

A new tactic for *Triatoma infestans* control: fabrics impregnated with beta-cypermethrin

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ABSTRACT

Triatoma infestans is the most important vector of Chagas' disease in Argentina. Experiences from Argentine national campaigns have shown indoor spraying to control triatomine bugs is both expensive and difficult. An alternative control method may be the use of pyrethroid-impregnated fabrics (PIFs), which could be both a practical and cheap complement to conventional control measures. In this study, a formulation of emulsifiable concentrate of β -cypermethrin [α -cyano-3-phenoxybenzyl-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate] pyrethroid was used to impregnate different fabrics. The fabrics were then tested for their efficacy and persistence in killing *Triatoma infestans*. Insects attempting to penetrate or grasp the fabric in search of blood meals were poisoned by the exposure to the absorbed pyrethroid.

Laboratory bioassays showed that the insecticidal effect of the PIFs with a dosage of 0.2 g/m² lasted longer on wool than on cotton or rayon. Eight months after impregnation, a residual efficacy of 100% was found for wool, 80% for rayon-cotton fabrics, and 50% for rayon-polyester fabrics. In addition, β -cypermethrin-impregnated fabrics showed a better repellency effect than did fabrics impregnated with either deltamethrin or cypermethrin.

For field trials, fabrics were impregnated either in the laboratory or "in situ" at a dosage of 1 g β -cypermethrin/m² and then dried. The PIFs were placed inside homes, either under the roof or under the bed mattress. The field trials showed the PIF approach to be very effective in keeping dwellings free of triatomine bugs for at least one year and found a high degree of acceptability among the houses' residents.

Nets are being used worldwide to protect people from biting insects (1). Impregnation of bednets with pyrethroids represents the most important advance in malaria vector control since the invention of residual house spraying (2, 3).

Different formulations of permethrin and deltamethrin, as well as different netting, have been used to establish

which combinations of pyrethroid and netting are the most effective in mosquito control (4–6). Field trials have been performed in Africa, Asia, and such Western Hemisphere countries as Mexico, Venezuela, Guyana, and Suriname (7). Since 1982, the World Health Organization has recommended extensive field trials with pyrethroid-impregnated nets (8, 9).

According to MacCormack (10), experiences in China and India showed that impregnating nets was acceptable and people were willing to use them. The primary health care system in many countries should be well suited to the organization of such operations.

In Argentina, *Triatoma infestans* Klug (Hemiptera: Reduviidae) is the most important vector of Chagas' disease (11). Experiences from the national campaigns in that country have shown that indoor spraying is not only expensive but also sometimes difficult due to organizational problems and many residents' lack of acceptance of the practice. Houses are generally far from one another, and sometimes there are no roads for the vehicles from the spraying task forces to reach the dwellings. Inhabitants dislike conventional chemical vector control because they have to take all of their personal belongings out of their houses

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during the spraying. Therefore, alternative methods of control are necessary, especially those that can be implemented through community participation (12, 13).

Pyrethroid-impregnated fabrics (PIFs) are a completely new application of pyrethroids for Chagas' disease vector control and provide a practical and cheap complement to conventional control measures.

β -cypermethrin has been evaluated in our laboratory and compared with deltamethrin and other pyrethroids being used in the Argentine national campaigns for Chagas' disease vector control (14). Comparing the 50%-mortality lethal dose (LD_{50}) amounts, β -cypermethrin is three times more effective than deltamethrin for nymphs I and twice as effective for nymphs V, but slightly less effective for adult insects of *Triatoma infestans* (15). β -cypermethrin could thus be a promising alternative for Chagas' disease vector control.

In this research, various kinds of fabrics, including wool, cotton, polyester, and rayon, were assayed to find the best combination of fabric support and β -cypermethrin for satisfactory triatomaticidal activity. The research demonstrated the efficacy of PIFs in terms of mortality and repellency, in both the laboratory and the field.

MATERIALS AND METHODS

Fabrics, chemicals, and biological material

Eight different fabrics were assayed. The fabrics were: 58% cotton and 42% polyester, 100% pure wool, 43% rayon and 57% cotton, 51.2% rayon and 48.8% polyester, 100% cotton, 100% cellulose acetate ("taffeta"), 100% polyester (a wide-mesh fabric called "tulle"), and 100% nylon ("voile").

