Demographic effects of deltamethrin resistance in the Chagas disease vector *Triatoma infestans*

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Abstract. *Triatoma infestans* (Heteroptera: Reduviidae) Klug is the main vector of Chagas disease in Latin America. Resistance to deltamethrin was reported in Argentina and recently associated with reproductive and longevity trade-offs. The objectives of the present study were to describe the demographic consequences of deltamethrin resistance in *T. infestans* and to establish possible target stages for chemical control in susceptible and resistant colonies. A stage-classified matrix model was constructed based on the average stage length for susceptible, resistant and reciprocal matings' progeny. The differences between colonies were analysed by prospective and retrospective analysis. The life table parameters indicated reduced fecundity, fertility and population growth in resistant insects. The retrospective analysis suggested the latter was associated with lower reproductive output and increased fifth-instar nymph stage length. The prospective analysis suggested that the adult stage should be the main target for insecticide control. Although, fifth-instar nymphs should also be targeted when resistance has been detected. The presented results show demographic effects of deltamethrin resistance in *T. infestans*. While the older stages could be the main targets for chemical control, this approach is impeded by their higher tolerance to insecticides. It is concluded that the different mode of action insecticides would be more effective than a dose increase for the control of deltamethrin-resistant *T. infestans*.

Key words. *Triatoma infestans*, Chagas disease, deltamethrin resistance, demography, elasticity, matrix population models, population growth, retrospective analysis, sensibility, vector control.

Introduction

Insecticide resistance has long been regarded as an evolutionary response to selection of chemical compounds, and hundreds of species are known to have developed resistance to at least one pesticide. A vast literature is available regarding resistance spread and intensity, as well as its consequences on pest management. Insecticide resistance has also been demonstrated for disease vector insects and is currently considered an obstacle for effective vector control and disease management (Hemingway & Ranson, 2000; Rivero *et al.*, 2010). The resistance to an insecticide generally improves survival in an insecticide-pressured environment, although the physiological investment done to maintain its mechanisms does not go without cost. Indeed, when the resistant populations are raised in an untreated environment, biological costs are frequently expressed in fecundity or lifespan, among other factors (Carriere *et al.*, 1994; Boivin *et al.*, 2001; Sayyed *et al.*, 2008; Martins *et al.*, 2012). The variation in life-history traits can often be understood as a trade-off between survival and reproduction when environmental conditions are disadvantageous (in this case insecticide treatment). In the case of pests, the possible changes in population structure and growth associated with insecticide resistance could result in unexpected chemical control outcome and even translate into rapid population size compensation after insecticide spraying. In fact, the population structure at the time of toxicant exposure has been proven to have an impact on treatment effect (Stark & Banken, 1999; Stark & Banks, 2003), which suggests that differences

in the population structure of susceptible and resistant insects may lower or intensify the consequences of resistance in the field.

The resistance to the pyrethroid deltamethrin has been extensively studied in *Triatoma infestans*, the main vector of Chagas disease in Latin America (Picollo *et al.*, 2005; Lardeux *et al.*, 2010; Germano *et al.*, 2010a,2012). This trait has been detected in several areas of the Gran Chaco region where this insecticide is the primary chemical control tool, and several mechanisms are involved in its expression (Santo Orihuela *et al.*, 2008; Fabro *et al.*, 2012; Capriotti *et al.*, 2014; Mougabure-Cueto & Picollo, 2015). Recent findings demonstrated that fecundity and longevity costs are associated with deltamethrin resistance in *T. infestans* (Germano & Picollo, 2015). These studies also suggested that the recently described developmental delay of this species could be more frequent in resistant individuals, posing as a possible bet-hedging strategy in the pressured environment (Menu *et al.*, 2010; Germano & Picollo, 2015). Even although *T. infestans*' population dynamics after insecticide spraying have been previously studied, none of these studies have considered the differences between insecticide susceptible and resistant insects and their possible implications for chemical control (Gorla & Schofield, 1989; Gorla, 1992; Cecere *et al.*, 2004).

In the present study, the population growth of deltamethrin-resistant *T. infestans* will be measured in comparison to susceptible individuals, using a demographic approach based on matrix population models. A life table will be constructed, and parameters will be calculated considering previous findings of the authors, to achieve a stage-structured model of population growth. This information will also be used to identify key developmental stages for chemical control effectiveness. The causes and consequences of the findings will be discussed in the context of insect control.

Materials and methods

Insect sampling sites

Insects were sampled at areas of current infestation, as reported by the authorities of the Health Ministry of Argentina. The two collection sites 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 part of the Gran Chaco ecoregion and present a semiarid climate and vegetation (Cabrera & Willink, 1973). The average temperature range and annual rainfall are (13.3–27.7)°C and 596 mm at Termas de Río Hondo and (15.6–27.5)°C and 970 mm at Aguaray (data obtained from the National Meteorological Service of Argentina). Typical domestic structures are made of adobe with thatched roofs. Peridomestic structures such as goat corrals and chicken coops are generally made of wood and adobe. Deltamethrin spraying had been conducted at both sites by the Health Ministry of Argentina, and no chemical treatment had been applied during the previous year of the field collection. A dislodging agent was used to facilitate insect detection (Tetramethrin 0.2%; Icona, Buenos Aires, Argentina).

