

Short Communication

Spray and Pour-On Acaricides Killed Tennessee (United States) Field-Collected *Haemaphysalis longicornis* Nymphs (Acari: Ixodidae) in Laboratory Bioassays

R. A. Butler,^{1,3} J. G. Chandler,¹ K. M. Vail,¹ C. J. Holderman,² and R. T. Trout Fryxell¹

¹Department of Entomology and Plant Pathology, The University of Tennessee, Knoxville, TN, USA, ²Central Life Sciences, Dallas, TX, USA, and ³Corresponding author, e-mail: rbutle25@vols.utk.edu

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Abstract

Haemaphysalis longicornis Neumann (Asian longhorned tick) is an exotic and invasive tick species presenting a health and economic threat to the United States (U.S.) cattle industry due to its ability to transmit pathogens and infest hosts in large numbers. The objective of this study was to evaluate available products at causing *H. longicornis* mortality in a laboratory bioassay. The efficacy of products was evaluated at label rates using *H. longicornis* nymphs collected from a cattle farm in eastern Tennessee in two different bioassays (spray or dip) against untreated controls. After exposure, ticks were transferred to clean petri dishes and checked for mortality at 0, 1, 2, 3, 4, 21, 24, and 48 h post exposure. No mortality occurred in the untreated controls, whereas all treated ticks were dead within 24 h of exposure ($P < 0.0001$). These findings support the hypothesis that currently available spray and pour-on products are effective at causing *H. longicornis* mortality. We conclude that these acaricides can be used as a component to prevent *H. longicornis* dispersal and for control in the U.S.

Key words: acaricide, management, bioassay, *Haemaphysalis longicornis*

Haemaphysalis longicornis Neumann (Asian longhorned tick) (Acari: Ixodidae) is an exotic and invasive pest recently discovered in the United States (U.S.), with populations now established in several eastern states (Egizi et al. 2020). This species feeds on a variety of wildlife, companion animal, livestock, and human hosts (Schappach et al. 2020; USDA-APHIS, 2020). Recently, this economically important tick species has been found with *Theileria orientalis* genotype Ikeda which is associated with diseased cattle (Oakes et al. 2019; Thompson et al. 2020) and newly invasive populations of this tick can transmit this pathogen (Dinkel et al. 2021). Knowing the U.S. strain of *H. longicornis* has a parthenogenetic reproductive strategy (Hoogstraal et al. 1968; Qiu et al. 2020), it is speculated that the strategy can increase the ticks invasive potential and cause significant blood loss causing death from anemia (Rainey et al. 2018; Oda et al. 2019; Egizi et al. 2020). Current *H. longicornis* management strategies are adopted from the ticks' native range or

include those used for North American tick species (Schappach et al. 2020). In the United States, there are no established or evaluated *H. longicornis* management programs and there are limited data on acaricide efficacy (Schappach et al. 2020).

Previous studies in other countries evaluated systemic and topical products for *H. longicornis* control. Otaki et al. (2018) found lotilaner (Credelio, Elanco, Greenfield, IN), a veterinary drug commonly used to treat companion animal ectoparasites, effectively killed *H. longicornis* adults when administered to dogs as a chewable tablet. Similarly, afoxolaner (Nexgard, Merial, Duluth, GA) canine chewable tablets killed 100% of *H. longicornis* 48 h after first exposure (Kondo et al. 2014). Amitraz, commonly formulated as a spray and cattle pour-on (PO), significantly reduced the number of female *H. longicornis* on calves (Heath et al. 1980). Topical imidacloprid and permethrin were 100% effective at killing female *H. longicornis* ticks within 4 d after feeding for 3 h on treated dogs (Hagimori et al. 2005).

While systemic products are designed to kill ticks during blood feeding, a topical acaricide can control ticks on contact and prevent biting and blood feeding. El-Tokhy et al. (2019) found lambda-cyhalothrin 10% capsule suspension (CS) sprayed with a portable electric ultra-low volume (ULV) sprayer to the cattle's surrounding environment (e.g., bedding material, wall, etc.) reduced *Hyalomma marginatum* Koch (Acari: Ixodidae) ticks on cows by 97.5% after just 24 h of exposure. Concentrations of 0.05% Phosmet evaluated in a dipping vat management study caused 100% mortality in *Rhipicephalus (Boophilus) annulatus* Say (Acari: Ixodidae) ticks on cattle (Ahrens et al. 1995). Diflubenzuron was found to be an effective acaricide for *R. microplus* Canestrini (Acari: Ixodidae) larvae control on cattle (Andreotti et al. 2019). Topical acaricides with contact activity could be highly effective for controlling *H. longicornis* populations, especially since this tick species is frequently recovered from cattle and dogs (Egizi et al. 2020; Thompson et al. 2020; USDA-APHIS, 2020).

