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Spatial distribution of acaricide profiles (*Boophilus microplus* strains susceptible or resistant to acaricides) in southeastern Mexico

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Abstract

The ability of *Boophilus microplus* strains to be susceptible (−) or resistant (+) to amidines (Am), synthetic pyrethroids (SP), and/or organo-phosphates (OP) (or acaricide profiles) was investigated in 217 southeastern Mexican cattle ranches (located in the states of Yucatán, Quintana Roo, and Tabasco). Three questions were asked: (1) whether acaricide profiles varied at random and, if not, which one(s) explained more (or less) cases than expected, (2) whether the spatial distribution of acaricide profiles was randomly or non-randomly distributed, and (3) whether acaricide profiles were associated with farm-related covariates (frequency of annual treatments, herd size, and farm size). Three acaricide profiles explained 73.6% of the data, representing at least twice as many cases as expected ($P < 0.001$): (1) Am−SP–, (2) Am+SP+, and (3) (among ranches that dispensed acaricides ≥6 times/year) Am−OP+SP+. Because ticks collected in Yucatán ranches tended to be susceptible to Am, those of Quintana Roo ranches displayed, predominantly, resistance to OP/SP, and Tabasco ticks tended to be resistant to Am (all with $P \leq 0.05$), acaricide profiles appeared to be non-randomly disseminated over space. Across states, two farm-related covariates were associated with resistance ($P \leq 0.02$): (1) high annual frequency of acaricide treatments, and (2) large farm size. Findings supported the hypothesis that spatial acaricide profiles followed neither random nor homogeneous data distributions, being partially explained by agent- and/or farm-specific factors. Some profiles could not be explained by these factors. Further spatially explicit studies (addressing host-related factors) are recommended.

Keywords: *Boophilus microplus*; Acaricide resistance; Mexico; Spatial analysis

1. Introduction

The dichotomy related to the expression of infectious disease versus lack of disease has classically been attributed to interactions that include a triad: the host, the agent, and the environment. In parasitology, this paradigm translates as the ability of the agent to be susceptible or resistant to acaricides, outcome that may

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be explained by factors pertaining to the agent, the animal, the ranch, or combinations involving two or more of these factors.

These considerations apply to parasitic diseases, such as the cattle infestation caused by the Southern cattle tick *Boophilus microplus*. This is a widely distributed, one-host parasite. The tick is responsible for severe economic losses caused by blood loss, injection of toxins and tick-borne diseases (Solorio et al., 1999). While, in other diseases, cost-benefit analysis of treatments performed against ectoparasites that develop resistance against therapies have been conducted (Kolaczinski and Hanson, 2006), no such study has been reported in relation to *B. microplus*. In southeastern Mexico, acaricide resistance is a major problem for the cattle industry (Rodríguez-Vivas et al., 2005).

Acaricides, including amidines (Am), organophosphates (OP), and synthetic pyrethroids (SP) play a major role in controlling *Boophilus* ticks in Mexico (Taylor, 2001; Aguilar-Tipacamu and Rodríguez-Vivas, 2003). The intensive use of these chemicals has led to the development of resistant tick populations (Fragoso et al., 1995). Resistance is defined as the ability of a tick strain to tolerate doses of a toxicant that, in a typical population of the same species, would prove lethal to most individuals (Kunz and Kemp, 1994). The farm prevalence of *B. microplus* resistance to Am, OP, and SP varies from 19% to 95% (Rodríguez-Vivas et al., 2005; Rodríguez-Vivas et al., 2006a,b).

The assessment of acaricides profiles (*B. microplus* strains susceptible or resistant to acaricides) is a major strategy used in prevention campaigns against this tick (Mekonnen et al., 2002; White et al., 2004). While the use of geographical information systems (GIS) has expanded the array of tools available to investigate the spatial distribution of *B. microplus* (Estrada-Peña et al., 2006; Estrada-Peña and Venzal, 2006) and/or interactions between climate change and economic losses attributed to this ixodida (White et al., 2003), spatial analysis of acaricide profiles have not yet been conducted. Such studies, if conducted at spatial point-basis (e.g., using individual ranches as units of study) could uncover profiles not detected in studies that do not assess space or focus on larger spatial units, such as municipalities, states or countries. Spatially local assessments have facilitated, in other diseases, case-specific (local or regionalized) decision-making (Shirley et al., 2003; Ducrot et al., 2005; Rivas et al., 2006).

The simultaneous assessment of multiple acaricides within a given acaricide profile and location helps to elucidate whether resistance to one acaricide may be compensated by susceptibility induced by another acaricide, and whether that outcome is a spatially independent event (whether the profile observed in one ranch is independent of the profile observed in another ranch), or whether they are spatially associated. Both pieces of information may be valuable for policy-making.

