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Response of Mexican *Rhipicephalus* (*Boophilus*) *microplus* Ticks to Selection by Amitraz and Genetic Analysis of Attained Resistance

[1]H. Fragoso-Sanchez, [2]Z. Garcia-Vazquez, [3]G. Tapia-Perez, [4]A. Ortiz-Najera, [2]R. Rosario-Cruz and [5]I. Rodriguez-Vivas

[1]Universidad Autónoma Metropolitana, Unidad Xochimilco, Calzada del Hueso 1100, Col. Villa Quietud, Delegación Coyoacán, C. P. 04960 México, D.F.

[2]Instituto Nacional de Investigaciones Forestales, Agrícola y Pecuario, Centro Nacional de Parasitologia Veterinaria (CENID-PAVET-INIFAP), Apartado Postal 206, CIVAC, C.P. 62550, Morelos, México

[3]Facultad de Medicina Veterinaria y Zootecnia. Universidad Nacional Autónoma de México, Departamento de Genética, Av. Universidad No. 2000, Ciudad Universitaria, Col. Coyoacán, C.P. 04150, México, D.F.

[4]Centro Nacional de Servicios de Constatación en Salud Animal. SAGARPA. Km. 11.5 Carretera Federal Cuernavaca Cuautla. Colonia Progreso, C.P. 62550. Jiutepec, Morelos, México

[5]Facultad de Medicina Veterinaria y Zootecnia. Universidad Autónoma de Yucatán. Yucatán, México

Corresponding Author: Zeferino Garcia-Vazquez, Instituto Nacional de Investigaciones Forestales, y Agropecuarias. Centro Nacional de Parasitologia Veterinaria (CENID-PAVET-INIFAP). Apartado Postal 206. CIVAC. C.P. 62550, Morelos, México Tel: +52-777-319-28-40 Fax: +52-777-320-55-44

ABSTRACT

The aim of this study was to describe the genetics of amitraz resistance evolution and to obtain the independent genes number involved. Three Mexican *R. microplus* tick strains were used, one susceptible to all acaricides and two amitraz-resistant. Larvae were reared on isolated naïve-heifers and maintained nine generations in laboratory conditions. From each generation and each strain, the amitraz LC_{50} was chosen as the selection concentration for each strain. The control population of susceptible *R. microplus* ticks was maintained throughout the study. Genetic estimators were obtained. Selection of nine generations produced a 29-fold increase in resistance. Estimated h^2 to amitraz resistance were 0.3057, 0.4348, 0.4863 with effective number of factors (genes) 6.31, 5.93, 6.69 and the numbers of generations required for 10-fold increase in LC_{50} were 10, 8 and 4 for susceptible Media Joya, Palenque and San Alfonso amitraz-resistant strains respectively. The increase in resistance in all the three selected strains showed that a part of the variation in amitraz resistance in *R. microplus* is additive. It is suggested that insecticide resistance against amitraz is controlled by almost completely recessive more than one gene. But these differences may not necessarily translate to field performance of this acaricide.

Key words: Cattle tick, acaricide resistance, selection response, heritability, resistance gene number

INTRODUCTION

Rhipicephalus (*Boophilus*) *microplus* is an important ectoparasite of cattle and the key vector of bovine babesiosis in many tropical and subtropical regions of the world (Friedhoff and Smith, 1981; Bram *et al*., 2002). The cattle tick *R. microplus* is the principal ectoparasite that affects cattle in México and tick control is carried out by the use of acaricides such as organophosphates,

pyrethroids and amidines which have been used intensive and widely for tick control (Foil *et al*., 2004).

In Mexico, resistance to OP acaricides first diagnostic was made in 1980s and resistance to pyrethroids subsequently developed in the 1990s. Amitraz, along with pyrethroids, was introduced to control OP-resistant ticks in Mexico in 1986 (Soberanes *et al*., 2002).