Considering the potential economic impact of *H. longicornis* infestations to livestock and that there is a potential for these ticks to transmit pathogens to cattle, the purpose of this study was to assess the efficacy of several spray and PO acaricides for tick control at label rates. Identifying effective products for controlling *H. longicornis* nymphs, which are hard to detect and numerous, can prevent further accidental dispersal and establishment of this species. The objective of this study was to evaluate available products applied in a laboratory bioassay and their impact on *H. longicornis* mortality.

Materials and Methods

Tick Collection

H. longicornis were collected from an angus cow-calf farm in Cocke County, Tennessee with 0.6 × 0.9 m (2 × 3 ft) corduroy drag cloths (June–September 2020). Collected ticks were identified using taxonomic keys (Egizi et al. 2019) and maintained in sand-filled vials in a Haier refrigerator (Rapid City, SD) with a mean ± SD relative humidity of 71.6 ± 7.76 and mean ± SD temperature of 17.6°C ± 2.04. Prior to use in bioassays, ticks acclimated to room temperature and were held at room temperature throughout the study.

Product Selection

Spray and PO products were evaluated, and untreated control treatments were used in both bioassays. Specifically, RF2301 LPD PO (0.5% lambda-cyhalothrin, 2.5% piperonyl butoxide, 3% diflubenzuron, currently under environmental protection agency (EPA) registration review), Starbar E-PRO (36.8% permethrin, EPA Reg. No. 89459–66), and Prolate Lintox-HD (11.75% phosmet, EPA Reg. No. 2724-262) were used in a spray bioassay and Y-TEX Brute (10% permethrin, EPA Reg. No. 3039-7), Y-TEX Gardstar EC (40% permethrin, EPA Reg. No. 3039-8), Martin's FLY-BAN (7.4% permethrin, EPA Reg. No. 53883-74), Martin's Permethrin 1.0% (1.0% permethrin, EPA Reg. No. 53883-76), and Martin's Permethrin 1.0% Synergized (1.0% permethrin, 1.0% piperonyl butoxide, EPA Reg. No. 53883-75) were used in a dip bioassay. Acaricides, concentrations, dose on label or proposed label, and amount of acaricide used per treatment to expose ticks are shown in Table 1. Each of the treatments and an untreated control (no acaricide, but ticks were handled in the same manner) had five replicates with 10 nymphs each in each bioassay method (one experimental unit was 10 nymphal ticks).

Spray Application of Acaricides

Nymphs were held at room temperature in cardboard containers with a volume of 56.75 cm³. A 1 ml pipette (Rainin, San Diego, CA) was used to dispense mixed products into the reservoir of an air-brush (Paasche model D500SR-50, Kenosha, WI) that was used to spray the entire contents of each product onto an experimental unit (10 nymphal ticks in the cardboard cup). Application rates of each product were rate equivalents to each product being sprayed over the area of a 1,000 lb animal (Berman 2003). Label-recommended application rates were scaled from the size of 1,000 lb animal with a 4.57 m² surface area to the size of 56.75 cm² paper cups used to spray ticks as suggested by label rates.

After 10 min of exposure to each treatment, ticks were transferred from treated containers to clean petri dishes (100 mm diameter) using separate live insect forceps for each treatment and control. Two replicates for each product were performed on 15 September 2020 and the remaining three replicates were performed on 25 September 2020. Between treatments, the air-brush gun was rinsed by flushing it with 20 ml of tap water into a beaker

Table 1. Trade names, active ingredients with concentrations, application rate, dilution concentration, and the amount of product used in each treatment for acaricides used in bioassay trials to test the efficacy on Asian longhorned tick (*Haemaphysalis longicornis*) nymphs

Trade name	Active ingredients	Label application rate	Product type	Dilution concentration	Volume applied per replicate (ml)
RF2301 LPD PO	0.5% lambda-cyhalothrin 2.5% piperonyl butoxide 3% diflubenzuron	3.0 ml/100 lb	Spray	3.2%	1.212
StarBar E-PRO	36.8% permethrin	1 quart diluted product (0.05%) per animal	Spray	0.14%	1.175
Prolate Lintox-HD	11.75% phosmet	1 quart diluted product (0.01%) per animal	Spray	0.43%	1.175
Y-TEX Brute	10% permethrin	3.0 ml/200 lb	RTU pour-on	NA	1.175
Y-TEX GardStar EC	40% permethrin	1–2 quarts per animal (0.05%)	Spray	0.125%	1.175
Martin's FLY-BAN	7.4% permethrin	15 ml/100 lb	RTU pour-on	NA	1.175
Martin's Permethrin 1.0%	1.0% permethrin	15 ml/100 lb	RTU pour-on	NA	1.175
Martin's Permethrin 1.0% Synergized	1.0% permethrin 1.0% piperonyl butoxide	15 ml/100 lb	RTU pour-on	NA	1.175

RTU, ready-to-use; NA, not applicable.

and the reservoir was thoroughly cleaned with tap water and dish detergent and rinsed six times to ensure that no chemical residue remained.

