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Determination of the Discriminating Concentration Towards Permethrin for Surveying Resistance in *Amblyomma americanum*

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Subject Editor: Dina Fonseca

Abstract

Amblyomma americanum Linnaeus (Ixodida: Ixodidae) is ubiquitously present throughout the southeastern United States and is capable of vectoring several pathogens. White-tailed deer are the main host for adult *A. americanum*. However, this tick species is a generalist that will feed on most vertebrates, including humans, deer, livestock, and pets. Management of this species can be challenging due to a lack of cost-effective strategies. Acaricides are often utilized, however, this may lead to pesticide resistance. The Food and Agriculture Organization of the United Nations (FAO) larval packet test (LPT) was performed on susceptible *A. americanum* to determine the lethal concentration (LC) and discriminating concentration (DC) values for permethrin. The FAO LPT was used at these pre-established values to compare levels of resistance in ticks collected from a captive deer farm and wild areas representing high and low permethrin exposure settings, respectively. Resistance ratios (RR) calculated from the LC values for the ticks collected from farmed and wild deer ranged between 1 and 2. *A. americanum* collected from farmed and wild deer were not found to be resistant, however, some samples had slightly elevated RRs as compared to the susceptible laboratory strain, which may suggest tolerance development. Although the *A. americanum* sampled in this study were not resistant to permethrin, the DC calculated in this study will allow for rapid evaluation of resistance in a permethrin resistance monitoring program such that alternate management strategies can be adopted if resistance is detected.

Key words: lone star tick, acaricide resistance, cervid, larval packet test

The lone star tick, *Amblyomma americanum* Linnaeus (Ixodida: Ixodidae), is a commonly encountered tick species throughout the southern United States that has impacts on humans, companion animals, and livestock. Lone star ticks are competent vectors of several *Rickettsia* species, *Francisella tularensis* (McCoy & Chapin; Thiotrichales: Francisellaceae), an important human pathogen that is the causative agent of tularemia, as well as *Ehrlichia chaffeensis* Anderson (Rickettsiales: Anaplasmataceae) and *Ehrlichia canis* Donatien & Lestoquard (Rickettsiales: Anaplasmataceae), which cause disease in humans and dogs, respectively (Goddard and Varela-Stokes 2009). Feeding activity of lone star ticks has been estimated to reduce productivity in purebred cattle, whereby the impact of 40 continuously feeding female ticks across a 100 day tick season could reduce weight gain by 37 kg (Ervin et al. 1987). However, the

preferred host for all life stages of the lone star tick are white-tailed deer. The captive cervid industry in Texas had a direct economic impact of $893.5 million in 2007 (Anderson et al. 2007), with considerable growth in the intervening years. Therefore, if weight gain and performance are similarly affected in cervids as they are in bovids, this tick species presents an economic risk to this emerging industry.

There are a variety of management strategies that are implemented to reduce tick population densities, including the use of acaricides and landscape management (Pérez de León et al. 2014). Chemical tools are commonly utilized for tick management due to consistent results, ease of application, and relatively low cost (Stafford III 2007). Permethrin, which is frequently used in tick management programs, is registered for use in mosquito control, as well as managing pests on domestic pets, livestock, and agricultural commodities (EPA 2011). Approximately

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1,356,000 kg of permethrin are applied annually in the United States for use in the public health, residential, and agricultural sectors (EPA 2011). Permethrin is a synthetic compound created to emulate naturally occurring pyrethrins, which are compounds produced in *chrysanthemum* flowers (Asterales: Asteraceae) (Davies et al. 2007). Permethrin and pyrethrins act by disrupting the nervous system of arthropods by preventing the depolarization of voltage-gated sodium channels and causing hyperexcitation of the nerve (Davies et al. 2007). Permethrin can be applied as a residual spray product for homeowners to apply on residential land or to treat wildlife hosts through passive delivery systems, such as bait stations (Stafford III 2007, Pérez de León et al. 2014). Permethrin resistance has been documented in *Rhipicephalus microplus* Canestrini (Ixodida: Ixodidae) (Miller et al. 2007) and *Rhipicephalus sanguineus* Latreille (Ixodida: Ixodidae) (Eiden et al. 2015), but not in *A. americanum* to date.