Amitraz has played a critical role in the control of the cattle tick, *Rhipicephalus* (*Boophilus*) *microplus* in countries where resistance to both organophosphate (OP) and pyrethroid acaricides reached unacceptable levels (Kunz and Kemp, 1994), the first case reported in Mexico of amitraz resistance in cattle tick was in 2002 (Soberanes *et al*., 2002).

The presence of acaricide resistance to the different compounds in Mexico has made tick control difficult. It is generally believed that large scale uniform acaricide treatment of cattle exerts high selection pressure on targeted ticks and accelerates the development of resistance. It is believed that large scale selection of ticks with amitraz created the problem of amitraz resistance in Mexico as described by Soberanes *et al*. (2002) and Rosado-Aguilar *et al*. (2008).

Heritability (h^2) is one of the most important character values in quantitative genetics and the predictive role of heritability in quantitative genetic study is an important function (Guo, 1993). Direct resistance development comparisons among selection experiment treatments can be problematic because of different selection intensities and other selected factors. Quantitative genetic techniques can often be used to reduce such problems (Via, 1986; Falconer, 1989). Estimates of heritability and related parameters can be useful for understanding and managing evolution of resistance (Tabashnik, 1992). For example, estimates of realized heritability, the proportion of phenotypic variation accounted for additive genetic variation has been used for the assessment of resistance risk in insect pests (Falconer, 1989; Tabashnik, 1992; Lu *et al*., 2004). Estimates of resistance heritability are based on laboratory selection experiments (Ru *et al*., 1997; Lu *et al*., 2004).

The aim of this study was to describe the genetics of amitraz resistance evolution, obtain the independent genes number involved and their dominance degree. We report a laboratory-selected resistance to amitraz in three strains of *R. microplus* ticks and present an analysis of the heritability of such resistance.

MATERIALS AND METHODS

Tick strains: Three Mexican *R. microplus* ticks strains were used in this study. The San Alfonso was an amitraz-resistant tick strain collected from a ranch in the municipality of Emiliano Zapata in the state of Tabasco, Mexico in 2001 and was maintained at the National Center of Parasitology Laboratory, Jiutepec, Morelos, Mexico. The Palenque strain was obtained from a ranch in the municipality of Palenque in the state of Chiapas, Mexico and was amitraz-resistant too. The Media Joya strain was a susceptible laboratory strain established at the National Institute of Forestry, Agriculture and Livestock (INIFAP) in Jiutepec, Morelos in 2001 from cattle infested with *R. microplus* ticks in the municipality of Tapalpa in the state of Jalisco, Mexico. The Media Joya strain was susceptible to all major classes of acaricides, therefore, was used as the susceptible reference strain to compare with the amitraz-resistant San Alfonso and Palenque strains in this study.

Host animals: A total of 30 Angus heifer calves approximately 6-9 months of age and approximately 250 kg were used in this study. The individually tagged calves had no prior exposure

to *R. microplus* and were randomly assigned treatment groups throughout the study. The heifers were individually stanchioned in a covered barn. Each heifer was used only once and removed from the stanchion after all female ticks reached repletion and were collected.

Parental strains: Three heifers were infested with 0.5 g (ca. 10,000 individuals) of 15 d old larvae from the Media Joya, San Alfonso or Palenque strains, respectively, by gluing the vials containing the larvae to the back of each animal. After 21 d, engorged females were collected in groups of 20 and placed in Petri dishes in separate incubators at 28±2°C, 90% RH and a photoperiod of 0:24 (L:D) h. After each group of females completed oviposition (20 d), the females were discarded and their egg masses were mixed and weighed. One gram of the egg mass from each strain was added to a vial and the resulting F_1 larvae were used for bioassays.

Acaricide: Formulated amitraz (Taktic, 12.5% EC) was used in this study provided by Intervet laboratory, Mexico.