Dip Application of PO Acaricides

Ready-to-use products were applied undiluted to represent an assumed maximum exposure that would be encountered by the ticks on a treated bovine in the field. Treatments were applied in October 2020. For each treatment, 10 nymphs were individually dipped into plastic disposable cups filled with the measured treatment volume (Table 1) for 60 s and immediately transferred to a product-dedicated petri dish (100 mm diameter) following previously published procedures (Lee et al. 2015). Ten ticks per replicate were handled with live insect forceps and a different pair of forceps was used for each of the five treatments.

Tick Mortality Assessment

Mortality in each replicate was assessed at 0, 1, 2, 3, 4, 21, 24, and 48 h post-treatment. Mortality was verified using a light microscope by gently prodding individual ticks with forceps. Mortality was defined as ticks not moving 1 min after stimulation. Live ticks were defined as ambulatory or moving any portion of their body when viewed under a light microscope.

Data Analyses

The experimental unit was the individual unit (10 nymphs). Separate statistical tests were conducted for each bioassay (spray and dip). Repeated measures analysis of variance (ANOVA) using PROC GLIMMIX with a Poisson distribution and a two-tailed analysis ($\alpha = 0.05$) was conducted separately for spray and dip treatments using SAS (ver. 9.4, Cary, NC). Treatment, time, and their interaction were used as main effects.

Results

Spray Application of Acaricides

No mortality occurred in the untreated control and all test materials caused 100% mortality within 24 h of exposure (Table 2). Treatment ($F = 389.51$; $df = 3, 16$; $P < 0.0001$), time ($F = 223.23$; $df = 7, 112$; $P < 0.0001$), and the interactive effects were significant ($F = 39.77$; $df = 21, 112$; $P < 0.0001$). There was no significant difference in efficacy between RF2301 LPD PO and StarBar E-PRO ($P = 1.00$), but Prolate Lintox-HD took significantly more time to cause 100% mortality than the RF2301 LPD PO and StarBar E-PRO treatments ($P < 0.0001$).

At 1 h post-treatment exposure, RF2301 LPD PO and StarBar E-PRO caused 100% mortality. Only 44% of the nymphs sprayed with Prolate Lintox-HD died after 1 h, but percent mortality increased over time and was 100% by 24 h post-treatment.

Dip Application of PO Acaricides

No mortality occurred in the untreated control and all test materials caused 100% mortality within 1 h after treatment in nymphal *H. longicornis* and were significantly different from the untreated control ($F = 317.80$; $df = 5, 24$; $P < 0.0001$) (Table 3). There were statistically significant differences between time points or mortality on ticks ($F = 162.14$; $df = 7, 203$; $P < 0.0001$), such that ticks exposed to any of the products were dead 1 h after exposure. While products significantly killed more ticks than the control ($P < 0.0001$) and across time ($P < 0.0001$), there were no differences in the ability

Table 2. Mean percent mortality \pm standard deviation for spray products applied to Asian longhorned tick (*Haemaphysalis longicornis*) nymphs at 0, 1, 2, 3, 4, 21, 24, and 48 h post exposure

Trade name	Mean % tick mortality \pm standard deviation							
	0 h ^P	1 h ^C	2 h ^C	3 h ^{BC}	4 h ^{BA}	21 h ^A	24 h ^A	48 h ^A
RF2301 LPD PO	0.00 \pm 0.000 ^P	100.00 \pm 0.000 ^A	—	—	—	—	—	—
StarBar E-PRO	0.00 \pm 0.000 ^P	100.00 \pm 0.000 ^A	—	—	—	—	—	—
Prolate Lintox-HD	0.00 \pm 0.000 ^P	44.00 \pm 20.73 ^C	56.00 \pm 31.30 ^C	72.00 \pm 25.88 ^C	92.00 \pm 13.03 ^B	96.00 \pm 8.94 ^{AB}	100.00 \pm 0.000 ^A	—
Control	0.00 \pm 0.000 ^P	0.00 \pm 0.000 ^P	0.00 \pm 0.000 ^P	0.00 \pm 0.000 ^P	0.00 \pm 0.000 ^P	0.00 \pm 0.000 ^P	0.00 \pm 0.000 ^P	0.00 \pm 0.000 ^P

Times with similar letter groupings represent no difference, while different letter groupings signify differences in the time for products to control ticks. Mean \pm standard deviation with similar letter groupings represents no difference, while different letter groupings signify differences in time and product interactions.

