High Resistance to Pyrethroid Insecticides Associated with Ineffective Field Treatments in *Triatoma infestans* (Hemiptera: Reduviidae) from Northern Argentina

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**ABSTRACT** Field populations of *Triatoma infestans* Klug were collected during 2002 from four villages in northern Argentina (El Chorro, La Toma, El Sauzal, and Salvador Mazza), after application of deltamethrin and other pyrethroids was ineffective. High levels of resistance to the pyrethroid insecticides deltamethrin, β-cypermethrin, β-cyfluthrin, and lambda-cyhalothrin were detected in all of the evaluated populations. The resistance ratio to pyrethroids determined by topical application ranged from 50.5 (deltamethrin, El Sauzal) to 667.6 (β-cyfluthrin, Salvador Mazza). None of the pyrethroid-resistant insects was resistant to the organophosphorus insecticide fenitrothion. Topical application of piperonyl butoxide to the most deltamethrin-resistant population (Salvador Mazza) led to slight reduction in levels of resistance. Activity of P450 monooxygenase, measured in individual insects through ethoxycoumarine-O-deethylase, showed a slight but noticeable difference in the distribution of activities between susceptible and resistant populations. The total percentage of insects below 0.48 pmol of 7-OH coumarine/min/insect was 36.4 for Salvador Mazza population and 64.3 pmol of 7-OH coumarine/min/insect for CIPEIN strain. Whereas a low level of resistance to deltamethrin was previously related to monooxygenase activity in *T. infestans*, the high levels of resistance shown by these populations seem to involve monooxygenase in combination with other resistance mechanisms, for example, insensitivity of nervous membrane. Research on *T. infestans* resistance is in progress to improve Chagas vector control programs in Latin America and to implement resistance management strategies.

**KEY WORDS** *Triatoma infestans*, insecticide resistance, resistance ratios, monooxygenases

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**Triatoma infestans** Klug (Hemiptera: Reduviidae) is the major vector of American trypanosomiasis or Chagas disease in Argentina. Pyrethroid insecticides have been used to control this pest during the past 20 yr (Zerba 1999). Possible development of resistance in field populations exposed to vector control programs with insecticides has been evaluated in our laboratory since 1997. Low resistance levels (resistance ratios, RRs) to deltamethrin were initially found (resistance ratio ranged from 2.0 for San Luis colony to 7.9 for Salta colony) that were not correlated with failures of chemical control in the field (Picollo et al. 2000). Laboratory studies on the population with highest deltamethrin resistance (Salta) in comparison with the susceptible strain (CIPEIN), suggested enhanced degradative activity by cytochrome P450 monooxygenases (González Audino et al. 2004).

In 2002, deltamethrin-resistant field populations were detected in several localities close to where the Salta colony was collected. The new region with detected resistance is located in northern Argentina (San Martín Department, Salta Province), in the area bordering Bolivia. At the same time, the Health Authorities for Vector Control reported ineffectiveness of the application of deltamethrin and other pyrethroid insecticides in infested houses of this endemic area. To evaluate the resistance profile and the resistance mechanisms in these field populations from four villages in this region (El Chorro, La Toma, El Sauzal, and Salvador Mazza), we bioassayed the insecticides recommended for the chemical control of this vector against susceptible and collected resistant populations. The monooxygenase synergist piperonyl butoxide (PBO) and the esterase synergist triphenyl phosphate (TPP) were used to evaluate the relative role of these enzymes in the resistance. The enhanced activity of cytochrome P450 microsomal monooxygenases was further characterized using biochemical measurements.
Materials and Methods

Study Sites. Field T. infestans were collected in November 2002 from infested houses of an endemic area in northern Salta Province. The vector control campaign intervention based on pyrethroid insecticides was considered ineffective in this area due to presence of insects assessed by household and official reports a week after the intervention. In this area, insecticides have been intensively used for T. infestans and mosquito chemical control. Four sites located in the San Martín Department (22°5’S, 63°7’E) were chosen. Sites were El Chorro, La Toma, and El Sauzal, which are rural areas, and Salvador Mazza, an urban area. They are small villages located 50 km from Tartagal city (Fig. 1). A high percentage of infested houses was detected in this area since 2002. Data on the evolution of dwelling infestation and the insecticides used are summarized in Table 1.

Insects. T. infestans nymphs and adults were individually collected with entomological forceps from the infested houses of the four localities. We found an average of 30 insects per house. Further generations of the field insects were bred in the laboratory. CIPEIN is an insecticide-susceptible strain maintained in our laboratory since 1975 without any exposure to insecticides (Picollo et al. 1976). Insects were reared at 28°C, 50% RH, and a photoperiod of 12:12 (L:D) h and were fed on pigeons weekly. From all colonies, 3-d-old first instars, starved since eclosion (mean weight 1.2 ± 0.2 mg), were selected for toxicity tests according to the World Health Organization protocol (1994) and for the measurement of the enzymatic activity (González Audino et al. 2004).

