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Resistance to deltamethrin in *Triatoma infestans*: microgeographical distribution, validation of a rapid detection bioassay and evaluation of a fumigant canister as control alternative strategy

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Abstract

Triatoma infestans (Klug) (Hemiptera: Reduviidae) is the main vector of Chagas disease in the Southern Cone of America and resistance to pyrethroid insecticides has been detected in several areas from its geographical distribution. Pyrethroid resistance presents a complex geographical pattern at different spatial scales. However, it is still unknown if the toxicological variability is a common feature within villages of the Gran Chaco were high resistance was descripted. The objectives of this study were to determine: (a) the microgeographical distribution of the deltamethrin-resistance in insects from Pampa Argentina village, (b) the performance of the insecticide impregnated paper bioassay to evaluate deltamethrin-resistance in field collected insects and (c) the lethal activity of the fumigant canister containing DDVP against insects resistant to deltamethrin. High survival of *T. infestans* exposed to discriminant dose was observed in the samples of all the evaluated dwellings, suggesting that the resistance to deltamethrin is homogeneous at the microgeographical level. Resistance determination by impregnated paper bioassay was similar to traditional topical determination, highlighting the use of this rapid methodology in field large-scale monitoring. The fumigant canister was not effective against resistant insects, remarking the need to develop suitable formulations that ensure minimal toxicological risk and high effectivity.

Introduction

The individual susceptibility to an insecticide, also called toxicological phenotype, is the expression of multiple processes that occur during the toxicokinetic/toxicodynamic phases of the insect–insecticide interaction, each one determined by genetic and environmental factors. Due to the variation in these multiple factors, the individual susceptibility is randomly distributed among the individuals of a population (Mougabure-Cueto and Sfara, 2016). On the genetic variation that underlies this distribution can operate the insecticide through its differential toxic action promoting a natural selection processes and shifting the distribution of susceptibilities towards higher doses, i.e. the proportion of the less-susceptible or resistant individuals is incremented in the population. The result of this evolutionary process is a population resistant to insecticides (McKenzie, 1996).

The insecticide resistance is considered one of the main causes of chemical control failures in insects. In Triatominae (Hemiptera: Reduviidae), the subfamily that groups the Chagas disease vectors, insecticide resistance has been reported in *Triatoma infestans*, *T. sordida*, *Rhodnius prolixus* and *Panstrongylus herreri* (Mougabure-Cueto and Picollo, 2015). *T. infestans* is the main vector in the Southern Cone of America and resistance to pyrethroid insecticides has been detected in several areas from its geographical distribution (mainly in Argentina and Bolivia) including village where the spraying were ineffective (Picollo *et al.*, 2005; Toloza *et al.*, 2008; Germano *et al.*, 2010*b*, 2012; Fronza *et al.*, 2016). Although far from the complete understanding of the phenomenon, insecticide resistance in *T. infestans* has received much attention and was studied in its different aspects from various methodologies and theoretical frameworks. So, the studies approached the resistance mechanisms, cross-resistance patterns, heritance and heritability, expression during ontogeny, pleiotropic effects (e.g. adaptive costs), macro and micro-geographical distribution and environmental influence (González-Audino *et al.*, 2004; Santo Orihuela *et al.*, 2008; Germano *et al.*, 2010*a*; Roca-Acevedo *et al.*, 2013; Gomez *et al.*, 2015; Germano and Picollo, 2015, 2018; Bustamante-Gomez *et al.*, 2016; Sierra *et al.*, 2016; Lobbia *et al.*, 2018, 2019*a*; Fronza *et al.*, 2019).

