

Reproductive and developmental costs of deltamethrin resistance in the Chagas disease vector *Triatoma infestans*

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Received 3 September 2014; Accepted 2 December 2014

ABSTRACT: Effective chemical control relies on reducing vector population size. However, insecticide selection pressure is often associated with the development of resistant populations that reduce control success. In treated areas, these resistant individuals present an adaptive advantage due to enhanced survival. Resistance can also lead to negative effects when the insecticide pressure ceases. In this study, the biological effects of deltamethrin resistance were assessed in the Chagas disease vector *Triatoma infestans*. The length of each developmental stage and complete life cycle, mating rate, and fecundity were evaluated. Susceptible and resistant insects presented similar mating rates. A reproductive cost of resistance was expressed as a lower fecundity in the resistant colony. Developmental costs in the resistant colony were in the form of a shortening of the second and third nymph stage duration and an extension of the fifth stage. A maternal effect of deltamethrin resistance is suggested as these effects were identified in resistant females and their progeny independently of the mated male's deltamethrin response. Our results suggest the presence of pleiotropic effects of deltamethrin resistance. Possible associations of these characters to other traits such as developmental delays and behavioral resistance are discussed. *Journal of Vector Ecology* 40 (1): 59-xxx. 2015.

Keyword Index: *Triatoma infestans*, deltamethrin resistance, fitness cost, fecundity, life cycle, developmental delay.

INTRODUCTION

Effective vector control using chemicals implies full or strong reduction of insect population size and often acts as a selection factor for insecticide resistance. Due to enhanced survival, resistant insects incur an adaptive advantage relative to susceptible ones while in the pressured environment. Consequently, treatment success is endangered by residual populations that resist the insecticide application and increase in frequency. Nevertheless, pleiotropic effects of insecticide resistance are frequent, since genes which increase in frequency as a consequence of environmental change and adaptation generally present an adaptive cost in the population's original environment (Carriere et al. 1994).

The vector of Chagas disease, *Triatoma infestans* (Heteroptera: Reduviidae), has been successfully controlled through insecticide spraying for over 20 years. However, the development of resistance to deltamethrin, the most widely used insecticide, currently puts control effectiveness in danger (Germano et al. 2010b, Lardeux et al. 2010, Picollo et al. 2005). Even though pyrethroid resistance mechanisms and consequences on *T. infestans* control are well studied (Fabro et al. 2012, Germano et al. 2010a, Germano et al. 2014, Pedrini et al. 2009, Santo Orihuela et al. 2008), no studies are available regarding its effects on this insect's development and life traits. One of the commonly described effects of resistance is the variation in the life cycle duration, which is generally expressed as an extension of certain stages of development. Such effect has been described for several species of insects, including pyrethroid-resistant *Heliothis virescens* (Lepidoptera: Noctuidae) and *Aedes aegypti* (Diptera: Culicidae), and Bt resistant *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) (Campanhola et al. 1991, Martins et al. 2012, Trisyono and Whalon 1997). In addition, reproductive

costs have been demonstrated in several economically relevant resistant insects. Insecticide-resistant *Myzus persicae* (Homoptera: Aphididae) presented a low reproductive rate in comparison to their susceptible populations, as demonstrated by Ghadamyari et al. (2008). As well, deltamethrin-resistant *H. virescens* presented a low fecundity and fertility, together with an extended preimaginal development (Sayyed et al. 2008).

Considering the available information regarding the biological effects of insecticide resistance, the objectives of this work were to establish the possible fitness costs of deltamethrin resistance in *T. infestans*, covering the areas of development and reproduction.