Toxicity bioassay: The Soberanes technique was performed as previously described (Soberanes *et al.*, 2002). Briefly, formulated amitraz was diluted to specific concentrations with distilled water (dH_2O). Ten milliliters of each concentration was put into a 10-cm-diameter glass Petri dish containing a 9-cm-diameter piece of Whatman no. 1 filter paper (Whatman, Maidstone, United Kingdom). Approximately 400 larvae (14 day old) were placed onto the wet filter paper and a second piece of filter paper (9 cm in diameter) was placed on top. The larvae were held between these pieces of filter paper for 10 min and then they were removed in groups of approximately 100 into three untreated packets made of Whatman no. 1 filter paper (7.6 by 8.9 cm) folded in half and sealed on the sides with steel paper clips (Bulldog, Boston Clip No. 2, Hunt Manufacturing Co., Statesville, NC) to form a packet. After the larvae were introduced to the packet, a third clip was used to seal the top. The larvae were held in an environmental chamber at 27°C, 85-90% RH and a photoperiod of 12:12 (L: D) h. After 72 h, the packets were removed from the environmental chamber, opened and numbers of live and dead larvae were recorded. Each concentration of the treatment was replicated three times.

***Rhipicephalus microplus* tick populations and selection:** *Rhipicephalus microplus* F_0 generation adults were collected from San Alfonso, Palenque and Media Joya strains. Larvae were reared on isolated heifers. Subsequent generation adults were self-crossed throughout the study after selection. The selection concentrations used were based in the results of bioassay. From each generation and each strain, the LC_{50} was selected as the selection concentration. Fifteen d old larvae from each strain were treated by larval immersion with amitraz and the surviving larvae were reared on a heifer to generate the next generation. This process was repeated nine generations. A control population of susceptible *R. microplus* ticks Media Joya was maintained throughout the study. In Palenque resistant strain F_2 and F_3 was not challenged due to a schedule conflict. Because older larvae were used for selection at F_5, the challenging dose was reduced to 0.0004% amitraz, a dose which would kill 100% of larvae from the susceptible strain in San Alfonso strain.

Amitraz bioassays were performed to monitor the change of resistance to amitraz in all of the generations.

Data analysis: The concentration-mortality responses of all amitraz bioassays were analyzed using the POLO-PC program (Le Ora Software, 1987), with correction for control mortality

(Abbott, 1925). Mortality data of all three replicates of each concentration were included in the probit analysis. Resistance ratios (RR) were calculated by dividing the LC_{50} of the selected generations (F_1-F_{10}) of the San Alfonso and Palenque strains, with the LC_{50} of the reference Media Joya strain at F_0 non-selected generation. LC_{50} values were considered significantly different if their 95% confidence limits did not overlap.

Resistance heritability: Threshold trait analysis was used for the estimation of resistance heritability (h^2) of *R. microplus*, as described previously (Tabashnik, 1992; Tabashnik and McGaughey, 1994).

$$h^2 = R/S \quad (1)$$

where, R is the response of selection and S is the selection differential (Falconer, 1989; Hartl, 1988). The response of selection (R), the difference in mean phenotype between the offspring of the selected parents and the whole parental generation before selection (Falconer, 1989), is given by:

$$R = [\log (\text{final } LC_{50}) - \log (\text{initial } LC_{50})]/n = \log (\text{resistance ratio})/n \quad (2)$$

where, final LC_{50} is the LC_{50} of the offspring after 'n' generations of selection and initial LC_{50} is the LC_{50} of the parental generation before 'n' generations of selection. The difference between LC_{50}s was calculated on a logarithmic scale because the logarithm of tolerance was assumed to be normally distributed while the numerator of above equation for R estimates the cumulative responses to selection over n generations.