The resistance to pyrethroids in *T. infestans* shows a complex geographical pattern. At the macro-geographical level, the resistance was detected in several areas spaced from a few tens of kilometers to over a thousand kilometers across two countries, Argentina and Bolivia (Mougabure-Cueto and Picollo, 2015). These resistant foci showed differences between countries and within countries in resistance levels, cross-resistance pattern, ontogeny of resistance and resistance mechanisms (Santo Orihuela *et al.*, 2008; Toloza *et al.*, 2008; Roca-Acevedo *et al.*, 2011; Germano *et al.*, 2012; Sierra *et al.*, 2016). At a smaller geographical level (i.e. villages within a department), a mosaic of toxicological phenotypes categorized as susceptible, low, and highly resistance was described at Güemes Department of Chaco province of Argentina (Fronza *et al.*, 2016). Finally, at the microgeographical level (i.e. houses within a locality), the resistance foci do not appear to be toxicologically homogeneous. Germano *et al.* (2013) demonstrated that the insects from different dwellings of the village La Esperanza (Argentinean province of Chaco) present different susceptibility to deltamethrin showing that some houses host resistant insects and other houses host susceptible insects. According to the authors, these differences could reflect the micro-spatial distribution of susceptibilities and suggest a high degree of genetic structure at the micro-level on which the insecticide could exert its selective pressure. Regarding the later, a significant genetic difference between *T. infestans* from different houses in the same locality was reported through the analysis of microsatellite loci (Marcet *et al.*, 2008; Pérez de Rosas *et al.*, 2008). However, it is not known if this variation pattern of the toxicological phenotype is a common feature in the villages of the Chaco eco-region. In general, variation in toxicological phenotypes in a given geographical scale might be originated from non-homogenous insecticide pressure, diversity of resistance mechanisms, high genetic structure and/or the influence of environmental variables (Mougabure-Cueto and Picollo, 2015).

The evolution of insecticide resistance leads to the inefficiency of the chemical control strategy that was used successfully until that moment. In triatomines, the current control is based on pyrethroid insecticides, mainly formulated as a suspension concentrate. However, the shortage of available insecticides and formulations available for Chagas vectors make difficult to implement an alternative chemical control strategy. The organophosphates fenitrothion as wettable powder and malathion as emulsifiable concentrate, and the carbamate bendiocarb as wettable powder were the alternatives used successfully for the control of pyrethroids resistant foci of *T. infestans* in Argentina and Bolivia (Programa Nacional de Chagas, 2009; Gurevitz *et al.*, 2012; Zaidenberg, 2012; Germano *et al.*, 2014). However, the toxicological and eco-toxicological risk of these insecticides and the low quality and bad receptivity by residents of their formulations are the main reasons for questioning it use in public health. In this context and considering that all populations studied of the resistant focus of Güemes Department of Argentine Chaco province were susceptible to organophosphates (Fronza *et al.*, 2016), the use of fumigant canister containing dichlorvos (DDVP) emerges as a possible alternative to control of resistant insects. The fumigant canister was developed in Argentina approximately 30 years ago and is a smoke-generating device that releases insecticides (Gonzalez-Audino *et al.*, 1999; Zerba, 1999). This tool was initially designed to be used during the surveillance phase of chemical control of triatomines, mainly in areas where domiciliary vectors are present, but there are not studies about of its lethal activity on the *T. infestans* resistant to pyrethroids.

Finally, when insecticides are used in pest control, a resistance management strategy should be implemented oriented to detect the resistance evolution at the lowest possible level and to interrupt the selection process (Mougabure-Cueto and Picollo, 2015). The basis of this strategy is the toxicological monitoring, i.e. the follow up in time of insecticide susceptibility in pest populations subjected to chemical control. Currently, the toxicological monitoring in triatomines use the protocol developed by the World Health Organization (WHO, 1994). This protocol established the topical bioassay as the methodology of exposure to the insecticide and requires standardized insects under laboratory conditions, equipment, and trained technicians that make it difficult to carry out a decentralized monitoring at the regional level. Remón *et al.* (2017) developed a simple bioassay based on insecticide-impregnated papers for *T. infestans* and proposed a protocol to carry out toxicological monitoring working in field. This protocol implies a first phase using field-collected insects evaluated by discriminant concentration (DC) through the impregnated paper and a second phase using laboratory-reared insect evaluated by dose-response assays through topical application to confirm the resistance status. The authors also established a DC of deltamethrin in filter paper for all developmental stages of *T. infestans* and evaluated the bioassay in the laboratory, which showed high sensitivity in the discrimination of resistance (Remón *et al.*, 2017). However, a field validation is required to propose this bioassay for the routine protocol in toxicological monitoring in *T. infestans*.

The objectives of this study were to determine the microgeographical distribution (i.e. between dwellings) of the susceptibility and resistance to deltamethrin in *T. infestans* from Pampa Argentina village; to evaluate the performance of the insecticide impregnated paper methodology to determine deltamethrin-resistance in *T. infestans* collected in field; and to determine the lethal activity of the fumigant canister containing dichlorvos against *T. infestans* resistant to deltamethrin.

