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Control of experimental *Triatoma infestans* populations: effect of pour-on cypermethrin applied to chickens under natural conditions in the Argentinean Chaco region

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Abstract. Among peridomestic structures, chicken coops are sites of major importance for the domestic ecology of *Triatoma infestans* (Hemiptera: Reduviidae). The aim of this study was to evaluate in an experimental context the effects of a cypermethrin pour-on formulation applied to chickens on blood intake, moulting and mortality in *T. infestans*, under the natural climatic conditions of a region endemic for Chagas' disease. Experimental chicken huts were made of bricks and covered with plastic mosquito nets. Ninety fourth-instar nymphs were maintained in each hut. The study used a completely random design in which chickens in the experimental group were treated with a cypermethrin pour-on formulation. Five replicates (= huts) of the experimental and control groups were conducted. The number of live *T. infestans*, blood intake and moults to fifth-instar stage were recorded at 1, 5, 20, 35 and 45 days after the application of cypermethrin. Cumulative mortality was higher in nymphs exposed to treated chickens ($> 71\%$) than in control nymphs ($< 50\%$) ($P < 0.01$). Blood intake and moulting rate were lower in nymphs fed on treated chickens than in control nymphs ($P < 0.05$). Pour-on cypermethrin was able to cause significant mortality, although it did not eliminate the experimental population of *T. infestans*.

Key words. *Triatoma infestans*, Chagas' disease, cypermethrin, pour-on, vector control.

Introduction

Chagas' disease, the most important endemic disease in Latin America, is caused by *Trypanosoma cruzi* (Kinetoplastida: Trypanosomatidae), which is mainly transmitted to humans inhabiting rural communities in the Southern Cone countries of South America by its vector *Triatoma infestans* (Klug) (Dujardin *et al*., 2004). Standard vector control methods based on pyrethroid insecticides have shown high efficacy in the elimination of intradomestic infestations of *T. infestans* (Gorla *et al*., 2010). However, the same standard application of pyrethroid insecticides was found to have less efficacy in controlling peridomestic populations of the vector in the Gran Chaco region (Gürtler *et al*., 2007; Porcasi *et al*., 2007). Pyrethroid-based suspension concentrate formulations applied using traditional spraying techniques have been reported as less efficacious in peridomestic habitats in which exposure to the environmental conditions of peridomestic structures decreases the residual lethality of the insecticide, favouring the survival of residual *T. infestans* populations after the application of insecticide (Gürtler *et al*., 2004; Cecere *et al*., 2006). Active or passive dispersal from these residual foci may represent the most probable mechanisms of the subsequent domiciliary re-infestation of rural settlements that have been treated with pyrethroid insecticides (Schofield, 1985; Abrahan *et al*., 2011; Hernández *et al*., 2013).

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The structural complexity that usually characterizes the peridomestic habitat, of which the chicken coop is a key component, makes it difficult to completely eliminate *T. infestans* populations (Cecere *et al*., 1997). A number of different approaches to and techniques for controlling or eliminating peridomestic populations of *T. infestans* have been considered as alternatives or complements to the traditional application of pyrethroid-based insecticides. Among them, use of a pour-on formulation (a solution for external transcutaneous administration, typically containing active ingredient, a co-solvent and a spreading agent) of cypermethrin is an approach that may complement pyrethroid spraying. This formulation is recommended for the control of ectoparasites affecting cattle, pigs and sheep, although it is not specific for chickens. In previous studies carried out under laboratory conditions, we established that 2 mL of cypermethrin formulated as a pour-on with 6% active ingredient (a.i.) represents the most effective dose to be applied to chickens (Amelotti *et al*., 2009; Amelotti, 2012) and that adding piperonyl butoxide (PBO) as a synergist does not enhance the effectiveness of the insecticide (Amelotti *et al*., 2010; Amelotti, 2012).