The selection differential (S), the difference in mean phenotype between the selected parents and the entire parental generation (Falconer, 1989; Hartl, 1988), was estimated as:

$$S = i\sigma_p$$

where, i is the intensity of selection and σ_p is the phenotypic standard deviation. The intensity of selection: (i) (Lu *et al*., 2004) was estimated as $i=1.583-0.0193336p + 0.0000428p^2 + 3.65194/p$ ($10<p<80$) p = average surviving×100.

The phenotypic standard deviation (σ_p) was estimated as the reciprocal of the mean of the estimated slopes of probit regression lines (Finney, 1971), from the parental selection before insecticidal selection (initial slope) and the offspring after n generations of selection (final slope) (Sethi *et al*., 2008).

$$\sigma_p = \left[1/2\,(\text{initial slope} + \text{final slope})\right]^{-1}$$

From Eq. 1, we can see that, assuming S is constant across acaricides; lower h^2 values will produce lower R values, which will produce slower resistance development. Because S is the product of i and σ_p, S is constant across insecticides for a particular percentage mortality only if the slope of the probit regression line (and thus σ_p) is constant across insecticides. In practice, however, this is often not the case. Thus, Tabashnik (1991) define the response quotient (Q) as $Q = R/i$. Assuming that the mean percentage mortality is constant across acaricides, the acaricide for which the population has the lower Q will have the slower resistance development.

Resistance risk assessment: The number of generations (G) required for a 10-fold increase in LC_{50}, is the reciprocal of R (Sethi *et al*., 2008):

$$G = R^{-1}$$

The number of independent genes with additive effects that contribute to the expression of a trait (such as acaricide resistance) was estimated from the mortality data obtained in successive generations selected with three acaricides as per Lande (1981):

$$n_E = \left[\sum_{i=1}^{n} \sigma_i^2\right]^2 / \sum_{i=1}^{n}\left(\sigma_i^2\right)^2$$

where, σ_i^2 is the genetic variance of the acaricide-selected strain at generation i, estimated as ($slope^{-1}$) and n is the number of generations.

Degree of dominance (of resistant component): This was estimated separately for the three selected strains with the following formula (Sethi *et al*., 2008):

$$D = \frac{(2Y_3 - Y_2 - Y_1)}{(Y_2 - Y_1)}$$

Where:
D = Dominance of the examined character (resistance)
Y_1 = Log_{10} of the LC_{50} from generation F_0 of the unselected control strain
Y_2 = Log_{10} of the LC_{50} from generation F_n (last) of the insecticide-selected strain
Y_3 = Log_{10} of the LC_{50} from generation F_1 of the insecticide-selected strain

This formula will result in-1 value if resistance is completely recessive, 0 if there is no dominance and 1 if resistance is completely dominant.

RESULTS

Response of *R. microplus* to amitraz: The LC_{50}s of the non-selected susceptible strain did not show significant change during the period of selection than the other strains (Table 1), where as selection of nine generations produced a 29-fold increase in resistance. Ticks did not show appreciable change in the resistance to amitraz during the first five generations (LC_{50} ranged from 0.00002-0.00007%). The resistance ratio during this period was 7 fold (Table 1). Maximum increase in LC_{50}s was seen between generations 6 (0.0020%) and 9 (0.00029%), 19.75-fold. The results did not show appreciable increase in the values of slope in the successive generations in spite of continued selection pressure. The slope values related to Media Joya susceptible selected strain, ranged from 1.206 at F_0 generation to 4.007, indicating considerable heterogeneity in the response of these ticks to amitraz, suggesting the development of different levels of resistance (Table 1).

