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Carbaryl Resistance in Mexican Strains of the Southern Cattle Tick (Acari: Ixodidae)

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ABSTRACT Susceptibility to carbaryl in six Mexican strains of the southern cattle tick, *Boophilus microplus* (Canestrini), was evaluated with the Food and Agricultural Organization larval packet test. Tick strains from the cattle fever tick quarantine zone in Texas were more susceptible to carbaryl than to coumaphos or diazinon. Compared with the susceptible reference (Gonzalez) strain, Mexican tick strains demonstrated 10.9–59.5-fold resistance to carbaryl. Significant cross-resistance was found between carbaryl and the organophosphate acaricides coumaphos and diazinon. Bioassay results with synergists suggested that metabolic detoxification mechanisms did not play a major role in carbaryl resistance. Resistance to carbaryl was likely conferred by insensitive acetylcholinesterase. The implications of carbaryl resistance in tick eradication and control also are discussed.

KEY WORDS acaricide, carbaryl, resistance, cattle tick, *Boophilus microplus*

CARBARYL IS A BROAD-SPECTRUM carbamate pesticide that has been used to control various ectoparasites of animals and humans (Schulze et al. 1992, Hoelscher et al. 1994, Burridge et al. 2002, Downs et al. 2002). An early study in Australia demonstrated that carbaryl was highly effective in controlling the southern cattle tick, *Boophilus microplus* (Canestrini) (Roulston et al. 1965). Carbaryl also was also to reduce the reproductive potential of ticks by inhibition of female oviposition and egg hatching at sublethal doses (Mansingh and Rawlins 1979). This acaricide has been widely used to control *B. microplus* in many countries, including Rhodesia, India, Jamaica, New Caledonia, and Indonesia (Rawlins and Mansingh 1980, Sharma and Gupta 1982, Brun et al. 1984, Basu and Halder 1994), as well as the blue tick, *Boophilus decoloratus* (Koch) infesting cattle (Matthewson and Wilson 1976). It also has been used to control ticks that infest dogs (Pathak and Gaur 1985) and other tick species of medical importance, including the blacklegged tick, *Ixodes scapularis* Say, the vector of the disease agent that causes Lyme disease (Stafford 1991, 1997; Schulze et al. 1992).

B. microplus is endemic to Mexico and causes severe economic damage to Mexican ranchers. This tick was eradicated from the United States in 1961, and the United States Department of Agriculture (USDA) has maintained an active tick eradication program to prevent the reintroduction of this pest and disease vector to the United States from Mexico (Graham and Hourrigan 1977, George 1996). The control of *B. microplus* relies primarily on chemical acaricides, and *B. microplus* has demonstrated a high capacity to develop resistance to many of these compounds. In Mexico, *B. microplus* developed resistance to organophosphate (OP) acaricides in the 1980s, and pyrethroid resistance emerged in the 1990s (Aguirre and Santamaría 1986, Miller et al. 1999). *B. microplus* also has developed resistance to amitraz in recent years, as a result of more frequent use of this acaricide to control OP- and pyrethroid-resistant ticks in Mexico (Li et al. 2004). The development of acaricide resistance in Mexican populations of *B. microplus* poses a serious threat to the USDA's Cattle Fever Tick Eradication Program (CFTEP). This program prevents the reintroduction of *B. microplus* into the United States via cattle importation from Mexico. The core of CFTEP is the treatment of all cattle for importation in dipping vats charged with the OP acaricide coumaphos (0.3% [AI]) to kill any ticks that may have escaped inspection by tick inspectors at importation facilities (George 1996). Resistance to coumaphos or other acaricides with the same mode of action may allow tick larvae to survive the dipping treatment and therefore endanger CFTEP (Davey and George 1999).

Previously, we have determined the levels of resistance to the OP acaricides coumaphos and diazinon and characterized the mechanisms of resistance in various Mexican strains of *B. microplus* (Li et al. 2003). The objective of the current study was to measure the susceptibility/resistance of these tick strains to carbaryl, to determine the mechanisms of resistance, and to test the cross-resistance pattern between carbaryl and other OP acaricides.

This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation by the USDA for its use.