Insect rearing and toxicological profile

Captured insects and their progeny were raised in a conditioned chamber at the laboratory. The following environmental conditions were used: temperature (26 ± 1°C), humidity (60–70% RH) and photoperiod (12 h day – 12 h night). A pigeon was weekly provided as a source of blood.

The toxicological response to deltamethrin was evaluated with bioassays that are described in a previous work from the authors (Germano & Picollo, 2015). These bioassays established susceptibility to deltamethrin for the insects collected in Termas de Río Hondo (S colony) and high resistance to the insecticide for the insects collected in Aguaray (R colony, resistance ratio ≈ 340x). Reciprocal crossings (see below) presented intermediate values of resistance.

Assay design

Susceptible and resistant adult *T. infestans* were mated both intra and inter-colony. This procedure led to four experimental colonies: susceptible (S), resistant (R), susceptible female × resistant male (R♂S♀) and resistant female × susceptible male (R♀S♂). A cohort of 100 eggs was raised per experimental group, and the length of stage duration was measured. Briefly, laying, moulting and death dates were recorded daily for each individual, and sex was determined based on external adult morphology (a full description of this methodology is available at Germano & Picollo, 2015). Those insects that moulted to the adult stage were immediately set in couples and a number of eggs laid was counted and used to calculate the average amount of eggs laid per individual (m_x) and per female. If the male died, it was replaced by a second male within 48 h. Colony fertility was defined as the average egg hatching percentage. To this aim, five batches of 100 eggs were randomly selected per colony, and the number of hatched/non-hatched eggs was counted. The hatching of eggs was established by the observation of an open operculum and the absence of the embryo.

The survival probability to stage x (l_x) was calculated as the proportion of individuals alive at stage x, with respect to the original amount of individuals in the cohort. These values and the m_x were used to calculate the basic net reproductive rate, i.e. the average amount of offspring produced per individual:

$$R_0 = \sum l_x m_x \qquad (1)$$

The fecundity of the colonies was compared by a one way ANOVA and Duncan test, with a 0.05 significance level. ANOVA assumptions were verified using Shapiro–Wilks and Levene tests ($P > 0.05$). Colonies' fertility was compared using a generalised linear model (GLM) with binomial distribution and DGC contrasts. The sex ratio of the colonies was compared by a chi-squared test (χ^2). These analyses were conducted using R and Infostat software (Di Rienzo *et al.*, 2012).

Model construction and parameter estimation

The life cycle of *T. infestans* was analysed as a stage-classified matrix model (Lefkovitch, 1965). Considering a weekly projection time, the life cycle that is shown in Fig. 1 was constructed

representing the species developmental stages, i.e. the egg, five immature instars and the adult stage. In this life cycle, the survival probability in each stage splits into two different processes: survival within the stage (Pi, $1 \leq i \leq 7$) and moulting to the following stage given survival (Gi, $1 \leq i \leq 6$). Adults are the only reproductive stage, and contribute to the egg stage with a weekly fecundity called F. The proportion of females in the colony was taken into account through the correction of fecundity by the female proportion (ρ), calculated among the individuals that reached the adult stage.

The model used was of the form:

$$n(t+1) = An(t) \qquad (2)$$

where n(t) is a vector giving the number of individuals in each stage at time t, and A is a population projection matrix constructed as shown below (Caswell, 2001):

$$A = \begin{pmatrix} P_1 & 0 & 0 & 0 & 0 & 0 & F\rho \\ G_1 & P_2 & 0 & 0 & 0 & 0 & 0 \\ 0 & G_2 & P_3 & 0 & 0 & 0 & 0 \\ 0 & 0 & G_3 & P_4 & 0 & 0 & 0 \\ 0 & 0 & 0 & G_4 & P_5 & 0 & 0 \\ 0 & 0 & 0 & 0 & G_5 & P_6 & 0 \\ 0 & 0 & 0 & 0 & 0 & G_6 & P_7 \end{pmatrix} \qquad (3)$$

Parameters of the projection matrix A were estimated based on the average stage length for each colony, fully described by Germano & Picollo (2015). In summary, the average stage length among colonies was not significantly different for eggs (19 days), first instar nymphs (29–40 days), fourth instar nymphs (63–73 days) and adults (187–224 days). In the case of second instar nymphs, no differences were found between the S and the reciprocally mated colonies (55–70 days), although the R colony presented a significantly shorter second instar length (45 days), except in comparison to the R♀S♂ colony. The R colony's third instar nymph presented a significantly shorter duration (52 days) than the other colonies (63–70 days). By contrast, the fifth instar nymph was significantly longer in the R colony (119 days). The shorter length of this instar was found for the S colony (70 days), which did not differ from the R♂S♀ colony (81 days). The latter was also not significantly different from the R♀S♂ colony (88 days).