Evaluations of acaricide profiles in relation to space involve the simultaneous assessment of multiple dimensions. That is so because each acaricide being evaluated may result in two outcomes, which may vary throughout space. When two acaricides are compared (e.g., A and B) and each can be described by susceptible (−) or resistant (+) tick sub-populations, four outcomes may be observed (A−B−, A−B+, A+B−, and A+B+). If a third acaricide is included in the assessment, the number of possible profiles to be observed increases to eight (e.g., A+B+C+, A−B+C+, A−B−C+, A+B−C+, A−B−C−, ...). If, in addition to acaricides, other covariates are investigated (for instance, the annual frequency of acaricide treatments), even if each covariate is investigated in terms of only two ranks (e.g., ''low'' and ''high''), the total number of composite profiles would be multiplied by two with each added covariate (e.g., A+B+C-low treatment frequency, A+B+C+ high treatment frequency, A−B+C+ low treatment frequency, A−B+C+ high treatment frequency, ...). Such complexity is further increased when space is investigated: each composite profile may differ in magnitude (the number of ranches) and/or space (regions where it is observed). Based on the expectation that uncovering the inherent complexity of the spatial distribution of acaricide profiles may open a new opportunity toward ranch- (or region-) and acaricide profile-specific decision-making, this study was set: (1) to explore whether all acaricide profiles explained a similar percentage of cases and, if not, to identify those explaining more cases than expected, (2) to assess the spatial location of each observed acaricide profile, and (3) to investigate whether acaricide profiles were associated with farm-specific covariates (frequency of annual acaricide treatments, farm size, and herd size).

2. Materials and methods

2.1. Study design

Research questions were addressed through (a) an *in vitro* survival test of *B. microplus* larvae exposed to (Am, OP, SP) acaricides, and (b) a questionnaire that assessed the magnitude (low/high, small/large) of three farm-specific covariates (treatment frequency, herd size, farm size).

2.2. Study area

B. microplus ticks were sampled in southeastern Mexico (the states of Yucatán, Quintana Roo and Tabasco), where the climate varies from sub-humid to humid tropical. In this region the mean relative humidity is 80%, 83%, and 90%, and the annual rainfall (mm) is 950, 105, and 1400, in Yucatán, Quintana Roo, and Tabasco, respectively (INEGI, 2002). The predominant livestock-production system in the three investigated states is semi-intensive (beef farms), mainly based on a year-round grazing on improved pastures (e.g., Guinea grass (*Panicum maximum*) and Star grass (*Cynodon plectostachyus*)), with supplementary feeding during the dry season. Most farms in this region use acaricides to control ticks (Rodríguez-Vivas et al., 2005).

2.3. Study population

The sample size in each state was calculated, considering (with a confidence level of 95% and an error of 10%) an expected prevalence of 50% in Yucatán, 70% in Quintana Roo, and 75% in Tabasco (Rodríguez-Vivas et al., 2005). From a list provided by the Cattlemen's Association of each state, 96 (Yucatán), 66 (Quintana Roo) and 55 (Tabasco) ranches were randomly selected. Logistical difficulties to sample 21 of those ranches led to their replacement by 21 other ranches, also randomly selected. Each ranch was visited 1–2 times to collect *B. microplus* adult engorged females.

2.4. In vitro studies

From each ranch, 20–30 *B. microplus* engorged females were collected from at least 10 bovines. They were transported to, and analyzed at, the Parasitology Laboratory of the Yucatán State College of Veterinary Sciences at the Yucatan State Autonomous University. Upon arrival, engorged females were placed into Petri dishes and incubated in the dark at 27 ± 1.5 °C, with 85–86% relative humidity (Cen et al., 1998). To allow larval eclosion, eggs laid were transferred into two 10 ml glass tubes with a cotton cap. Seven to 14-day-old larvae were analyzed as described elsewhere (Kemp et al., 1998). The modified larval packet test (Stone and Haydock, 1962) was used to test OP and SP resistance. The modified larval immersion test was used for Am resistance (FAO, 1984). For both tests, a discriminat dose (DD) of technical grade acaricide (SP and OP), or emulsifiable concentrate formulation (amitraz, Taktic® 12.5%, Intervet, Mexico) were used. The DD was calculated by doubling the mean lethal dose 99.9% (LD 99.9%) derived from the series of tests conducted with a susceptible strain (Kemp et al., 1998). Two replicates of the acaricide and a control were used. Larvae treated with Am were exposed for 72 h, while those treated with either SP or OP were exposed for 24 h. After exposure, the numbers of live and dead larvae were counted to calculate the percentage of larval mortality. If one or more larva(e) was(were) found alive, the strain was considered to be resistant. The DD used for Am, OP, and SP compounds was: 0.2% (coumaphos, chlorfenvinphos), 0.08% (diazinon, Aguirre and Aburto, 1983), 0.01% (flumethrin), 0.009% (deltamethrin), 0.05% (cypermethrin, Santamaría, 1992), or 0.0002% (amitraz, Soberanes et al., 2002). A positive case of an acaricide family was considered when a ranch was positive to any member of the family compound (Am, OP, or SP).

2.5. Farm survey

A questionnaire on farm descriptors (farm size [small or <50 ha/large or ≥50 ha], herd size [small or <50 animals/large or ≥50 animals], and annual frequency of acaricide treatments [low or <6/high or ≥6]) was submitted to all farmers or managers.

2.6. Creation of a geo-referenced database

Spatial data (latitude and longitude of each farm, the *in vitro* results to exposure to each acaricide tested, and the associated farm-specific covariates [treatment frequency, farm size and herd size]) were geo-referenced using *Arc View GIS* 3.3 and *Arc View* 8.0 (ESRI, Redlands, California, USA). The *query* command of the GIS package was used to quantify the number of cases explained by each acaricide profile. The same command was also used to create *shapefiles* that included individual ranches that shared farm covariate categories (e.g., those where acaricides were dispensed ≥6 times/year).