Three chemicals were used in this research. One was deltamethrin, from Agrevo (Argentina); as a 5% emulsifiable concentrate (EC) it is called DECIS. The second chemical was cypermethrin, from Chemotécnica S.A. (Argentina), as a 25% EC. The third chemi-

cal, β -cypermethrin, is a mixture of isomers of cypermethrin (α -cyano-3-phenoxybenzyl-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate) containing 38% 1Rcis S + 1Scis R and 57.5% 1Rtrans S + 1Strans R. As a 5% EC formulation, it is called Chinmix (Chemotécnica S.A., Argentina). β -cypermethrin provides a wide efficacy spectrum but with a relatively low environmental risk (16). A literature review shows that β -cypermethrin is slightly more toxic than pyrethrin I and belongs to the cost-effective group of pyrethroids (17). It is not hazardous to humans at the low recommended dosages (18).

The biological material consisted of a susceptible strain of *Triatoma infestans* that was reared from a colony in our laboratory and that was maintained at 28–30 °C and 50–60% RH (19). The insects selected for toxicological tests were first-instar nymphs (3–5 days old) that had been starved since eclosion (20).

Laboratory trials

A number of laboratory trials were done to characterize the commercial fabrics and to standardize them before the field trials. To assess the water absorption of the fabrics, fabric rectangles of 12.5 × 25 cm were placed in a graduated cylinder containing 50 mL of water at 25 °C. After 30 min, the fabric was removed, the excess water allowed to drip off for 5 min, and the amount of water the fabric absorbed was measured. Absorbency was defined as the amount of water absorbed per square meter during 30 min at 25 °C.

The analysis of the chemical composition of the fabrics was done by the Textile Investigation Center (INTI, Buenos Aires, Argentina), using established techniques (21).

A Nikon (Japan) model SKe optical microscope was used to examine pieces of fabric, and the number of fibers per cm^2 were counted using an ocular micrometer (40x).

For impregnation testing, rectangles of fabrics 12.5 × 25 cm were immersed for 1 h at a concentration of 0.2 g/m² of

β -cypermethrin in water. Fabrics were then naturally dried lying flat on a polyethylene sheet for 24 h, so that any liquid that dripped out of the fabric was reabsorbed. Control fabrics were treated only with water.

PIF samples that were 10 × 10 cm underwent acetone extraction several times in an ultrasonic bath for 1 h each time. Acetone extracts were mixed and volume made up to 50 mL. The concentration of β -cypermethrin was determined by gas-liquid chromatography (Shimadzu 6AM Chromatograph, Japan) using a glass column filled with Silicone DC QF-1 1.5% on Chromosorb W (AW-DMCS) mesh 60–80 (Shimadzu, Japan) at 220 °C and a flame detector at 250 °C, against a standard of β -cypermethrin 0.02%, according to internal guidelines Chemotécnica uses for quality control.

Four different bioassays were done. The first assessed insecticidal activity for all eight of the fabrics. On each piece of treated PIF, 10 first-instar nymphs were exposed for 1, 3, 6, or 10 min at room temperature (25 °C), confined inside a glass ring with an inner diameter of 4.8 cm. The nymphs were then transferred to a disposable clean flask and kept at 25 °C. Mortality was assessed 24 h posttreatment. Median lethal time (LT_{50}) values were calculated with a computer software program specially designed in our laboratory based on probit analysis using the Litchfield and Wilcoxon method (22). Fabrics treated only with water were used as control assays.

To evaluate fabrics that had been used in the field, 10 first-instar nymphs were exposed for 3, 6, 9, or 12 min to PIF samples from treated dwellings and were then transferred to a clean flask. Mortality was assessed 24 h after exposure. LT_{50} was calculated with a computer software program specially designed in our laboratory based on probit analysis using the Litchfield and Wilcoxon method (22).

Residual activity was measured with PIFs that had been held in indoor environmental conditions (25 °C); their insecticidal activity was determined at 7, 15, 30, 90, 120, 240, and 365 days after impregnation.