MATERIALS AND METHODS

Insect sampling and rearing

Insects were sampled at areas of current infestation after deltamethrin field application, as reported by the authorities of the Health Ministry of Argentina. The selected areas were Aguaray, province of Salta, Argentina (22°14'S 63°43'W) and Termas de Río Hondo, province of Santiago del Estero, Argentina (27°29'S 64°52'W). Both areas are characterized by semi-arid climate and vegetation, and are part of the Gran Chaco ecoregion (Cabrera et al. 1973). Average temperature range and annual rainfall are 13.3 to 27.7° C and 596 mm at Termas de Río Hondo and 15.6 to 27.5° C and 970 mm at Aguaray. Typical domestic structures are made of adobe with thatched roofs. Peridomestic structures are generally made of wood and adobe and mainly represent goat corrals and chicken coops. Both sites had been subjected to deltamethrin spraying conducted by the Health Ministry of Argentina and had received no chemical treatment during the previous year of the field collection.

A dislodging agent was used to facilitate insect detection.

Captured insects and their progeny were raised in a conditioned chamber at the laboratory. Environmental conditions consisted of controlled temperature ($26 \pm 1^\circ \text{C}$), humidity (60-70% RH) and photoperiod (12h day – 12h night). A pigeon was provided weekly as a source of blood.

Deltamethrin response determination

Bioassays were conducted on the first generation raised at the laboratory. First nymphs were exposed to 0.2 μl of increasing doses of deltamethrin in acetone, delivered to the dorsal abdomen by means of a 10 μl syringe provided with automatic dispenser (Hamilton, Reno, NV) (WHO 1994). Nymphs were five to seven days old and had been starved since eclosion (mean weight $1.2 \pm 0.2 \text{ mg}$). At least four doses in a range that produced between 10 and 90% mortality were applied, and control groups received only pure acetone. Mortality was evaluated 24 h after the treatment and defined as the inability to walk to the border of an 11 cm diameter filter paper, with or without the need of mechanical stimulation.

As the Termas de Río Hondo colony was determined to be susceptible to deltamethrin in comparison to the laboratory strain CIPEIN, it was chosen as the susceptible reference for subsequent assays (Table 1). Semi-dominant inheritance of resistance was verified in the crossed colonies, as previously described (Germano et al. 2010a). Briefly, the LD_{50} of the crossed progenies was calculated and used for the calculation of the degree of dominance (DD) as proposed by Stone (1968). This index ranges from -1 (complete recessivity) to 1 (complete dominance).

Fecundity and longevity assays

Susceptible (Termas de Río Hondo colony) and resistant (Aguaray colony) insects were reciprocally crossed to evaluate reproductive compatibility, fecundity, and the possible maternal effects of resistance. Virgin adults were randomly selected such that three couples were formed and kept during eight weeks. This procedure led to four experimental colonies: susceptible (S), resistant (R), susceptible female x resistant male ($\text{R}\sigma\text{S}\phi$), and resistant female x susceptible male ($\text{R}\phi\text{S}\sigma$). Each couple was held in a double bottom plastic flask (8 cm diameter x 13 cm height). The superior bottom was made of a metallic mesh that allowed the collection of eggs in the bottom base. Flasks included absorbent paper for spermatophore adhesion, and a cotton gauze cover was provided to allow humidity dissipation. Average weight of insects at the assay onset was (302.9 ± 17.7) mg and (306.7 ± 22.5) mg for susceptible and resistant insects, respectively. This weight was not significantly different between males and females nor between colonies (Anova, $p > 0.05$; $n = 10$).

Each week the amounts of spermatophores and eggs laid per couple were counted as a reference of reproductive affinity and fecundity. A cohort of 100 eggs was identified and numbered for each colony and raised in individual acrylic flasks (4 cm diameter x 6 cm height) that included absorbent paper and a cotton gauze cover for humidity dissipation. Nymphs were kept under the mentioned environmental conditions and the feeding conditions were standardized such that a pigeon was weekly offered during 30 min for first to third nymphs, 40 min for fourth and fifth nymphs, and 1 h for adults. Laying, molting, and death dates were recorded daily for each individual, and sex was determined based on external adult morphology. Fifty individuals that reached the adult

stage in each colony were randomly chosen for the comparison of stage duration and overall longevity so that the information corresponds to complete life cycles.