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Materials and Methods

Tick Strains. Six strains of *B. microplus* originating from various locations in Mexico and two outbreak strains of ticks within the cattle fever tick quarantine zone along the United States–Mexico border in Texas were included in this study. The Coatzacoalcos and Tuxapn strains were collected from Veracruz in southeastern Mexico and were established at the Cattle Fever Tick Research Laboratory (CFTRL) in Mission, TX, in 1994. The San Felipe strain was collected from a ranch in the state of Tamaulipas, Mexico, and was established at CFTRL in 1996. The San Roman and Caporal strains were collected from two separate ranches in the area of Champoton, Campeche, Mexico, and were established at CFTRL in 1998. The Pesqueria strain was collected in 2000 at the U.S. port of entry in Reynosa, Tamaulipas, Mexico, by USDA Veterinary Service inspectors from cattle originating from Pesqueria, Nuevo Leon, Mexico. The Gonzalez and Munoz strains are acaricide-susceptible tick strains that were established at CFTRL in 1994 and 1999, respectively, from outbreaks of ticks in Zapata County, Texas. They are susceptible to all major classes of acaricides. The Gonzalez strain was used here as the reference strain to determine the level of susceptibility in other tick strains. The procedures for rearing ticks on cattle, maintaining the nonparasitic stages in the laboratory, and challenging larvae with coumaphos were the same as described by Davey et al. (1980). The Mexican strains were challenged either with coumaphos (Tuxpan, San Roman, Caporal, and Pesqueria) or permethrin (Coatzcolcos and San Felipe) to maintain their resistance to the respective acaricide. Resistance profiles of these tick strains to other acaricides have been described in previous accounts (Miller et al. 1999; Li et al. 2003, 2004).

Chemicals. The formulated carbaryl (Sevin, 80% WP) used in this study is a product of the Union Carbide Corporation (Houston, TX). Carbaryl was tested in bioassays with and without one of three synergists: triphenylphosphate (TPP), an inhibitor of esterases (Aldrich, Milwaukee, WI); piperonyl butoxide (PBO), an inhibitor of oxidases (Aldrich); and diethyl maleate (DEM), an inhibitor of glutathione S-transferases (Aldrich).

Bioassay. Carbaryl bioassays were conducted between April 2001 and October 2002. The FAO larval packet test (LPT) (FAO 1971) was used to determine the susceptibility to carbaryl and the effect of synergists on the toxicity of carbaryl in all tick strains. Larvae used for all bioassays were between 12 and 16 d old. Stock solutions were made by dissolving formulated carbaryl (Sevin 80% WP) in trichloroethylene (Sigma, St. Louis, MO). The highest concentration was prepared by adding a volume of the stock solution to a mixture of trichloroethylene and olive oil (Sigma) with a final 2:1 ratio. Serial dilutions were prepared using a diluent of 2 parts trichloroethylene and 1 part oil. When the effect of a synergist on acaricide toxicity was evaluated the synergist was added to the diluent at a constant rate of 1%, except when 2% synergist was used for the Caporal strain. A volume of 0.7 ml of each dilution was applied to Whatman No. 1 filter papers (7.6 by 8.9 cm, Whatman, Maidstone, Kent, United Kingdom). Three papers were prepared for each concentration. The treated filter papers were placed in a fume hood for 2 h to allow trichloroethylene to evaporate before being folded in half and sealed with bulldog clips on both sides. Approximately 100 larvae were placed into each packet, and the top was sealed immediately with another bulldog clip. Packets were then held in an environmental chamber at 27 ± 2°C, 90% RH for 24 h. After this time, packets were removed from the environmental chamber, and the mortality determined by counting live and dead larvae.

Data Analysis. The POLO-PC program (LeOra Software 1987) was used to analyze the dose-mortality response of all bioassays. Resistance ratios (RR) were calculated by dividing the LC_{50} of a particular tick strain with the LC_{50} of the reference Gonzalez strain. The ratios of synergism (SR) caused by a synergist were calculated by dividing the LC_{50} of the bioassay using the acaricide alone with the LC_{50} using both the acaricide and synergist. Differences between LC_{50} estimates were designated as significant ($P = 0.05$) when their 95% confidence intervals (CIs) did not overlap (Robertson and Preisler 1992). The correlation between carbaryl LC_{50} and synergism ratios, and the correlation between carbaryl LC_{50} and the LC_{50} of the OP acaricides from a previous study (Li et al. 2003) were analyzed with JMP software (SAS Institute 2000).

Results

Resistance to Carbaryl. Toxicity data for carbaryl and the levels of resistance and synergism of the eight strains of *B. microplus* are summarized in Table 1. The Munoz strain was as susceptible as the Gonzalez strain. The LC_{50} estimates of the Mexican strains ranged from 0.0340 to 0.1844%. In comparison with the Gonzalez strain, the resistance ratios of these strains ranged from 10.97 to 59.48. The Caporal strain was the most resistant to carbaryl.

Effects of the Synergists. Although there were significant differences in carbaryl LC_{50} estimates for some tick strains when a synergist was added to carbaryl, correlation analysis for all tick strains combined found no significant correlation between carbaryl LC_{50} estimates and synergism ratio for any of the synergists used (Table 1). Data for the Caporal strain were excluded from analysis due to the higher synergist concentration used for this tick strain.