In this work, a constant probability of moulting from stage i to i + 1, independent of the time spent on the stage, was considered. This lead to the following estimation of survival probabilities (Pi and Gi) (Brault & Caswell, 1993; Caswell, 2001):

$$P_i = \sigma_i (1 - \gamma_i) \qquad (4)$$

$$G_i = \sigma_i \gamma_i \qquad (5)$$

where

$$\sigma_i = 1 - \gamma_i \frac{\# \, dead \, individuals \, during \, stage \, i}{\# \, individuals \, entering \, stage \, i} \qquad (6)$$

$$\gamma_i = \frac{1}{average \, length \, of \, stage \, i} \qquad (7)$$

The average fecundity of the adult stage (F) was calculated in base of the survival probability for the adults (P_7) and the egg stage (P_1 and G_1), and the weekly number of offspring per female (m) (Caswell, 2001):

$$F = \sqrt{(P_1 + G_1) \frac{(1 + P_7) \, m}{2}} \qquad (8)$$

The corresponding dominant eigenvalue which represents the per capita growth rate [population growth ($\lambda > 1$) or decay ($\lambda < 1$)] and the right and left eigenvectors, i.e. the stable stage distribution of the population and the reproductive value of each stage, respectively, were calculated (Caswell, 2001). The reproductive value was also weighted by the proportion of individuals in each stage of the stable stage distribution (Rabinovich, 1972a). The intrinsic growth rate (r) was calculated as ln (λ). By contrast, the rate of convergence to the stable stage distribution was calculated as the ratio between the dominant eigenvalue and the magnitude of the first subdominant eigenvalue (damping ratio). Generation time (T) was calculated as the quotient between ln (R_0) and r (Caswell, 2001).

The contribution of the life history parameters to the differences in population growth between the S and the R colony was evaluated through a retrospective analysis. For the susceptible population matrix A^s and the resistant population matrix A^r, the population growth for the R colony was defined as (Caswell, 1996):

$$\lambda^r = \lambda^s + \sum_{kl} \left(a_{kl}^r - a_{kl}^s \right) \left. \frac{\partial \lambda}{\partial a_{kl}} \right|_{A^m} \qquad (9)$$

where A^m is the mean matrix between the resistant colony's matrix and the susceptible colony's matrix. The matrices of differences ($D^r = A^r - A^s$) and contributions [$C^r = D^r$ o S (A^m)] were calculated according to Caswell (1996,2001).

A prospective analysis was conducted to define the impact of possible changes in the parameters of each population matrix and to identify possible target instars for chemical control. The sensitivity of the a_{kl} elements of the population projection matrix was calculated as follows (Caswell, 2001):

$$S = \frac{\partial \lambda}{\partial a_{kl}} \qquad (10)$$

The elasticity of the a_{kl} elements of the population projection matrix was calculated as (Caswell, 2001):

$$e_{kl} = \frac{\partial \log(\lambda)}{\partial \log(a_{kl})} \qquad (11)$$

Matrix analysis was conducted using MATLAB R2011B software.

Results

The survival probabilities (l_x) are presented in Fig. 2. For immature stages, the lowest survival probabilities were observed in the R colony. The S colony presented higher survival probability than the latter, although the reciprocally mated colonies presented higher survival probabilities. However, the survival
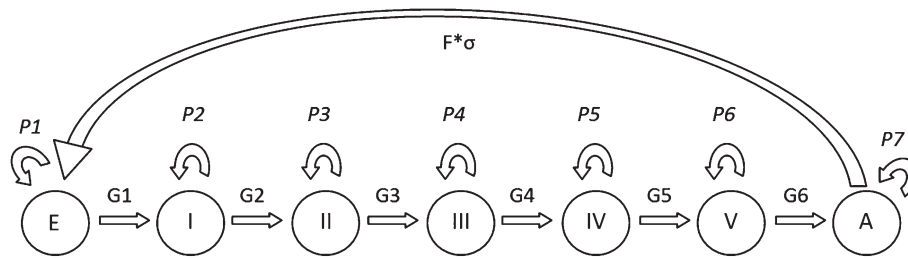
Fig. 1. Diagram of the components and processes of population growth model for *T. infestans*. Circles represent stages: eggs (E), five immature instars (I–V) and the adult stage (A). Arrows indicate the probability of survival within the stage (Pi; $1 \leq i \leq 7$) or the probability of moulting to the next stage given survival (Gi; $1 \leq i \leq 6$). Fecundity (F) weighed by female proportion (ρ) corresponds only to the adult stage, and affects only the egg stage.
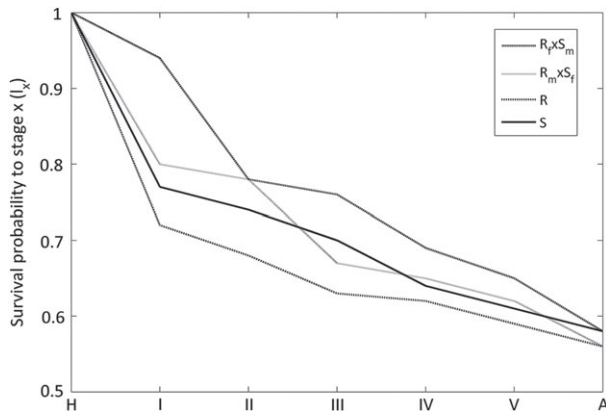