Permethrin is one of a few commonly utilized acaricides in tick management programs. In Florida, pyrethroids including permethrin are the most frequently applied chemicals for pest management on deer farms (Harmon et al. 2020). The first indication of pesticide resistance often is observed as the failure of treatments to effectively manage the pest. If tools to screen populations for resistance existed, then actions could be taken before full treatment failure occurs, allowing the mitigation of resistance development. Once calculated, the discriminating concentration (DC), which is two times the concentration that would kill 99% of ticks susceptible to that pesticide, could be implemented as a screening mechanism because it can be used quickly and efficiently to test for resistance in small samples of the population (FAO 2004).

The purpose of this study was to determine the DC for permethrin in susceptible lone star ticks and evaluate it against field-collected *A. americanum*. Engorged, female ticks were collected from deer ranging on sites with high exposure to pesticides (on deer farms) and perceived low exposure to pesticides (wild areas) and the emergent larvae were screened for permethrin resistance at the lethal concentration (LC) and DC values.

Materials and Methods

Susceptible Colony Ticks

Larvae, which hatched from eggs laid by pesticide susceptible female *A. americanum*, were used to determine the LC and DC values. Pesticide susceptible engorged/gravid female *A. americanum* were purchased from Oklahoma State University (OSU, Stillwater, OK). The OSU laboratory colony was established in 1976 and wild ticks were introduced to the colony every year. The wild ticks were collected annually from the same cattle herd that is not treated with any pesticides. After collection and before introduction to the colony the ticks are tested for the presence of pathogens. At OSU, the laboratory colony ticks were held at approximately 23°C, 90% relative humidity, under a 15:9 (L:D) hour light cycle and fed on rabbits and sheep. Upon receipt to the University of Florida (UF), each engorged female tick was placed into 26 ml (7 dram) glass vial, the opening of the vial was covered with a piece of organdy fabric and a plastic cap, which was modified by replacing the center of the cap with metal screening to allow ventilation. Tick-containing vials were stored in an incubator at 25°C, 92% relative humidity and under a 12:12 (L:D) hour light cycle to promote oviposition. After eggs were laid and had begun to lighten in color, which signified readiness to hatch, the dead females were removed. Larvae were exposed to permethrin 14 to 16 d after hatching.

Field-Collected Ticks

Ticks tested in this study were obtained either directly from live or dead white-tailed deer or from environmental sampling using tick drags or CO_2-baited traps. Engorged, female *A. americanum* were collected from white-tailed deer *Odocoileus virginianus* (Zimmermann; Artiodactyla: Cervidae), located on a single farm (UF IACUC #201609412) and in wildlife management areas (UF IACUC #201508838), from October to December 2017 and from March to October 2018. Additional farm-sourced ticks were obtained in 2018 as flat adults by dragging in tick habitat at this site and brought into the laboratory where they were subsequently reared on cattle (UF IACUC #201702142) to increase the number of larvae for resistance screening. Tick collections were performed at two additional deer farm sites by dragging and use of CO_2-baited traps between March and July 2020. In all three farms, deer were routinely treated with oral ivermectin for parasite control. Deer on the Alachua County farm received no more anti-parasitic interventions. In the Gadsden County farm, a permethrin plus piperonyl butoxide solution was applied as a mist daily to areas with penned animals and weekly in free-roaming areas throughout the summer. In the Levy County farm, a fog formulation of permethrin and piperonyl butoxide was applied 1–2 times a month.

Ticks collected from wild deer were collected from hunter-harvested deer at wildlife check stations located in Joe Budd Wildlife Management Area, Gadsden County during May through August 2017, and February through March 2018 and from Florida Fish and Wildlife Conservation Commission (FFWCC) agents from deceased deer submitted to the FFWCC station in Gainesville, Florida throughout 2017 and 2018. County information was collected for deceased deer to allow for resistance comparisons by county.

Female ticks were placed into individual 26 ml (7 dram) glass vials for oviposition as previously described. Newly hatched larvae were maintained under the same parameters mentioned previously for 14 to 16 d posthatch before use in bioassays.

Chemicals and Concentrations

A series of bioassays were conducted with technical grade permethrin (98.8%, 40.1:58.7 cis:trans, ChemService Inc., West Chester, PA) diluted in trichloroethylene (Sigma-Aldrich, St. Louis, MO) and olive oil (Spectrum, Gardena, CA) to optimize a range of concentrations that would cause 25–99% mortality in susceptible *A. americanum*. Initial chemical concentrations for these packets were based on previous work with *R. sanguineus* performed by Eiden et al. (2015). Based on preliminary tests, the highest concentration tested was 1.50% with a 40% dilution strategy yielding 0.90%, 0.54%, 0.32%, and 0.19%. The 1.50% stock solution was freshly prepared for each replicate. This enabled the calculation of the LC values at 50%, 90%, and 99% mortality, where each respective value represents the concentration needed to kill that percentage of the sample, and the DC.