2.7. Statistical analysis

Results were expressed as categorical data, where susceptible, low frequency, small farm size and small herd size were denoted as 0; resistance, high frequency, large farm size and large herd size were expressed as 1. The proportion of cases explained by each acaricide profile (across states and on state basis), was assessed by the χ^2 test. The median percents of cases explained by individual acaricide profile were evaluated with the Mann–Whitney test. Statistical tests were conducted using a commercial package (*Minitab 14.1*, Minitab, State College, PA, USA). For all tests, values of P values <0.05 were considered significant.

3. Results

The location of the 217 investigated ranches is indicated in Fig. 1A. Across states, the median percent of ranches with ticks susceptible to amidines (Am) was

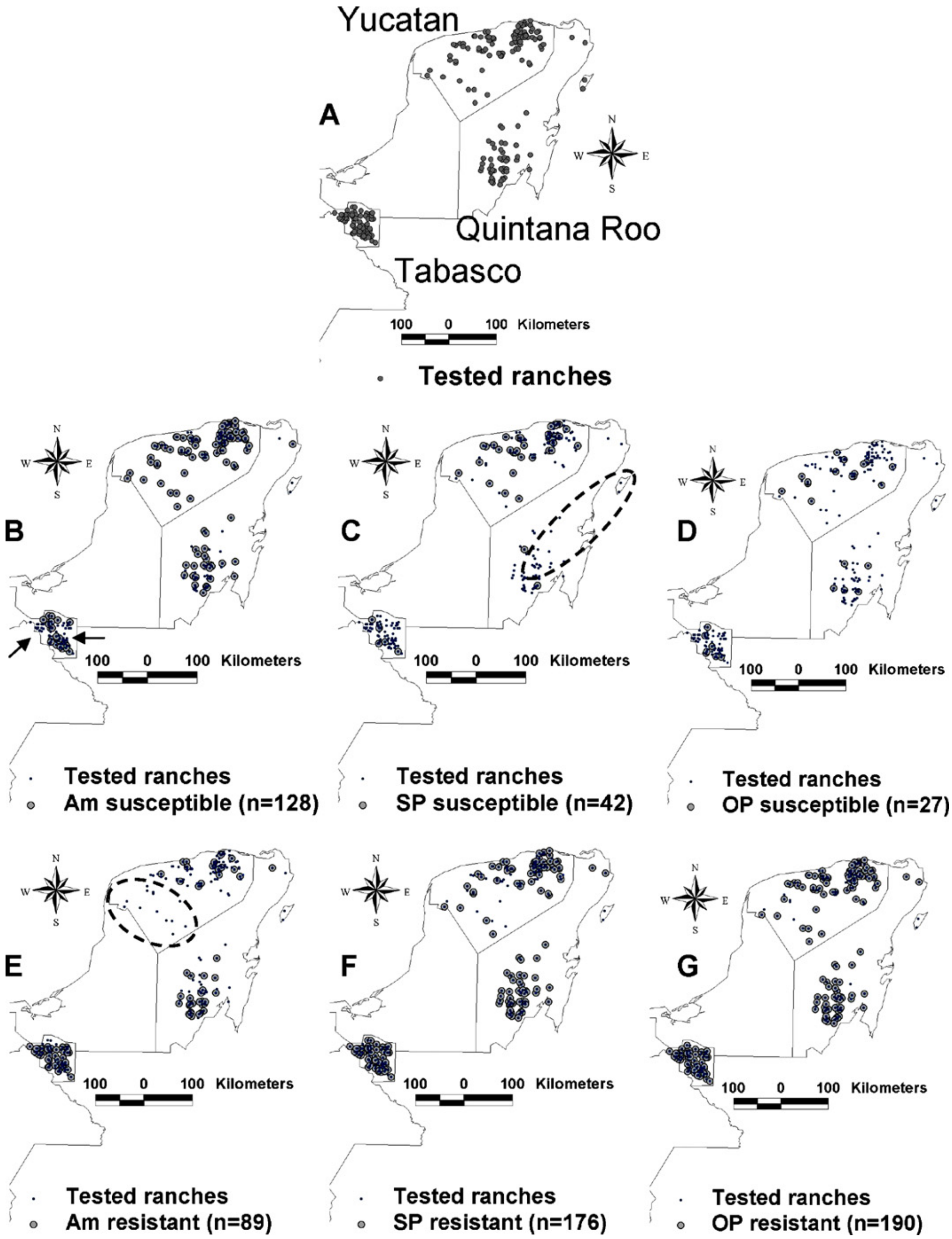

Fig. 1. Location of ranches and single acaricide profiles. (A) Ranches tested in Southeastern Mexico ($n = 217$). Ranches with ticks susceptible to: Am (B), SP (C), and OP (D). Ranches with ticks resistant to: Am (E), SP (F), and OP (G). Ovals and arrows indicate a region/state that differs significantly in relation to the remaining states/region (e.g., the proportion of ticks susceptible to SP is lower in Quintana Roo ranches, Fig. 2C).

