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Research paper

Cattle fever tick, *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae): Potential control on pastures by the application of urea fertilizer

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ABSTRACT

The southern cattle fever tick, *Rhipicephalus (Boophilus) microplus*, spends as much as 80–90% of its life cycle as a larva questing for a host. Standard control methods are limited to on-host applications, leaving a need for methods directed at the pasture infesting stages. Reports from Brazil indicate that pasture fertilization can reduce tick numbers. Granular urea was tested using standard pesticide efficacy methods in both the laboratory and field trials to determine if there was a significant impact on adult reproduction and larval survival. Under the conditions of this present study, there was no detectable effect on either female adults or larval stages. Ammonification in the soil may be a key factor limiting the impact of fertilizer treatments.

1. Introduction

The southern cattle fever tick, *Rhipicephalus (Boophilus) microplus* (Canestrini), was eradicated from the United States in 1943 (Graham and Hourrigan, 1977) but infestations periodically recur along the border area of south Texas (Lohmeyer et al., 2011) and thus this area is the focus of intensive re-eradication efforts. These one-host ticks are important arthropods posing an economic threat due to their capacity as vectors of *Babesiosis* and *Anaplasmosis* (Kocan et al., 2000; Uilenberg, 2006; Pérez de León et al., 2014). Although cattle are a highly suitable host, potential hosts also include: horses, white-tail deer, and nilgai (an introduced exotic antelope species) (Teel et al., 1996). The primary control treatments are acaricides applied directly to the domestic host leaving wild hosts as a source of re-infestation. Thus, there is also an arguably greater need for a control method for the off-host stages. The cattle fever tick spends as much as 80–90% of its life cycle off-host (Needham and Teel, 1991). Replete females lay their eggs on the ground around vegetation, and the larvae cluster together on grasses and other pasture plants questing for a host (Ivancovich, 1975; Nuñez et al., 1985). Populations of this tick can survive in harsh semi-arid environments with long drought seasons and high temperatures, conditions prevalent in south Texas. According to Teel et al. (1997), plants that provide high cover are essential to the survival of the ticks. Yet, a recent study found that fertilized pastures have fewer fever ticks concluding that urea fertilizer negatively impacted adults and larval tick populations in Brazilian savannahs (Pinto da Cunha et al., 2010). For the current study, granular urea was tested *in vitro* and *in vivo* to determine if fertilization would have an effect on tick populations in arid south Texas pastures.

2. Materials

2.1. Study site

All experiments were conducted at the USDA-ARS Cattle Fever Tick Research Laboratory at Moore Air Field located near Edinburg, TX, 26.3871°N, 98.3376°W, elevation 66 m.

2.2. Rearing of ticks

Experimental colonies of *R. microplus* were maintained following the procedures described by Davey et al. (1982, 1994). Briefly, ticks were reared on stanchioned cattle at the USDA quarantine facility (in accordance with in house IACUC protocols [established 17 October 2015]) until females were engorged and dropped from the host. These females were collected and held in petri dishes at optimal conditions (27 ± 1 °C, 80 ± 5 R.H.) for oviposition. Some of these reared females were used for the laboratory (adult immersion) testing and others for the field trials. Females were used prior to oviposition, usually within 24 h of engorgement and dropping from the host. Larvae used for the *in vitro* immersion test were taken from the colony at an age of 14 days post-eclosion. The strain designated as "*Deutch*" in the F_{59}, F_{60}, and F_{61} generations of colonization were used throughout both laboratory and field trials.

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2.3. Granular Urea

Fertilizer grade granular urea (Wilbur-Ellis, Edinburg, TX) was used for all applications. Fertilizer grade urea contains 97% urea (22.58% nitrogen) and 3% formaldehyde (manufacturer's material safety data sheet). Urea has a water absorbency of 94x its own weight (Weston et al., 1994; Guo et al., 2005). When compared to commercial fertilizers, granular urea provides significant plant health benefits.