Permethrin concentrations, percentage of active ingredient (w/v), that were used to screen ticks from wild and farmed deer were based on those calculated from the pesticide susceptible ticks and consisted of the control (0.00%), LC_{90} (1.11%), the LC_{99} (2.27%), the DC (4.54%), and two times the DC (9.08%). These dilutions were prepared from a stock solution of 9.08%, which was prepared fresh for every replicate.

Bioassay

The Food and Agriculture Organization of the United Nations (FAO) larval packet test (LPT) procedure, as described in FAO (2004) and Kaplan et al. (2020), was used to determine the DC for permethrin. Permethrin was added to a 50 mL flask. Two parts trichloroethylene and one part olive oil were added in sequence, vortexing after each addition. The control solution contained 2:1 trichloroethylene to olive oil with no pesticide added. For each concentration and control,

1 ml of solution was applied to four pieces of chromatography paper (7.6 × 8.9 cm; Whatman, Maidstone, UK), starting from the lowest to the highest concentration. The treated papers were suspended and left to dry in a fume hood for two hours. Packets were formed by folding the treated papers in half, treated side inwards, and sealing the side edges with Boston Number 2 bulldog clips (Sparco, Atlanta, GA).

A vial containing 14–16 d old larvae was opened inside double containment, approximately 100 larvae were added to each packet. The open edge of the packets was then closed with a third bulldog clip and the packet was suspended on a metal rod on a wooden rack until all the packets were filled. Once filled, the packets were placed inside an aquarium with a saturated potassium nitrate solution covering the bottom to achieve 92% relative humidity inside an incubator set at 25°C, for 24 h.

The packets were removed from the incubator after 24 h and individually opened over a lightbox. A modified Pasteur pipette attached to a vacuum pump was used to collect live larvae from the packets. The Pasteur pipettes were modified by covering the wide end of the pipette with a piece of organdy fabric attached with masking tape. Larvae were considered dead after displaying no response to exposure to human breath twice and being disturbed with a paint brush twice as described by Kaplan et al. (2020). The number of dead and live ticks were recorded in each packet at each concentration.

Statistics

To determine the LC values for permethrin from an acaricide susceptible colony, analysis of the mortality data was completed using the software PoloPlus (LeOra Software Company, Petaluma, CA). Control mortality was not corrected and was used to calculate the natural response slope and standard error. For each chemical, concentration-response curves were generated and the LC values at which 50%, 90%, and 99% of the treated ticks died (LC_{50}, LC_{90},

and LC_{99}, respectively) were calculated along with the corresponding 95% confidence intervals for each LC value. The discriminating concentration (DC) was calculated from the LC_{99}.

The ticks collected from farmed and wild deer were tested at the LC and DC values calculated using the pesticide susceptible ticks. The mortality data from these assays were used to calculate resistance ratios and to establish LC values for these field-collected ticks as described above. The LC values between different groups were compared by confidence intervals; values with non-overlapping confidence intervals were considered to be different. Resistance ratios (RR) for the LC_{50}, LC_{90}, and LC_{99} were calculated by dividing the respective value for the field-collected ticks by the corresponding LC values for the pesticide susceptible tick strain (RR_{50}, RR_{90}, and RR_{99}, respectively). The differences in magnitude of the RR for ticks collected from farmed and wild deer were compared. Ticks were scored as susceptible if the RR was less than two, tolerant if their RR was between two and 10, and resistant if the RR was greater than 10. Mortality data collected over the three seasons, 2017, 2018, and 2020 were pooled when calculating the LC values to better represent each treatment group (farm vs wild).

The LC and RR values of ticks collected from the three farms were compared. Additionally, LC and RR values from ticks collected from the deer submitted to the FFWCC were compared by county using the same procedure as mentioned previously.