Material and methods

Study area, insects sampling, and rearing

Triatoma infestans were collected in dwellings located in Pampa Argentina (PA) village, General Güemes Department, Province of Chaco, Argentina (25°53′59″ S, 60°29′36″ W). The insects from PA were toxicologically characterized by Fronza *et al.* (2016) grouping insects from different dwelling (i.e. a pool of the village) as highly resistant [lethal dose 50 (LD_{50}) >200; resistance ratio (RR) >1000]. PA is geographically located 15 km to the northeast of J.J. Castelli (Head of department) and it is composed of 121 inhabited households, while 95% of the population is of from the Qom ethnic group. A household demographic and entomological survey was conducted during September of 2017. The collection of insects was performed through active searches in intradomicile and peridomicile by technicians from the National and Provincial Chagas Programs of Argentina. The sampling technique follows the protocol established by the National

Chagas Program (NCP) for resistance monitoring under which a minimum number of 15 males and 15 females were collected of each house. Once insects were collected were maintained separated by dwelling (i.e. each sample), transported to laboratory and maintained in rearing conditions. Of each sample two groups were formed. The insects of one group were reared to obtain first instar nymphs for topical bioassay and the insects of other group were exposed to insecticide impregnated paper. The reference susceptible strain (S) originated from the village of 25 de Mayo in the Quitilipi Department, Province of Chaco, Argentina (26°52′42″ S, 60°13′52″ W), and first-generation descendants of field-collection insects were used. This control strain was toxicologically characterized by Lobbia *et al.* (2018) [$LD_{50} = 0.5$ (0.17–0.91); RR = 2.7 (0.9–6.2)]. Field and reference population insects were raised at the laboratory under controlled temperature (26 ± 1°C), humidity (50–70%), and a photoperiod of 12:12 (L:D) h. A chicken was weekly provided as a blood meal source. Chickens were reared and handled in accordance with resolution 1047/2005 of the National Council of Scientific and Technical Research (CONICET) on the National Reference Ethical Framework for Biomedical Research with Laboratory, Farm, and Nature Collected Animals, and National Law 14,346 on Animal Welfare.

Chemicals

Technical grade deltamethrin (94.4% purity) (Sigma-Aldrich Co., St Louis, MO, U.S.A.), analytical grade acetone (Merk, Buenos Aires, Argentina), silicone oil (Tetrahedron – Laboratorio Andes, Mendoza, Argentina), and analytical grade chloroform (Dorwil, Química analítica, Buenos Aires, Argentina) were used. Fumigant canister Musal contains 7.0% dichlorvos, 2.0% permethrin, and 1.3% of beta-cypermethrin (Chemotecnica SA, Buenos Aires, Argentina).

Design study and bioassays for resistance determination

The toxicological status (i.e. susceptible or resistant) of each sample was determined by topical bioassay and by impregnated paper bioassay and according to the percentage of mortality of groups of insects exposed to the discriminant dose (DD) or DC, depending of the bioassay. In order to evaluate the performance of the impregnated paper bioassay, the percentages of mortality obtained for each sample with the two bioassays were compared. The dose or concentration that causes 99% mortality of individuals of the susceptible strain (DL or CL_{99}) was used as DD or DC, respectively.

Topical bioassay

The topical bioassay according to the World Health Organization protocol was used (WHO, 1994). Briefly, a volume of 0.2 µl of deltamethrin diluted in acetone was applied on the dorsal abdomen of first instar nymphs (5–7 days old, mean weight 1.3 ± 0.2 mg) starved since hatching. The application was performed with a 10-µl Hamilton syringe (Hamilton, Reno, NV) provided with a repeating dispenser (Hamilton PB-600-1). Ten insects were used for each replicate and each sample was replicated at least three times. Control groups received only pure acetone. The DD used for deltamethrin was of 2 ng per insect (i.e. a concentration of 0.01 mg of deltamethrin/ml of acetonic solution) (Picollo *et al.*, 2005). After exposition, insects were kept at the previously mentioned laboratory conditions for 24 h, and then mortality was evaluated. Criterion for mortality was the inability to walk from the center to the border of a circular 11-cm diameter filter paper. Only those nymphs that were able to reach the filter paper border, with or without mechanical stimulation with forceps, were considered alive (WHO, 1994).