However, applications in the field involve a high number of factors that may influence the efficacy of the insecticide application. These particularly include environmental factors (Gorla, 1991) and the ability of each developmental stage to metabolize the insecticide (Alzogaray & Zerba, 2001). Therefore, the present study was conducted to evaluate the effects of a pour-on cypermethrin treatment in a system that resembles the chicken hut under natural environmental conditions. Such experimental structures have been widely used in triatomine research for the study of the population dynamics of *T. infestans* (Gorla & Schofield, 1989; Gorla, 1991, 1992; Cecere *et al*., 2003). The main objective of this study was to evaluate the effects on blood intake, moulting and mortality in *T. infestans* fed on chickens that were treated with a pour-on formulation of cypermethrin. We also quantified how much residual active ingredient of the insecticide remains on the feathers of treated chickens over time. Additionally, different methods of applying the pour-on formulation were compared on the assumption that coverage of a greater area of the surface of the chicken body with the insecticide would lead to a higher degree of protection against infestation.

Materials and methods

The study was carried out during three consecutive warm seasons (October–March), between January 2009 and March 2011, at the Centro Regional de Investigaciones Científicas y Transferencia Tecnológica de La Rioja (CRILAR) experimental field, La Rioja, Argentina (28°47' S, 66°57' W). During the study period, the environmental conditions of temperature, rainfall and wind were measured using a weather station (Weather Monitor II; Davis Co., Baltimore, MD, U.S.A.).

The cypermethrin [(RS)-α-cyano-3-phenoxybenzyl (1RS)-cis, trans-3-(2,2-dichlorovinyl)-2,2-methylcyclopropanecarboxylate] was formulated as a pour-on with 6% a.i. by Biogénesis SA (Buenos Aires, Argentina). The formulation is registered in Argentina for veterinary purposes. Treated mammals intended for meat production are subject to a withdrawal period of 6 days.

The insects used in this study were provided by the breeding facility of the Coordinación Nacional de Control de Vectores in Punilla (Córdoba, Argentina), bred under controlled conditions and fed on chickens (*Gallus* sp.). The specimens were F3 offspring of *T. infestans* collected in the northern region of the province of San Luis in Argentina. Fourth-instar nymphs used in the experiments were 15 days post-moulting on average. The insects were unfed for 2 weeks and kept in appropriately labelled plastic jars under controlled temperature (26–28 °C) and relative humidity (RH 50–70%) conditions.

The chickens were fed with controlled and equivalent quantities of corn grain. They were weighed at the start and end of each assay using an electric balance accurate to 2 g.

The experimental design included two groups of five randomly selected chickens. These represented, respectively, one control group and one experimental group treated with 2 mL cypermethrin (= 0.12 g a.i. per chicken). Each chicken was individually identified using a numbered plastic ring that was retained during the whole experiment. The chickens were maintained in separate and fenced huts made of cooked red clay bricks (see Gorla & Schofield, 1985), with doors and roofs of wood. A nylon cover was applied over the roofs to protect the structures from rain. Each hut measured 60 × 60 × 60 cm, was built on a cement base and was covered with a plastic mosquito net in order to prevent the movement of insects between huts. Each hut was located within a small compound (1.0 × 1.5 m) that was fenced to prevent the movement of chickens among replicates. Chickens were allowed to remain within the small individual fenced compounds during the day and were re-introduced into their nests at night. During the night, *T. infestans* nymphs were free to feed on the chickens, and the chickens were free to predate on the nymphs. As an additional barrier to prevent the dispersal of bugs, the 10 replicates (five treatment and five control groups) were located within a large fenced compound (5 × 10 m) that was completely covered (walls and top) with mosquito netting.

The effects of the insecticide were studied by placing an experimental group of 90 fourth-instar nymphs within each of the 10 chicken huts (900 nymphs in each assay). Nymphs remained inside the chicken huts for 45 days. Huts were dismantled at days 5, 20, 35 and 45 in order to count the number of nymphs. (In Assay 3, we were unable to collect data on day 35.) At each census, all nymphs present were collected and processed in the nearby laboratory. Numbers of live and dead specimens, and of moulted nymphs were counted. We also estimated the nutritional status of each nymph using a semi-qualitative approach in which each nymph was classified as unfed, fed or engorged according to the size of its abdomen. Blood intake was estimated as the difference between the average weight of nymphs found in each hut at each census occasion and the average weight of nymphs measured at the start of the assay. The average weight was calculated by dividing the weight of the group of nymphs

present in each hut by the number of nymphs found alive in each hut at the same census. Nymphs were weighed as a group and not individually in order to avoid excessive manipulation that might artificially increase their rate of mortality.