In an assessment of repellency, fabrics impregnated with β -cypermethrin were compared with ones impregnated with deltamethrin and cypermethrin, at a sublethal dose of 8.3 mg/m² as a target dosage for all three pyrethroids. Groups of 10 first-instar nymphs were held in a transparent plastic container (15 cm \times 30 cm) for 30 min to become acclimated before placing two small dark shelters simultaneously inside the plastic container. One of the shelters contained the fabric impregnated with pyrethroid. The other shelter, the control, contained the same fabric but without pyrethroid. The dark shelters were small plastic vessels (100 cm³), with openings in the lower part of each of them. Both shelters were put in place at the same time, separated by not more than a few centimeters. After 60 min, there was no mortality of insects at the assayed dosage. The number of insects inside the two dark shelters was counted and compared. This simple methodology suitably quantified the nymphs' hiding movements, which mimicked the natural behavior of triatomines searching for dark shelter. Repellency was defined as a negative taxis, with a lower ratio meaning a higher repellency effect (23). A simple calculation was used for repellency calculations. Each value was the mean of five replicates \pm SD ($n = 10$). Significance was determined by the nonparametric Mann-Whitney test ($P < 0.005$).

Field trials

For the field trials, two of the fabrics, 100% wool and the rayon-cotton combination, were impregnated in the laboratory by immersing pieces that were 60 m \times 1.5 m for 60 min in a vessel containing 60 L of a mixture of 5% EC β -cypermethrin, for a target dosage of 200 mg a.i./m². The fabric was then wound on a wooden pole and placed in a draining rack with a grate below it to collect the runoff. After the excess liquid had dripped off, the fabric was left to dry for 24 h on lines outdoors but not in direct sunlight. After that, the fabric was folded and stored in

polyethylene bags until it was to be used in the field.

The rayon-polyester fabric was impregnated in the field ("in situ"), rather than in the laboratory. Fabric pieces of 5 m \times 1.5 m, folded to 0.31 m \times 0.37 m, were immersed for 60 min inside a polyethylene bag that was 0.95 m \times 0.50 m and 30 μ thick and that contained 4 L of a solution of β -cypermethrin (EC 5%) in water, for a final concentration of 1 g a.i./m². After being soaking in the closed plastic bag, the fabric was wrung out, allowing the excess insecticide to drip back into the plastic bag. The folded fabric was placed flat on a polyethylene sheet for drying for at least 24 h, with any liquid dripping out of the fabric being reabsorbed during this process. Impregnated fabrics were stored in polyethylene bags until they were to be used inside the dwellings.

In the houses, samples of the impregnated fabrics were nailed up under the roof or attached to camp beds. Using small tacks the PIFs were nailed to the horizontal wooden tie beams that supported the straw and mud roof of each home. The tacks held the fabric in place just below the straw where the adult bugs and large nymphs hid, covering the whole roof area and hindering the triatomines' movement. In the case of the camp beds the impregnated fabric was nailed to wooden pieces supporting the mattress, so as to impede the movement of small nymphs that hid in holes in those wood pieces.

Every two months a health official visited the dwellings and looked for live insects or signs of triatomine bugs, such as eggs, exuvias, or fecal deposits on the walls and in the María Sensor Box, as described by Wisnivesky-Colli (24). Dwellings in the same area and with similar characteristics, but without PIFs installed, were used as controls. Both the PIF and control dwellings had been infested before the treatment program began.

Preliminary field trials using PIFs with a target dosage of 200 mg a.i./m² were started in 1993 in different localities of the province of Santiago del Estero, Argentina. This program was

done in cooperation with the Dr. M. Fata Chaben Institute for the Diagnosis and Treatment of Chagas' Disease of Argentina and the National Program for Chagas' Disease Control of the Ministry of Public Health of Argentina.

A first field experiment was performed with two fabrics that had been impregnated in the laboratory, with 100% wool fabric in 11 dwellings and with the rayon-cotton combination fabric in 5 dwellings. The PIFs remained in place for 1 year. Five infested houses were used as controls.

In 30 dwellings a second experimental cycle was carried out with rayon-polyester fabrics impregnated in field conditions, at a target dosage of 1 g a.i./m². The PIFs were left in place for more than a year. Five infested houses were used as controls.