Table 1
Single or double (similar) acaricide profiles

	All ranches, $n = 217$ (%)	Yucatán, $n = 96$ (%)	Quintana Roo, $n = 55$ (%)	Tabasco, $n = 66$ (%)
(A) Ranches with ticks susceptible to[a]				
Am	128 (59.0)	79 (82.3)	28 (50.9)	21 (31.8)
SP	42 (19.3)	34 (35.4)	3 (5.5)	5 (7.6)
OP	27 (12.4)	15 (15.6)	5 (9.1)	7 (10.6)
Am and SP	31 (14.3)	27 (28.1)	1 (1.8)	3 (4.5)
Am and OP	19 (8.7)	11 (11.5)	3 (5.5)	5 (7.6)
OP and SP	6 (2.8)	4 (4.2)	1 (1.8)	1 (1.5)
(B) Ranches with ticks resistant to[a]				
Am	89 (41.0)	17 (17.7)	27 (49.1)	45 (68.2)
SP	176 (81.1)	62 (64.6)	53 (96.4)	61 (92.4)
OP	190 (87.6)	81 (84.4)	50 (90.1)	59 (89.4)
Am and SP	79 (36.4)	10 (10.4)	26 (47.3)	43 (65.2)
Am and OP	82 (37.8)	13 (13.5)	27 (49.0)	42 (63.6)
OP and SP	155 (71.4)	51 (53.1)	49 (89.1)	55 (83.3)

[a] Yucatán ranches showed a significant higher proportion of ticks susceptible to Am, SP, and Am and SP; Quintana Roo ranches showed a significant lower proportion of ticks susceptible to SP, Am and SP, OP, and Am and OP; Tabasco ranches showed a significant lower proportion of ticks susceptible to Am; Yucatán ranches showed a significant lower proportion of ticks resistant to Am, SP, Am and OP, OP and SP, and Am and SP, than the remaining ranches (all with $P < 0.05$).

at least three times greater (59.0%) than that of ranches susceptible to organo-phosphates (OP) or synthetic pyrethroids (SP) (12.4 and 19.3%, respectively, $P < 0.001$, Mann–Whitney test, Table 1A, Fig. 1B–D). While ranches with susceptible ticks predominated in Yucatán (where a cluster of farms with ticks susceptible to Am was noticed in its southwestern sub-region), ranches with ticks resistant to acaricides prevailed in Quintana Roo and Tabasco (Fig. 1B–G).

Ranches with ticks susceptible to Am tended to have ticks susceptible to other acaricides as well: 31 of the 42 ranches with ticks susceptible to SP also displayed *in vitro* tick susceptibility to Am (Table 1A, Figs. 1C and 2A), while 19 of the 27 ranches with tick susceptibility to OP showed tick susceptibility to Am (Table 1A, Figs. 1D and 2B). In contrast, ranches with ticks susceptible to SP did not reveal, on average, tick susceptibility to OP: only six ranches (2.8%) showed ticks susceptible to both acaricides (Fig. 2C). In four of six inter-state comparisons, Quintana Roo ranches displayed the lowest tick susceptibility. In contrast, in six comparisons involving single or double acaricide treatments, Yucatán state showed the highest percentage of ranches with ticks susceptible to acaricides (Table 1A).

While the number of ranches with ticks resistant to individual acaricides was the mirror image of those with ticks susceptible to a single chemical, the resistance profiles displayed against two acaricides differed markedly from its counterpart. For instance, only 6 of 217 (2.8%) ranches showed ticks susceptible to both OP and SP, while 155 ranches (71.4%) displayed tick resistance to both acaricides (Table 1B).

Across states, an association between resistance to both OP and SP was suggested by the data: 88% of all ranches with tick resistance to SP (155 of 176 ranches) also showed tick resistance to OP (Fig. 2D, Table 1B). In contrast, the remaining double resistance profiles (Am+SP+ and Am+OP+) represented about half of the OP+SP+ resistance (Fig. 2E and F). Across states, double resistance including Am was approximately half of that against OP/SP ($\leq$37.8% versus $\geq$71.4%, respectively, $P < 0.001$, Mann–Whitney test, Table 1B).

Among the six double opposite profiles, the larger profile included ranches with ticks susceptible to Am: Am−OP+ and Am−SP+ profiles were observed in 109 and 97 ranches, respectively (Fig. 3A and B, Table 2A). In contrast, the remaining four double opposite profiles explained $\leq$36 of all cases (Fig. 3C–F).

When triple acaricide profiles were considered, triple resistance (74 ranches) and Am−OP+SP+ ($n = 81$) explained 155 cases (71.4% of all ranches), while the remaining 6 triple acaricide profiles were observed in $\leq$28 ranches (Table 2B). Across states, tick resistance to Am and OP (82 ranches, Fig. 2F) tended to be associated with triple resistance ($n = 74$ ranches, Table 2B).

While ticks collected in southwestern Yucatán ranches showed no (or marginal) double/triple resistance, those from Quintana Roo and Tabasco ranches displayed double and triple resistance (Fig. 2D–F and Table 2B). While the highest resistance involving Am