Data analysis

Dose-mortality data were subjected to probit regression analysis to estimate the lethal dose to kill 50% of the population (LD_{50}) and the resistance ratio (RR) (Robertson et al. 2007). Both analyses were conducted using POLO PLUS software (LeOra 2007). Lethal dose values are expressed in nanograms per insect (ng/i).

The numbers of spermatophores and eggs laid were compared between colonies using univariate analysis of variance (ANOVA), together with a Duncan test ($\alpha = 0.05$). A repeated measures analysis of variance was conducted to compare stage length between males and females within a colony. As no significant differences were found ($p > 0.05$), males and females were pooled and stage length was analyzed for each colony. Between colonies comparison was conducted using a multivariate analysis of variance (MANOVA) applied to repeated measures. A discriminant analysis allowed determining the variables that mostly contributed to between colonies differences, and univariate analysis of variance was used to compare stages. A Duncan test was applied to define within stage differences, considering a Bonferroni correction of the significance level to account for multiple comparisons ($\alpha = 0.0125$). Statistical analysis was conducted using Infostat software.

Chemical compounds

Tetramethrin 0.2% was used as a dislodging agent in insect field collections (Icona, Buenos Aires, Argentina). Technical grade deltamethrin (99.5%) used for bioassays was obtained from Dr. Ehrenstorfer GmbH, Augsburg, Germany. An analytical grade of acetone was used for dilutions.

RESULTS

The toxic response to deltamethrin was analyzed in field-collected insects. Bioassay results showed that the Termas de Río Hondo colony was susceptible to deltamethrin, as its LD_{50} did not differ from the laboratory reference strain CIPEIN (Table 1). Hence, this field colony was used as reference for further studies. Table 2 presents the bioassay results taking the Termas de Río Hondo colony as susceptible reference. The Aguaray colony was highly resistant to deltamethrin ($\text{RR} \approx 340\times$). Progeny of reciprocally crossed insects presented intermediate lethal doses and resistance levels. Using this information, the calculation of the dominance degree verified the previously described codominance of the resistance character in this species ($\text{DD} = -0.21$).

Susceptible and resistant insects were able to successfully reproduce. The weekly amount of copulation events was not significantly different either between pure and reciprocally crossed couples ($p > 0.05$). However, the fecundity of couples did significantly differ. Both the weekly and the total amount of eggs laid was significantly higher in susceptible than resistant couples ($p < 0.05$). This effect was not so noticeable in reciprocally crossed individuals, whose fecundity was intermediate to the parental colonies. In this case, the number of eggs laid did not differ from the susceptible colony, although evidence of a maternal

Table 1. Deltamethrin toxicity in field colony Termas de Río Hondo and laboratory reference CIPEIN.

	Colony	n ^a	Slope \pm SE ^b	LD ₅₀ (ng/i) ^b (CL 95%)
Laboratory reference	CIPEIN ^c	125	2.1 \pm 0.66	0.13 (0.12-0.15)
Santiago del Estero	Termas de Río Hondo	150	1.8 \pm 0.32	0.11 (0.04-0.22)

^a Number of insects used for bioassays.^b Slope and Lethal Dose 50% (LD₅₀) with respective Standard Errors (SE) and Confidence Limits (CL) calculated following Robertson et al. (2007).^c Data from Picollo et al. (2005).

Table 2: Deltamethrin toxicity in susceptible, resistant, and reciprocally crossed colonies.

Colony	n ^a	Slope \pm SE ^b	LD ₅₀ (ng/i) ^b (CL 95%)	RR ^b (CL 95%)
S	150	1.8 \pm 0.3	0.1 (0.04-0.22)	-
R	183	1.2 \pm 0.17	38.8 (23.98-69.75)	343.2 (73.11-1611.53)
R♂xS♀	129	1.7 \pm 0.4	0.8 (0.43-1.32)	7.1 (3.79-13.33)
R♀xS♂	100	2.5 \pm 0.7	2.5 (1.61-3.96)	21.7 (11.63-40.00)

^aNumber of insects used for bioassays.^bSlope, lethal dose 50% (LD₅₀), and resistance ratios (RR) with respective standard errors (SE) and confidence limits (CL) calculated following Robertson et al. (2007).Table 3: Weekly and total amount of copulation events and eggs laid (\pm standard error) by susceptible, resistant, and reciprocally crossed colonies.