RESULTS

The characteristics of all the tested fabrics are shown in Table 1. For dilute EC solutions, the uptake of emulsified pyrethroid is considered similar to the uptake of water (5). Thus, water absorbency was used to calculate the amount of EC (mL) needed to provide the required target pyrethroid dosage for a given area of fabric.

The chemical composition of the fibers was also established, in order to standardize the different type of fabrics, as well as the mesh, by measuring the number of fibers/cm² by microscopy (Table 1).

Table 1 also shows which combinations of pyrethroid and fabric material were the most effective, as indicated by the LT₅₀ data for first-instar nymphs. Four materials gave values below 3 min: wool, rayon-polyester, rayon-cotton, and tulle. Analysis of the impregnated fabrics, given by the target dosage, shows that the amount of insecticide absorbed depends on the type of fabric used. Such fabrics as cotton give high target dosage values but, as can be seen in the mortality data, the insecticide is not bioavailable. Other fabrics, such as tulle, have low target dosages but give high initial mortality (LT₅₀ = 2.1 min).

TABLE 1. Fabric characteristics and insecticidal activity, Argentina, 1993–1995

Fabric	Chemical composition	Fibers/cm ²	Water absorption (mL/m ²)	Target dosage (mg/m ²)	Median lethal time (min) ^{a,b}
Wool	100% wool	800	230 ± 10	170 ± 10	0.6 (0.3–1.3)
Cotton	100% cotton	1 100	290 ± 10	305 ± 21	16.5 (7.7–35.0)
Rayon-polyester	51.2% rayon + 48.8% polyester	1 100	240 ± 10	260 ± 10	2.8 (2.1–3.5)
Cotton-polyester	58% cotton + 42% polyester	1 200	230 ± 10	235 ± 10	3.0 (2.3–3.9)
Rayon-cotton	43% rayon + 57% cotton	400	360 ± 20	93 ± 18	1.7 (1.1–2.6)
Tulle	100% polyester	200	32 ± 5	23 ± 18	2.1 (1.6–2.8)
Voile	100% polyamide	1 600	64 ± 5	25 ± 18	8.6 (5.7–13.1)
Taffeta	100% cellulose acetate	600	48 ± 5	26 ± 4	9.8 (5.4–17.2)

All values are the mean of at least two independent determinations.

^a Controls of fabrics without insecticide showed 0% mortality in all cases.

^b Median values with 95% confidence intervals.

TABLE 2. Residual efficacy of β-cypermethrin-impregnated fabrics, Argentina, 1996

Fabric	Mortality (%) ^{a,b}						
	7 days	15 days	30 days	90 days	120 days	240 days	365 days
Wool	100	100	100	100	100	100	90
Rayon-cotton	100	100	100	100	100	80	50
Rayon-polyester	100	100	100	90	80	50	—
Cotton-polyester	70	0	—	—	—	—	—
Tulle	0	—	—	—	—	—	—
Taffeta	0	—	—	—	—	—	—
Voile	0	—	—	—	—	—	—

^a The values are the mean of at least two independent determinations.

^b Controls of fabrics without insecticide did not show any mortality in any cases.

Table 2 shows the residual efficacy data. Only wool, rayon-cotton, and rayon-polyester produced high mortality (> 80%) for more than 3 months. Because its initial mortality was so low, the 100% cotton fabric was not investigated for residual activity.

Table 3 shows that for the three pyrethroids tested on fabrics, β-cypermethrin had the lowest repellency ratio and thus the greatest repellency effect.

Preliminary field trials that were performed using wool fabrics in 11 dwellings and rayon-cotton fabrics in 5 dwellings showed no infestation 1 year after PIF installation (Table 4). In

the second cycle, performed with rayon-polyester fabric in 21 dwellings, there was no triatomine infestation almost 1 year after the PIFs were installed (Table 4). Another 9 dwellings in which rayon-polyester fabrics were impregnated in field conditions were also negative after a similar length of time. Meanwhile, the 5 infested houses used as controls for each trial did not show any change in infestation (Table 4). All the evaluations of infestation were done by trained personnel using the María sensor boxes. Bioassays performed in the laboratory with fabric samples taken from the dwellings 1 year after installation showed that

those PIFs remained active in comparison with PIFs kept in the laboratory at 25 °C for the same period of time (Table 5).