Cross-Resistance with OPs. Analysis of correlation between carbaryl LC_{50} estimates and $LC\ C_{50}$ estimates reported previously for coumaphos and diazinon (Li et al. 2003) revealed significant cross-resistance patterns with the two OP acaricides (Fig. 1.). Carbaryl resistance in those tick strains was more strongly correlated with resistance to diazinon ($Y = -0.504 + 1.067{*}X$; $F = 59.66$, $r^2 = 0.909$, $P = 0.002$) than with resistance to coumaphos ($Y = -0.748 + 1.243{*}X$; $F = 6.77$, $r^2 = 0.530$, $P = 0.041$).

Table 1. Results of probit analysis of dose-mortality responses of various strains of *B. microplus* to carbaryl with and without synergists

Strain and treatment	*n*	Slope (SE)	LC_{50} (95% CI)[b]	χ^2 (df)	RR[c]	SR[a]		
						TPP	PBO	DEM
Gonzalez								
carbaryl	2,500	2.46 (0.18)	0.0031 (0.0025–0.0037)	25.3 (19)	1			
Munoz								
carbaryl	1,808	2.78 (0.17)	0.0025 (0.0020–0.0030)	78.2 (19)	0.81			
+ TPP	2,011	2.14 (0.12)	0.0009 (0.0005–0.0012)	138.9 (19)		2.78		
+ PBO	1,977	3.13 (0.15)	0.0032 (0.0024–0.0042)	205.7 (19)			0.78	
+ DEM	2,171	2.70 (0.11)	0.0029 (0.0026–0.0032)	36.5 (19)				0.86
San Roman								
carbaryl	2,372	4.07 (0.27)	0.0340 (0.0287–0.0385)	84.1 (19)	10.97			
+ TPP	3,530	1.95 (0.09)	0.0300 (0.0264–0.0350)	65.0 (19)		1.13		
+ PBO	2,811	6.22 (0.47)	0.0272 (0.0228–0.0304)	137.3 (19)			1.25	
+ DEM	2,705	3.60 (0.22)	0.0260 (0.0226–0.0292)	81.5 (19)				1.31
Coatzacoalcos								
carbaryl	1,682	3.88 (0.24)	0.0461 (0.0399–0.0525)	59.8 (19)	14.87			
+ TPP	1,809	2.95 (0.16)	0.0199 (0.0172–0.0228)	51.8 (19)		2.32		
+ PBO	1,764	29.32 (—)	0.0394 (—)	68.9 (19)			1.17	
+ DEM	1,962	5.34 (0.37)	0.0413 (0.0378–0.0449)	30.2 (19)				1.12
San Felipe								
carbaryl	787	10.07 (0.86)	0.0532 (0.0457–0.0583)	37.6 (7)	17.16			
+ TPP	1,843	38.53 (—)	0.0431 (—)	1.6 (22)		1.23		
+ PBO	964	9.07 (0.63)	0.0539 (0.0455–0.0600)	59.3 (7)			0.99	
+ DEM	2,023	8.08 (1.07)	0.0982 (0.0625–0.1109)	159.8 (22)				0.54
Tuxpan								
carbaryl	3,084	8.87 (0.54)	0.0879 (0.0793–0.0949)	169.8 (25)	28.25			
+ TPP	2,881	13.20 (1.41)	0.0454 (0.0424–0.0477)	59.9 (25)		1.94		
+ PBO	2,621	8.24 (0.49)	0.0590 (0.0535–0.0645)	215.4 (25)			1.49	
+ DEM								-
Pesqueria								
carbaryl	2,344	9.86 (1.18)	0.1172 (0.0995–0.1262)	116.1 (22)	37.8			
Caporal[d]								
carbaryl	2,215	5.11 (0.20)	0.1844 (0.1677–0.2030)	125.3 (22)	59.48			
+ TPP	2,337	9.42 (0.81)	0.0613 (0.0572–0.0647)	43.9 (22)		3.10		
+ PBO	2,373	9.60 (0.60)	0.0693 (0.0646–0.0743)	14.1 (22)			2.66	
+ DEM	2,289	13.72 (1.25)	0.0964 (0.0884–0.1028)	82.5 (22)				1.91

[a] SR = LC_{50} of carbaryl alone divided by LC_{50} of carbaryl with a synergist of the same strain.
[b] LC_{50} estimates are presented as percentage (AI).
[c] RR = LC_{50} of test strain divided by the LC_{50} of the susceptible reference (Gonzalez) strain.
[d] Synergists (2%) were used for the Caporal strain, and 1% was used for all other strains.