Colony	Average weekly copulation events \pm SE	Average weekly egg laying \pm SE*	Average total copulation events \pm SE	Average total egg laying \pm SE*	Eggs/Copulation event \pm SE
S	1.4 \pm 0.13	6.1 \pm 0.87 ^a	10.8 \pm 1.11	48.0 \pm 7.70 ^a	4.7 \pm 1.04
R	1.3 \pm 0.44	2.7 \pm 0.63 ^b	10.7 \pm 3.48	20.7 \pm 4.37 ^b	2.8 \pm 1.18
R♀xS♂	1.3 \pm 0.57	4.9 \pm 1.19 ^{a,b}	10.5 \pm 4.50	39.5 \pm 9.50 ^{a,b}	4.1 \pm 0.87
R♂xS♀	1.4 \pm 0.19	6.5 \pm 0.60 ^a	11.0 \pm 1.68	50.0 \pm 4.74 ^a	4.9 \pm 0.74

*Different letters indicate significant differences between colonies (Anova - Duncan test, $p < 0.05$).

effect could be present given that the reciprocal mating $R\sigma S\phi$ laid significantly more eggs than the resistant colony, which was not the case for the $R\phi S\sigma$ colony (Table 3). On the other hand, the amount of eggs laid respective to the copulation events was the lowest in the resistant colony, which suggested a decrease in copula efficiency ($p > 0.05$).

Considering the susceptible colony as a reference, the egg was the shorter stage, followed by the first nymph. The length of central stages (nymph II to V) was similar, and the adult was the longest stage. Full life cycle duration was 535.8 ± 20.1 days for the susceptible colony, 559.7 ± 20.9 days for the resistant colony, 531.2 ± 20.3 days for the $R\phi S\sigma$ colony, and 514.4 ± 15.7 days for $R\sigma S\phi$ colony. The statistical analysis showed that the colonies significantly differed in the length of their stages (Pillai's trace, $p < 0.05$), although differences seemed to be compensated as the total life cycle length was not different among colonies (Pillai's trace, $p > 0.05$).

Discriminant analysis showed that 73.0% of the variability between colonies is explained by the fifth nymph length. Length of the first, second, and third nymphs further explain 21.3% of variation between colonies. Univariate analysis of variance demonstrated that the susceptible and the resistant colonies significantly differ in the length of the second, third, and fifth nymph. The progeny of the reciprocally mated individuals only differed in the length of the second nymph and significantly differed from the resistant colony for the third and fifth nymphs. Among the reciprocal matings' progeny, the only stage that differed from the susceptible colony was the fifth in the $R\phi S\sigma$ colony (Bonferroni-corrected $\alpha = 0.0125$) (Figure 1). In addition, the fifth stage variability was higher in the resistant colony, suggesting a wider dispersion of molting times in this colony. In fact, the maximum length of this stage was 246 days in the resistant colony, while it was 126 in the S colony, 168 in the $R\phi S\sigma$ colony, and 136 in the $R\sigma S\phi$ colony.

DISCUSSION

The two sites of insect collection differed in their response to deltamethrin. This indicated that even though high resistance to deltamethrin is associated with field control failure (Picollo et al. 2005), an ineffective treatment does not necessarily imply that resistance has developed. This result is in accordance with previous findings of the authors, which showed that the resistant area is circumscribed to the northern border of Argentina, and that deltamethrin remains as an appropriate control tool in other geographic areas of the country (Germano et al. 2010b).