Fig. 2. Survival probabilities to stage x (l_x) for the susceptible (S), the resistant (R) and the reciprocally mated colonies ($R_f \times S_m$ and $R_m \times S_f$).

probability to the adult stage was similar for the four colonies. For the initial cohort of 100 eggs, 58 individuals in the S and R♀S♂ colonies, and 56 individuals in the R and the R♂S♀ colonies reached the adult stage. The sex ratios (♀ : ♂) measured among the individuals that moulted to the adult stage were 1 : 1.1 (S colony); 1 : 1.3 (R colony); 1 : 1.8 (R♀S♂ colony); 1 : 1.2 (R♂S♀ colony). Despite apparent differences in the colonies' sex ratios, none differed from the 1 : 1 expected ratio (χ^2_1; $P > 0.05$).

A summary of the population growth parameters is presented in Table 1. The S colony presented a significantly higher fecundity than the R and the reciprocally crossed colonies ($P < 0.05$). The S colony's fertility was significantly higher than the R colony's ($P < 0.05$). However, the R♀S♂ did not show effects on its fertility, although this effect was present for the R♂S♀ colony, whose fertility was intermediate to parental insects. The per capita and intrinsic growth rates, and the net reproductive rate were higher in the S colony. All of these parameters showed intermediate values for the reciprocally mated colonies. Further differences were found in generation time, which was shorter for the susceptible than for the resistant colonies. The population demographic projections are presented in Fig. 3. All the colonies presented positive population growth, although the S colony presented a higher population size than the other colonies after a projection time of 50 weeks.

The stable stage distribution was similar between the four colonies (Table 2). Minor differences were observed among the youngest stages (egg to nymph III), as 80% of susceptible individuals belong to this group, and only 69% of the resistant individuals do. The proportion of individuals per stage in the reciprocally crossed colonies was intermediate to the S and R colonies. In every case, the proportion of adults was low, although it was higher in the R colony. The rate of convergence to the stable stage distribution was similar between the S and R colonies, although the reciprocal crosses presented lower convergence ratios (Table 1).

Figure 4 presents the reproductive value of each colony (by definition, the reproductive value is 1 in the egg stage), and the reproductive values weighted by the proportion of individuals in the stage. As expected, the reproductive values increased with the insect's stage. The S colony presented the highest reproductive values for each of the developmental stages, except for the first nymph. The R colony generally had the lowest per-stage reproductive values, and the reciprocal crosses tended to present intermediate values. The weighed reproductive values followed the same pattern, except the fifth instar nymph, whose higher reproductive value was found in the R colony.

The S and R colonies were compared through a retrospective analysis of the differences in their matrix parameters and the contributions to the differences in growth rate (Fig. 5). The probabilities of survival and moulting tended to be lower in the R colony, but the differences in the parameters tended to compensate within stages as the higher the Pi, the lower the Gi, and vice-versa. Nevertheless, the differences in the parameters suggested lower survival and decreased fecundity in the R colony (ΔPi = −0.0340; ΔGi = 0.0146; ΔTotal = −0.0194; ΔFec = −0,852). In accordance with the differences found, the contributions of the Pi and Gi parameters to the differences in the growth rate were generally compensated within stages, except for the egg stage which presented a total negative contribution. The greatest contribution to the differences in λ between the S and R colonies was done by the fecundity (contribution = −0.0486) and the fifth nymph parameters (total contribution = −0.0123).

Sensitivity and elasticity analysis is presented in Fig. 6. The sensitivity of λ to changes in the probabilities of moulting to the following stage (Gi) was generally higher than those to the probabilities of survival within the stage (Pi). Also, the sensitivity to Pi increased with the stage of the insect, although they were similar between colonies within the stage.

Table 1. Population growth parameters estimated for susceptible (S), resistant (R) and reciprocally crossed colonies (R♀S♂ and R♂S♀).

