Molecular and biochemical diagnosis of esterase-mediated pyrethroid resistance in a Mexican strain of *Boophilus microplus* (Acari: Ixodidae)

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Abstract. We examined pyrethroid resistant Mexican strains of *Boophilus microplus* using biochemical and molecular tests to determine the mechanisms conferring resistance. Permethrin hydrolysis assays and esterase activity gels indicated enhanced esterase-mediated metabolic detoxification in the Cz strain, while one other pyrethroid resistant strain, SF, and two pyrethroid susceptible strains had lower levels of permethrin hydrolysis. Results from assays using a PCR-based test to detect a pyrethroid target site resistance-associated mutation in the tick sodium channel gene found only low levels of mutations in the Cz strain, while the SF strain had a high level of the mutated sodium channel alleles. A specific esterase, designated CzEst9, believed to be responsible for the esterase-mediated pyrethroid resistance in the Cz strain was purified, and the gene encoding CzEst9 cloned.

Key words: pyrethroid resistance mechanism, sodium channel mutation, polymerase chain reaction, diagnostic assay, metabolic esterase

Introduction

The southern cattle tick, *Boophilus microplus* (Canestrini), was eradicated from the United States during a 55-year program coordinated by the United States Department of Agriculture (Graham and Hourrigan, 1977) and the US remains free of *B. microplus* through a USDA-APHIS/VS program established at the border of the United States and Mexico. Large numbers of cattle are imported annually into the United States from Mexico and the continued ability to control tick populations on the ranches of Mexico will be a major factor in the United States remaining *Boophilus*-free. Mexican cattle producers have made extensive use of pyrethroid-based pesticides in attempting to control *B. microplus* and problems with pesticide resistance have begun to intensify.

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There are several mechanisms which can lead to pyrethroid resistance in arthropods. The target of pyrethroids is the sodium channel and several mutations in arthropod sodium channel gene coding regions have been shown to cause pyrethroid resistance (Vais et al., 2000; Tan et al., 2002). In *B. microplus*, a nucleotide substitution in the sodium channel gene coding region leading to a Phe→Ile amino acid substitution in the S6 transmembrane segment of domain III has been associated with pyrethroid resistance (Guerrero et al., 2001). However, toxicological studies of several pyrethroid resistant Mexican strains of *B. microplus* indicated that, in addition to target site insensitivity, metabolic resistance to pyrethroids was also an important mechanism (Miller et al., 1999). Esterases are a family of enzymes, which can be involved in metabolic-based pesticide resistance through their ability to sequester or hydrolyze various substrates. Jamroz et al. (2000) examined general esterase activities in Mexican strains of *B. microplus* and reported a pyrethroid resistant strain, Coatzacoalcos (Cz), which had elevated activity of a specific esterase designated CzEst9. The toxicological profile of the Cz strain had indicated that an esterase-mediated mechanism was involved in pyrethroid resistance instead of target site insensitivity. We have utilized a PCR assay to test for the pyrethroid resistance-associated amino acid substitution in the sodium channel gene of the Cz strain and a target site-mediated pyrethroid resistant strain, San Felipe (SF). Permethrin hydrolysis assays and esterase activity gels were utilized to assay for metabolic esterase-based resistance. The metabolic esterase CzEst9 was purified and the gene encoding CzEst9 cloned.

**Materials and Methods**

Rearing of ticks and bioassays were done at the USDA-ARS Cattle Fever Tick Research Laboratory (CFTRL) in Mission, TX as described by Davey et al. (1980). The sources, establishment and toxicological characterization of the strains were described by Miller et al. (1999). Bioassays were performed using the FAO standard larval packet test (FAO, 1984). Probit analysis, including probit transformation of percentage mortality and natural logarithm transformation of dose, was performed using the Polo-PC Program (LeOra Software, 1987). Resistance ratios were determined relative to bioassay data from the reference pyrethroid susceptible Gonzalez strain. To separate bioassay ‘alive’ and ‘dead’ larvae in the Cz and SF resistant strains, bioassays were conducted using five concentrations of permethrin, with each concentration repeated three times. When bioassays were read, ‘alive’ and ‘dead’ larvae at each concentration were separated with vacuum and immediately frozen at −70°C for molecular assays.