Exposure to insecticide-impregnated filter papers

The exposure to impregnated surfaces according to Remón *et al.* (2017) was used. Briefly, the bioassay was based on circular filter papers (Qualitative Filter Paper 102 Moderate, 9 cm diameter; Xinxing, Zhejiang, China) in which was distributed 1 ml of deltamethrin diluted in a mixture of silicone oil (non-volatile solvent) and chloroform (volatile solvent) in proportion 1:3 (oil:chloroform). The papers were impregnated using a pipette and in a spiral form towards the center ensuring a homogeneous distribution of the solution with the insecticide (WHO, 1994). Chloroform allows a homogenous distribution of solution on the paper and the oil improves the bioavailability and the absorption of the insecticide. The chloroform was allowed to evaporate during 24 h and then the insects were exposed to the impregnated papers during 1 h (i.e. the insects could walk on papers for 1 h). A plastic container (diameter: 9 cm; height: 7 cm) disposed on paper with its opening downwards was used to prevent insects from escaping the paper. Between five and ten fifth instar nymphs or adults were exposed to a paper impregnated with DC (i.e. each replicate) and each sample was replicated at least three times. Insects exposed to papers impregnated only with silicone oil and chloroform mixture were used as a control. The DC used was 0.36% w/v of deltamethrin in oil (Remón *et al.*, 2017). At the end of the exposure, the insects were removed from the paper and were placed in plastic containers (diameter: 9 cm; height: 7 cm) which were kept in controlled laboratory conditions for 72 h. The mortality was registered at 24, 48, and 72 h post-exposure. The criterion for mortality was the inability to walk from the center to the border of a circular 11 cm diameter filter paper. Only those nymphs that were able to reach the filter paper border, with or without mechanical stimulation with forceps, were considered alive (Picollo *et al.*, 2005).

Evaluation of fumigant canister

In order to evaluate the fumigant canister containing dichlorvos (DDVP) as an alternative control tool for *T. infestans* resistant to pyrethroids and susceptible to organophosphates (Fronza *et al.*, 2016), a semi-field assay was carried out. For this, groups of 15 fifth instar nymphs placed inside the plastic jar with the opening covered with a voile were exposed to two canister in a room of 3 × 4 × 5 m according to the NCP protocol. In each replicate, two jars were exposed to smoke: one jar containing resistant insects obtained of the PA laboratory strain (first-generation of laboratory) and one jar containing susceptible insects obtained of the S laboratory strain (second-generation of laboratory). Once the fumigant canister was lit, the room was kept closed during 3 h after which it was ventilated. Once there was no trace of smoke, the jars with the insects were removed, the insects were transferred to other jars and kept in breeding conditions until the observation of mortality at 24, 48, and 72 h. The mortality criterion was the same as that described for the bioassays.

Data analysis

Mortality data were corrected by the eventual mortality of controls using Abbott's formula (Abbott, 1925). The comparisons of the percentage of mortality between samples or between resistance determination bioassays were based on the 95% confidence interval (CI) of the groups that showed variation. The CI 95% were obtained with the InfoStat statistical software, version 2017 (Di Rienzo *et al.*, 2017).

Results

The entomological evaluation covered the 87.6% of dwellings (106 of 121 total dwellings) and 46.2% of them showed infestation by *T. infestans* (49 of 106 dwellings evaluated). Insect abundance was variable among the inspected dwellings, therefore enough insects were collected for resistance assessment in 20.8% of the household evaluated (22 of 106 dwellings evaluated).

Table 1 shows the results of the topical and impregnated papers bioassays with DD or DC, respectively, of deltamethrin for each dwellings/sample evaluated. For each dwelling, the number of replicates performed for the impregnated paper bioassay was always less than the number of replicates performed for the topical bioassay, and the average mortality percentage did not differ significantly between bioassays ($P > 0.05$). In the case of the impregnate papers bioassay, only was possible to perform one replicate in some households and the sample of two dwellings (95 and 114) was not sufficient to carry out the evaluation. Respect to mortality data, for topical bioassay, the 50% of the samples evaluated showed no mortality and in the rest of the samples the average mortality ranged from 0.77 to 3.3%. The percentages of mortality of these samples differed significantly from the mortality of the reference (100%) ($P < 0.05$) and did not differ significantly from 0% ($P > 0.05$). When all samples with all their replicates were considered together, only 17 dead insects of 1908 exposed insects were recorded. For impregnated papers bioassay, the 80% of the evaluated samples did not shown mortality and in the rest of the samples the average mortality ranged from 3.3 to 10%. The percentages of mortality of these samples differed significantly from the mortality of the reference (100%) ($P < 0.05$) and did not differ significantly from 0% ($P > 0.05$). When all samples with all their replicates were considered together, only seven dead insects of 426 exposed insects were recorded.