All living bugs were returned within the same day to their respective huts. When the number of nymphs (dead or alive) did not add up to the number that had been counted in the previous census, missing nymphs were assumed to have been eaten by the chickens.

Two different methods of insecticide application were evaluated: (a) 2 mL (0.12 g a.i/chicken) of the insecticide formulation was applied to each treated chicken under the wings using a needleless syringe, and (b) 2 mL of the insecticide formulation was diluted in 13 mL of water and sprayed on to the chicken's belly and under its wings. For each assay, the control group was manipulated similarly to the experimental group but did not receive the insecticide. The sites of insecticide application were chosen because they were considered to represent the warmest areas of the chicken's body and therefore to have the highest probability of attracting insects (Ferreira *et al*., 2007; Catalá, 2011), and to afford the highest degree of protection against the degradation of the insecticide. The method found to be most effective was then repeated in two consecutive warm seasons.

The residual amount of active ingredient was estimated from feather samples taken at random from three treated chickens at 1, 15 and 40 days after the application of insecticide. Feathers were taken from the site of insecticide application below the chickens' wings. These feather samples were weighed using an analytical balance and transferred to a conical centrifuge with tube capacity of 15 mL. Ethyl acetate (2.0 mL) was added to the samples and stirred for 10 min before extraction. An aliquot of the organic extract was passed through a filter (0.45 μm). Ten microlitres were injected into a high performance liquid chromatography (HPLC) injector (RP-18; Rheodyne LLC, Rohnert Park, CA, U.S.A.) with a 125 × 4.6-mm column. The mobile phase used methanol : acetonitrile : water (60 : 25 : 15 v/v). Detection was performed at ultraviolet (UV) of 210 nm with a flow of 0.90 mL/min. The cypermethrin retention time was 3.54 min [unpublished protocol based on Bottomley & Baker (1984)]. The calibration curve for the estimation of the extract concentrations was prepared with reference standards (Biogénesis SA).

Data were analysed using parametric analysis of variance (ANOVA) when variance heterogeneity was rejected (Levene's test). Cases with heterogeneous variances were analysed with the Kruskal–Wallis test. All statistical analyses were carried out using Infostat 2011 (Di Rienzo *et al*., 2011) and STATISTICA Version 7.1 (StatSoft, Inc., Tulsa, OK, U.S.A.). Kaplan–Meier survival curves were calculated for the different treatments. A log-rank test was used to compare survival curves between the different treatments.

Results

Weather variables

Temperature and rainfall during the four study seasons are summarized in Table 1. During the warm season of 2010–2011, total rainfall was higher (173 mm) than in previous periods (range: 0.4–51.4 mm). Average temperatures were similar among all four study seasons.

Weight of chickens

Cypermethrin-treated chickens and chickens in the control groups were of similar weight in all assays (results not shown).· Treated and control chickens maintained good health during the experimental periods and no adverse effects were observed.

Comparison of the different methods of cypermethrin application

The mortality of *T. infestans* nymphs in both treatment groups (application of the undiluted cypermethrin formulation under the chicken wings vs. application of the diluted cypermethrin formulation around the ventral body and wings) was higher than that in the respective control groups ($P < 0.05$). However, nymph mortality differed significantly between the two cypermethrin application methods. At 20 days after treatment, cumulative mortality of nymphs was higher in the groups in which the pure formulation had been applied ($70.0 \pm 6.3\%$ vs. $55.5 \pm 9.6\%$; $P < 0.01$). This difference persisted for 45 days post-application ($83.3 \pm 6.9\%$ vs. $71.3 \pm 11.5\%$; $P = 0.02$). Numbers of newly moulted fifth-instar nymphs did not differ between the two cypermethrin application methods (Fig. 1). As this partial result showed the pure cypermethrin formulation to be more effective than the diluted formulation, two new assays were carried out in order

Table 1. Average, maximum and minimum temperatures and total rainfall during each assay.

