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Comparison of the reproductive biology between acaricide-resistant and acaricide-susceptible *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae)

Ronald B. Davey [a,*], John E. George [b], Robert J. Miller [a]

[a] *USDA, ARS, Cattle Fever Tick Research Lab., Moore Air Base, Bldg. 6419, 22675 N. Moorefield Rd., Edinburgh, TX 78541, USA*
[b] *USDA, ARS, Knipling-Bushland U.S. Livestock Insects Lab., 2700 Fredricksburg Rd., Kerrville, TX 78029, USA*

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Abstract

The reproductive fitness of *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini) strains resistant to organophosphate (OP), pyrethroid (P), or formamidine (F) acaricides was compared to an acaricide-susceptible (SUS) strain to determine whether the acquisition of resistance affected reproductive fitness in the resistant strains. The SUS strain females had a 3.0 days preoviposition period, a 12.1 days oviposition period, a 22.5 days egg incubation period, a mean of 3670 eggs per female, and a mean percentage egg hatch of 78.1%, which were all remarkably similar to these same parameters reported for this species throughout the world. The reproductive biology of the P-resistant strain (PYR) and the F-resistant strain (FOR) were, for the most part, similar to those of the SUS strain. In the few instances where statistical differences did occur there was little evidence that the variation had any biological basis that could be attributed to a reduction in fitness related to resistance to P or F acaricides. Although the comparison of reproductive parameters of the OP-resistant strain (OPR) and the SUS strain identified statistical differences between the mean egg incubation and oviposition periods, the magnitude of the differences was not sufficient to conclude that the OPR strain was biologically less fit than the SUS strain. However, the OPR strain produced 30% fewer eggs (2562 eggs per female) than the SUS strain (3670 eggs per female) indicating the acquisition of resistance placed the OPR at a selective disadvantage relative to the SUS strain. This coupled with a lower, though non-significant, egg hatch was used to predict there would be a reduction of at least 34.1% in larval numbers available to potentially re-infest subsequent cattle than were available from the SUS strain. These data may aid the development of management strategies that can be used to control OP-resistant ticks.

Keywords: *Rhipicephalus*; *Boophilus*; Reproduction; Resistance

1. Introduction

The United States Cattle Fever Tick Eradication Program (CFTEP) has faced many challenges during its 100-year history, but perhaps the greatest challenge

* Corresponding author at: USDA, ARS, SPA, Cattle Fever Tick Research Lab., Moore Air Base, Bldg. 6419, 22675 N. Moorefield Rd., Edinburgh, TX 78541, USA. Tel.: +1 956 580 7262; fax: +1 956 580 7261.
E-mail address: Ronald.Davey@ars.usda.gov (R.B. Davey).

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has been the development of widespread acaricide resistance to the major classes of pesticides that have been used to control *Rhipicephalus* (*Boophilus*) spp. in Mexico during the past 30 years. Considerable research in the U.S. in recent years has focused on control technologies, characterization of resistance mechanisms, and development of molecular assay techniques associated with acaricide-resistant *R.* (*B.*) *microplus* (Canestrini) ticks (Davey and George, 1998, 1999; He et al., 1999a,b,c, 2002; Miller et al., 1999, 2002; Guerrero et al., 2001; Davey et al., 2003, 2004; Li et al., 2003, 2004, 2005a,b; Temeyer et al., 2004). However, few studies conducted anywhere in the world have specifically investigated the impact that resistance has on biological factors, such as reproduction. The reproductive biology of *R.* (*B.*) *microplus*, consisting of the preoviposition and oviposition periods and fecundity of engorged females, along with the incubation period and fertility of eggs was documented several decades ago in widely divergent parts of the world, such as Australia, Cuba, and the USA, using acaricide-susceptible ticks (Hitchcock, 1955; Cerny and de la Cruz, 1971; Bennett, 1974a; Davey et al., 1980a). But, with the exception of a single Australian study (Bennett, 1974b) there appears to be little specific information on the effect of resistance on the reproductive processes of this species, even though acaricide resistance seriously threatens to undermine chemical control strategies used against the species. Although it has been reported that fitness reduction in pesticide-resistant arthropods is likely to occur in the absence of pesticide pressure (Roush and Daly, 1990), it is difficult to associate fitness disadvantages specifically with resistance.

The purpose of this study was to compare the reproductive fitness, as measured by oviposition, fecundity, and fertility, of acaricide-resistant strains of *R.* (*B.*) *microplus* with those of a tick strain that was susceptible to the major classes of acaricides used to eradicate or control the species. The rationale for the study was based on the assumption that any selective disadvantages in reproductive capacity of acaricide-resistant ticks that could be demonstrated under laboratory conditions could potentially explain the occurrence of unusual reproductive patterns, such as lower egg production or reduced egg viability that might occur in naturally occurring resistant tick populations. In addition, demonstration of selective disadvantages associated with acaricide-resistant ticks might provide insight for the development of strategies that could be used to manage resistant tick populations.

2. Materials and methods

2.1. Tick strains

One strain of *R.* (*B.*) *microplus* that was susceptible to acaricides and three strains that were resistant to either organophosphate (OP), pyrethroid (P), or formamidine (F) acaricides were evaluated in the study. Each of the four strains had been maintained in the laboratory for multiple generations using standard rearing techniques (Davey et al., 1982). The acaricide-susceptible strain (SUS) used in the study, to which all of the resistant strains were compared, was originally collected from an outbreak of ticks discovered in Zapata Co., TX in 1999. No acaricidal pressure has ever been applied to the ticks since its laboratory colonization. However, larvae from most generations were subjected to laboratory bioassay tests with OP, P, and F acaricides using the larval packet test method described by FAO (Anonymous, 1971) to track the susceptibility level of the strain. The OP-resistant strain (OPR) used in the study was originally obtained from a ranch located in Champoton, Campeche, MX in 1998. The strain was selectively pressured during most generations of laboratory colonization with the OP acaricide coumaphos to maintain or increase the level of OP resistance. The P-resistant strain (PYR) was collected from a ranch located near Soto la Marina, Tamaulipas, MX in 1995 and colonized at our laboratory in 1996. The strain was selectively pressured during many generations of colonization with the P acaricide permethrin to maintain or increase the level of P resistance, and was subjected to laboratory bioassay tests (FAO method) to track the level of P resistance. The F-resistant strain (FOR) was originally collected from a ranch in Tabasco, MX in 2001, and was colonized in our laboratory in 2002. The strain was selectively pressured during numerous generations since colonization with the formamidine acaricide amitraz to maintain or increase the level of resistance. Laboratory bioassays (FAO method) were conducted on numerous generations to track the level of F resistance in the strain.