DISCUSSION

This study is the first to evaluate the effectiveness of fabrics impregnated with β-cypermethrin to control the Chagas' disease vector in Argentina by placing the PIFs under the roof as a sanitary ceiling and under bed mattresses. The immersion method was chosen as the most convenient method of impregnating fabrics based on previous experience (25, 26). The emulsified concentrate was preferred for impregnation instead of a flowable or a wetting powder because the EC provides better adhesion to the fiber and does not leave a powdery residue.

In earlier studies, our laboratory evaluation of β-cypermethrin against *T. infestans* nymphs and adult insects showed a performance similar to that of deltamethrin (15). The vector control and the cost-effectiveness ratio were very promising for this new pyrethroid. Taking into account preliminary assays that gave 100% mortality in all stages of *T. infestans* (unpublished results), the target dosage of 200 mg a.i./m² was chosen for the impregnation of fabrics for laboratory bioassays.

Evaluation of the different types of fabrics indicates that only wool, rayon-cotton, and rayon-polyester showed both good triatomocidal activity and long residuality. Previous studies showed that cotton is a very porous and absorbent material, thus it could pick up more insecticide than synthetic fibers. However, a substantial portion of the insecticide would not be bioavailable, and the water absorbed produces longer drying times after impregnation (27). In contrast, pyrethroids have almost the same polarity as such synthetic fabrics as polyester, so diffusion into the fabric would occur during impregnation with subsequent slow release (28). Consequently, the bioavailability of the insecticide is more dependent on the composition of the fabric, such as

TABLE 3. Repellency effect of fabrics impregnated with various pyrethroids toward 1st-instar nymphs of *Triatoma infestans*, Argentina, 1996

Compound	% of sheltered insects after 60 min ^a	Repellency ratio
β-cypermethrin	28.0 ± 4.5 ^b	0.36
Deltamethrin	54.0 ± 11.4 ^b	0.65
Cypermethrin	52.0 ± 16.4 ^b	0.70
Control	88.8 ± 14.8	1

^{a,b} Each value is the mean of five replicates ± SD ($n = 10$). Superscript letter ^b indicates significant difference with respect to the control groups ($P < 0.005$). Significance was determined by the Mann-Whitney nonparametric test.

supervision of a health care agent, in contrast to conventional spraying done by an experienced, professional team. Second, new methods of pest management may come from the changes in the bugs' behavior due to the repellency action that was found. Compared with deltamethrin and cypermethrin, the two other pyrethroids currently used in national campaigns in Argentina, β-cypermethrin showed twice as much repellency effect in the tested fabrics.

In field trials, wool, rayon-cotton, and rayon-polyester fabrics impregnated with β-cypermethrin, either used as a sanitary ceiling under the roof or placed under the mattress, showed very good efficacy and good residuality for Chagas' disease vector control. The dwelling residents also accepted the PIFs well and were, in fact, delighted to receive them.

To ensure adequate residuality in field conditions, a dose of 1 g a.i./m² was chosen, a concentration that was five times the one used in the laboratory.

In the field all three fabrics tested performed well and had equivalent costs; the major expense was the insecticide, not the fabric.

Installing the PIFs did not present difficulties and had added benefits beside their triatomocidal activity. The fabric nailed to the tie beams completely concealed the roof area and also kept dust from the roofing materials from falling on the residents. And given that the insecticide was only targeted to the ceiling, it was likely that human exposure was very low.

There has previously been a failure to develop safe, effective, longlasting prophylactic agents to reduce Chagas' disease. This study has shown that an effective method to decrease Chagas' disease is by integrating the use of impregnated fabrics within large-scale primary health care programs. This vector control method proved to be appropriate in terms of both acceptability and achievability in rural areas of Argentina.