The LC_{50} values of the amitraz-resistant Palenque population selected to amitraz also showed perceptible change during the course of ten generations. The LC_{50} values ranged between 0.00001-0.00014 and 0.0002-0.0006%, respectively in the F_1-F_5 and F_6-F_{10} generations of selection

Table 1: Resistance increase in Media Joya susceptible strain of *Rhipicephalus microplus* ticks selected with amitraz

Media joya strain	n	Slope (SE)	CL_{50} (CI 95%)	RR*	Challenge doses (% AI)	Surviving proportion
Original	1206	1.423 (0.091)	0.00001 (0.00-0.00001)	1.0	0	0.24
Selected						
F_1	2380	2.446 (0.092)	0.00002 (0.00002-0.00003)	2.0	0.0000125	0.61
F_2	3960	1.780 (0.069)	0.00007 (0.00005-0.00009)	7.0	0.00002	0.62
F_3	2124	1.713 (0.107)	0.00001 (0.00001-0.00002)	1.0	0.00003	0.10
F_4	4007	1.542 (0.044)	0.00003 (0.00001-0.00011)	3.0	0.0000125	0.60
F_5	3710	0.752 (0.023)	0.00005 (0.00003-0.00009)	5.0	0.00008	0.56
F_6	2779	2.250 (0.071)	0.00020 (0.00012-0.00036)	20.0	0.0002	0.62
F_7	3514	1.22 (0.033)	0.00011 (0.00007-0.00018)	11.0	0.0003	0.68
F_8	2938	1.516 (0.047)	0.00019 (0.00014-0.00026)	19.0	0.0003	0.68
F_9	1517	1.461 (0.060)	0.00029 (0.00019-0.00043)	29.0	0.0002	0.55
F_{10}	2850	2.368 (0.075)	0.00010 (0.00007-0.00015)	10.0	0.0002	0.40
Mean		1,704		10.7		0.54

* RR: Resistance ratios were calculated by dividing the LC_{50} of the Media Joya susceptible strain selected generations (F_1-F_{10}), with the LC_{50} of the reference Media Joya strain at F_0 nonselected generation

Table 2: Resistance increase in Palenque resistant strain of *Rhipicephalus microplus* ticks selected with amitraz

Palenque strain	n	Slope (SE)	CL_{50} (CI 95%)	RR*	Challenge doses (% AI)	Surviving proportion
Original	1508	2.995 (0.131)	0.00004 (0.00002-0.00012)	4.0	0	0.52
Selected						
F_1	1609	1.535 (0.066)	0.00002 (0.00002-0.00003)	2.0	0.00005	0.43
F_2	3910	1.759 (0.057)	0.00013 (0.00011-0.00016)	13.0	0.00003	0.65
F_3	3774	1.830 (0.063)	0.00001 (0.00001-0.00001)	1.0	§	0.3
F_4	3391	1.309 (0.060)	0.00001 (0.00000-0.00001)	1.0	§	0.31
F_5	4071	1.870 (0.058)	0.00014 (0.00010-0.00021)	14.0	0.00010	0.67
F_6	3216	1.604 (0.045)	0.0002 (0.00016-0.00025)	20.0	0.00020	0.5
F_7	3586	0.600 (0.022)	0.00063 (0.00029-0.00194)	63.0	0.00030	0.74
F_8	2997	0.748 (0.030)	0.00038 (0.00021-0.00076)	38.0	0.00040	0.66
F_9	2002	2.104 (0.073)	0.00045 (0.00039-0.00052)	45.0	0.00060	0.85
F_{10}	2079	1.518 (0.054)	0.00060 (0.00042-0.00085)	60.0	0.00060	0.47
Mean		1.304		25.7	0	0.56

§Not challenged. * RR: Resistance ratios were calculated by dividing the LC_{50} Palenque strain, with the LC_{50} of the reference Media Joya strain at F_0 nonselected generation

(Table 2). With the development of resistance over the generations, this strain showed little heterogeneity in the response, slope values when selected with amitraz range 0.60-1.87.

The LC_{50} values of the amitraz-resistant San Alfonso population selected to amitraz also showed a perceptible change during the course of eight generations. The LC_{50} values of selected generations ranged between 0.000200-0.00107% (Table 3), compared with the original F_0 LC_{50} (0.00009%). The relaxation of selection pressure in the F_5 led to a decrease of the resistance ratio in F_6. Although, the selection pressure was equal to or above the original amitraz concentration (0.0002%) after F_6, the resistance ratio declined and then stabilized in F_8. The high slope value point to the establishment of highly homozygous population is F_8 with regard to the resistance trait.