Table 2 shows the average mortality after exposure to fumigant canister for resistant and susceptible strain at 24, 48, and 72 h post-exposure. The average mortality of susceptible reference strain was always higher than 88% and did not differ significantly from 100% at each post-exposure time ($P > 0.05$). On the other hand, the average mortality of deltamethrin-resistant strain was always less than 10% and did not differ significantly from 0% at each post-exposure time ($P > 0.05$).

Discussion

The present study determined the toxicological status to deltamethrin of *T. infestans* from different dwellings of a village of the Argentine Chaco. In addition, a new bioassay based on impregnated papers was compared with the methodology of topical application, the historically used bioassay in toxicological studies in triatomines. Finally, the fumigant canister containing dichlorvos was evaluated against resistant insects. The study showed a very high survival of insects at DD in the samples of all the evaluated dwellings. The two bioassays used revealed the same toxicological status for each sample. Finally, the fumigant canister was not effective against the resistant insects from Pampa Argentina village.

The high survival observed in all samples indicate the presence of resistant insects in all evaluated dwellings of Pampa Argentina village and suggest that, in this village, the resistance to deltamethrin present homogeneity at the microgeographical level (i.e. between dwellings). This result can be compared with the only previous study concerning of the susceptibility to insecticides in *T. infestans* from different households of the same village (Germano *et al.*, 2013). The authors demonstrated that *T. infestans* from different dwellings of Argentine village of La Esperanza in the province of Chaco (close to Pampa Argentina village) presented different toxicological phenotypes where some households host insects resistant to deltamethrin and other households host susceptible insects. However, the same study did not found differences in the susceptibility to deltamethrin between insects from different dwellings of Argentine village of Acambuco in the province of Salta, where all houses harbored resistant insects.

It would be very interesting to determine if the descripted microgeographical distribution of resistance in *T. infestans* varies over time. There are no previous data on the susceptibility/resistance to deltamethrin evaluated in each dwelling in Pampa Argentina and there are no studies of this type in any other village. However, the history of chemical control in the village and the evaluation of resistance without discriminating by dwelling (i.e. insects from different dwellings pooled and evaluated as a single sample) carried out in different years allows to propose a hypothesis to evaluate in future studies. In Pampa Argentina, five chemical control events with pyrethroids were carried out in the 11-years old period 2005–2015 (control events in 2005, 2008, 2010, 2013, and 2014) (unpublished data). In this period, two evaluations of pyrethroid resistance in *T. infestans* were performed. The 2014 evaluation showed a high level of resistance (RR >1000) (Fronza *et al.*, 2016) and the 2017 evaluation reported in this manuscript showed low mortality by DD (mortality <1%), suggesting a very high resistance sustained over time. This background allows us to propose that the toxicological homogeneity between the dwellings within the Pampa Argentina village could also be sustained over time, at least since the very high resistance was detected in 2014.

Insecticide-resistant populations can be present in several areas of the geographical distribution of an insect species constituting resistance foci. The resistance foci express a resistance profile according to the type and amount of resistance mechanisms involved in each one. The occurrence of several resistance foci might be due the scattering of an ancestral focus, i.e. a single selection process with insecticide and subsequent spread, or the development of independent selection processes (Mougabure-Cueto and Picollo, 2015). This disquisition has practical relevance in resistance management because the evolutionary scenario determines the response of each focus to a specific alternative control action and, consequently, the resistance management strategy to be implemented. Therefore, if each focus had an independent origin, each would probably have a different resistance profile (although not necessarily) and each would probably require a particular control strategy. On the contrary, if all foci were descendants of an ancestral resistant population, all would have the same resistance profile and the same strategy could be applied to each one (Mougabure-Cueto and Picollo, 2015). In this context, the results of the present study and its comparison with the previous ones show that the geographical distribution of resistance to pyrethroids in *T. infestans* is complex and does not shown a common pattern through the geographical