Assay	Cypermethrin formulation	Period	Average temperature, °C	Maximum temperature, °C	Minimum temperature, °C	Total rainfall, mm
1	Undiluted	05/01/09–20/02/09	22.2	33.9	13.2	45.8
2	Diluted with water	01/11/09–12/12/09	20.2	34.1	11.2	0.4
3	Undiluted	01/12/10–14/01/11	22.6	33.6	11.6	51.4
4	Undiluted	24/01/11–11/03/11	18.9	31.8	14.9	173

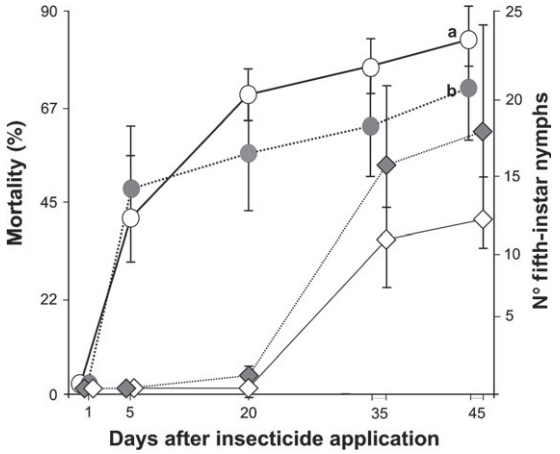

Fig. 1. Comparison between different cypermethrin application methods. Mortality (%) (circles) and number of fifth-instar nymphs (diamonds) at each time-point post-insecticide application in *Triatoma infestans* fourth-instar nymphs fed on chickens treated with the diluted (grey symbols) and non-diluted formulations of cypermethrin (white symbols). Vertical lines represent standard deviations. Different letters indicate significant differences (Kruskal–Wallis test, $P < 0.05$).

to confirm the effect of this method of cypermethrin application and to study its performance under different environmental conditions. A 'year effect' attributed to the differences in total precipitation recorded in the various experimental periods was detected ($P < 0.04$) and therefore the results could not be aggregated (Table 1).

Nymph mortality and cypermethrin concentration

Nymphs exposed to chickens treated with the insecticide showed the highest mortality at 5 days after the application of insecticide, as shown by Kaplan–Meier survival curves for the treated and control groups in the two independent assays. Kaplan–Meier survival estimates showed overall significant differences between the control and treated groups (log-rank test, $P < 0.01$). Nymphs in the treated groups had a probability of 0.1 of surviving for 20 days after the application of insecticide (Fig. 2A, B). Nymph mortality at each time-point after the application of insecticide showed a positive association with the concentration of cypermethrin found in the chickens' feathers (Fig. 3). Fifteen days after the application of insecticide, the cypermethrin concentration was found to be about four times lower than the initial concentration (837 p.p.m.), resulting in the mortality of 50% of the nymphs. Forty days after the application of insecticide, only 6.6% of the original cypermethrin remained in feathers (276 p.p.m.). This concentration was not high enough to kill the nymphs under the assay conditions (Fig. 3).

Blood intake

Despite the differences in environmental conditions and in the methods of cypermethrin application, the blood intake of