Results

Over 20,000 pesticide susceptible larvae were tested in 10 concentration finding bioassays to calculate the LC values and the DC for permethrin. The LC_{50}, LC_{90}, LC_{99}, and DC values for permethrin were 0.46%, 1.11%, 2.27%, and 4.54% for the OSU colony ticks (Table 1). These values were used to compare permethrin sensitivity

Table 1. Calculations for the lethal concentration (LC) values for pesticide susceptible lone star tick larvae challenged against permethrin at each larval packet test

Test	n	slope (SE)	LC_{50} (95% CI)	LC_{90} (95% CI)	LC_{99} (95% CI)	X^2 (df)	DC
1	1,292	2.305 (0.188)	0.337% (0.176–0.581%)	1.211% (0.683–3.919%)	3.440% (1.535–24.978%)	131.830 (18)	6.880
2	1,609	3.234 (0.162)	0.686% (0.511–0.969%)	1.709% (1.167–3.315%)	3.587% (2.114–9.773%)	182.170 (18)	7.174
3	2,793	5.082 (0.183)	0.570% (0.527–0.616%)	1.019% (0.916–1.166%)	1.635% (1.395–2.023%)	78.813 (18)	3.270
4	2,587	3.518 (0.123)	0.551% (0.501–0.608%)	1.257% (1.086–1.521%)	2.461% (1.962–3.342%)	88.696 (18)	4.922
5	1,930	3.251 (0.139)	0.413% (0.337–0.450%)	1.023% (0.900–1.199%)	2.144% (1.748–2.791%)	42.141 (18)	4.288
6	1,369	1.894 (0.145)	0.368% (0.234–0.504%)	1.746% (1.063–5.989%)	6.215% (2.619–62.777%)	153.340 (18)	12.430
7	2,789	3.568 (0.120)	0.427% (0.399–0.456%)	0.975% (0.885–1.094%)	1.914% (1.646–2.299%)	38.854 (18)	3.828
8	2,308	1.885 (0.078)	0.344% (0.294–0.407%)	1.648% (1.236–2.429%)	5.907% (3.745–11.107%)	102.070 (26)	11.814
9	1,492	1.709 (0.110)	0.109% (0.079–0.138%)	0.612% (0.450–0.971%)	2.499% (1.440–6.118%)	93.437 (26)	4.998
10	2,484	3.056 (0.317)	0.258% (0.208–0.288%)	0.677% (0.565–0.986%)	1.487% (1.012–3.383%)	76.178 (26)	2.974
Pooled	20,653	3.060 (0.061)	0.460% (0.424–0.496%)	1.100% (1.011–1.225%)	2.270% (1.964–2.713%)	2,149.400 (213)	4.540

The discriminating concentration (DC) is calculated as two times the LC_{99}.

Lethal concentration (LC) determined using Probit analysis. Values represent percentage of active ingredient (w/v) applied to treated filter papers 7.5 × 8.5 cm²·

n, number of larvae tested; SE, standard error; CI, confidence interval; df, degrees of freedom.

Table 2. Assessment of potential tolerance development towards permethrin in Florida *Amblyomma americanum*

Ticks	n	Slope (SE)	LC$_{50}$ (95% CI)	LC$_{90}$ (95% CI)	LC$_{99}$ (95% CI)	X^2 (df)	RR$_{50}$[a]	RR$_{90}$[a]	RR$_{99}$[a]
Susceptible	20,653	3.060 (0.061)	0.460% (0.424–0.496%)	1.10% (1.011–1.225%)	2.27% (1.964–2.713%)	2,149.40 (213)	n/a	n/a	n/a
Deer farm[b]	28,795	2.453 (0.050)	0.513% (0.457–0.567%)	1.71% (1.601–1.834%)	4.56% (4.021–5.285%)	1,406.40 (246)	1.12	1.55	2.01
Wild deer[c]	25,481	3.528 (0.097)	0.750% (0.622–0.854%)	1.73% (1.588–1.920%)	3.42% (2.886–4.391%)	3,110.00 (214)	1.63	1.57	1.51

The lethal concentration (LC) values were based on pooled mortality data collected from exposing the deer farm and wild ticks to permethrin concentrations calculated from the pesticide susceptible tick colony in the larval packet test.

[a]RR calculated as the LC value of a field-collected sample divided by the respective LC value of the pesticide susceptible colony.

[b]Pooled data from ticks collected off deer living on farms in Alachua, Gadsden, and Levy Counties from 2017 to 2020.

[c]Pooled data from ticks collected off wild deer from Gadsden, Lake, Sumter, and Volusia Counties during 2017 and 2018.