Table 1. Results of the topical and insecticide-impregnated papers bioassays with DD or discriminant concentration, respectively, of deltamethrin in *Triatoma infestans* of each dwellings/sample evaluated

	Topical bioassay				Impregnated paper bioassay			
Sample	Number of exposed insects	Number of replicates	Dead insects	Mortality % Mean (CI 95%)	Number of exposed insects	Number of replicates	Dead insects	Mortality % Mean (CI 95%)
Reference	70	7	70	100	60	6	60	100
Dwelling								
03	106	11	1	0.91 (−1.12 to 2.93)	40	5	0	0
40	75	7	0	0	26	4	0	0
42	101	10	3	2.91 (−0.45 to 6.27)	45	8	2	4.20 (−5.69 to 14.02)
51	52	5	0	0	3	1	0	0
54	130	13	0	0	17	3	0	0
59	81	8	2	2.53 (−1.53 to 6.58)	8	1	0	0
61	128	13	2	1.54 (−0.73 to 3.81)	38	4	2	5.00 (−10.91 to 20.91)
63	106	10	1	0.77 (−0.97 to 2.51)	20	3	0	0
64	93	10	1	1.00 (−1.26 to 3.26)	5	1	0	0
70	55	6	1	1.52 (−2.38 to 5.42)	25	4	0	0
74	100	10	2	2.00 (−1.02 to 5.02)	30	4	0	0
89	72	7	0	0	22	4	2	10.00 (−21.82 to 41.82)
90	62	6	0	0	18	3	0	0
91	91	9	0	0	29	5	0	0
108	60	6	0	0	6	1	0	0
115	61	6	0	0	30	3	1	3.33 (−11.01 to 17.68)
168	120	12	0	0	12	3	0	0
201	90	9	1	1.11 (−1.45 to 3.67)	32	5	0	0
328	88	9	0	0	16	3	0	0
335	96	10	0	0	4	1	0	0
95	55	6	1	3.33 (−5.24 to 11.90)	–	–	–	–
114	86	9	2	2.22 (−1.17 to 5.61)	–	–	–	–
Total	1908	192	17	0.92 (0.48 to 1.35)	426	66	7	1.57 (−0.12 to 3.25)

Table 2. Mortality after exposure to fumigant canister for resistant and susceptible *Triatoma infestans* at 24, 48, and 72 h post-exposure.

	Mortality % Mean (CI 95%)		
Strain	24 h	48 h	72 h
Reference	97.78 (88.21 to 107.34)	95.56 (76.44 to 114.67)	88.89 (41.09 to 136.69)
Pampa Argentina	8.33 (−1.82 to 18.49)	10.00 (−0.61 to 20.60)	8.33 (−1.82 to 18.49)

distribution of the species. At the macro-geographical level, the resistant foci detected in Argentina and Bolivia showed differences both between countries and within countries. For example, the pyrethroid-resistant insects from Bolivia were resistant to phenylpyrazole insecticide fipronil but this did not occur in the pyrethroid-resistant insects from Argentina (Toloza *et al*., 2008; Roca-Acevedo *et al*., 2011); some Bolivian resistant populations showed susceptible eggs while the Argentinean resistant populations showed resistant eggs (Toloza *et al*., 2008); enhanced pyrethroid-esterase activity occurred in resistant insects from Argentina but not in resistant insects from Bolivia (Germano *et al*., 2012); the L1014F substitution in voltage-gated sodium channel was detected in Bolivia and north of Argentina whereas the L925I substitution was detected in the center of Argentina (Sierra *et al*., 2016). At the lower geographical level (i.e. variation between villages within a department), the susceptibility to deltamethrin in *T. infestans* from Güemes Department of the Argentine Chaco province was highly variable with villages host insects with high resistance (36%), villages host insects with low resistance (41%) and villages host susceptible insects (23%) (Fronza *et al*., 2016). In this focus, the investigation of the resistance mechanisms showed that the L925I substitution in the sodium channel was detected in all the resistant villages studied while the enhanced metabolism was only detected in some of the resistant villages studied (Fronza *et al*., 2020). Finally, at the microgeograhical level (i.e. houses within a locality), the distribution of the resistant insects depended of the resistant focus. As was discussed above, there are the case of Acambuco village described by Germano *et al*. (2013) and the Pampa Argentina in the present study where the insects of each evaluated dwelling were resistant to deltamethrin, and there is the case of La Esperanza where the insects from different dwellings showed different toxicological phenotypes (Germano *et al*., 2013). The differences in the resistance profiles between the different resistant foci of *T. infestans* suggest the occurrence of independent evolutionary processes at different geographical levels.