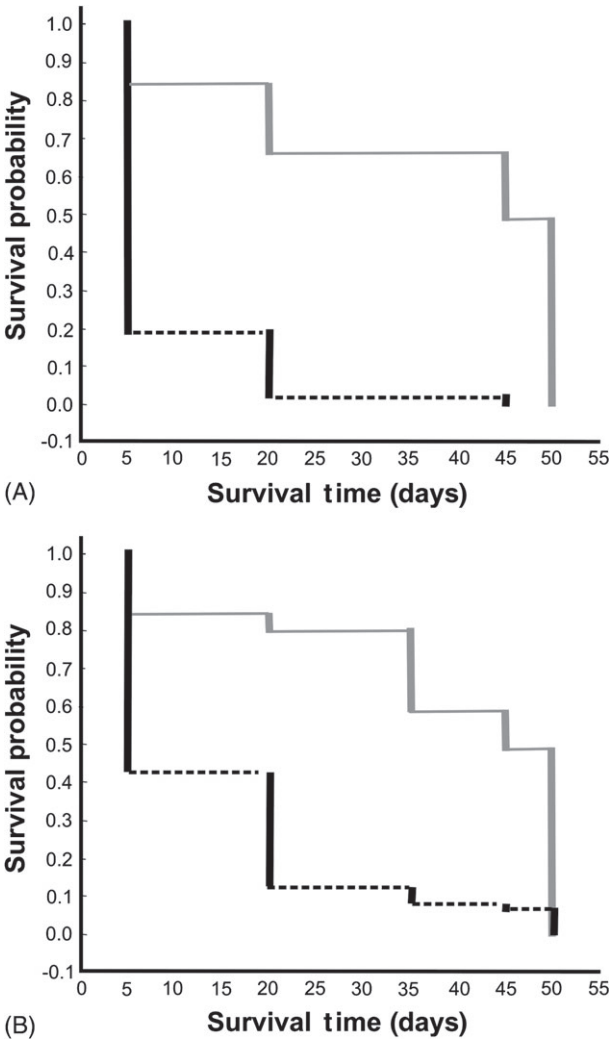

Fig. 2. Kaplan–Meier estimates of cumulative proportions of surviving *Triatoma infestans* nymphs fed on treated (undiluted formulation) chickens for each post-insecticide application interval during (A) Assay 3 (December 2010 to January 2011) and (B) Assay 4 (January–March 2011). Grey lines represent the control group; black lines represent the treatment group. In each assay, the curves differ significantly (log-rank test, $P < 0.01$).

nymphs in the treatment groups was always lower than that of nymphs in the control groups (Table 2). Numbers of engorged nymphs in the treatment groups were significantly lower than in the control groups ($P < 0.01$). Five days after the application of insecticide, the mean ± standard deviation (SD) percentage of engorged nymphs in the treatment groups was 8.5 ± 6.8%, whereas that in the control groups was higher at 58.5 ± 18.6%. After 45 days, the mean ± SD biomass (weight of all insects alive in each hut) of *T. infestans* collected from control huts was almost three times higher than that of *T. infestans* collected from huts with treated chickens (13.79 ± 2.9 g vs. 3.77 ± 1.2 g; $P < 0.05$). The number of nymphs in the control groups and their average body mass was higher than those observed in the treatment groups.

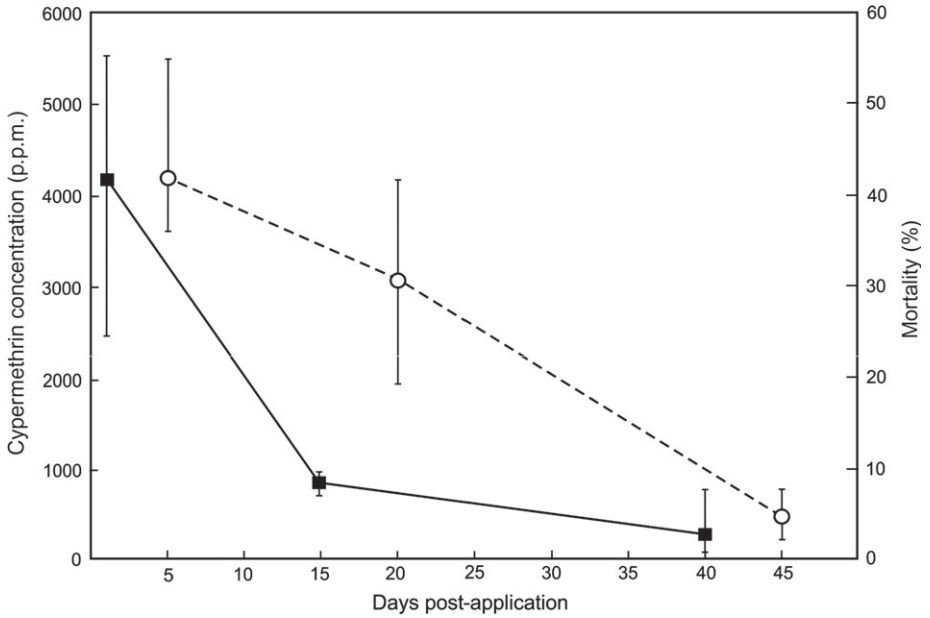

Fig. 3. Comparison between cypermethrin concentrations (p.p.m.) in the feathers of treated chickens and *Triatoma infestans* nymph mortality (%) during Assay 1 (January–February 2009) at different time-points post-insecticide application. ■, cypermethrin concentration; ◯, mortality. Vertical lines indicate the minimum and maximum values for each variable.