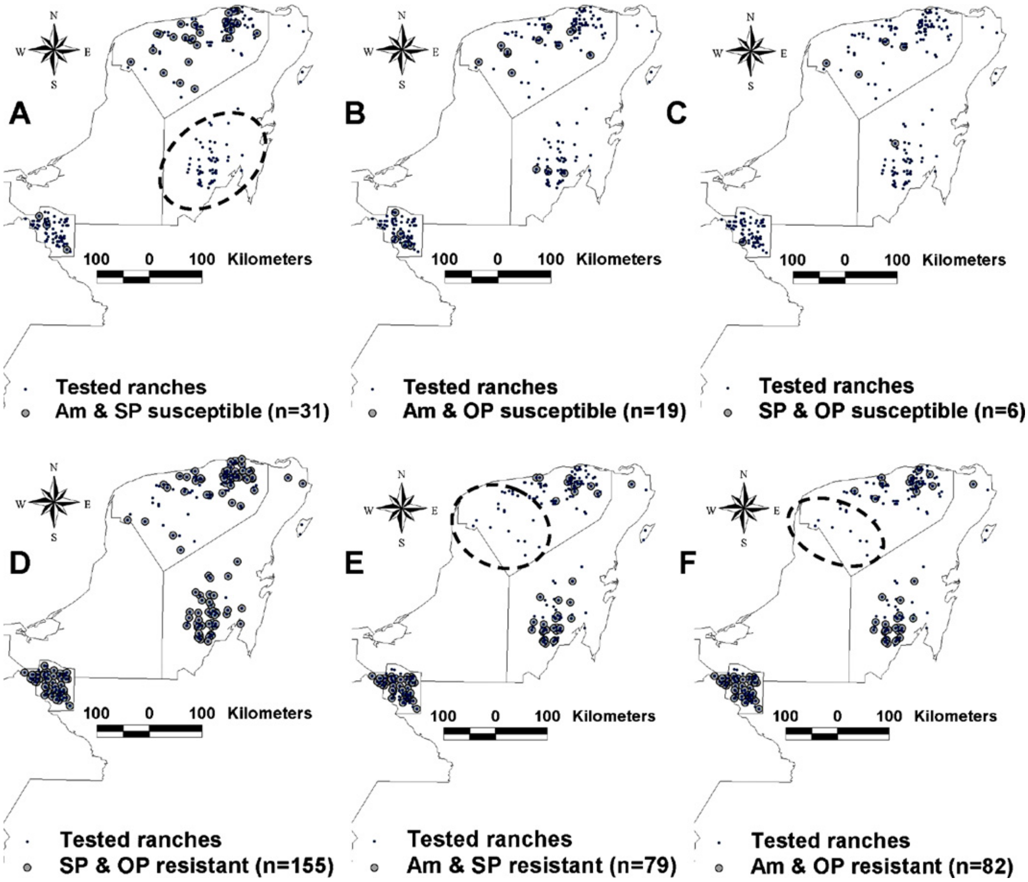

Fig. 2. Double (similar) acaricide profiles. Ranches with ticks susceptible to Am and SP (A), to Am and OP (B), and to SP and OP (C). Ranches with ticks resistant to OP and SP (D), to Am and SP (E), and to Am and OP (F). Ovals indicate a state or region differing in the reported profile from the remaining states/regions.

was shown in the state of Tabasco (where at least 68.2% of the tested ranches showed ticks with single, double, or triple resistance, $P < 0.01$), the highest (single or double) resistance to OP and SP was seen in Quintana Roo ranches ($P < 0.001$, Tables 1B and 2B). Ticks found in Tabasco ranches displayed greater (double and triple) resistance to any acaricide combination that included Am than those collected in other states (four of the six drug combinations, Table 1B). In contrast, Yucatán ticks revealed lower resistance to all treatments. Even when they displayed resistance to one acaricide, ticks from Yucatán ranches were more susceptible to another acaricide than those of other states, as shown in Am− and OP+ (70.8% in Yucatán

Table 2A
Double (opposite) acaricide profiles

Ranches with ticks[a]		All states, $n = 217$ (%)	Yucatán, $n = 96$ (%)	Quintana Roo. $n = 55$ (%)	Tabasco $n = 66$ (%)
Resistant to	Susceptible to				
OP	Am	109 (50.2)	68 (70.8)	25 (45.5)	16 (24.2)
SP	Am	97 (44.7)	51 (53.1)	28 (50.9)	18 (27.3)
OP	SP	36 (16.6)	31 (32.3)	1 (1.8)	4 (6.1)
SP	OP	21 (9.7)	11 (11.5)	6 (10.9)	4 (6.1)
Am	OP	8 (3.7)	4 (4.2)	2 (3.6)	2 (3.0)
Am	SP	11 (5.1)	7 (7.3)	2 (3.6)	2 (3.0)

[a] Yucatán ranches showed a higher proportion of ticks susceptible to Am (whether resistant to OP or SP) and of ticks resistant to OP but susceptible to SP than non-Yucatán ranches ($P < 0.05$).