Table 3. Mean percent mortality \pm standard deviation for pour-on products applied as a dip to Asian longhorned tick (*Haemaphysalis longicornis*) nymphs at 0, 1, and 48 h post exposure

Trade name	Mean % tick mortality \pm standard deviation		
	0 h	1 h	48 h
Y-TEX Brute	0.00 \pm 0.000	100.00 \pm 0.000	100.00 \pm 0.000
Y-TEX GardStar EC	0.00 \pm 0.000	100.00 \pm 0.000	100.00 \pm 0.000
Martin's FLY-BAN	0.00 \pm 0.000	100.00 \pm 0.000	100.00 \pm 0.000
Martin's Permethrin 1.0%	0.00 \pm 0.000	100.00 \pm 0.000	100.00 \pm 0.000
Martin's Permethrin 1.0% Synergized	0.00 \pm 0.000	100.00 \pm 0.000	100.00 \pm 0.000
Control	0.00 \pm 0.000	0.00 \pm 0.000	0.00 \pm 0.000

of each product to control ticks or in the amount of time that it took for ticks to die when exposed to each product.

Discussion

With the recent invasion and establishment of *H. longicornis* in the United States, this may be the first published report to show existing cattle products in the United States that can control this tick. All test materials caused 100% mortality in *H. longicornis* nymphs within 24 h of exposure. These products will be an important tool for limiting tick abundance and preventing initial tick establishment at new sites, since populations should not be able to disperse or feed and then establish from treated hosts (Frisch 1999; Barré and Uilenberg 2010; Benelli et al. 2016). On-host acaricides have not been extensively evaluated yet for *H. longicornis* collected in the United States for on-animal control (Cisak et al. 2012; Schappach et al. 2020), but on-animal testing is the next logical step to confirm our results.

Permethrin-based products are commonly used in North America for tick control (Mencke 2006; Mitchell et al. 2020). The six permethrin products evaluated in this study all caused 100% mortality within 1 h of exposure. Permethrin products have been used in the Texas Cattle fever tick eradication program to treat white-tailed deer (Currie et al. 2020); however, in the United States there are permethrin-resistant *R. sanguineus* (Latreille) (Acari: Ixodidae) and *R. microplus* populations (Miller et al. 2007; Eiden et al. 2015). It will be imperative to monitor these ticks for permethrin resistance.

In the present study, Prolate Lintox-HD (11.75% phosmet) caused 100% *H. longicornis* mortality 24 h after exposure, which is similar to previous reports where *R. microplus* were killed on cattle with a PO formulation of phosmet at 40 mg/kg and produced 93–99% control of ticks after exposure (Loomis et al. 1972) and *Amblyomma americanum* (L.) (Acari: Ixodidae) on canines with infused-collars (Koch and Popham 1987). Although Prolate Lintox-HD didn't work as quickly as the other products in this study, its ability to cause 100% mortality within 24 h indicated that it is an effective tick control product and can be used by producers.

Though no direct published comparisons exist for RF2301 LPD PO, the active ingredients evaluated in this laboratory study produced similar results to previous field studies. Lambda-cyhalothrin was applied to vegetation in a grassland habitat in Korea and resulted in 100% *H. longicornis* mortality 3 d post-treatment (Park et al. 2019). In addition, lambda-cyhalothrin applied to vegetation at an *H. longicornis*-infested site in New Jersey (United States) effectively controlled multiple life stages (Bickerton et al. 2021).

Integrated pest management (IPM) strategies for *H. longicornis* and other economically and medically important tick species still need to be developed. For example, up to 96% of lone star tick control can occur with proper habitat management, acaricide application, and host management (Bloemer et al. 1990). Reliance on

repeated acaricide use has resulted in tick resistance to several active ingredients (George et al. 2004). Some degree of resistance to active ingredients used in this study has been reported for different tick species (Roulston et al. 1977; Eiden et al. 2015; Ziapour et al. 2016); thus, it is imperative to periodically test the resistance of field strains of *H. longicornis* on commonly used products.

Field-collected *H. longicornis* nymphs were highly susceptible to the products evaluated in this study. While there was a significant time effect noted in this study, knowing that all ticks exposed to all products were dead within 24 h is the most important information to convey to producers. This finding could help explain why *H. longicornis* are found sporadically on farms with different management practices as well as on untreated animals (e.g., dog from an animal shelter). Future investigations should evaluate the effectiveness of commercially available products on hosts located in infested pastures over time. Additionally, knowing if *H. longicornis* is susceptible to available products can serve as the first step in building an IPM plan for controlling *H. longicornis* on livestock and to prevent their further spread and establishment.

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