Resistance risk assessment: Estimate of realized heritability (h^2) is that proportion of phenotypic variation accounted for by additive genetic variation. Estimated h^2 to amitraz was 0.26, 0.31 and 0.77 for susceptible Media Joya, Palenque and San Alfonso amitraz-resistant strains, respectively

Table 3: Resistance increase in San Alfonso resistant strain of *Rhipicephalus microplus* ticks selected with amitraz

San alfonso strain	n	Slope (SE)	CL50 (CI 95%)	RR*	Challenge doses (%AI)	Surviving proportion
Original	2032	1.20 (0.05)	0.00009 (0.00006-0.00012)	10	0	0.37
Selected						
F1	3693	1.90 (0.06)	0.00031 (0.00025-0.00039)	31	0.0002	0.68
F2	3577	1.86 (0.06)	0.00020 (0.00017-0.00023)	20	0.0004	0.58
F3	4009	1.22 (0.04)	0.00063 (0.00049-0.00082)	63	0.0006	0.69
F4	4378	1.23 (0.04)	0.00094 (0.00048-0.00242)	94	0.0008	0.78
F5	4194	1.36 (0.04)	0.00061 (0.00046-0.00085)	61	0.0004	0.68
F6	3008	1.38 (0.04)	0.00034 (0.00024-0.00047)	34	0.0008	0.59
F7	3173	1.07 (0.03)	0.00030 (0.00020-0.00043)	30	0.0006	0.60
F8	3759	2.34 (0.08)	0.00107 (0.00090-0.00127)	107	0.0008	0.67
Mean		1.53		55		0.66

RR: Resistance ratios were calculated by dividing the LC_{50} San Alfonso strain, with the LC_{50} of the reference Media Joya strain at F_0 nonselected generation

Table 4: Estimation of realized h^2 and number of genes contributing to amitraz resistance in the three strains of *R. microplus*[a]

		Estimate of mean response					Estimate of mean selection differential				Estimate of heritability and No. of genes			
Strains	No. of generations selected (n)	Initial †LC_{50} (log)	Final LC_{50} (log)	R	†p	i	Initial slope	Final slope	σ_p	S	h^2	Q	G	n_E
*S	F_0-F_{10}	-5.0	-4.00	0.100	54	0.74	1.42	2.37	0.53	0.39	0.26	0.13	10	6.31
§P	F_0-F_{10}	-3.22	-4.39	0.12	56	0.70	2.99	1.52	0.54	0.38	0.31	0.17	8.5	5.93
#SA	F_0-F_8	-4.04	-3.00	0.24	66	0.55	1.20	2.34	0.56	0.31	0.77	0.43	4.1	6.69

*S is the susceptible strain Media Joya; §P is the Palenque resistant strain; #SA is the San Alfonso resistant strain; n is the selection generation; R is the response of selection R = [log (final LC_{50}) - log (initial LC_{50})]/n; p is (average surviving proportion)×100 = average surviving percentage; i is the intensity of selection; $i = 1.583-0.0193336p + 0.0000428p^2 + 3.65194/p$ ($10<p<80$); σ_p is the phenotypic standard deviation; $\sigma_p = [1/2 \text{ (initial slope + final slope)}]^{-1}$; S is the selection differential; $S = i\sigma_p$; Q is the response quotient; Q = R/i; G are the number of generations required for a 10-fold increase in LC_{50} $G = R^{-1}$; n_E the number of independent genes with additive effects that contributing to the expression of resistance: $n_E = \left[\sum_{i=1}^{n} \sigma_i^2\right]^2 / \sum_{i=1}^{n}\left(\sigma_i^2\right)^2$; †$LC_{50}$ and surviving proportion values from tables 1-3

(Table 4). This reflects that high levels of resistance to amitraz can only be realized after long periods of selections at the LC_{50} (eight-ten generations) in the field *R. microplus* tick populations.