Colony	Average female fecundity $\pm$ SE*	Average fertility (%) $\pm$ SE†	Net reproductive rate (R_0)	Intrinsic growth rate (r)	Finite growth rate (λ)	Generation time (weeks)	Damping ratio‡
S	165.1 ± 16.14^a	73.5 ± 1.00^a	38.32	0.063	1.065	58.2	1.083
R	86.1 ± 22.04	59.2 ± 0.73^b	18.92	0.045	1.046	65.8	1.083
R♀S♂	100.7 ± 12.25	72.2 ± 1.60^a	20.13	0.048	1.049	62.8	1.077
R♂S♀	85.0 ± 20.89	66.2 ± 1.94^c	19.66	0.047	1.048	63.0	1.075

*Average female fecundity with respective standard errors (SE). Different letters indicate significant differences (ANOVA – Duncan test; $p < 0.05$).
†Average colony fertility with respective standard errors (SE). Different letters indicate significant differences (GLM – DGC test; $p < 0.05$).
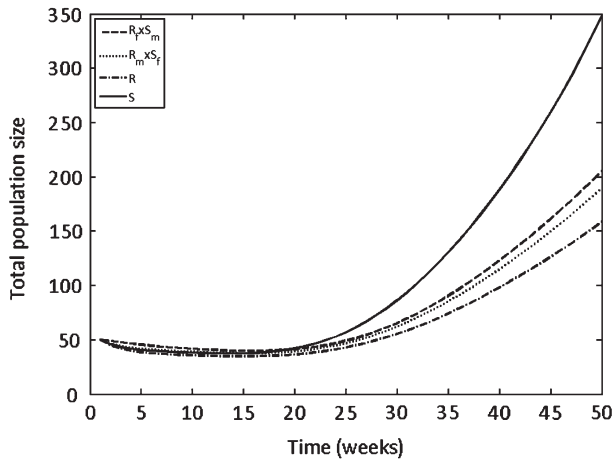‡Rate of convergence to the stable stage distribution.

Fig. 3. Population growth projection based on the stage-structured matrix model and iterated for 50 weeks. Lines represent the susceptible (S), resistant (R) and reciprocally crossed colonies ($R_f \times S_m$ and $R_m \times S_f$) projections.

Table 2. Stable stage distribution for susceptible (S), resistant (R) and reciprocally crossed colonies (R♀S♂ and R♂S♀).

Developmental stage	S	R	R♀ × S♂	R♂ × S♀
Egg	18.19	14.96	15.60	14.86
Nymph I	20.87	16.25	22.47	18.40
Nymph II	25.84	21.18	21.28	26.16
Nymph III	15.09	16.32	16.87	15.92
Nymph IV	9.16	12.59	10.26	10.64
Nymph V	6.07	11.63	8.12	8.24
Adult	4.77	7.07	5.40	5.78

Values expressed in relative percentage of individuals per developmental stage.

The changes in the survival parameters of the adult stage would present the highest effect on the population growth rate in all the colonies, although this effect would be similar to the fifth instar nymphs of the R colony. In the case of the S colony, the changes in moulting probabilities of immature instars would cause similar effects in the population growth, although a different pattern was observed in the R colony because the highest effect of changes in Gi would be associated to the fifth nymph. By contrast, the sensitivity to fecundity was lower than that of the Pi and Gi in all of the colonies, namely 0.05 for the S and R♀S♂ colonies, 0.06 for the R♂ × S♀ colony, and 0.07 for the R colony. The proportional changes in the probabilities of survival increased with the stage, such that the adult stage presented the highest elasticity to the survival parameters. The elasticity of λ to moulting probabilities was the same at every stage, and equal to those of fecundity ($e_S = 0.08$; $e_R = 0.07$; $e_{R♀\times S♂} = 0.06$; $e_{R♂\times S♀} = 0.07$). In general, the S colony presented higher elasticity to the Pi than the R and the reciprocally mated colonies. The exception to this was that the elasticity of λ to the survival probability of the fifth-instar nymph (P_6) was higher in the R colony.

Discussion

Life history parameters were measured and used to model the population growth of deltamethrin susceptible and resistant *T. infestans*. In agreement with previous findings of the authors (Germano & Picollo, 2015), the fecundity of the susceptible colony was significantly higher than that of the resistant colony and was related to the former's higher reproductive value. For the first time a decreased fertility was described for resistant *T. infestans*, establishing an additional reproductive cost of this trait. While several studies have demonstrated fertility effects associated with resistance (Boivin *et al.*, 2001; Abbas *et al.*, 2012; Martins *et al.*, 2012), the mechanisms through which this effect is achieved remain uncertain. The mating rate positively correlates with egg fertility in several insect groups (Arnqvist & Nilsson, 2000), but this mechanism cannot account for differences found in this work because the copulation rate between the colonies was not significantly different (Germano & Picollo, 2015). Male characters such as spermatophore quality may be contributing to these differences, considering that the fertility of the R and the R♂ × S♀ colonies was lower than that of the S and the R♀S♂ colonies. Further studies, although, would be necessary to establish a possible role of the spermatophore quality on resistant *T. infestans* reproductive success.