Discussion

Results of this study indicated that the Gonzalez and Munoz strains of *B. microplus* were highly susceptible to carbaryl, and their LC_{50} estimates (0.0031 and 0.0025%) were similar to that of the susceptible tick strain Yeerongpilly (0.0016%), used in Australia (Roulston et al. 1968). In comparison with previous studies (Li et al. 2003), carbaryl was more toxic to *B. microplus* larvae than the organophosphate acaricides diazinon and coumaphos. The relative toxicity of these acaricides obtained in our studies was consistent with a previous report (Rawlins and Mansingh 1978).

Resistance to carbaryl was reported in a number of *B. microplus* strains resistant to organophosphates in Australia in the 1960s and 1970s (Roulston et al. 1968, Schnitzerling et al. 1974) and also was reported later in Jamaica, South Africa, and New Caledonia (Rawlins and Mansingh 1978, Baker et al. 1979, Brun et al. 1984). In India, *B. microplus* developed resistance to carbaryl after only 3 yr of use (Basu and Halder 1997). RRs to carbaryl in various *B. microplus* strains were measured at 7.5–92. However, a higher level (RR = 218) of resistance to carbaryl was reported in a closely related tick species, *Boophilus decoloratus* from Rhodesia (Matthewson and Wilson 1976). In our study, we found 10.9–59.5-fold resistance to carbaryl among the Mexican tick strains. The Caporal, Pesqueria, Tuxpan, and San Roman strains demonstrated relatively high resistance to OPs, as a result of challenges with coumaphos during their laboratory colonization.

Resistance to carbaryl and the cross-resistance patterns between carbaryl and OPs suggest the involvement of similar resistance mechanisms. Both the carbamate and OP acaricides exert their toxic effect on insects or ticks by inhibiting acetylcholinesterase (AChE), a key enzyme critical to the function of the nervous system of invertebrates. Resistance to OP and carbamate pesticides was found to be conferred by insensitive AChE in many pest species (Siegfried and Scharf 2001). Insensitive AChE has been implicated to play a major role in OP resistance in *B. microplus* (Pruett 2002). In addition, a *cytP450*-mediated oxidative detoxification also has been implicated specifically in resistance to coumaphos, but not to diazinon (Li et al. 2003). The results of the current study indicated that resistance to carbaryl was likely conferred mainly by insensitive AChE. Carbaryl has not been used extensively in Mexico for the control of *B. mi-*

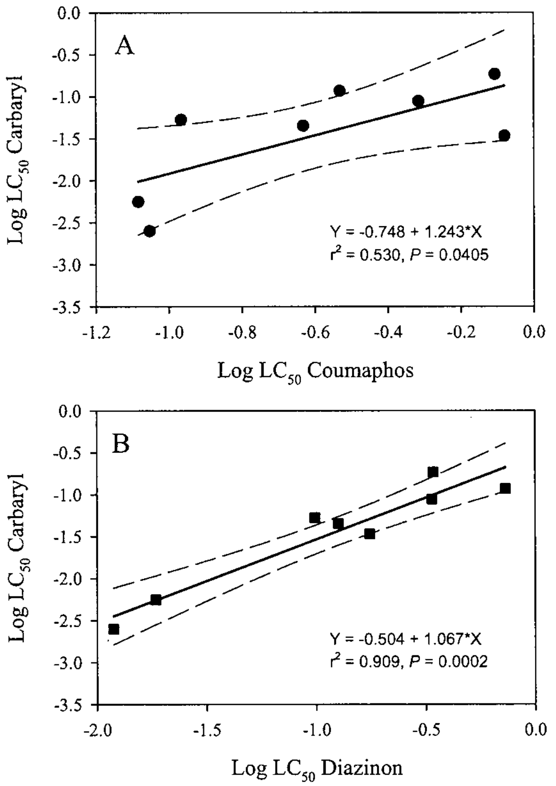

Fig. 1. Results of correlation analysis between log transformations of carbaryl LC_{50} estimates and coumaphos LC_{50} estimates (A), and between log transformations of carbaryl LC_{50} estimates and diazinon LC_{50} estimates (B).

croplus, and none of the tick strains have been exposed to carbaryl during their laboratory colonization. Resistance to carbaryl demonstrated by this study was likely a result of cross-resistance to coumaphos, which has been widely used in Mexico for controlling *B. microplus* and also has been used to challenge OP-resistant strains in the laboratory. Nevertheless, the finding of carbaryl resistance and its cross-resistance to OPs have important implications for both the control of *B. microplus* in Mexico and the USDA's CFTEP. Due to the cross-resistance with OPs, it is unlikely that carbaryl will be effective in controlling OP-resistant *B. microplus.* The knowledge of carbaryl resistance and cross-resistance would help in choosing the right acaricides to control OP-resistant ticks in Mexico or to eradicate outbreaks of cattle ticks within the quarantine zone along the U.S.–Mexican border in Texas.

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