Projected rates of resistance development: The number of generations (G) required for 10-fold increase in LC_{50} at the LC_{50} selection rate was estimated to be 10, 8.5 and 4.1 for susceptible Media Joya, Palenque and San Alfonso amitraz-resistant strains, respectively (Table 4).

Estimation of the number of genes involved in insecticide resistance: The number of genes involved in insecticide resistance against amitraz with the use of Lande's (1981) formula for effective number of factors (genes) segregating across eight to ten generations was estimated to be 6.31,5.93 and 6.69 for the insecticide resistance in susceptible Media Joya, Palenque and San Alfonso amitraz-resistant strains, respectively.

Estimation of degree of dominance for the resistant characteristic: The values of dominance for San Alfonso susceptible strain, Palenque resistant strain and San Alfonso resistant

Table 5: Resistant component degree of dominance estimation in three *R. microplus* strains

Strains	Y_1‡	Y_2	Y_3	$D = (2Y_3-Y_2-Y_1)/(Y_2-Y_1)$
*S	Log (0.00001) = -5	Log (0.0001) = -4	Log (0.00002) = -4.699	-0.397
§P	Log (0.00004) = -4.4	Log (0.0006) = -3.22	Log (0.00002) = -3.88	-0.140
*SA	Log (0.00009) = -4.04	Log (0.00107) = -2.97	Log (0.00031) = -3.5	-0.028

*S is the susceptible strain Media Joya; §P is the Palenque resistant strain; *SA is the San Alfonso resistant strain; ‡ Y1, Y2, Y3 obtained from Tables 1-3. $Y_1 = Log_{10}$ of the LC_{50} from generation F_0 of the unselected control strain. $Y_2 = Log_{10}$ of the LC_{50} from generation F_n (last) of the acaricide-selected strain. $Y_3 = Log_{10}$ of the LC_{50} from generation F_1 of the acaricide-selected strain

strain were-0.397,-0.14 and-0.028 respectively, indicating a nearly completely recessive resistance to amitraz (Table 5).

DISCUSSION

The increase in resistance in all the three selected strains indicates that a part of the variation in amitraz resistance in *R. microplus* is additive (Falconer, 1989). One of the important uses of heritability estimates is the prediction of future response (Falconer, 1989; Hartl, 1988). The purpose of pesticide resistance studies is to predict the rate of development of resistance in response to pesticide application (Via, 1986; Firko and Hayes, 1990). Although, laboratory experiments cannot completely reflect real field conditions, the estimated h^2 values still could provide evidence for the potential for resistance development. The estimated h^2 values from selection experiments may be higher than those in the field, because of lower environmental variation in the laboratory. Ru *et al.* (1997) assumed that h^2 was half of the experimental value when they predicted the resistance risk of field *H. armigera* to pyrethroids. They obtained satisfactory predicted results. Heritability estimate after one generation of selection is often a reliable approximation of the heritability of the trait in the parental population because the laboratory environment has minimal effects (Bloch and Wool, 1994). As the mean response for the first three generations was high in case of San Alfonso-resistant strain, the risk for development of resistance was higher as compared to Media Joya susceptible strain and Palenque resistant strain. Ten generations were required to develop a 10-fold increase in resistance in case of the Media Joya susceptible strain and almost nine generations were required for the Palenque resistant strain *versus* only four generations in the case of San Alfonso strain. The different number of generations required to develop 10-fold increase in LC_{50} among strains,