3. Methods

3.1. Stage I. In vitro studies

3.1.1. Larval Immersion

The larval immersion test followed the methods of Klafke et al. (2006). Packets containing approximately 100 larvae were prepared in three to five replicates at each concentration. Concentrations were based on the studies of Pinto da Cunha et al. (2010). Granular urea was dissolved in distilled water at three dosages: high (15 g/L), medium (7.5 g/L), and low (3.75 g/L) treatments. Test tubes containing each solution (high, medium, low, and water control) were used in each replicate, placing clusters of larvae into the tubes using a toothpick. Following five minutes of immersion, larvae from each tube were transferred onto filter papers then into pre-prepared packets using a small paint brush. Packets were sealed with clips and incubated at a constant temperature and humidity regime [27 ± 1 °C, 80 ± 5% R.H.]. After 24 h treated larvae were removed from the packets and scored for survivorship.

3.1.2. Adult Immersion

For the adult immersion test, ten engorged females were treated per replicate with a total of five replicates. The same dosages were used as in the larval packet test. Each replicate was immersed at each dosage (high, medium, low, and control) in a glass scintillation vial for five minutes. Females were poured off through a strainer, dried with paper towels, and transferred to petri dishes lined with dry filter paper and sealed with tape. After seven days, females were scored for survivorship and mortality. Eggs oviposited were weighed and held in one dram vials sealed with cotton. The vials with the egg masses were held in an incubator [27 ± 1 °C, 80 ± 5% R.H] then scored for larval eclosion (hatch) at 28 days post-treatment.

3.2. Stage II. Field trial

Study arenas (tick gardens) consisted of numbered galvanized metal tubs filled with soil and a buffelgrass, *Pennisetum ciliare* (L.), plant. The plants with crown, root mass, and soil were transplanted from pastures located at the site (Fig. 1). Buffelgrass is a dominant pasture grass in south Texas (Hanselka, 1988) and northern Mexico (Franklin et al., 2006), thriving in areas with low precipitation (20–40 cm/year) (Cox et al., 1988). The trial consisted of three experiments (designated I, II, and III in results) each containing ten tubs per trial with a total of two engorged females per tub. Five tubs were treated by evenly spreading 14 g of granular urea and adding 1.8 L of water and five tubs were used as controls with 1.8 L of water only, similar to the study of Pinto da Cunha et al. (2010) using potted plants. Soil pH levels were measured 10 days after the application of urea using a soil master pH meter (Mosser Lee, Millston, WI) following manufacturer's instructions. Soil temperatures were measured with a small dial model HH bimetal thermometer (Reotemp instruments, San Diego, CA).

For experiment I each tub was infested with females one month after the application of urea. For experiment II each tub was infested 24 h after application, and for experiment III each tub was infested two weeks before application. Corresponding to previous reports (*i.e.*, Nuñez et al., 1985) the released preoviposition females were observed to ensconce themselves deep in the base of the plant to lay their eggs.

Fig. 1. "Tick Garden" containing buffelgrass for experimental treatments.

Thereafter, the plants and females were not disturbed. Ticks were sampled daily until the population of each cohort was depleted. Larvae were sampled by dragging a white flannel cloth (25 × 20 cm) over the grass, dragged in opposite directions to simulate contact of a potential host (Wilkinson, 1961). The flannel cloths were placed in individual numbered zip-lock bags corresponding to each tub. Clear adhesive tape was used to collect larvae attached to the cloth, then mounted on a tick data sheet following the recording methods of Wilkinson (1961).

3.2.1. Data analysis

For comparison between controlled and treated groups a one-way analysis of variance (ANOVA) and/or Pair-wise t-test were used to test differences between means. Abbott's Corrected mortality formula (Abbott, 1925) was used prior to the statistical analysis. Data was tested for normality using a Shapiro-Wilks test prior to statistical comparison (Shapiro and Wilk, 1965).

4. Results

The results of the *in vitro* and *in vivo* (field) experiments showed that urea treatments had no effect on the survivorship of the larvae or adults.

4.1. In vitro studies

4.1.1. Larval immersion test

At 24 h post-treatment the larval immersion packets were scored for survivorship. Survivorship in all treatments was higher than in the controls (100% *vs.* 99.3%, $n = 2153$) (Table 1). Abbott's corrections and statistical comparison was unnecessary inasmuch as there was no mortality in the treatment groups.

4.1.2. Adult immersion test

After seven days treated females were scored for survivorship. The survivorship means were: 85% (Control), 86% (Low), 92% (Medium),