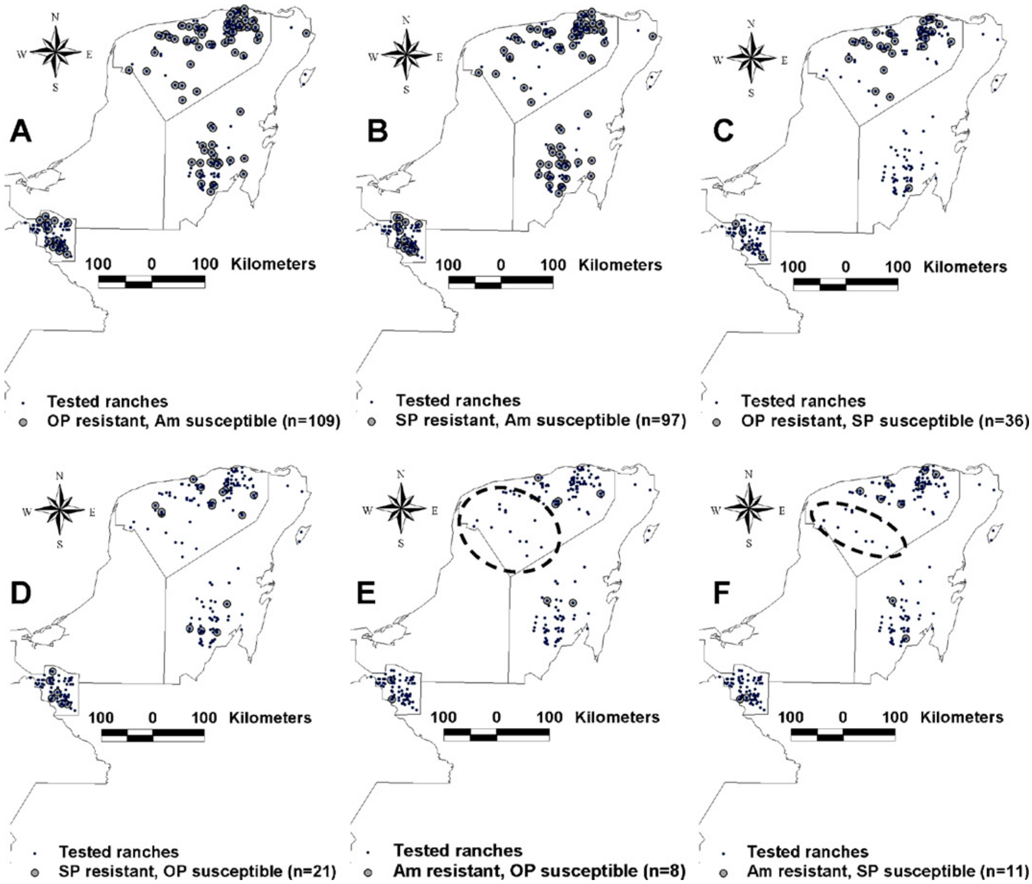

Fig. 3. Opposite (double) acaricide profiles. Ranches with ticks resistant to OP and susceptible to Am (A), resistant to SP and susceptible to Am (B), resistant to OP and susceptible to SP (C), resistant to SP and susceptible to OP (D), resistant to Am and susceptible to OP (E), and resistant to Am and susceptible to SP (F).

ticks versus ≤45.5% in non-Yucatán ticks), in Am− and SP+ (53.1% in Yucatán versus ≤50.9 in non-Yucatán ticks); OP+ and SP− (32.3% in Yucatán versus ≤6.1% in non-Yucatán ticks, Table 2A and Fig. 3A-F, all with $P < 0.05$, χ^2 tests).

Across states, resistance to Am was associated with high annual frequency in the number of acaricide treatments. While single and double resistance profiles (involving OP and/or SP) were not statistically associated with high frequency of acaricide treatments,

Table 2B
Triple acaricide profiles

Acaricide profiles[a] (ticks susceptible to [−]/resistant to [+])	All states, $n = 217$ (%)	Yucatán, $n = 96$ (%)	Quintana Roo, $n = 55$ (%)	Tabasco, $n = 66$ (%)
Am−OP+SP+	81 (37.3)	43 (44.8)	25 (45.5)	13 (19.7)
Am+OP+SP−	7 (3.3)	5 (5.2)	1 (1.8)	1 (1.5)
Am+OP−SP+	5 (2.3)	3 (3.1)	1 (1.8)	1 (1.5)
Am+OP−S−	3 (1.3)	1 (1.0)	1 (1.8)	1 (1.5)
Am−OP−SP−	3 (1.4)	0 (0.0)	0 (0.0)	3 (3.1)
Am+OP+SP+	74 (34.1)	42 (63.6)	25 (45.4)	7 (7.3)
Am−OP+SP−	28 (12.9)	25 (26.0)	0 (0.0)	3 (4.5)
Am−OP−SP+	16 (7.4)	8 (8.3)	3 (5.5)	5 (7.6)

[a] Yucatán ranches showed a higher proportion of ticks with triple resistance (Am+OP+SP+) and of ticks susceptible to Am and SP but resistant to OP than the remaining ranches (all with $P < 0.05$).

Table 3A
Association of farm-related covariates with resistance

Resistance to single (or multiple) acaricide(s)	High treatment frequency	Large herd size	Large farm size
Am	0.001	0.27	0.48
SP	0.12	0.53	0.95
OP	0.17	0.49	0.81
Am and OP	IDCT[a]	0.90	0.28
Am and SP	0.43	0.18	0.14
OP and SP	IDCT[a]	0.11	0.02
OP, SP, and Am	0.001	0.64	0.12

Cells indicate the P value associated with each χ^2 test ($n = 217$ ranches).
[a] IDCT, insufficient data to conduct a test (one or more cells with less than five counts).

triple resistance was associated with six or more annual acaricide treatments ($P = 0.001$, χ^2 test, Table 3A). Across states, ticks collected in 144 ranches (where acaricide therapy was practised with high frequency) displayed double resistance to OP/SP (Fig. 4A). While the effect of high treatment frequency could not be determined to be associated with OP/SP resistance (because of insufficient data of ranches applying acaricides with low frequency, Table 3A), approximately one third (51/144) of ranches where tick resistance to OP/SP was observed and acaricides were frequently used, displayed ticks susceptible to Am (Fig. 4B). Although resistance to Am was not associated with farm size, that to OP and SP was ($P < 0.02$, χ^2 test, Table 3A). In Quintana Roo, even where acaricides were dispensed with high frequency and resistance to OP/SP was reported, ranches with ticks susceptible to Am were observed (Fig. 4B).