Susceptible and resistant insects were able to successfully mate, indicating that resistance does not present an effect in mate recognition or selection. The highly resistant colony presented a significant reduction in reproductive output, which was expressed to a lesser extent in the reciprocal mating of a female resistant parent. This result was expected, since the investment done to maintain resistance traits such as target-site mutations and increased detoxification is likely to be sustained through a trade-off with other traits such as reproduction. In the context of chemical control, a physiological investment in survival at the cost of lower fecundity can be understood as a convenient trait, as an effective control would result in null survival. However, the insect's physiological commitment to face the challenge represented by insecticide exposure would result in a lower fitness when these resistant individuals are set in an untreated environment. Several examples of resistance costs have been demonstrated in the past, as genes that are rapidly selected as advantageous in an environment under selective pressure tend to present disadvantages in the original environment (Holloway et al. 1990). Pyrethroid resistant *H. virescens* and *Ae. aegypti*, among others, have presented lower fecundity and extended larval developmental time in comparison to insecticide-susceptible strains (Brito et al. 2013, Campanhola et al. 1991, Sayyed et al. 2008). On the other hand, the reduction in

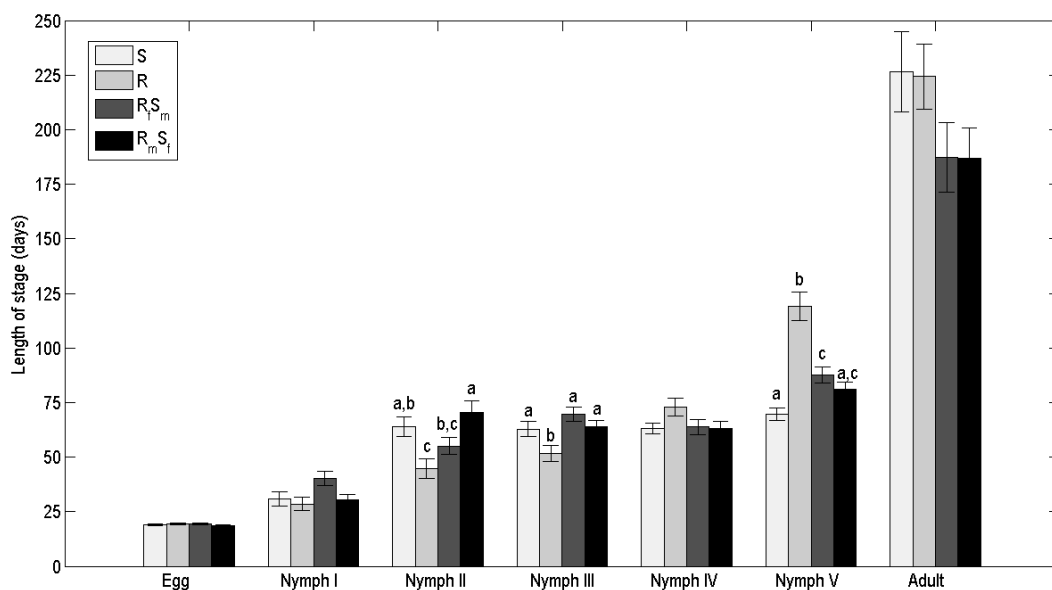


Figure 1. Average length in days (\pm standard error) of each developmental stage in the susceptible (S), resistant (R), resistant female x susceptible male ($R_f S_m$), and resistant male x susceptible female ($R_m S_f$) colonies. Different letters indicate significant differences between colonies, within stage (Anova - Duncan test, Bonferroni corrected $\alpha = 0.0125$).

fecundity found in resistant *T. infestans* could be associated with reduced blood ingestion in the reproductive female, a pleiotropic effect of deltamethrin resistance that has been previously described in hematophagous disease vectors (Martins et al. 2012). Further studies, though, are necessary to test this hypothesis and its possible relationship to *T. infestans*' copula efficiency and vectorial capacity.