The reduction in survival and the described trade-offs in reproduction and longevity of resistant insects were translated into lower population growth rates (Germano & Picollo, 2015). The retrospective analysis conducted for the R colony in reference to the S colony suggested that the most contributing factors to these differences in growth rate were the lower fecundity and the fifth-instar nymph survival parameters. Regarding the structure of the colonies' populations, we found that the distribution
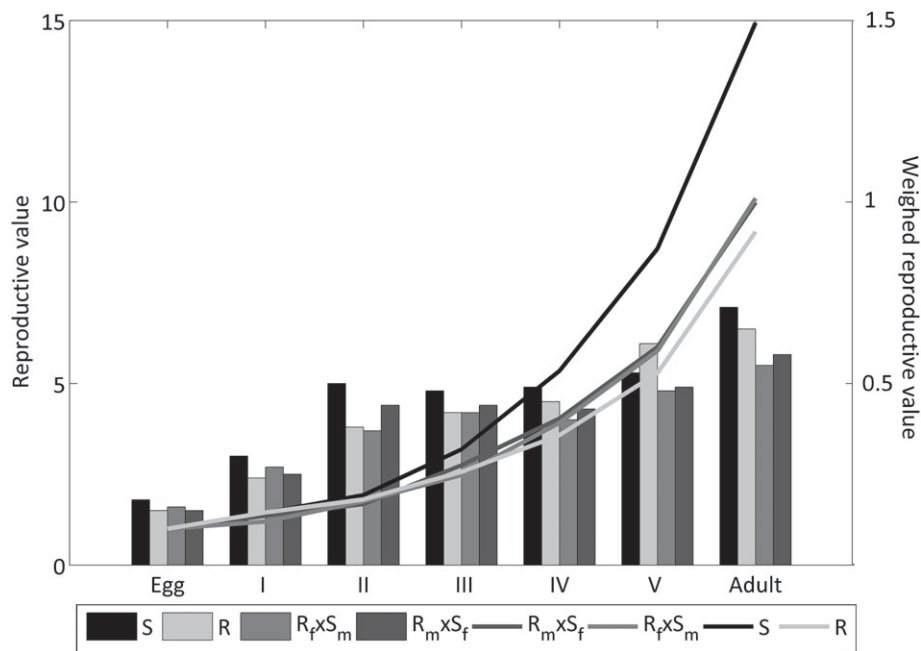
Fig. 4. Reproductive (line plotted) and weighed reproductive (bar plotted) value of susceptible (S), resistant (R) and reciprocally mated colonies ($R_f \times S_m$ and $R_m \times S_f$).

of individuals per stage in the S colony was similar to previous reports, with a majority of individuals in younger stages and their proportion decreasing up to the adult stage (Rabinovich, 1972b). Some differences were found between the susceptible and the resistant colonies, which presented an increment in the proportion of older stages (IV instar nymphs to adult), at the expense of younger ones. This change in the stable stage distribution is probably a consequence of the described reproductive trade-offs as changes in fecundity can have more impact on this aspect than changes in survival, as found by Carey (1982) in his studies about *Tetranichus urticae* (Acari: Tetranychidae) C. L. Koch. In contrast, a tendency to a higher proportion of males was observed in the resistant colonies. Considering that males have been established as the main dispersive sex in *T. infestans* (Vazquez-Prokopec *et al.*, 2004), this brings the question as to whether higher dispersion rates may be present in resistant populations. If this was the case, the increased dispersion of resistant males may more frequently lead to mid-level resistant offspring in susceptible areas, possibly contributing to the microgeographical patching of deltamethrin resistance (Germano *et al.*, 2010b,2013).