Finally, it can be concluded that pyrethroid-impregnated fabrics are very effective in reducing exposure to

TABLE 4. Field trials with β-cypermethrin-impregnated fabrics for *Triatoma infestans* control, Argentina, 1993–1997

No. of dwellings, fabric type	Date fabric installed	Initial infestation ^a	Date evaluated	Subsequent infestation ^a
11 dwellings, wool	July 1993–March 1994	Yes	April 1995	No
5 dwellings, rayon-cotton	April 1994–Jan 1995	Yes	December 1995	No
5 dwellings, control	(control)	Yes	1993–1995	Yes
21 dwellings, rayon-polyester	Jan 1996–May 1996	Yes	December 1996	No
9 dwellings, rayon-polyester	May 1996–December 1996	Yes	July 1997	No
5 dwellings, control	(control)	Yes	1996–1997	Yes

^a "Infestation" is measured by the presence or absence of triatomines in the María sensor boxes.

TABLE 5. Insecticidal activity of PIFs taken from field trials 1 year after impregnation, Argentina, 1995–1997

Dwelling	Characteristic of fabric	Median lethal time ^a (min)
1	a) clean fabric	4.2 (2.9–6.0)
	b) smudged fabric	8.9 (6.9–11.5)
2	clean fabric	21.0 (15.7–28.1)
3	PIF painted by residents	
	a) external painted side	> 40
	b) internal unpainted side	34.9 (25.6–47.5)
Control	Impregnated fabric held in laboratory conditions (25 °C)	4.8 (3.4–6.7)

^a Median values with 95% confidence intervals.

rayon-polyester or cotton, than on the target dosage. Therefore, taking into account their long residuality and local availability, wool, rayon-cotton, and rayon-polyester fabrics were chosen for the field trials.

Our interest in testing the repellency of PIFs in Chagas' disease vectors

using the simple methodology we devised was based on two concerns. First, there was a need to know the actual performance, in both laboratory and field conditions, of this new approach. This approach could be called "non-professional," in that it is carried out with community participation and the

bites from triatomines and other nuisance insects. Whether this reduction has an effect on Chagas' disease prevalence rates and morbidity will depend on a variety of local factors. Among these are seasonal fluctuations in the level of endemicity of Chagas' disease, and the immune status of the population. In areas with low levels of Chagas' disease transmission, PIFs appeared to be effective in reducing parasite rates, particularly as indoor treatments that are part of a surveillance program. In highly endemic areas, PIFs should be supplemented by other

Chagas' disease vector control methods. This is especially true for peridomesticated triatomines, which could be eradicated by conventional insecticide spraying.

This PIF approach represents a real possibility as a complementary insecticidal tool in vector control programs.

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 β -cipermetrina: una nueva
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*Triatoma infestans***

RESUMEN

Triatoma infestans es el principal vector de la enfermedad de Chagas en Argentina. La experiencia adquirida en las campañas nacionales de este país ha demostrado que la fumigación de los domicilios para controlar los triatómidos es cara y difícil. Un método de control alternativo que podría ser un complemento práctico y barato de las medidas de control convencionales sería el empleo de telas impregnadas de piretroides. Los insectos que intentaran penetrar la tela o caminaran sobre ella en busca de comida serían intoxicados por la exposición al piretroide absorbido.

En este estudio se utilizó un concentrado emulsionado del piretroide β -cipermetrina [α -ciano-3-fenoxibencil-3-(2,2-diclorovinil)-2,2-dimetil-ciclopropano-carboxilato] para impregnar diferentes telas y después se investigó su eficacia y persistencia para matar a *Triatoma infestans*.

Los bioensayos de laboratorio revelaron que, con una dosis de β -cipermetrina de 0,2 g/m², el efecto insecticida era más duradero en la lana que en el algodón o el rayón. Ocho meses después de la impregnación, se registró una eficacia residual de 100% en la lana, de 80% en las telas de rayón y algodón y de 50% en las de rayón y poliéster. Además, las telas impregnadas de β -cipermetrina presentaron mayor efecto repelente que las impregnadas de deltametrina o cipermetrina.

En los estudios de campo, después de ser impregnadas in situ con una dosis de β -cipermetrina de 1 g/m² y secadas, las telas fueron colocadas en el interior de las casas, debajo de los colchones o clavadas al techo. Estos estudios de campo demostraron que el método era muy eficaz para mantener los domicilios sin triatómidos durante al menos un año y que tenía un alto grado de aceptación por parte de los moradores.

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