Chemicals. Technical grade insecticides used for bioassay were deltamethrin (97%, Bayer, Buenos Aires, Argentina), β-cypermethrin (99.4%, Chemotec-
400-nm excitation and 440-nm emission filter. Fluorescence reader (Packard Fluorocount), with 1998). Fluorescence was determined using microplate (ECOD) on intact tissue on microplate (Bouvier et al. were mechanically stimulated (WHO 1994). Mortality was assessed at 24 h after insecticide treatment, were used for blanks.

Statistical Analysis. Mortality data were corrected using Abbott’s formula (Abbott 1925). Bioassay data from each T. infestans population were pooled and analyzed based on probit analysis (Litchfield and Wilcoxon 1949). Doses giving 50% lethality (LD50 values) obtained in probit were expressed as nanograms of insecticide per insect. To compare lethal dose and estimate whether the LD50 and the 95% CL for the ratio were calculated. If the 95% confidence interval includes 1, then the LD is not significantly different (Robertson and Preisler 1992).

The biochemical data were plotted as the percentage of individuals responding within a particular range of values of enzyme activity (Sokal and Rohlf 1980). A threshold between susceptible and resistant colonies was established, and the total percentage of insects below the threshold was calculated for both populations.

Results

Resistance ratios to deltamethrin were assessed in T. infestans first instars whose parents were collected in houses that has been unsuccessfully treated with pyrethroid insecticides. The toxicity data estimated for resistant (El Chorro, La Toma, El Sauzal, and Salvador Mazza) and susceptible (CIPEIN) insects are shown in Table 2.

All field populations exhibited high degrees of resistance compared with the insecticide-susceptible strain. The resistance ratios ranged from 50.5 to 133.1. These high levels of resistance were not previously observed in T. infestans or any other vector of Chagas disease. There was no difference between the resistance ratios to deltamethrin of the four resistant populations (the confidence limits overlapped).

The resistance status of each population used in this study toward β-cypermethrin, β-cyfluthrin, lambda-cyhalothrin, and fenitrothion is shown in Table 3. All the deltamethrin-resistant populations also were resistant to β-cypermethrin, β-cyfluthrin, and lambda-cyhalothrin, three pyrethroids that had been used for chemical control on T. infestans in the field. The results showed high degrees of resistance, similar to those

<table>
<thead>
<tr>
<th>Population</th>
<th>n</th>
<th>Slope ± SE</th>
<th>LD50 ng/insect (95% CL)</th>
<th>RR (95% CL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIPEIN*</td>
<td>125</td>
<td>2.10 ± 0.66</td>
<td>0.13 (0.12–0.15)</td>
<td></td>
</tr>
<tr>
<td>El Chorro</td>
<td>220</td>
<td>1.90 ± 0.14</td>
<td>12.8 (11.0–14.9)</td>
<td>99.0 (78.2–125.3)</td>
</tr>
<tr>
<td>La Toma</td>
<td>100</td>
<td>1.90 ± 0.14</td>
<td>11.3 (6.1–23.3)</td>
<td>86.9 (68.1–111.0)</td>
</tr>
<tr>
<td>El Sauzal</td>
<td>90</td>
<td>1.47 ± 0.11</td>
<td>6.5 (2.3–19.4)</td>
<td>50.5 (30.7–83.1)</td>
</tr>
<tr>
<td>S. Mazza</td>
<td>90</td>
<td>1.37 ± 0.11</td>
<td>31.1 (12.9–84.7)</td>
<td>133.1 (105.6–167.7)</td>
</tr>
</tbody>
</table>

* Susceptible strain.
found for deltamethrin. Topical application of β-cypermethrin gave the highest resistance ratios for all samples, ranging from 270.1 to 451.2.

None of the pyrethroid-resistant populations was resistant to fenitrothion, an organophosphorus insecticide that had not been used in Argentina for controlling *T. infestans* for the past 20 yr. The resistance ratios ranged from 1.29 for El Chorro to 1.78 for El Sauzal.

Topical application of cytochrome P450 inhibitor PBO to the most resistant population (Salvador Mazza) before deltamethrin led to slight reduction in the levels of resistance. At LD50, PBO reduced the resistance ratios from 133.1 to 101.1 (Table 4). The topical application of the esterase inhibitor TPP did not increase the toxicity of the insecticide, suggesting that these enzymes were not involved in deltamethrin resistance of Salvador Mazza population (Table 4).