Both deltamethrin-susceptible and -resistant colonies presented similar rates of convergence to a stable stage distribution, which may imply that an insecticide spraying conducted in heterogeneous areas would act on both types of populations at this stage distribution. However, the stable age/stage distributions are hardly ever reached in natural conditions, so the presented results should be treated cautiously and as an approximation of reality only. As an approximation to the possible results of spraying cycles in different populations of *T. infestans*, we conducted a prospective analysis. This analysis showed that changes in the survival of different instar nymphs would present a similar outcome in population growth of the S colony. Although, the changes in survival that would most affect population growth are related to the fifth instar nymph in the case of the R colony. The conducted elasticity analysis allowed targeting possible control-vulnerable stages (Benton & Grant, 1999). In all the colonies the adult stage was the one whose changes would present the highest impact on population growth, which suggests this stage should be the main focus of this vector's control. Some differences observed between the susceptible and resistant colonies suggest that the control strategies may have to be adjusted depending on the treated area. A relevant finding is that the youngest instars of the S colony present a higher contribution to population growth than those of the R colony. As younger stages tend to respond to lower doses of insecticide, it is possible that a decreased effect of changes in these instars in the R colony would contribute to its control failure. The resistant fifth instar nymph presented a high contribution to the population growth rate, which suggests that this stage should be targeted by the chemical control in addition to the adult stage. This novel finding brings concern on the adjustments that may be needed for resistant *T. infestans* control, as the fifth nymph has been described as the most resistant stage in the Triatoma genera, both to insecticides as to fasting (Corrêa & Schiavi, 1954; Costa & Perondini, 1973; Zerba *et al.*, 1997; Costa & Marchon-Silva, 1998). Moreover, the resistant colony presented a higher proportion of delaying fifth instar individuals that could survive the unfavourable season produced by deltamethrin spraying (Germano & Picollo, 2015). As a consequence, surviving fifth instar nymphs could quickly compensate the population mortality, through the moulting to the adult stage and a boost of the reproductive rate caused by a decrease in resource competition (Stark *et al.*, 1997). Previous work has established that the
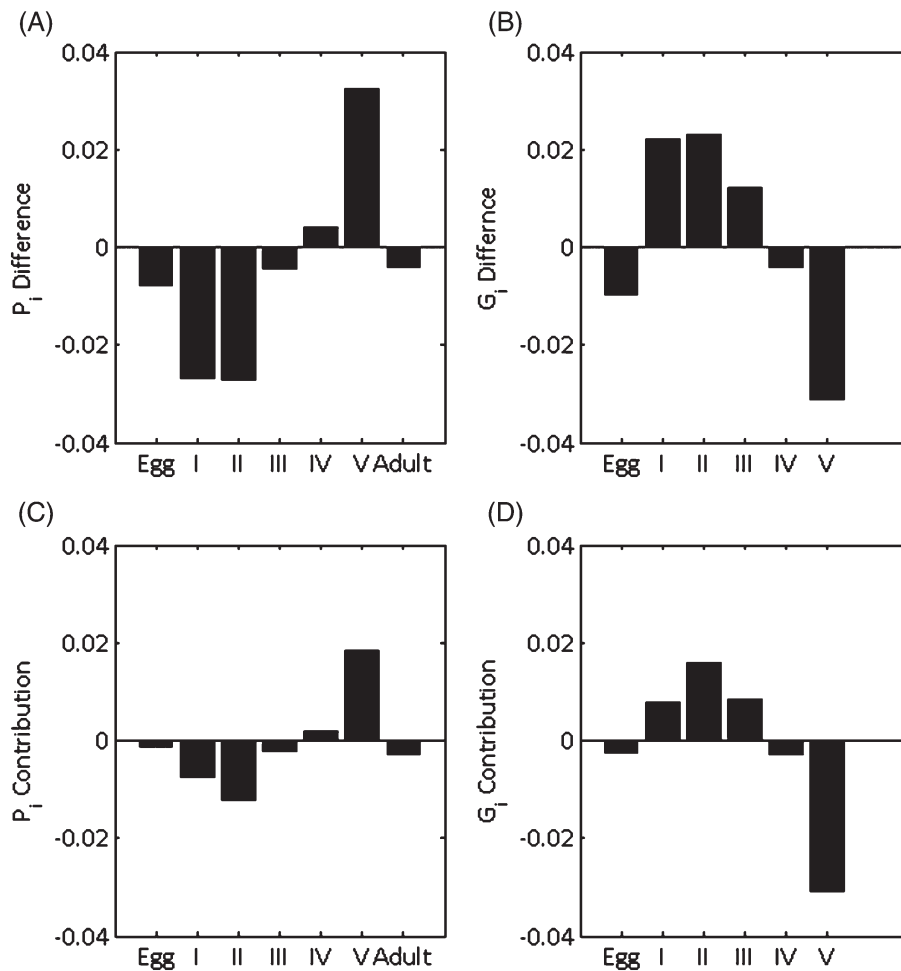
Fig. 5. Retrospective analysis of matrix parameters for the resistant colony (R) in reference to the susceptible colony (S). (A,B) differences in life parameters between resistant (R) and susceptible (S) colonies. (C,D) contributions of the differences in life parameters to the resistant (R) colony's per capita growth rate λ.

increment in reproductive value of a certain stage, as observed here, could contribute to a compensation of the population size after insecticide treatment, which reinforces the hypothesis that surviving fifth nymphs could quickly compensate the population reduction caused by insecticide application (Rabinovich, 1972a). This may help explain the rapid development of deltamethrin resistance in this species, which was considered to be unable to develop this trait owing to a low population growth among other characteristics (Dias & Schofield, 1999; Picollo *et al.*, 2005; Mougabure-Cueto & Picollo, 2015).