Considering the high degree of population structure in *T. infestans* (Pérez de Rosas *et al*., 2007; Marcet *et al*., 2008; Pizarro *et al*., 2008), the dissimilar resistance profiles could be the consequence of the diverse regimes of selection with insecticides occurred in the different endemic areas acting on the variable population genetic backgrounds. In this scenario, the hypothesis of environmental variables other than insecticide as possible selective factors of resistant insects should not be ruled out. This hypothesis could include both the possible modulating effect of environmental variables on the toxic effect of the insecticide and the selection, by environmental variables, of resistant individuals with certain phenotypic characters determined by genes that confer resistance but different from resistance mechanisms (Mougabure-Cueto and Picollo, 2015). The latter are understood as pleiotropic effects of resistant genes with positive adaptive consequences in the natural environment. While most pleiotropic effects of resistance reported in several species were interpreted as adaptive costs for the natural environment (i.e. adaptive resistance costs) (Rivero *et al*., 2011; Kliot and Ghanim, 2012), including in resistant *T. infestans* (Germano and Picollo, 2015; Lobbia *et al*., 2018, 2019*a*, 2019*b*), pleiotropic effects with positive adaptive consequences were described for *Tribolium castaneum* and *Musca domestica* (Arnaud *et al*., 2005; McCart *et al*., 2005). In triatomines, Lobbia *et al*. (2019*b*) studied the reproductive efficiency after dispersal in susceptible and resistant *T. infestans* and showed that the dispersed resistant females had a higher reproductive efficiency than the dispersed susceptible females and the non-dispersed resistant females. The authors suggested that the resistant insects could have an adaptive advantage over the susceptible ones if both toxicological phenotypes are dispersed. On the other hand, Bustamante-Gomez *et al*. (2016) and Fronza *et al*. (2019) studied the association between the distribution of populations of *T. infestans* susceptible or resistant to pyrethroids and environmental variables and proposed that indicators of temperature and precipitation are good descriptors of the insecticide resistance. In addition, Fronza *et al*. (2019) showed that spraying variables did not contribute to the explanation of the toxicological heterogeneity and proposed that the environmental variables explain part of the resistance distribution because they modulate the selection pressure exerted by the insecticide.

Moreover, both the type of construction and the materials of human dwellings can affect the effective dose to which the insects are exposed after spraying (for example, affecting the bioavailability or the residuality of the insecticide formulation) and, by therefore, determine the selection regime of the less susceptible individuals by the insecticide. Many studies demonstrated the differences in lethal activity on triatomines and the residuality of insecticide formulations applied to different housing construction materials (e.g. wood, mud and lime, brick, glass, etc.) (Cichero *et al*., 1983; Ferro *et al*., 1995; Guillén *et al*., 1997; Rojas de Arias *et al*., 2003, 2004; Gurtler *et al*., 2004; Germano *et al*., 2014). In this way, the variation in the structural characteristics of the dwellings could promote different resistance profiles, and this variation between the dwellings within the same village could promote toxicological heterogeneity in the village. Specific studies are needed to consistently determine the role of these variables in the evolution of insecticide resistance in *T. infestans*. Finally, the geographical structure of susceptibility and resistance to pyrethroids in *T. infestans* does not exclude the possibility of dispersal processes that could spread resistance to nearby areas in which there were only susceptible insects (Lobbia *et al*., 2019*a*, 2019*b*), which probably explains the local extension and toxicological homogeneity of some foci (e.g. Acambuco and Pampa Argentina villages).