Table 2. Mean ± standard deviation (SD) blood intake of fourth-instar *Triatoma infestans* nymphs fed on chickens treated with 6% cypermethrin pour-on and on chickens without insecticide (control) at different intervals after insecticide application.

Assay: group	Post-application blood intake, mg, mean ± SD			
	Day 5	Day 20	Day 35	Day 45
1: Control	84.2 ± 9.0^a	90.6 ± 3.8^a	164.6 ± 33.3^a	210.4 ± 15.9^a
1: Treated	30.9 ± 5.2^b	71.5 ± 4.4^b	102.3 ± 15.7^b	184.2 ± 18.8^b
2: Control	96.6 ± 7.8^a	98.5 ± 5.6^a	236.9 ± 17.8^a	245.5 ± 16.2^a
2: Treated	42.8 ± 20.2^b	82.9 ± 6.2^b	158.7 ± 36.5^b	181.2 ± 41.7^b
3: Control	54.4 ± 16.0^a	83.3 ± 3.4^a	ND	252.9 ± 18.0^a
3: Treated	15.4 ± 7.4^b	31.5 ± 48.7^b	ND	36.52 ± 53.3^b
4: Control	58.0 ± 11.0^a	70.4 ± 5.8^a	56.6 ± 16.6^a	86.6 ± 24.4^a
4: Treated	12.0 ± 10.6^b	23.7 ± 23.4^b	37.4 ± 22.9^a	35.0 ± 12.3^b

Different letters indicate significant differences at $P < 0.05$.
ND, no data.

Moulting rate

The first moults to fifth-instar nymphs were recorded at 20 days after the beginning of the experiment. From that date to the end of the experiment the moulting rate of nymphs exposed to treated chickens was lower than that of control nymphs (Table 3). During Assays 3 and 4, the experimental field was exposed to abundant rain (Table 1) affecting both the control and treatment groups. In comparison with those apparent in previous dryer seasons (Assays 1 and 2), a lower proportion of moulted nymphs was observed. At 35 days after the beginning of the experiment, the treatment group showed $< 21.3\%$ of moulted nymphs, whereas higher proportions ($> 47\%$) were found in all control groups (Table 3).

Table 3. Mean ± standard deviation (SD) percentages of fourth-instar *Triatoma infestans* nymphs moulting (N5 at each date/initial number) when fed on chickens treated with a 6% cypermethrin pour-on formulation or untreated chickens (control) at different intervals after insecticide application.

Assay: group	Post-application fourth-instar nymphs moulting, %, mean ± SD			
	Day 5	Day 20	Day 35	Day 45
1: Control	0	0	47.3 ± 7.6^a	50.7 ± 10.3^a
1: Treated	0	0	12.3 ± 6.2^b	12.8 ± 8.3^b
2: Control	0	16.1 ± 6.0^a	53.1 ± 5.0^a	55.1 ± 0.4^a
2: Treated	0	1.3 ± 0.8^b	21.3 ± 8.7^b	21.8 ± 9.1^b
3: Control	0	7.5 ± 9.0^a	ND	47.3 ± 7.6^a
3: Treated	0	1.5 ± 0.6^b	ND	1.1 ± 1.1^b
4: Control	0	0	12.8 ± 8.3^a	20.0 ± 11.4^a
4: Treated	0	0	1.1 ± 0.0^b	2.4 ± 2.3^b

Different letters indicate significant differences at $P < 0.05$.
ND, no data.

Discussion

The present results expand the knowledge reported in previous studies of the effects on *T. infestans* of cypermethrin pour-on formulations applied to chickens. By contrast with previous assays carried out under laboratory conditions (Amelotti *et al*., 2009, 2010), the present study evaluated the effects of the insecticide using hosts that were unrestricted in their movement and insects that were able to choose when and where to eat, and exposed the insecticide to unfavourable environmental factors such as dust, rain, sun radiation and different temperatures (Gürtler *et al*., 2004). The data show that, under semi-field

conditions, the cypermethrin pour-on formulation reduced survival, blood intake and moulting in *T. infestans*.

In order to achieve a greater coverage of the chicken's body surface, an aqueous dilution of the cypermethrin pour-on was tested. However, this method was not as effective as the application of the undiluted insecticide formulation to a specific body area.