Calculations based on Lande (1981) formulas suggested that acaricide resistance against amitraz is controlled by more than one gene. This can further be substantiated by the significant increase in resistance ratios in response to the selection over many generations. Apparently, combinations of alleles responsible for tolerance not present in parental generations were produced in the succeeding generations (under continuous selection pressure) as a result of inbreeding among resistant individuals as suggested by Bloch and Wool (1994). Roush and McKenzie (1987) noted that most significant cases of resistance are caused by allelic variants at one or two loci. They reasoned that polygenic resistance is favored by laboratory regimes that select at moderate doses from small samples where as field applications that select at high doses from large populations favors monogene resistance by rare alleles. The results finding in this study are supported by Tapia-Perez *et al.* (2003) who reported more than one gene acting in *R. microplus* pyrethroids resistance using a reciprocal crossing experiments method to evaluate the mode of resistance

inheritance in this tick, also Li *et al.* (2005) reported with the same method that more than one gene is involved in amitraz resistance in the Santa Luiza strain of *R. microplus*, this was confirmed by Cossio-Bayugar *et al.* (2008) who concluded a multifactorial causative for organophosphate and pyrethroid resistant *R. microplus*. Although, a putative octopamine receptor cDNA was reported (Chen *et al.*, 2007) the real mechanism of resistance to amitraz is not know yet.

Similar to our RR values were found by Li *et al.* (2005) in a similar experiment with Santa Luiza Brazilian strain, but they had higher values for the slopes, maybe because they use more elevated doses of challenge than ours. They not calculated the genetic estimators of heritability.

Rosado-Aguilar *et al.* (2008) study of amitraz resistance in *R. microplus* populations from three cattle-producing farms of Mexico demonstrated RR values 1-23 amitraz resistance. A study of amitraz resistance in *B. microplus* populations from several major cattle-producing areas of Mexico demonstrated low-order (RR =1.68-4.58) amitraz resistance in 11 of the 15 Mexican strains surveyed (Li *et al.*, 2005). Using the modified Shaw's larval immersion test, Soberanes *et al.* (2002) reported an RR of 41.9 to amitraz in the San Alfonso strain of *B. microplus* in Mexico. Because different bioassay techniques were used in the two studies, our results only be directly comparable with Soberanes *et al.* (2002), they used the discriminant dose of amitraz (0.0002%), at same dose, in this study, for the San Alfonso strain RR was 31 (25-39 IC 95%), RR baseline here was 10, average RR of the three strains were 10.7, 27.9 and 55 (Media Joya susceptible, Palenque resistant and San Alfonso resistant strains respectively).

Li *et al.* (2005) found similar values of degree of dominance (-0.156 and-0.500) in a Brazilian strain of *R. microplus* to ours (-0.028,-0.140 and-0.397), equivalent recessive values has reported by Tapia-Perez *et al.* (2003) in pyethroid resistant *R. microplus* Aldama strain, Sethi *et al.* (2008) reported similar values in *Bermisia tabaci* resistant to three insecticides.

Present study may contribute to a thorough understanding of the mechanisms that confer resistance in *R. microplus* to amitraz in order to elucidate the dynamics of resistance in natural populations from Mexico. Information about the genetic basis of resistance can facilitate efforts to detect and monitor resistance, to assess the risk of resistance, to model the evolution of resistance and to delay resistance development in pests.

In conclusion, although extremely high levels of resistance were attained by these laboratory-selected populations, these differences may not necessarily translate to the reduction or loss of field performance of this acaricide, caution must be used when directly extrapolating results from laboratory experiments to the field situations (Denholm *et al.*, 1984). Populations found in the field are usually more heterogeneous and their response to acaricide pressure is more complex. Field responses result from the interactions of environment, population structure and selection intensity (Roush and McKenzie, 1987). The degree of dominance profoundly affects the strategies for managing insecticide resistance, because when resistance is dominant high doses of a bad management in the field may accelerates the development of resistance, but if resistance is recessive that doses can slow resistance in presence of susceptible pests (Ives and Andow, 2002).

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