Three acaricide profiles explained 74.2% (161/217 ranches) of the data: (1) Am− and SP− ($n = 31$ ranches); (2) Am+ and SP+ ($n = 79$); (3) (among ranches that dispensed acaricide therapy al least six times/year) Am−OP+SP+ ($n = 51$, Fig. 4C). Those profiles explained three times as many cases as the remaining five profiles did, and at least twice as many cases as expected (Table 3B). Some acaricide profiles seemed to be non-randomly distributed over space (they appeared to be clustered, as indicated in Table 4). While some farm-specific covariates (high acaricide treatment frequency and large farm size) were associated with resistance, other spatial differences in acaricide profiles could not be explained by farm-specific covariates (Table 4).

4. Discussion

This study reported two farm-specific covariates (high annual frequency of acaricide treatments and large farm size) to be associated with resistance. These findings, however, may be influenced, at least, by false negative results. Lack of statistically significant associations between farm-specific covariates and resistance (as observed in the case of OP+ and SP+ ranches where acaricides were dispensed at least six times/year, Fig. 4A) does not necessarily deny that high treatment frequency promotes resistance: it only reflects the insufficient number of ranches where acaridice treatments were performed with low frequency.

OP were introduced in Mexico before or at the same time amidines were introduced. However, amidines were scarcely used in Mexico for several years (Li et al., 2005). Because resistance to OP was first noticed in

Table 3B
Percentage of cases explained by major acaricide profiles

	Three profiles (Am+SP+, Am−SP−, Am−OP+SP+ and high treatment frequency		Other (5) profiles[a]		All profiles		P value (χ^2 test)
	Ranches	Percentage	Ranches	Percentage	Ranches	Percentage	
Expected	81	37.5	136	62.5	217	100	
Observed	161	74.2	56	25.8	217	100	<0.001

[a] Calculations were based on the assumption that only eight acaricide profiles could occur (e.g., those depicted in Table 2B). If they were randomly distributed, each profile would explain 1/8 of all cases (12.5% each). Hence, three profiles would be expected to explain 37.5% of all cases. However, that calculation did not consider farm-specific covariates (each resulting in two possible outcomes), which would increase six times that total (48 profiles). In that scenario each profile, if distributed with a similar probability, would explain 1/48 of all cases (2.08% each) and, therefore, three profiles would be expected to explain 6.24% of all cases). However, the listed three profiles actually explained 74.2% of all cases.