Lethal concentration (LC) determined using Probit analysis. Values represent percentage of active ingredient (w/v) applied to treated filter papers 7.5 × 8.5 cm^2.

n, number of larvae evaluated; SE, standard error; df, degrees of freedom.

between *A. americanum* collected from captive deer on farms to ticks collected from wild deer. Resistance ratios at the RR$_{50}$, RR$_{90}$, and RR$_{99}$ for ticks collected from farmed deer were 1.12, 1.55, and 2.01, respectively. In ticks sampled from wild deer, the RR$_{50}$, RR$_{90}$, and RR$_{99}$ values generated were 1.63, 1.52, and 1.51, respectively (Table 2). Overall, field-collected ticks displayed slightly less sensitivity towards permethrin compared to the pesticide-susceptible strain represented by non-overlapping 95% confidence intervals. Between field samples, there were no differences in the LC values of ticks collected from farmed deer and wild deer (Fig. 1).

In 2017 and 2018, ticks were sampled from the same farm site in Gadsden County; however, these data were pooled as no differences were found between permethrin LC values of ticks sampled in the two years (based on overlapping confidence intervals, data not shown). Ticks collected on the farm site in Gadsden County were not less sensitive to permethrin than the pesticide susceptible strain (Table 3). In 2019, ticks were sampled from two additional farms in Alachua and Levy County. Ticks from Alachua and Levy County were less susceptible to permethrin compared to the pesticide susceptible strain, at the RR$_{99}$ with values of 2.04 and 2.34, respectively (Table 3).

Larvae of 14 female ticks collected from deceased deer submitted to FFWCC were screened for acaricide resistance. The permethrin LC values in the 2018 field season for ticks collected from wild deer were different to the pesticide susceptible colony at the LC$_{50}$ and LC$_{90}$, which corresponded with RR$_{50}$ and RR$_{90}$ of 1.72 and 1.56, respectively (Table 4). The RR$_{50}$, RR$_{90}$, and RR$_{99}$ values for ticks collected from deceased deer originating in Gadsden, Lake, and Sumter Counties corresponded with values ranging between 1 and <2 (Table 3). However, ticks collected from Volusia County demonstrated lower sensitivity to permethrin, with a RR$_{50}$ of 2 (Table 4).

Discussion

Permethrin is a commonly utilized chemical tool for the control of many arthropod pests, including ticks. Permethrin use has documented success against ticks as a repellent and toxicant (Miller et al. 2011, Prose et al. 2018) and is recommended by the U.S. Centers for Disease Control and Prevention to prevent tick bites (CDC 2020). For example, in a study investigating permethrin-treated clothing worn by volunteers that walked through pre-determined tick habitat, treatment provided almost 100% protection against *A. americanum* feeding (Schreck et al. 1982). The current study has determined the
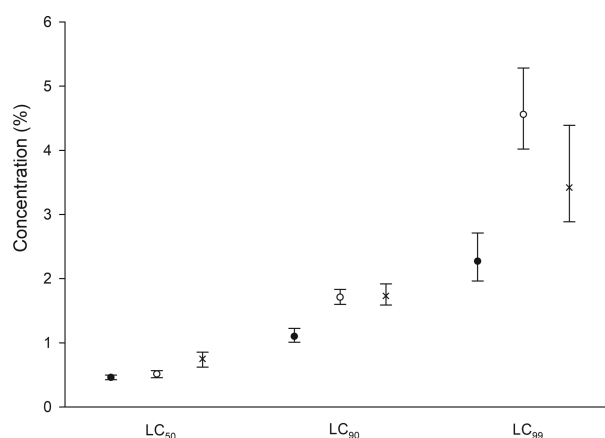

Fig. 1. Comparison of the lethal concentration (LC) values across the *Amblyomma americanum* tested from the pesticide susceptible colony (closed circle), ticks collected from deer farms (open circle), and ticks collected from wild deer (cross) between 2017 and 2020. The LC values screened against the two field groups were originally calculated from the concentration finding bioassays performed on pesticide susceptible ticks purchased from Oklahoma State University. Error bars represent 95% confidence intervals.

DC for permethrin in pesticide susceptible lone star ticks and evaluated it against *A. americanum* collected from deer ranging on sites with high exposure to pesticides (on deer farms) and perceived low exposure to pesticides (wild areas).