The susceptible colony was used as a reference for life cycle duration in this work. Previous studies conducted on *T. infestans* have shown shorter developmental times (Perlowagora-Szumlewicz 1969, Rabinovich 1972). However, as these authors conducted the assays in groups of insects and density dependence acts decreasing stage length in this species (Perlowagora-Szumlewicz 1969), differences could be accounted for by this factor. Colonies differed in the length of the second, third, and fifth nymph, although differences seemed to be compensated as total life cycle length was similar among colonies. Nevertheless, the fifth nymph explained the majority of variation between colonies and resulted in the longest and most variable stage in the resistant colony. A delay in molting to the adult stage could have detrimental consequences on survival and reproductive success, translated into a low survival of progeny in an unfavorable environment for growth and an excessive exposition to predation when in the extended larval stage. Nonetheless, the higher duration and variability of the last nymph stage in the resistant insects could indicate a higher frequency of an adaptive developmental delay or diapause, a well-studied bet-hedging strategy. Even though scarce evidence is available for this trait in *T. infestans*, recent studies conducted by Menu et al. (2010) established the presence of a developmental delay strategy in *T. infestans* and other triatomines. While it is not yet known if this finding is due only to phenotypic plasticity or to a true diapause, such a trait could be linked to an adaptation to the variable environments to which this insect is exposed. In fact, insecticide exposure represents a catastrophic event for susceptible populations, which decline and collapse after the spraying. However those nymphs that molt later can remain in refuges avoiding insecticide exposure and survive the unfavorable times, i.e., the insecticide residual activity. The difference found between the studied colonies could be associated with different control histories in different geographic areas, especially considering the increase in spraying frequency and hence the enhanced insecticide selection that resistant populations have experienced (Zaidenberg 2012). In fact, it is possible that a double selection mechanism acts as a strategy to survive the environmental stochasticity due to spraying cycles. On one hand, the insecticide application selects for resistant individuals and leads to resistant populations. On the other hand, the insecticide avoidance strategy could be highly favored in surviving individuals, allowing the delaying individuals to increase their frequency in the population. The fact that this character was identified in the fifth nymph could be related to the high resistance to starvation described for this stage in the *Triatoma* genera (Almeida et al. 2003, Costa and Marchon-Silva 1998), which would allow further times in refuges during the unfavorable season. Further studies are necessary to verify the possible relationship between a developmental delay and insecticide resistance in *T. infestans*. Previous studies, however, have indicated these traits are actually related in other pest species, for example in the lepidopteran

Choristoneura rosaceana (Lepidoptera: Tortricidae). In this species, testing for the relationship between insecticide resistance and diapause demonstrated an increased propensity to diapause after the repeated exposure to insecticide and the development of resistance in field populations (Carrière et al. 1995). Deltamethrin resistance has also been associated with an earlier beginning of the diapause in *Cydia pomonella* (Lepidoptera: Tortricidae) and explained by changes in its photoperiodic timing (Boivin et al. 2004).

While our results suggest the presence of deltamethrin resistance costs, life cycle differences could also be related to natural environmental conditions, as it has been described for a sylvatic/peridomestic Chagas disease vector (Martínez-Ibarra et al. 2012, Martínez-Ibarra et al. 2013). Both *T. infestans* collection sites present similar climate and vegetation, and assays were conducted under standardized laboratory environmental conditions. Moreover, *T. infestans* is a fully domiciliated species, and several studies have demonstrated that housing structures produce a dumping of temperatures and humidity that stabilizes conditions between and within domestic habitats throughout the year (Heger et al. 2006, Lorenzo et al. 2000, Vazquez-Prokopec et al. 2002). This information suggests the insects from different areas experience similar microclimate despite variations in external environmental conditions and, as such, the results presented in this work could well be understood in the context of insecticide resistance.

Variation in life cycle traits can be a key to enhance the management of resistant *T. infestans* in infested areas. The described differences in reproductive output and development could possibly lead to lower growth rates in the resistant insects, hence delaying resistance evolution or allowing its reversion in heterogeneous populations. Including such information in management programs could be decisive for insecticide rotation organization or a future reinstatement of pyrethroid spraying in areas where field control failures have been detected.

Acknowledgments

The authors thank Cynthia Spillmann and the technicians of the Coordinación Nacional de Control de Vectores of Argentina. This investigation received financial support from the UNICEF/United Nations Development Programme/World Bank/World Health Organization Special Programme for Research and Training in Tropical Diseases (TDR), and the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) of Argentina.

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