Finally, it is important to note that not all field control failures are a consequence of insecticide resistance, although high levels of resistance tend to be translated into control failure (Picollo *et al.*, 2005; Germano *et al.*, 2010a). By contrast, the residual effect of insecticides is usually short under natural climatic conditions, which suggests that the main effect of the spraying is obtained at its application (Rojas de Arias *et al.*, 2003; Germano *et al.*, 2014). This is a relevant factor for chemical control design because the natural differences in population structure could act as a contributing factor to treatment failure or success,

considering that the stage proportion at the time of insecticide application can be determinant for the result of the treatment (Stark & Wennergren, 1995; Stark & Banken, 1999). More research conducted on *T. infestans* field populations would provide evidence on the possible differences in susceptible and resistant population structure at the time of chemical control and their possible impact on its outcome. However, the estimations of non-toxicologically classified population structures in natural conditions have demonstrated that the fifth-instar nymphs and adults tend to increase in proportion as the summer starts, with younger stages present during autumn and winter (Cecere *et al.*, 2003). As explained by Gorla *et al.* (Gorla & Schofield, 1989; Gorla, 1992), the most effective time for insecticide spraying would be the onset of winter when the youngest instars are present, and climatic conditions act as a further denso-independent mortality factor. Although, younger nymphs tend to hide in refuges and be less accessible for insecticide contact, and the reduction in their survival has a decreased effect on resistant population dynamics than in susceptible dynamics. Surviving individuals, then, may lead to population
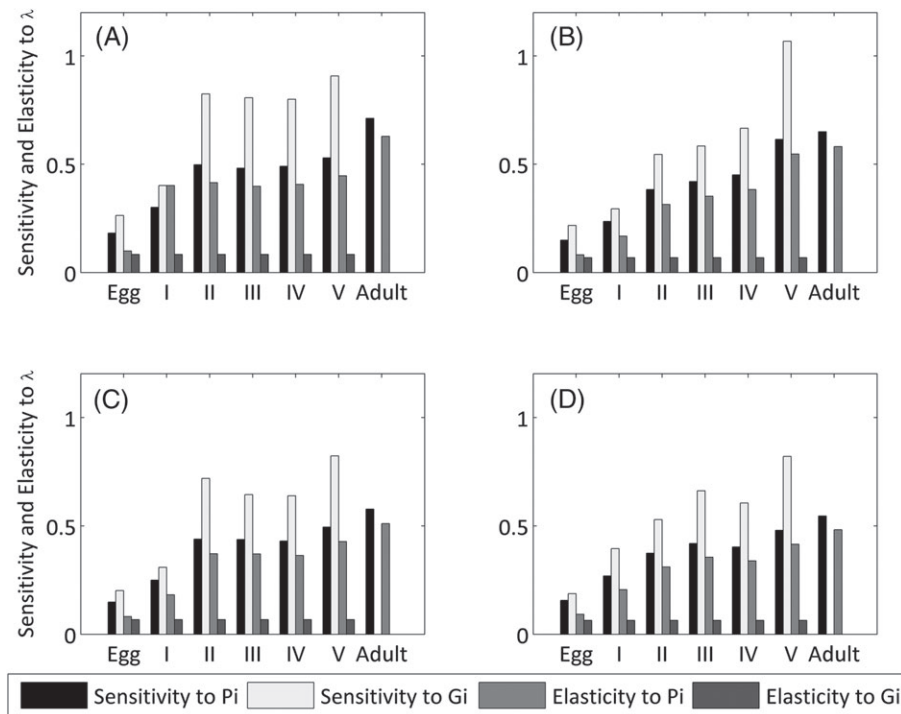
Fig. 6. Prospective analysis in terms of sensitivity and elasticity to survival (Pi) and moulting (Gi) probabilities for the (A) susceptible (S), (B) resistant (R) and reciprocally crossed colonies ((C) $R_m \times S_f$ and (D) $R_f \times S_m$).

size compensation as the summer begins. This complex scenario which implies that the most relevant and accessible individuals for insecticide treatment are also those which most tolerate the insecticide suggest that increasing insecticide dose for resistant insect control may not be effective. Instead, the best course of action when the resistance has been detected would be to move to using a different mode of action insecticide. As an example, a switch to the insecticide fenitrothion has been proven to be effective for the management of deltamethrin-resistant populations in several areas of Argentina (Zaidemberg, 2012; Germano *et al.*, 2014).

The results presented in this work show differences in the population dynamics of susceptible and resistant *T. infestans*. These differences related to the development of resistance in different areas of Argentina bring additional difficulties in conducting chemical control with deltamethrin. While target stages were identified, the fact that they are the most resistant to deltamethrin dissuades from focusing control management on these stages. In these areas, changing to a different insecticide would be necessary. Nonetheless, the possibility of targeting some stages, in particular, opens the possibility of trying alternative insecticides such as growth regulators that could act on them in particular, simplifying control measures and possibly decreasing the environmental impact of spraying cycles.

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