Pesticide susceptible lone star ticks were moderately sensitive to permethrin compared to the LC value described for fipronil (Kaplan et al. 2020), another pesticide that *A. americanum* might have exposure to through chemical pest control on domestic pets. Previous research supports a greater sensitivity to fipronil than permethrin but to varying degrees by species (see Table 5). In the current study, the LC values for laboratory-reared *A. americanum* towards permethrin were 0.46% at 50% mortality and 2.27% at 99% mortality, which is roughly 100-fold less sensitive than the values calculated in an earlier study for fipronil (LC$_{50}$: 0.00454% and LC$_{99}$: 0.01040%) using the same colony (Kaplan et al. 2020). In an earlier study using the same colony (OSU), toxicity of permethrin against adult lone star ticks was similar to that observed in larvae in this study, although dose-response curves could not be calculated, 50% mean mortality was observed between 0.19% and 0.39% permethrin (Machtinger and Yi 2017). White et al. (2004) reported LC$_{50}$ values

Table 3. Assessment of potential tolerance development towards permethrin in Florida *Amblyomma americanum* collected from deer farms

Ticks	n	Slope (SE)	LC$_{50}$ (95% CI)	LC$_{90}$ (95% CI)	LC$_{99}$ (95% CI)	X^2 (df)	RR$_{50}$[a]	RR$_{90}$[a]	RR$_{99}$[a]
Susceptible	20,653	3.060 (0.061)	0.460% (0.424–0.496%)	1.100% (1.011–1.225%)	2.270% (1.964–2.713%)	2,149.4 (213)	n/a	n/a	n/a
Gadsden	17,122	2.894 (0.101)	0.607% (0.480–0.716%)	1.683% (1.011–1.857%)	3.864% (3.325–4.984%)	1,122.3 (141)	1.32	1.53	1.70
Levy	5,797	2.467 (0.084)	0.607% (0.534–0.678%)	2.008% (1.806–2.269%)	5.324% (4.423–6.699%)	194.4 (63)	1.31	1.83	2.34
Alachua	5,970	2.078 (0.094)	0.352% (0.251–0.446%)	1.455% (1.234–1.771%)	4.631% (3.431–7.190%)	201.5 (38)	0.77	1.32	2.04

The lethal concentration (LC) values were based on pooled mortality data collected from exposing ticks to permethrin concentrations calculated from the pesticide susceptible tick colony in the larval packet test.

Lethal concentration (LC) determined using Probit analysis. Values represent percentage of active ingredient (w/v) applied to treated filter papers 7.5 × 8.5 cm^2.

[a]RR calculated as the LC value of a field-collected sample divided by the respective LC value of the pesticide susceptible colony.

n, number of larvae evaluated; SE, standard error; df, degrees of freedom.

Table 4. Assessment of potential tolerance development towards permethrin in Florida *Amblyomma americanum* collected from wild deer

Ticks	n	Slope (SE)	LC$_{50}$ (95% CI)	LC$_{90}$ (95% CI)	LC$_{99}$ (95% CI)	X^2 (df)	RR$_{50}$[a]	RR$_{90}$[a]	RR$_{99}$[a]
Susceptible	20,653	3.060 (0.061)	0.460% (0.424–0.496%)	1.100% (1.011–1.225%)	2.270% (1.964–2.713%)	2,149.4 (213)	n/a	n/a	n/a
Gadsden Co.	4,762	2.921 (0.215)	0.569% (0.182–0.829%)	1.562% (1.216–2.158%)	3.559% (2.449–11.018%)	545.7 (41)	1.24	1.42	1.42
Lake Co.	6,702	3.572 (0.219)	0.668% (0.544–0.768%)	1.542% (1.429–1.687%)	3.049% (2.596–3.877%)	135.1 (46)	1.45	1.40	1.24
Sumter Co.	3,945	2.939 (0.267)	0.590% (0.479–0.686%)	1.610% (1.495–1.747%)	3.650% (3.121–4.538%)	25.5 (30)	1.28	1.46	1.61
Volusia Co.	10,964	4.448 (0.159)	0.946% (0.848–1.027%)	1.836% (1.696–2.036%)	3.153% (2.713–3.914%)	566.4 (78)	2.00	1.67	1.39

The lethal concentration (LC) values were based on pooled mortality data collected from exposing ticks to permethrin concentrations calculated from the pesticide susceptible tick colony in the larval packet test.