The present study evaluated for the first time the insecticide-impregnated paper bioassay in field conditions and the results confirmed the good performance reported previously in laboratory conditions (Remón *et al*., 2017). Toxicological monitoring of resistance on a large geographical scale is usually carried out in two phases. Briefly, in the first phase, groups of insects of each population are exposed to a dose/concentration that theoretically kills 100% of susceptible individuals (i.e. DD/concentration). The population is considered susceptible if 100% mortality (or survival below a certain threshold) is recorded. By contrast, the population is considered resistant if survival (or survival above a certain threshold) is recorded and in the second phase a dose–response study is performed which allows the determination of the resistance level (ffrench-Constant and Roush, 1990). The topical bioassay was established by the World Health Organization as the methodology of exposure to insecticide for resistance monitoring in *T. infestans* (WHO, 1994). This bioassay requires standardized insects under laboratory conditions, equipment, and trained technicians. However, this makes it difficult to carry out monitoring at the regional level working in field or in laboratories without adequate infrastructure. Previous studies proposed the bioassay based on insecticide-impregnated papers to assess resistance to deltamethrin in *T. infestans* and implement it in large-scale geographic resistance monitoring (Lardeux *et al.*, 2010; Remón *et al.*, 2017). Remón *et al.* (2017) established a discriminant concentration of deltamethrin in filter paper for all stages of the development of *T. infestans* at different physiological states and proposed a protocol to carry out toxicological monitoring of populations using field-collected insects. The discriminant concentration allowed to discriminate in laboratory evaluation two colonies resistant to deltamethrin, one with high and one with low resistance, of the reference susceptible colony. The present study confirmed in field conditions the good performance of the insecticide-impregnated paper bioassay reported by Remón *et al.* (2017) in laboratory conditions. The insecticide-impregnated paper bioassay is easier to implement, it is suitable for field work as it is possible to distribute ready-to-use papers to the test sites, expedites the monitoring by evaluating insects collected from the field which, in turn, allows that the test sites do not need infrastructure for insect breeding.

Although for resistance studies it is recommended to evaluate the descendants of insects collected from the field (ffrench-Constant and Roush, 1990), field insects can be used by implementing the strategy in two phases. In this way, the field insects are exposed to DDs and then, if there is survival, the dose-response study is carried out on the descendants obtained in the laboratory. The results of phase two determine whether the original survival was due to environmental factors or inheritable factors. The present manuscript does not propose to replace the topical bioassay by the impregnated paper bioassay, but to expedite large-scale monitoring by using a bioassay as a screening test suitable for field work. The more precise topical bioassay is proposed for the quantification of toxicological parameters in phase II by specialized laboratories.

This was the first study that evaluated the fumigant canister containing dichlorvos (DDVP) for the management of resistance to pyrethroids in *T. infestans*. The fumigant canister showed low lethal activity against the resistant insects from Pampa Argentina village indicating that it is not an effective tool to control *T. infestans* resistant to pyrethroids. Considering that all populations studied of the resistant focus of Argentine Chaco province, which include the Pampa Argentina village, were susceptible to organophosphates (Fronza *et al.*, 2016), this result suggest that the low toxic activity of the fumigant canister was due to the low concentration of DDVP in the formulation. This study confirms the shortage of insecticides and formulations available to control *T. infestans* resistant to pyrethroids. The organophosphates fenitrothion as wettable powder and malathion as emulsifiable concentrate, and the carbamate bendiocarb as wettable powder were the alternatives used successfully for the control of pyrethroids resistant foci of *T. infestans* in Argentina and Bolivia (Programa Nacional de Chagas, 2009; Gurevitz *et al.*, 2012; Zaidenberg, 2012; Germano *et al.*, 2014). However, the toxicological and eco-toxicological risk of these insecticides promoted the questioning it use in public health and the consequent regulations. Currently, the only insecticide effective against *T. infestans* susceptible and resistant to pyrethroids approved in Argentine for use in public health is the organophosphates fenitrothion (Carvajal *et al.*, 2012; Germano *et al.*, 2014). Thus, it is necessary to continue the research and evaluation of new insecticides and the development of suitable formulations for optimal effectiveness and application, while allowing easy handling and ensure minimal toxicological risk to the operators performing applications.

In summary, this studied confirmed that the microgeographical distribution of the toxicological phenotypes in *T. infestans* depends on each village/location, some villages showing a heterogeneous distribution and other showing a homogenous distribution. On the other hand, the bioassay based on paper impregnated with insecticide showed a successfully performance in field conditions emerging as an alternative to the topical bioassay for the phase I of resistance monitoring in *T. infestans*. Finally, the fumigant canister containing an organophosphorus showed not to be a viable alternative to control deltamethrin-resistant *T. infestans*.

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