a number of residual individuals of the vector in the peridomestic environment, as reported in the southern part of the Chaco Region (Porcasi et al. 2006, 2007). These residual populations eventually re-colonise the domestic sites, re-establishing the domestic transmission cycle of *T. cruzi* (Gürtler et al. 2007). Complementary chemical control strategies include bed nets (Kroeger et al. 1995), pyrethroid-impregnated cur-

tains (Ferral et al. 2010) and a residual paint formulated as a micro-encapsulate containing an organophosphate and a juvenile hormone analogue (Alarico et al. 2010).

Although *T. cruzi* is transmitted by several species of triatomines, animals such as dogs, cats and chickens are the main domestic reservoirs of *T. cruzi* in the endemic areas of Chagas disease (Gürtler et al. 2009). Thus, Reithinger et al. (2006) found that deltamethrin-impregnated dog collars reduced the survival and fecundity of exposed kissing bugs on dogs. Similarly, a spot-on formulation of fipronil applied on dogs and a pour-on formulation of cypermethrin applied on chickens have been successfully tested against *T. infestans* (Rojas de Arias & Fournet 2002, Amelotti et al. 2009).

The imidacloprid Advantage G[®] spot-on formulation is recommended for treating cat fleas. The recommended dose ranges from 10-40 mg ai/kg. In this study, we tested doses from 4-160 mg ai/kg on pigeons. At a dose of 20 mg ai/kg, 50% of the nymphs were killed 24 h after the application. The doses of 80 and 160 mg ai/kg produced 100% mortality and had a high residual effect until seven days post-treatment.

Amelotti et al. (2009) studied the efficacy in chickens of a pour-on formulation containing cypermethrin. They found that after a week of initial exposure to the insecticide at a dose of 120 mg/chicken, 53% of the treated third-instar nymphs were killed, whereas at day 14, mortality had values similar to the controls (4.9%). This finding is similar to our results because a dose of 160 mg/kg between days 7-14 after initial exposure produced a mortality of 58% and 8%, respectively.

Rojas de Arias and Fournet (2002) studied the residual activity of fipronil with a contact test of the insecticide on filter paper against fifth-instar *T. infestans*. The authors reported a value of LC_{50} of 106 mg/m². Despite the subtle differences in methodology, the LC_{50} value of 960 mg/m² of imidacloprid suggest that this compound has a high contact activity that depends on the type of formulation. Thus, an approach involving the use of the spot-on formulation might complement traditional pyrethroid spraying, which has shown a low efficacy in the elimination of *T. infestans* peridomestic populations. These results support the idea of imidacloprid as an alternative insecticide to pyrethroids.

The effectiveness of imidacloprid against *T. infestans* is reinforced by its lower oral and dermal mammalian toxicity than fenitrothion, the current alternative to pyrethroids. For instance, the oral and dermal LD_{50} in rats for imidacloprid are 450 mg/kg and > 5,000 mg/kg, whereas the values for fenitrothion are 250 mg/kg and 2,500 mg/kg, respectively. Additionally, the no-observed effect level in rats is higher for a diet containing 300 mg/kg imidacloprid [based on a unit of 1 kg of body weight than for a diet containing 10 mg/kg fenitrothion (Tomlin 1997)]. Another advantage in comparison with fenitrothion is that imidacloprid is odourless. This concern is highly important if an insecticide must be used indoors and in a domestic environment.

Because of the increasing number of populations resistant to pyrethroids and the high mammalian toxicity of fenitrothion, it is essential that other insecticidal compounds,

especially those with alternative modes of action to pyrethroids and organophosphates, are rapidly made available for *T. infestans* control programmes in South America.

This study has indicated the potential of imidacloprid in the control of Chagas disease vectors. However, imidacloprid should be incorporated into an integrated pest management programme because its effectiveness is primarily restricted to domestic and peridomestic animals. Moreover, the type of formulation selected is essential in the function of the toxicokinetic and toxicodynamic processes by which the active ingredient (i.e., imidacloprid) affects the insect (*T. infestans*). A spot-on formulation appears to improve this interaction, resulting in increased mortality of the triatomine vector in the laboratory. Further studies are needed to test this type of formulation under semi-field or field conditions and to incorporate this formulation as a complementary strategy for triatomine control.

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