Ticks collected from Gadsden Co. were collected from hunter-harvested deer on wildlife management areas during 2017 and 2018. Ticks collected from the remaining counties were from deceased deer that were submitted to the Florida Fish and Wildlife Conservation Commission in Gainesville, Florida.

Lethal concentration determined using Probit analysis. Values represent percentage of active ingredient (w/v) applied to treated filter papers 7.5 × 8.5 cm^2.

[a]RR calculated as the LC value of a field-collected sample divided by the respective LC value of the pesticide susceptible colony.

n, number of larvae evaluated; SE, standard error; df, degrees of freedom.

Table 5. Lethal concentration to 50% (LC$_{50}$) and 99% (LC$_{99}$) mortality in previous studies as compared to the current study for permethrin and fipronil using the larval packet test

Species	Permethrin		Fipronil		Reference
	LC$_{50}$	LC$_{99}$	LC$_{50}$	LC$_{99}$	
Amblyomma americanum	0.46%	2.27%	0.00454%	0.01040%	Kaplan et al. 2020
Amblyomma americanum	0.0033%[a]	NC	0.0029%[a]	NC	White et al. 2004
Amblyomma americanum	0.000031%	NC	NC	NC	Koch and Burkwhat 1984
Rhipicephalus sanguineus	0.027%	0.093%	0.023%	0.074%	Eiden et al. 2015
Rhipicephalus sanguineus	0.000045%	NC	NC	NC	Koch and Burkwhat 1984
*Ixodes scapularis**	0.0162%	0.0808%	NC	NC	Burtis et al. 2021

NC, not calculated.

[a]values reported as EC$_{50}$ not LC$_{50}$, but mortality was measured.

*Say (Ixodida: Ixodidae).

for a susceptible *A. americanum* colony for permethrin that was equivalent to 0.0033% (84.53 µM) active ingredient. These values were 140-fold less than the value reported in this study. Comparably, a susceptible colony of *R. sanguineus* had LC$_{50}$ values to permethrin of 0.027% (LC$_{99}$: 0.093%) (Eiden et al. 2015), which was approximately 17-fold lower than the value reported in this study. The difference in permethrin sensitivity between susceptible colonies of both the same and different species suggests a lower sensitivity to

permethrin in the *A. americanum* colony tested in the current study than in previous research.

Previous baseline sensitivity data for the lone star tick towards permethrin that would cause mortality in 50% and 90% of the tick sample were 0.00017% and 0.00035%, respectively, using single field-collected permethrin-unexposed nymphs in a treated pipet tip (Barnard et al. 1981). Endris et al. (2003) evaluated two formulations of 65% permethrin diluted in diethylene glycol monomethyl ether (DGME) or propylene glycol monomethyl ether (PGME) applied to dogs and observed *A. americanum* mortality. The authors reported that permethrin diluted in DGME achieved over 90% mortality by the seventh post-treatment day (day 7) while the PGME formulation provided around 90% mortality by day 14 and continued to provide similar levels of control through day 21. Furthermore, permethrin caused 100% mortality in lone star ticks at concentrations of 0.01% through 0.0001% in bioassays using treated vials (Burridge et al. 2003). These studies demonstrate that *A. americanum* has historically been sensitive towards permethrin.

This study is the first quantified documentation of permethrin tolerance development in *A. americanum*. Although cyclodiene resistance has been reported in *A. americanum* collected in Oklahoma in 1955 (Brown 1971), this information was presented without quantifiable evidence. The permethrin RRs calculated in this study suggest that tolerance has begun to develop in both ticks collected from farmed and wild deer. Tolerance is defined as an RR ≥2 (Eiden et al. 2015), thus permethrin tolerant lone star ticks from farmed deer were collected in Alachua and Levy Counties (Table 3). While ticks on the farm in Levy County and Gadsden County were regularly exposed to permethrin, those on the farm in Alachua County were not. Therefore, it is not clear what is causing permethrin tolerance to develop on the farm in Alachua but not Gadsden County. However, permethrin is used to control a variety of medical, veterinary, and agricultural pests so it is possible the ticks on the farm in Alachua County were indirectly exposed. Overall, resistance ratio values for ticks collected from farmed deer were low and ranged between 1 and 2 (Table 3). It is likely that the low magnitude of the RRs is explained by the low frequency of resistance alleles in the population. Resistance mutations are generally present at a low frequency in wild-type populations. For example, Morgan et al. (2009) documented pyrethroid resistance in the domain II S 4–5 linker of the sodium channel in field-collected *R. microplus*. In populations that had 0% survival when challenged to the discriminating dose of cypermethrin, 94% of ticks were homozygous susceptible while 6% were heterozygous, however, the frequency of homozygous resistant alleles increased as survivorship towards the discriminating dose increased. This suggested that selection pressure increased the frequency of resistance genes. In *R. sanguineus*, the frequency of homozygous resistant alleles in permethrin resistant strains ranged from 88 to 100%, while heterozygous alleles were not detected in the susceptible reference strain (Tucker et al. 2017).