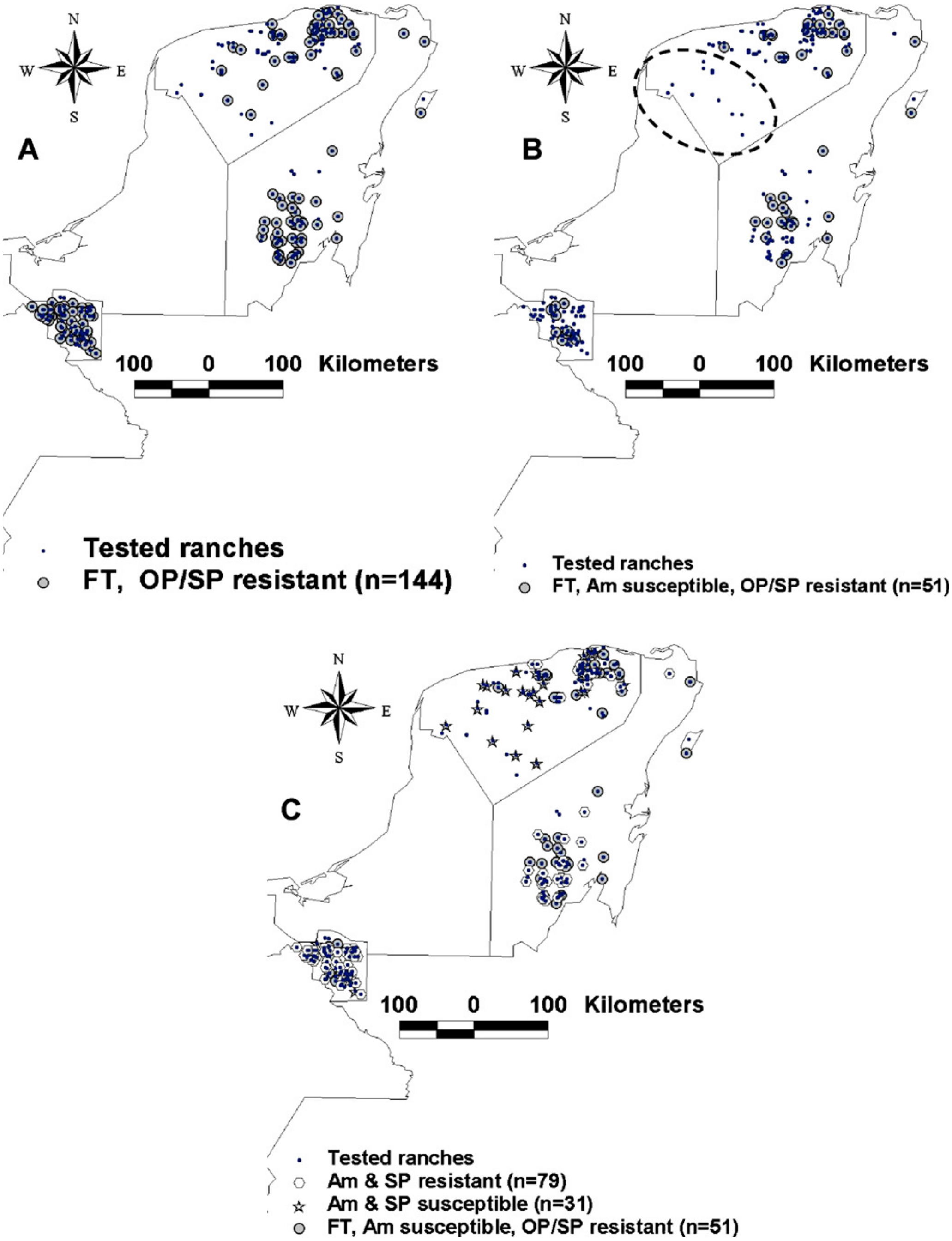

Fig. 4. Association of high annual frequency of acaricide treatments (FT) and spatial distribution of the three predominant acaricide profiles. (A) Ranches with ticks resistant to OP and SP, where acaridice treatments were dispensed at least six times/year (FT). (B) Ranches with ticks susceptible to Am (a sub-group of the profile shown in (A)). (C) Composite distribution of three non-overlapping observed profiles that explained 161 cases (74.2% of all ranches).

Mexico in the 1980s, while resistance to SP developed in the 1990s (Santamaría et al., 1999) and that to Am was first reported in Tabasco in 2001 (Soberanes et al., 2002), findings are in agreement with the time line of acaricide introduction: acaricides adopted earlier tend to induce earlier and/or more disseminated resistance.

The observed association between OP and SP resistance (which did not include resistance to Am in, approximately, one third of the cases), is in

Table 4
Regional differences in acaricide profiles

	Ranches with ticks[a] susceptible to Am	Ranches with ticks not susceptible to Am	All
Yucatán	79	17	96
Non-Yucatán	49	72	121
Total	128	89	217
	Ranches with ticks resistant to OP and SP	Ranches with ticks not resistant to OP and SP	All
Quintana Roo	49	6	55
Non-Quintana Roo	106	56	162
Total	155	62	217
	Ranches with ticks resistant to Am	Ranches with ticks not resistant to Am	All
Tabasco	45	21	66
Non-Tabasco	44	107	151
Total	99	128	217

[a] Yucatán ranches displayed a significantly greater proportion of ticks susceptible to Am, Quintana Roo ranches displayed a significantly greater proportion of ticks resistant to Am, and Tabasco ranches displayed a significantly greater proportion of ticks resistant to OP and SP than the remaining ranches (all with $P < 0.01$).

agreement with previous studies conducted in Australia and other countries (Foil et al., 2004). Once resistance to non-Am acaricides is reported, Am may be the choice chemical to be used (Foil et al., 2004).

Spatial differences in acaricide profiles (Table 4) may be due to differences in regional risk factors, including, but not limited to, ranch localization, type of application, fly control, and grazing management (Jonsson et al., 2000; Bianchi et al., 2003; Rodríguez-Vivas et al., 2006a). In addition, tick resistance to acaricide therapy may result from frequent ($\geq$6 times/year) treatments, as shown when SP are used (Rodríguez-Vivas et al., 2006a). The observed association between frequent administration of acaricides and resistance to Am provides support for the models of Sutherst et al. (1979), who indicated a much greater relative selective value for resistance alleles when six acaricide applications are administered per year.

A larger proportion of ranches with ticks susceptible to acaricides was observed in southwestern Yucatán, a region relatively warmer and drier than other regions evaluated in this survey, also characterized by different socio-economic conditions (smaller ranches, not oriented to commercial agriculture) than the remaining areas under study. Warmer and drier climate have been indicated as detrimental factors for the survival of *B. microplus* (Estrada-Peña et al., 2006; Estrada-Peña and Venzal, 2006) which, added to a presumed lower frequency of acaricide treatments and/or lack of exposure to acaricides, might explain, at least partially, the profile observed in southwestern Yucatán. While climate is an obvious, space-related factor, the frequency of acaricide treatments, as well as farm size, may be regarded to be management-, or socio-economic-related covariates (i.e., farm-specific factors).

However, neither spatial nor farm-specific factors, alone or combined, seemed to explain the differences observed in Quintana Roo versus Tabasco ranches (a greater tick resistance to OP and SP, observed in Quintana Roo ranches; a significantly greater tick resistance to Am, noticed in Tabasco ranches, Table 4). Those differences could be related to host-specific factors, which were not assessed in this study.

The statistically significant higher percentage of cases explained by a few acaricide profiles (which also differed in their spatial locations) strongly suggested that acaricide profiles (which are proxy indicators of disease profiles) may be spatially clustered. That hypothesis may bear theoretical and practical implications. If the chance of acaricide disease profiles is not normally distributed, then some epidemiological tenets (based on the assumption that all disease cases are identical) do not apply. When individuals (whether a cow or a ranch) are not identical, their chances for becoming infested are not identical, either. Then, some indicators based on the assumption of random disease occurrence (such as prevalence), may not be valid (Koopman, 2004). Unless shown otherwise, most diseases may be suspected to be clustered in space/time (Ward and Carpenter, 2000).

The practical implication of the previous proposition is that spatially explicit measures, not measures that ignore space (such as prevalence, in its most frequent usage), are needed to further improve our understanding on host–parasite–environmental interactions. From the policy-making viewpoint, that observation implies the need for creation (and updating) of detailed geo-referenced databases at the lowest possible spatial scale. Lack of digital data on farm parcels (farm size), herd size, standardized book-keeping, and animal-related factors (age, breed, nutrition, bovine cellular immune responses) may prevent optimal decision-making. To assess interactions that include host-specific factors and to facilitate data-based decision-making, further GIS-based studies are recommended.

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