The cumulative mortality of *T. infestans* observed 45 days after the application of insecticide varied between 71.3% and 96.4% in the different treatment groups. These percentages are certainly high, leading in some cases to the near elimination of all insects in the treatment groups. However, sources of mortality other than the insecticide were present in this experimental system. Discounting the mortality caused by factors other than the insecticide (e.g. rain, chicken predation and hut dismantling), the net effect of the insecticide (compared with the control group) was estimated to account for 44.2% (95% confidence interval 17.4–71.2%) of mortality. This value is similar to that obtained by Juan *et al*. (2013) in an analysis of the triatomicidal effect of a new spot-on formulation of β-cypermethrin applied to poultry under semi-field conditions. In both works, exposure to a pyrethroid-based formulation decreased the experimental *T. infestans* population.

A particular situation was observed in Assays 3 and 4, when the highest mortality recorded in the control and treatment groups coincided with periods of abundant rainfall (Table 1). The known knock-down effect of the cypermethrin (Amelotti *et al*., 2010) caused an additional increase in mortality among the nymphs because the mud that formed on the hut floor trapped several of the knocked-down nymphs, and made it harder for the insects to move, thereby increasing their risk for predation by chickens. Nymphs exposed to cypermethrin decreased their blood intake in almost all assay periods. Similar effects were reported in *T. infestans* nymphs exposed to dogs wearing deltamethrin-treated collars (Reithinger *et al*., 2006). This result may be attributable to a repellent effect of the cypermethrin (Wood *et al*., 1999). Although the anti-feeding effect produced by pyrethroids has not been demonstrated in *T. infestans*, exposure to cypermethrin has been shown to decrease food intake in other insects, such as larvae of *Pieris brassicae* (Lepidoptera: Pieridae) (Tan, 1981) and the beetle *Phaedon cochleariae* (Coleoptera: Chrysomelidae) (Hajjar & Ford, 1990). In *T. infestans*, this 'repellence effect' decreases with the washing out of the insecticide, but does not disappear completely until 45 days after application (Amelotti *et al*., 2010; Amelotti, 2012), and its effect on the ingestion of blood is one of the key factors regulating the population density of *T. infestans* (Gorla & Schofield, 1989). The amount of blood ingested also affects the development rate of nymphal stages and fecundity (Catalá de Montenegro, 1989). It is probable that, in nature, triatomines might avoid making contact with the insecticide because they have high sensibility to the scents of diverse chemical compounds (Cruz-Lopez *et al*., 2001; Barrozo & Lazzari, 2004) and they may be able to detect the cypermethrin before they are within the minimal distance required to kill them. As nutritional status is also a well-known trigger of *T. infestans* dispersal by flight (Lehane *et al*., 1992; Schofield *et al*., 1992; Abrahan *et al*., 2011), attention should be given to the possibility of their increased dispersal towards bedrooms in houses if this approach of xenointoxication is applied in a rural community within an endemic region. It was expected that higher quantities of cypermethrin would remain on the skin and feathers at the sites of application because the epidermis acts as a barrier to the absorption of most of the insecticide (Scott & Ramsey, 1987), causing the intoxication of the insects by contact. However, the concentration of cypermethrin on feathers was reduced by around four times at 15–18 days after the application (Fig. 3). Residual activity in these bioassays was shorter than in the majority of insecticide formulations so far tested against vectors of Chagas' disease in America, which typically show residual activity of 4–6 months and a much longer effect than is reported for pyrethroids applied on different surfaces (Rojas de Arias *et al*., 2003; Gürtler *et al*., 2004).

The results obtained in this study suggest that a cypermethrin pour-on formulation applied to chickens is not able to eliminate a *T. infestans* population, although it is able to produce significant mortality. The findings of this study suggest that pour-on insecticides could be considered to significantly complement the application of suspension concentrate formulations of pyrethroid insecticides in areas with high prevalences of peridomiciliary infestation by *T. infestans*. Validation of this eventual improvement on vector control efficacy should be carried out under experimental field conditions in order to compare the effects of traditional interventions using pyrethroid spraying with those of pyrethroid spraying plus pour-on applications on chickens.

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