Specific locality information below the county level often was not available for the FFWCC collected ticks. However, the deer from which these ticks were collected were animals that had mostly died because of vehicular collisions on public roads, making it unlikely that these animals were from farms. The only permethrin tolerant tick samples collected from wild deer were collected in Volusia County (Table 4). However, in general, like the ticks from farmed deer, RR values ranged between 1 and 2. The lack of differences between LC values of the ticks collected from the two field-collected tick groups (farmed and wild deer) was unexpected because ticks from the wild deer were expected to have low permethrin exposure versus the high exposure of ticks from farmed deer. This might be

in part due to the ubiquitous presence of permethrin in the environment as demonstrated by permethrin residues detected on wild deer fur (Harmon 2019).

The nature of how pesticide resistance is studied can provide challenges for extrapolating the significance of this study. Most often resistance is detected when commercial products fail to control a pest, however, when this occurs resistance is established. This limits the quantity of comparison studies to predict the future rate of resistance development (Miller et al. 2001, 2005, Reck et al. 2014). In contrast, resistance development and mechanisms in *R. microplus* is well documented (Rodriguez-Vivas et al. 2006, Rosario-Cruz et al. 2009, Lovis et al. 2013, Thomas et al. 2020) in part because of its economic importance. The difference in resistance prevalence in *R. microplus* compared to the *A. americanum* in this study are potentially influenced by human and biological factors. For example, Rodriguez-Vivas et al. (2006) reported that 88.8% of farmers surveyed applied acaricide against *R. microplus* more than six times a year. Additionally, *R. microplus* is a one-host tick that spends most of its lifecycle on the host, this coupled with the high frequency of acaricide applied on cattle is the cause of rapid resistance development on ranches. The lone star tick is a three-host tick that will feed on a wide variety of animals that are typically not confined to a defined area, so even in instances where acaricides are used, there is potential for migration of susceptible individuals to slow resistance development. However, the increase in deer farming, represents an opportunity to study resistance development in *A. americanum* because the main host of these ticks are kept in a defined area, with frequent pesticide applications and limited movement of susceptible individuals. In the above examples the LC and DC were both used in the LPT to help survey acaricide resistance, with the LC and DC defined in this study similar experiments can be conducted for *A. americanum*.

One limitation of the study was the use of a colony to calculate the susceptible LC and DC values where wild ticks are introduced annually. While this could create variation in the colony, we believe that this variation leads to a more natural population as well as reducing inbreeding, for this reason the practice of introducing wild material regularly is common among those that maintain tick colonies. One concern is that the wild material could vary in pesticide sensitivity. However, for the susceptible colony used in this study (OSU), the wild ticks that are introduced are collected every year from the same cattle herd that is not treated with pesticides.

These data provide a baseline for future research into this area, but also are a call to action. Although, tolerance is currently low it could progress to resistance in the future if populations are left unattended. Resistance does not develop in a linear fashion, it follows a more logarithmic trend. Waiting for the first signs of resistance (or a RR of >10) will result in the need for a much more aggressive mitigation plan, which may or may not be effective. Furthermore, higher concentrations of acaricide and more frequent applications may be necessary to eliminate pest populations on animals. This elevated usage can have impacts on the health of humans, livestock, pets and other animals, and the environment. The lone star tick is a known vector of several bacteria including *Rickettsia* and *Ehrlichia* species, which are pathogenic to animals including humans (Goddard and Varela-Stokes 2009). As a result, there is a need for effective management strategies that can help interrupt the transmission of these pathogens. Resistance monitoring should be continued in high and low exposure areas (i.e. ticks from deer farms, and wild deer, respectively) to help guide management practices that can slow the development of permethrin resistance, such as acaricide class rotations.

Acknowledgments

Funding

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