Activity of P450 monoxygenases showed a slight but noticeable difference in the distribution of 7-OH coumarine activities between susceptible and resistant populations (Fig. 2). Considering 0.48 pmol of 7-OH coumarine per minute as a reasonable threshold between the populations, the total percentage of insects below the threshold was 64.3 for CIPEIN and 36.4 for the resistant population.

### Discussion

This study reported the first case of pyrethroid resistance at a high enough level to cause control failures in field populations of *T. infestans* from northern Argentina. The laboratory toxicity tests indicated that resistance to topically applied deltamethrin existed at a high level in the field populations collected in four villages of the same area (El Chorro, La Toma, El Sauzal, and Salvador Mazza). The resistance ratios to deltamethrin estimated here (99.0, 86.9, 50.5, and 133.1 respectively) were consistently higher than those previously estimated for a field population of the same area collected in September 1999 (RR = 7.9 to deltamethrin), when chemical control was still successful. Based on these data, it can be assumed that RRs ≥ 50 measured by topical application are a signal of potential control failures in the field, whereas RRs ≤ 8 indicate that resistant populations are being successfully controlled. Although this information is not sufficiently complete to correlate laboratory test and field chemical control, it is valuable information in the development of practical resistance management strategies. Moreover, it will be useful for the study of variations in pyrethroid susceptibility of field populations of *Rhodnius prolixus* Stahl, another vector of...
Chagas disease without detected resistance to insecticides (Molina de Fernández et al. 2004).

Based on the records of the Vector Control National Programs, the resistant field insects have been successfully controlled with other pyrethroids (β-cypermethrin, β-cyfluthrin, and lambda-cyhalothrin) for the past 20 yr. Thus, resistance to these pyrethroids may be due to cross-resistance to deltamethrin, selection with each insecticide, or both. Our toxicity studies have indicated that the pyrethroid-resistant insects have not developed cross-resistance to fenitrothion and that the resistance was not completely reduced by pretreatment with PBO or TPP. The biochemical results found a slight but noticeable difference in the distribution of 7-OH-coumarine activities between susceptible and resistant populations. This result is not conclusive evidence but suggests monooxygenase involvement as a collaborative resistance mechanism.

Lack of PBO synergism also has been found in the presence of strong synergism by propynyl ethers in permethrin-resistant *Heliothis virescens* (Boddie) (Rose et al. 1995), suggesting that monooxygenase involvement in pyrethroid resistance should not be discarded when PBO fails to synergize toxicity. The authors suggested that monooxygenases present in a population might mean that a synergist capable of interacting with one isozyme may not be capable of interacting with another.

Resistance to pyrethroids in insects has been correlated with enhanced metabolism by enzymes (Oppenorth 1985), in particular, a higher cytochrome P450s activity was measured in pyrethroid resistance in house fly, *Musca domestica* L. (Lee and Scott 1989). Hung and Sun (1989), using ethoxycoumarine as a substrate, found that larval homogenates of fenvalerate-resistant diamondback moth, *Plutella xylostella* (L.), possessed 100-fold higher activity than susceptible insects. Bouvier et al. (1998) reported higher ethoxycoumarin deethylase activity measured at individual level in microtitration plates, in deltamethrin-resistant codling moth, *Cydia pomonella* (L.).

The role of monooxygenases was previously linked to deltamethrin resistance in *T. infestans* and *R. prolixus*, the two major vectors of Chagas disease in America. Vassena et al. (2000) demonstrated that the pyrethroid resistance in a Brazilian *T. infestans* (RR = 4.1).
7.0) and a Venezuelan R. prolixus (RR = 11.4) was decreased by piperonyl butoxide, suggesting oxidative metabolism as cause of resistance. Moreover, González Audino et al. (2004), using biochemical assays, established the role of enhanced detoxication in a deltamethrin-resistant colony of T. infestans (RR = 7.9) from Argentina. They found ECOD activities to be 1.8-fold higher in the resistant colony, with values 108.0 pg of 7-OH/min/insect for resistant colony and 61.3 pg of 7-OH/min/insect for susceptible strain. These values correspond to 0.67 and 0.38 pmol of 7-OH/min/insect, respectively.

However, all deltamethrin-resistant populations previously studied showed moderate resistance levels (RR ≤ 11.4). The high resistance to pyrethroid in the field populations studied here, suggests that a further mechanism, such as reduced nerve sensibility (i.e., kdr), may be involved in the resistance. The likely contribution of sodium channel mutations as an important resistance mechanism has been investigated in other hemipterans (Soderlund and Knipple 2003). Biochemical, electrophysiological, and molecular investigations are in advance to demonstrate this hypothesis. Meanwhile, fenitrothion could be used for chemical control of pyrethroid resistant populations, according to the low resistance ratios found in this study.

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